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NRG1 Cleavage Assay and Small Molecule Screen for Modulators of NRG1 Processing

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1 Abstract

Schizophrenia is a group of severe mental disorders, often with unknown pathological mechanisms. Genetic studies implied a connection between schizophrenia and Neuregulin-1 (NRG1) signaling, which might become an important drug target. A mutation has been identified within the *NRG1* gene, which impairs its intracellular cleavage. The aim of this study is to find potential substances or compounds that could modulate the intracellular cleavage of NRG1. The NRG1 cleavage assay was developed from live cell assays into 96-well format and finally into 384-well format using robotics. The drug library for screening was the NIH Clinical Collection, of which all compounds are already approved by the American Food and Drug Administration (FDA). These drugs have passed clinical trials, so it may accelerate clinical application of validated hits. In this study, promising compounds were identified that have been used in the treatment of psychiatric diseases, such as Citalopram as an activator, or Ifenprodil, Perphenazine, Pamelor, Annoyltin as inhibitors of NRG1 cleavage. Additional validation of these compounds will be conducted, and once all critical controls are performed, the compounds may be applied in mouse models of schizophrenia and even clinical studies.

Abstract

Schizophrenie ist eine Gruppe von schweren psychischen Störungen, oft mit unbekannten pathologischen Mechanismen. Genetische Studien ließen auf eine Verbindung zwischen Schizophrenie und Neuregulin-1 (NRG1) schließen, die ein wichtiges Drug-Target werden könnte. Eine Mutation wurde im *NRG1*-Gen identifiziert, welche seine intrazelluläre Spaltung beeinträchtigt. Das Ziel dieser Studie ist, potentielle Substanzen oder Compounds zu finden, die die intrazelluläre Spaltung von NRG1 modulieren können. Der NRG1 Spaltungs-Assay wurde vom Lebendzell-Assays in 96-Well-Platten und schließlich in 384-Well-Platten für automatisiertes Screening adaptiert. Die Medikamentendatenbank „NIH Clinical Collection“ enthält Substanzen, von denen alle bereits von der amerikanischen Food and Drug Administration (FDA) zugelassen sind. Diese Compounds haben klinische Studien durchlaufen, so dass eventuelle klinische Applikation von wirksamen Substanzen beschleunigt werden kann. In dieser Studie werden vielversprechende Hits identifiziert, die schon bei der Behandlung von psychiatrischen Erkrankungen verwendet werden, wie zum Beispiel Citalopram als Aktivator, oder Ifenprodil, Perphenazine, Pamelor, Annoyltin als Inhibitoren der NRG1-Spaltung. Zusätzliche Validierungen dieser Compounds sind notwendig, um diese dann in Mausmodellen der Schizophrenie und schlussendlich in klinischen Studien einzusetzen.

2 Introduction

2.1 Schizophrenia

Schizophrenia is a complex mental disorder that causes a range of different psychological symptoms, including positive symptoms, negative symptoms, and cognitive dysfunction (Tandon et al. 2013). Positive symptoms represent a change in behavior or thoughts such as hallucinations, delusions, or other reality distortions (Tandon, Nasrallah, and Keshavan 2009). Negative symptoms involve a withdrawal or lack of affective function so that people with schizophrenia often appears emotionless, flat, and apathetic (Tandon, Nasrallah, and Keshavan 2009). Cognitive dysfunction describes deficits in global intellectual performance or in cognitive abilities, such as learning disorder or memory impairment (Hegde et al. 2013; Aquila and Citrome 2015).

The diagnostic criteria for schizophrenia are based on the clinical manifestation, since there is no known pathognomonic biological marker (Tandon et al. 2013). Clinicians and researchers use the Diagnostic and Statistical Manual of Mental Disorder (DSM) as a manual to diagnose and classify mental illness (Table 1). In DSM-5, schizophrenia subtypes (paranoid, disorganized, catatonic, undifferentiated, and residual) no longer belong to the diagnostic criteria.

The causes of schizophrenia are multifactorial, involving neurobiological, psychological, social, environmental, and genetic factors (Paul J. Harrison and Owen 2003; Kuswanto et al. 2015). These exemplary factors, such as prenatal health issues (prenatal stress, prenatal infection, intrauterine malnutrition, brain hypoxia), infections, drugs (cannabis, hallucinogens, alcohol), smoking, and many other factors are shown to contribute to development of schizophrenia (Ameri 1999; Stathopoulou, Beratis, and Beratis 2013; Nielsen, Meyer, and Mortensen 2016). Evidence also supports elevated prenatal and family environmental disruptions in families with a first-degree relative with schizophrenia (Walder et al. 2014).

Genetics also play a significant role in the development of schizophrenia. Some studies suggested that several susceptibility genes have been identified, including neuregulin (*NRG*), *dysbindin, ERBB4, COMT, DISC1*, *RGS4, GRM3,* and *DAO* (Chumakov et al. 2002, 30; Williams et al. 2003; Stefansson et al. 2004; P. J. Harrison and Weinberger 2005; D. Li and He 2007, 30; Lu et al. 2010). Recently, a mutation has been found in the genes that encode NRG1 and its receptor ERRBB4 (Y. Chen et al. 2010; Lu et al.

2010). The identification of this mutation has provided a useful starting point to learn more about the pathological mechanism of schizophrenia (Mei and Xiong 2008).

Identification of risk loci that associated with schizophrenia is done with genome-wide association study (GWAS). In this case, GWAS examines the genetic variants (e.g. single-nucleotide polymorphism) and its connection with schizophrenia by comparing the DNA of two groups of different individuals. The participants in this study are the patients with schizophrenia and the healthy ones for control. If the genetic variant is more frequent in patients, then that variant might be associated with the disease. Research of these associated genes may lead to better understanding of the pathophysiological and etiological of the disease and the development of more effective treatments (Bergen and Petryshen 2012; Bush and Moore 2012; Ripke et al. 2014; Paul J Harrison 2015).

A genome-wide scan in Icelandic population provided the first genetic evidence, suggesting a linkage of schizophrenia to *NRG1* (Stefansson et al. 2002). The affected individuals were estimated to have 2.2 times the risk, as compared to controls (Stefansson et al. 2002). There is also a strong correlation between NRG1 and schizophrenia in Chinese population (Tang et al. 2003; Yang et al. 2003). However, the follow-up studies in different population, such as Scotland and Wales, showed lower ratio than that in Iceland (Stefansson et al. 2003; Williams et al. 2003). It was also confirmed that in Japanese population, there was no association between NRG1 and schizophrenia (Iwata et al. 2003). This highlights the fact that NRG1 is not necessarily related to all cases of schizophrenia. There are also other numerous genes that contribute in this complex disease (Corfas, Roy, and Buxbaum 2004).

Mutation of these genes results in disruption or dysregulation of the following pathways. Impaired NRG1-ERBB4 signaling affects both excitatory and inhibitory synaptic transmission in the adult brain (Mei and Xiong 2008; Mei and Nave 2014). The resulting abnormalities of altered neurotransmission and cortical function may cause psychotic symptoms and cognitive impairments during development, which can be observed in mouse models (Rimer et al. 2005; Arguello and Gogos 2006; Bjarnadottir et al. 2007).

Table 1. Diagnostic criteria for schizophrenia from DSM-5. Modified from (Tandon et al. 2013).

Criterion A	**Characteristic symptoms:** Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated). At least one of these should include 1-3 1. Delusions 2. Hallucinations 3. Disorganized speech 4. Grossly disorganized or catatonic behavior 5. Negative symptoms (i.e., diminished emotional expression or abolition)
Criterion B	**Social/occupational dysfunction** For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning, such as work, interpersonal relations, or self-care, are markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic, or occupational achievement).
Criterion C	**Duration of 6 months** Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or by two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).
Criterion D	**Schizoaffective and mood disorder exclusion** Schizoaffective disorder and depressive or bipolar disorder with psychotic features have been ruled out because either (1) no major depressive or manic episodes have occurred concurrently with the active phase symptoms; or (2) if mood episodes have occurred during active-phase symptoms, their total duration has been brief relative to the duration of the active and residual periods.
Criterion E	**Substance/general mood condition exclusion** Substance/general medical condition exclusion: The disturbance is not attributed to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition.
Criterion F	**Relationship to Global Developmental Delay or Autism Spectrum Disorder** If there is a history of autism spectrum disorder or other communication disorder of childhood onset, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least 1 month (or less if successfully treated).

2.2 Neuregulins and ERBB4

2.2.1 Neuregulin

Neuregulins (NRGs) are signaling proteins that comprise a family of epidermal growth factor (EGF)-like proteins (Mei and Nave 2014). The encoding gene for NRG1 is located on the short arm of chromosome 8 (Falls 2003). Neuregulins have multiple biological functions, which are essential for embryogenesis (Burden and Yarden 1997). Together with its receptors ERBB3-4, neuregulins play a role in the cardiac development by helping with the formation of the heart trabeculae (Gassmann et al. 1995; Burden and Yarden 1997). Neuregulins also influence the differentiation of Schwann cells and oligodendrocytes in the myelination of neurons (Vartanian, Fischbach, and Miller 1999). Furthermore, neuregulins contribute in neuromuscular synapse formation by stimulating the transcription of acetylcholine receptor (AchR) subunits (Burden and Yarden 1997).

The neuregulin family has four members: NRG1, NRG2, NRG3, and NRG4 (Falls 2003). NRG1 is the best characterized among these. Each *NRG* gene produces multiple isoforms by differential splicing and the use of alternative promoters and transcriptional start sites. For example, *NRG1* generates more than 30 different proteins (Mei and Xiong 2008) (Figure 1). Each type of NRG1 isoform has an EGF-like domain that activates ERBB receptor tyrosine kinases (Falls 2003).

Fully synthesized NRG1 isoforms are called pro-NRG1s. Most of them are single transmembrane proteins, with the EGF-like domain located in the extracellular region. NRG1 Type III is unique among other NRG1 proteins because it has not only one but two transmembrane domains, forming a so-called hairpin. Both its N- and C-terminal regions are located intracellular. NRG1 Type III also has a cysteine-rich domain (CRD), whose hydrophobic segment serves as the second transmembrane domain (N- terminal transmembrane domain) (Falls 2003).

When pro-NRG1s undergo proteolytic cleavage, a mature NRG1 (Ecto-NRG1) is released that contains the EGF-like domain and is soluble, except in the case of NRG1 Type III. Because of its hairpin nature, the EGF-like domain is still membrane-tethered with the N-terminal fragment of NRG1 Type III (NRG1-NTF) (Falls 2003). It was later revealed that the EGF-like domain of NRG1 Type III can be released from its NRG1-NTF (Fleck et al. 2013). Details of the proteolytic processing of NRG1 will be discussed below.

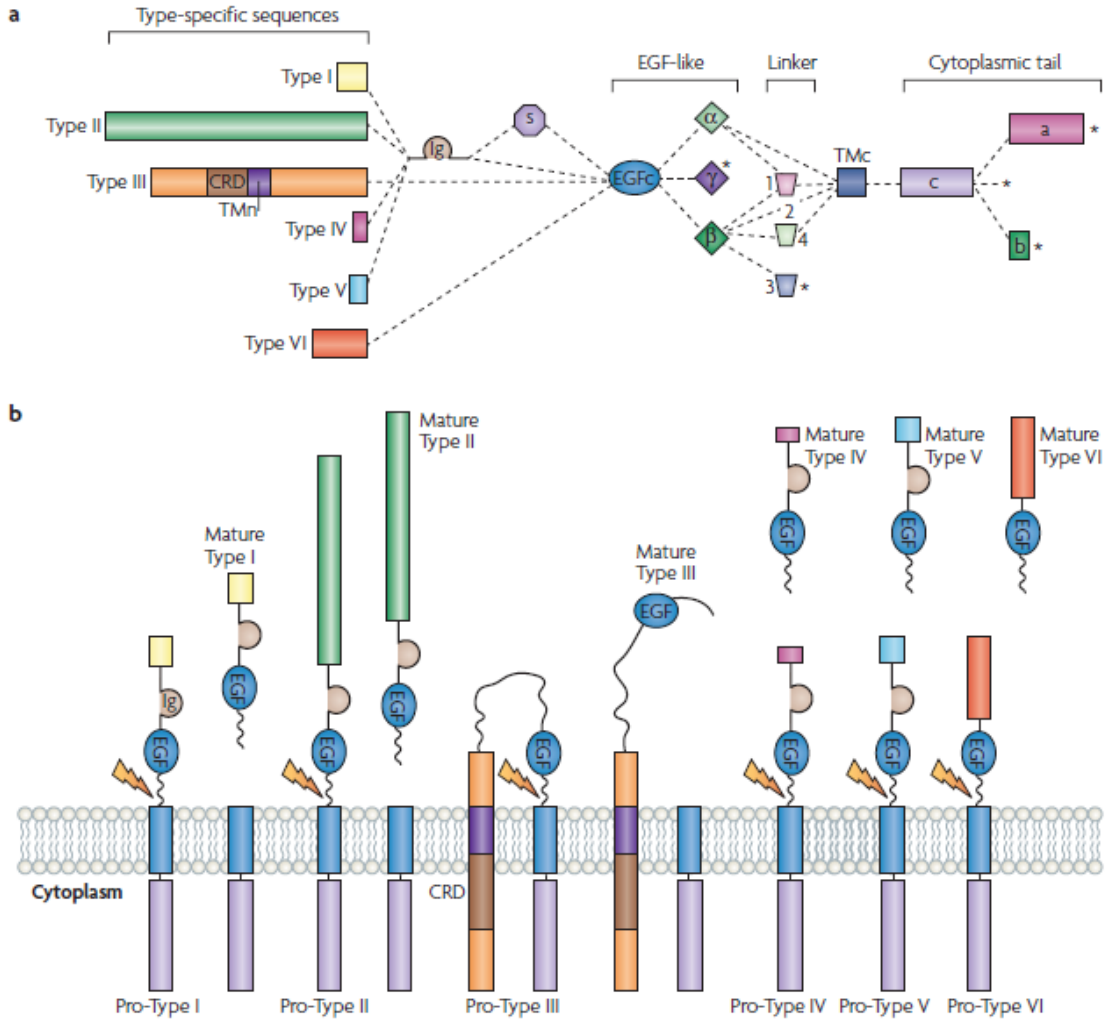

Figure 1: Different types of NRG1 isoforms. (a) The six types of NRG1 isoforms are classified according to their distinct amino-terminal sequences. Every single isoform has EGF-like domain. Type III NRG1 has a transmembrane domain within the cysteine-rich domain (CRD). Type I, II, IV, and V have immunoglobulin-like domain (Ig), with or without the spacer region (S). (b) Most NRG1 isoforms are synthesized as a transmembrane protein (pro-NRG1s) with the EGF-like domain located extracellular. Type III has both its N- and C-terminal fragment located intracellular. Extracellular cleavage by tumor necrosis factor-α converting enzyme (TACE) or β-site amyloid precursor protein cleaving enzyme 1 (BACE1) (indicated by the lightning arrow) generates a soluble mature NRG1, except for NRG1 Type III. The processing of NRG1 Type IV, V, and VI is still unclear, but is thought to resemble that of Type I and II. Adapted from (Mei and Xiong 2008).

2.2.2 ERBB4

ERBB proteins belong to the superfamily of receptor tyrosine kinases (RTKs). ERBB receptor is structurally similar to its founding member, epidermal growth factor receptor (EGFR), also known as ERBB1. ERBB family of proteins in humans includes HER1 (EGFR, ERBB1), HER2 (NEU, ERBB2), HER3 (ERBB3), and HER4 (ERBB4). Each member of the family has an extracellular ligand-binding domain, a single transmembrane region, and a cytoplasmic protein tyrosine kinase domain (Cho and Leahy 2002; Mei and Xiong 2008).

After ligands of the EGF family bind the extracellular domain of ERBB receptors, the ERBB proteins dimerize to form homo- or heterodimers. ERBB1 does not bind to NRG1, but can form a heterodimer with ERBB4. ERBB2 also does not bind to NRG1, but can form heterodimer with all other ERBBs. ERBB3 can bind to NRG1 but has impaired kinase activity, so it needs to form a heterodimer either with ERBB2 or ERBB4 in order to be functional. Only ERBB4 can bind to NRG1 as a homodimer. Dimerization triggers autophosphorylation of specific tyrosine residue in the cytoplasmic domain. The intracellular signaling cascade begins eventually (Olayioye 2000; Bublil and Yarden 2007; Mei and Xiong 2008). Figure 2 refers to the domain structure and proteolytic processing (that is very similar to the cleavage of NRG1) of ERBB4.

Among NRG1s receptors, ERBB4 is of particular interest due to its crucial role in neurodevelopment (Anton et al. 2004). Research has also found an association between a particular single nucleotide polymorphism (SNP) in the *ERBB4* gene and schizophrenia (Lu et al. 2010).

Loss of ERBB proteins results in embryonic or perinatal lethality in mice, depending on its type. It also contributes to cardiac, sexual, and neuronal development (Olayioye 2000). Overexpression of ERBB receptors, especially ERBB2/HER2, is found in many human cancers, especially in breast cancer. A monoclonal antibody against ERBB2/HER2 receptor, called Trastuzumab (Herceptin), is a choice of treatment for breast cancer (Shak 1999).

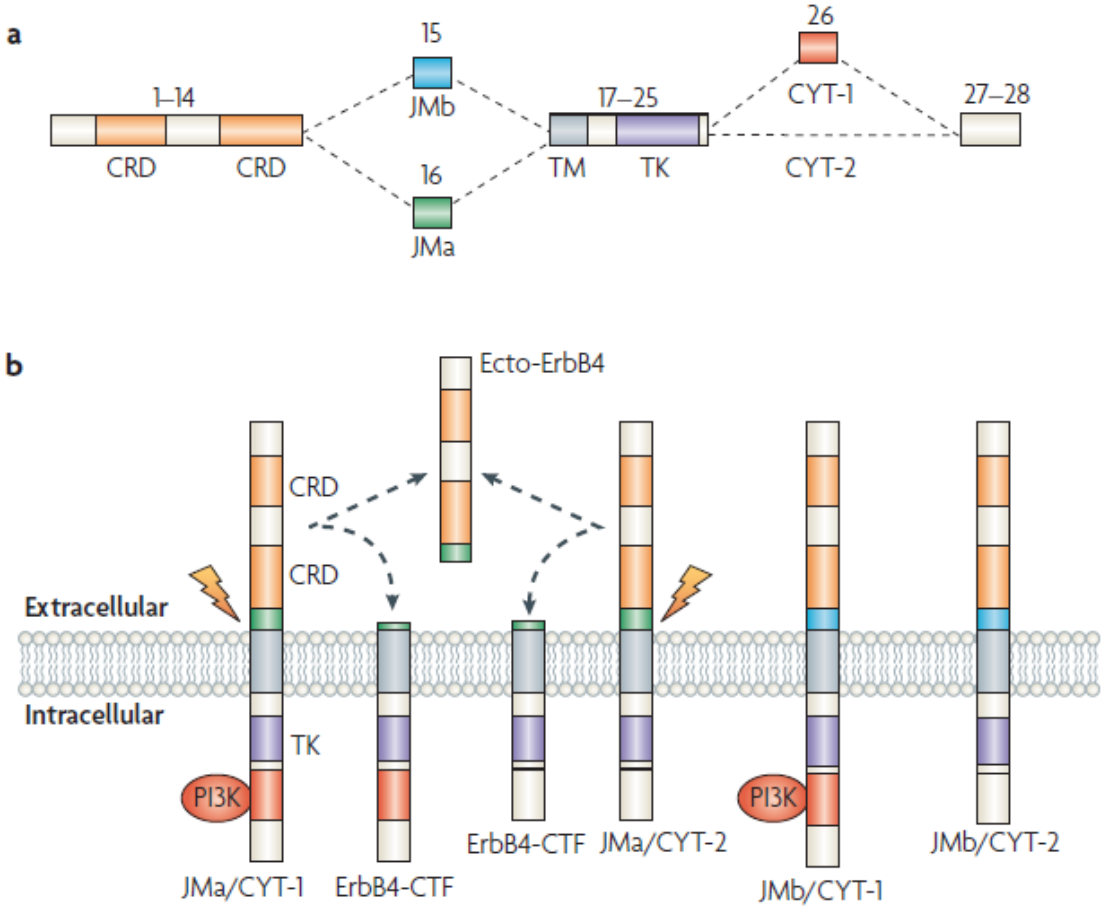

Figure 2: Domain structures of ERBB4. (a) Exon numbers are displayed above the domain structures. Inclusion of exon 26 generates CYT-1. Exclusion of it generates CYT-2. The extracellular juxtamembrane region is encoded either by exon 15 or exon 16 to produce JMb or JMa respectively. This combination makes four possible isoforms of ERBB4. (b) CYT-1 has the ability to bind to phosphoinositide 3-kinase (PI3K) and activates the signaling pathway (Junttila et al. 2000). Both JMa and JMb can be activated by NRG1, but only JMa can be cleaved by tumor necrosis factor-α converting enzyme (TACE) (indicated by the lightning arrow) (Rio et al. 2000). The cleavage produces a soluble Ecto-ERBB4 and a membrane-anchored C-terminal fragment of ERBB4 (ERBB4-CTF). CRD, cysteine-rich domain; TM, transmembrane domain; TK, tyrosine kinase domain. Adapted from (Mei and Xiong 2008).

2.3 NRG1 signaling

NRG1/ERBB4 signaling plays a critical role in neural development. Impairment of this signaling network has been thought to be connected to psychiatric diseases such as schizophrenia (Hahn et al. 2006). Studies also showed that abnormal NRG1/ERBB4 signaling is detected in postmortem brains of patients with schizophrenia (Hahn et al. 2006). There are three types of NRG1 signaling: canonical forward signaling, non-canonical forward signaling, and backward signaling. Therefore, signaling between NRG1 and ERBBs (and perhaps between RTKs and their ligands in general) should be considered as bidirectional.

2.3.1 Canonical forward signaling

The soluble Ecto-NRG1 contains the EGF-like domain, which is necessary for the activation of ERBB receptor tyrosine kinases. Upon stimulation by NRGs, the ERBB proteins form homo- or heterodimer (see above). The activation of the tyrosine kinase domains leads to phosphorylation of the intracellular domain, resulting in the initiation of signaling pathways. The main signaling cascades downstream of NRG1-ERBB4 are the mitogen-activated protein kinase (MAPK) pathways (e.g. via Raf-MEK-ERK) and the phosphoinositide 3-kinase (PI3K)/Protein Kinase B (PKB, also known as AKT) pathway (Earp et al. 1995; Y. Liu et al. 2007).

2.3.2 Non-canonical forward signaling

The extracellular cleavage of ERBB4 can be induced by binding with NRG1 as its ligand, or by protein kinase C (PKC) activation (Vecchi and Carpenter 1997; Ni et al. 2001). The cleavage requires a metalloprotease called tumor necrosis factor-α converting enzyme (TACE, or ADAM 17). It is an enzyme that belongs to the 'a disintegrin and metalloprotease' family (ADAM) (Rio et al. 2000). Only the extracellular juxtamembrane-a (JMa) isoform of ERBB4, not the juxtamembrane-b (JMb) isoform, can be cleaved by TACE (Rio et al. 2000). ERBB4 protein, cleaved by TACE, produces Ecto-ERBB4, which is soluble, and ERBB4-CTF, which is still membrane-anchored. The C-terminal fragment of ERBB4 (ERBB4-CTF) is further cleaved by an enzyme called γ-secretase in its transmembrane domain, leaving ERBB4-β and ERBB4-ICD. The soluble intracellular domain of ERBB4 (ERBB4-ICD) can translocate into the nucleus and regulate transcription (Ni et al. 2001; Sardi et al. 2006). This mechanism of sequential cleavage by TACE, BACE1, and γ-secretase is shared by

a number of proteins such as amyloid precursor protein (APP), voltage-gated sodium channel (VGSC) β, Interleukin-1 Receptor II (IL-1R2), and NRG1 (see below) (Wong et al. 2005; Kuhn et al. 2007).

2.3.3 Backward signaling

The pro-NRG1s undergo a similar proteolytic processing as the ERBBs (Mei and Xiong 2008). After the cleavage of the extracellular domain of pro-NRG1, the remaining membrane-anchored region (NRG1-CTF) can be further cleaved by γ-secretase to release the intracellular domain of NRG1 (NRG1-ICD) from the membrane. These proteolytic cleavages can also be stimulated by ERBB4 binding or neuronal membrane depolarization. Considering that the transmembrane isoforms of pro-NRG1 are bidirectional signaling function, the pro-NRG1 can act as a receptor for the ERBB4 ligand, either as a soluble Ecto-ERBB4 or as a transmembrane form (Bao et al. 2003; Mei and Xiong 2008; Canetta et al. 2011). Afterwards, the NRG1-ICD translocates to the nucleus to regulate gene expression (Bao et al. 2003; Mei and Xiong 2008) (Figure 3).

Signaling via the NRG1-ICD has not been fully characterized. There is some evidence that NRG1-ICD contributes to neural and cardiac development (X. Liu et al. 1998). Research also supports the signaling function of NRG1-ICD (Chong et al. 2008). Once in the nucleus, the NRG1-ICD represses expression of several regulators of apoptosis (Weinstein and Leder 2000; Bao et al. 2003). NRG1-ICD also forms specific complexes with cytoplasmic proteins, in particular with Lin11, Isl-1, & Mec-3 (LIM) kinase 1, which helps in controlling the organization of actin filaments (Wang et al. 1998; Sparrow et al. 2012). Another publication indicates that NRG1-ICD enhances the transcriptional activity of the PSD 95 promoter by binding to a zinc finger transcription factor, Eos (Bao et al. 2004).

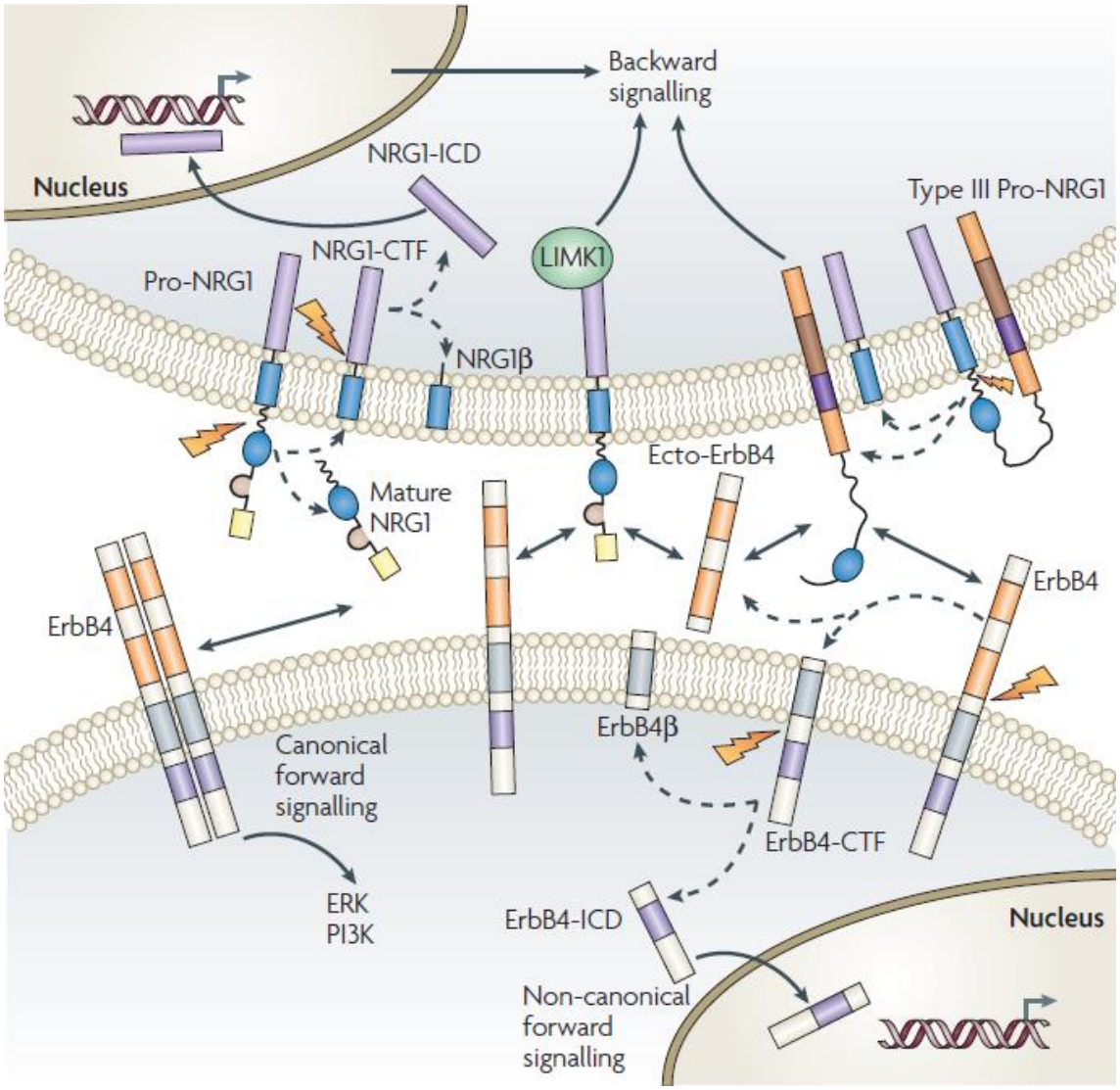

Figure 3: NRG1-ERBB4 signaling. In canonical forward signaling (bottom cell, left-hand pathway), binding of a mature NRG1 activates ErbB4 tyrosine kinase receptor to form a homodimer. The subsequent autophosphorylation of intracellular domain leads to signaling pathways, such as extracellular signal-regulated kinase (ERK) pathway or phosphoinositide 3-kinase (PI3K) pathway. In non-canonical forward signaling (bottom cell, right-hand pathway), ErbB4 is cleaved by TACE and produces soluble Ecto-ERBB4 and ERBB4-CTF. ERBB4-CTF is further cleaved by γ-secretase to generate ERBB4-β and ERBB4-ICD, which translocates to the nucleus to regulate gene expression. Backward signaling (top cell) by pro-NRG1 can proceed through enzymatic cleavage (left-hand pathway) and can be stimulated by ERBB4 binding (right-hand pathway). The released NRG1-ICD from NRG1-CTF relocates to the nucleus and regulates gene transcription. The cytoplasmic tail of pro-NRG1 can also interact with the protein kinase LIM kinase 1 (LIMK1) (Wang et al. 1998). Adapted from (Mei and Xiong 2008).

2.4 NRG1 cleavage

The pro-NRG1 can be extracellularly cleaved by β-site amyloid precursor protein cleaving enzyme 1 (BACE1), ADAM10, or TACE (ADAM17) (Fleck et al. 2012, 2013). BACE1 is a protease that plays a role in Alzheimer's disease. BACE1 cleaves extracellularly the amyloid precursor protein (APP), generating a soluble extracellular fragment and a membrane bound fragment. The γ-secretase cleaves at its transmembrane domain, releasing the intracellular domain of APP and amyloid-β peptide (Aβ). This accumulation of amyloid-β peptides are found in the brain of Alzheimer's patient. Considering the relationship of Aβ and BACE1, the inhibition of BACE1 should reduce the level of Aβ generation, which can be used as a treatment for patients of Alzheimer's disease (Cui et al. 2015; Hu et al. 2016). However, the side-effects due to many other substrates of BACE1 must be taken into account.

NRG1 has also been identified as BACE1 substrate, besides APP, type II α-2,6-sialyltransferase (ST6Gal-1), platelet selectin glycoprotein ligand-1 (PSGL-1), and interleukin receptor type II (Kitazume et al. 2001; Lichtenthaler et al. 2003; Hu et al. 2006; Willem, Lammich, and Haass 2009). Together, BACE1 and NRG1 are essential in cardiac and neuronal development. BACE1 is required for peripheral nerve myelination through NRG1 Type III processing (Hu et al. 2006; Willem et al. 2006). Because of this physiological function of BACE1, therapeutic enzyme inhibition as a therapy for Alzheimer's patient must be done with extreme caution.

Most of the NRG1 isoforms release a soluble Ecto-NRG1, which contain the EGF-like domain to stimulate paracrine signaling, and leaving a membrane-anchored domain called NRG1-CTF (C-terminal fragment). In the case of NRG1 type III, both the N-terminal fragment and the C-terminal fragment are still membrane bound after the extracellular cleavage because of its hairpin form, suggesting signaling in a juxtacrine (direct-contact) manner, whereas the other types of NRG1 are specialized in paracrine signaling (Falls 2003). Recent studies have shown that a dual cleavage by BACE1 and TACE liberates the EGF-like domain of NRG1 type III from the membrane anchor and also allows paracrine signaling (Fleck et al. 2013).

The NRG1-CTF is further cleaved by γ-secretase in its transmembrane domain, starting at the ε-like site and ending at the γ-site. This intramembrane cleavage creates NRG1-ICD, which is involved in backward signaling, and a NRG1-β peptide (Fleck et al. 2016) (Figure 4 and Figure 5).

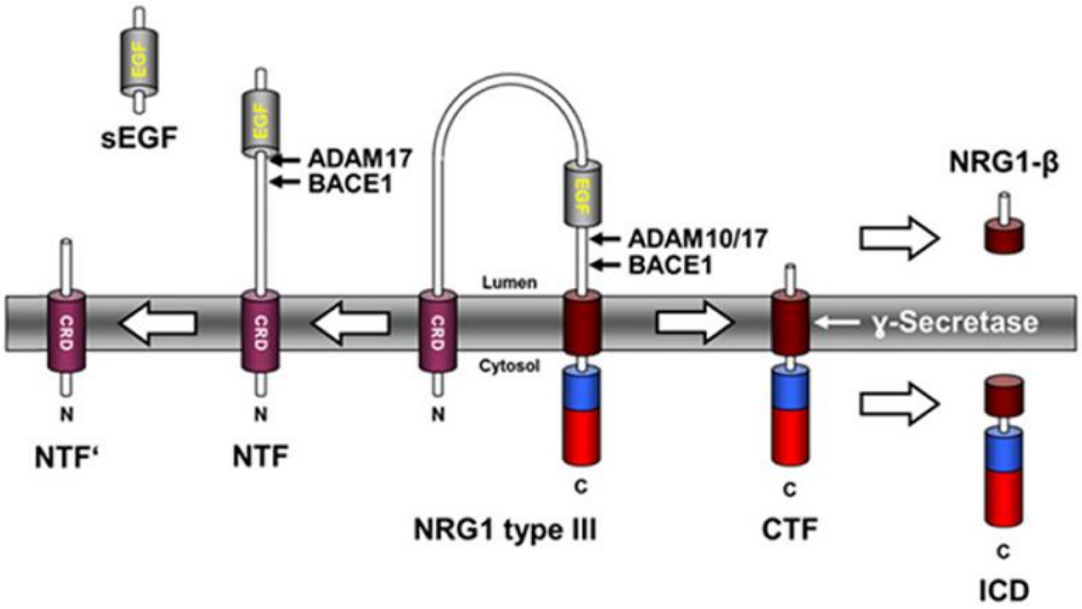

Figure 4: Cleavage processing of NRG1 Type III. Extracellular cleavage by ADAM10/17 generates NRG1-NTF (N-terminal fragment) and NRG1-CTF (C-terminal fragment). NRG1-NTF can be further cleaved by ADAM17 and BACE1 to liberate EGF-like domain from membrane-tethered NRG1-NTF. NRG1-CTF is cleaved by γ-secretase in the transmembrane domain and releases NRG1-β and NRG1-ICD. Modified from (Fleck et al. 2016).

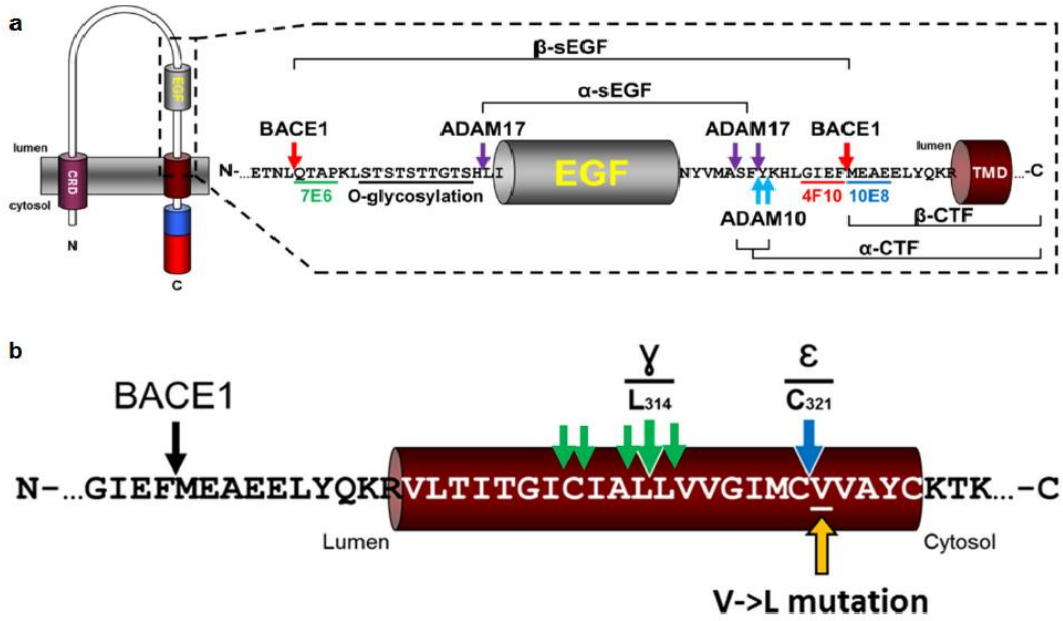

Figure 5: Identified cleavage sites of NRG1 Type III. (a) Cleavage sites by respective proteases are marked by arrows. (b) Intramembrane cleavage sites by γ-secretase at ε-like site (blue arrow) and at γ-like sites (green arrows). A single nucleotide polymorphism (Valine to Leucine) in the transmembrane domain is also represented (yellow arrow). CRD, cysteine-rich domain; EGF, epidermal growth factor; TMD, transmembrane domain. Adapted from *(Fleck et al. 2013, 2016)*

2.5 NRG1 mutation in schizophrenia

NRG1 has been identified as a susceptibility gene for schizophrenia in several populations (Stefansson et al. 2002, 2003). Research has found that there is a mutation within the transmembrane domain of NRG1 Type III. A particular SNP causes an amino acid change from valine to leucine at residue 321(Walss-Bass et al. 2006; Y. Chen et al. 2010). This valine-to-leucine substitution is associated with increased risk of schizophrenia in humans (Walss-Bass et al. 2006; Y. Chen et al. 2010). The mutation results in an accumulation of NRG1-CTF, suggesting that the γ-secretase cleavage is compromised (Fleck et al. 2016). Moreover, this mutation does not only reduce the generation of NRG1-ICD, but also affects the cleavage precision of γ-secretase (Fleck et al. 2016). The following defective backward signaling might impair a subset of NRG1 functions in cortical development and contribute to abnormal neuroconnectivity involved in schizophrenia (Y. Chen et al. 2010).

There are several reports indicating that overexpression of NRG1 in mice may contribute to the development of schizophrenia (Deakin et al. 2009; Weickert et al. 2012). Elevated level of NRG1-ICD protein is also found in the brain of schizophrenic patients (Chong et al. 2008). Other reports state that a decreased expression of NRG1 Type III may lead to hypomyelination in several brain regions (Taveggia et al. 2008; Makinodan et al. 2012) and some behavioral alterations that are also connected to schizophrenia (Y.-J. J. Chen et al. 2008). In behavioral studies, hypomorphic NRG1 mutant mice displayed hyperactivity in multiple tests, which can be suppressed by clozapine, an antipsychotic drug used to treat schizophrenia (Gerlai, Pisacane, and Erickson 2000; Stefansson et al. 2002). Another study said hypomorphic ErbB4 mutant mice showed hypoactivity syndrome (Golub, Germann, and Lloyd 2004). These results suggested an inverted U-shaped effect, in which both over- and under-expressing mice showed suspicious behavior related to schizophrenia (Deakin et al. 2009). This inverted U-shaped model has also been hypothesized for NRG1 signaling in relation to synaptic plasticity (Role and Talmage 2007).

2.6 NRG1 cleavage assay

NRG1 cleavage assay is focused on the backward signaling of NRG1, using a luciferase-based reporter gene assay. Reporter gene assays are used to analyze the NRG1 cleavage indirectly through luciferase expression (Figure 6). Luciferase is an enzyme that catalyzes a reaction with its substrate luciferin to produce bioluminescence. This luminescence can be measured using existing equipment.

The assay was established in a rat adrenal pheochromocytoma cell line called PC12 (Greene and Tischler 1976). The intracellular domain of NRG1 type I and type III is tagged with the Gal4-VP16 (GV) transcriptional activator. Gal4-VP16 is a hybrid protein that combines a DNA-binding fragment of the yeast activator (Gal4) and herpes simplex virus protein VP16 (also known as Vmw65) (Sadowski et al. 1988, 4). After the γ-secretase cleavage, the NRG1-ICD-GV will translocate to the nucleus and bind specifically to the clustered upstream activating sequence (UAS) (Duffy 2002). The UAS is fused with the reporter gene luciferase, activating its transcription (Webster et al. 1988) (Figure 6).

The luciferase activity was first measured in living cells with a LumiCycle 32-channel Luminometer (Actimetrics). The luciferase induction kinetics provided some crucial information, such as timing of optimal NRG1 cleavage and conditions of cleavage stimulation or inhibition. The assay was also adapted in both 96-well and 384-well microplates and could be measured using a microplate reader (Berthold Technologies). Potential constructs or compounds that were identified during the experiments were added as controls for the screen. The drug screen was then performed to find the modulators of NRG1 cleavage.

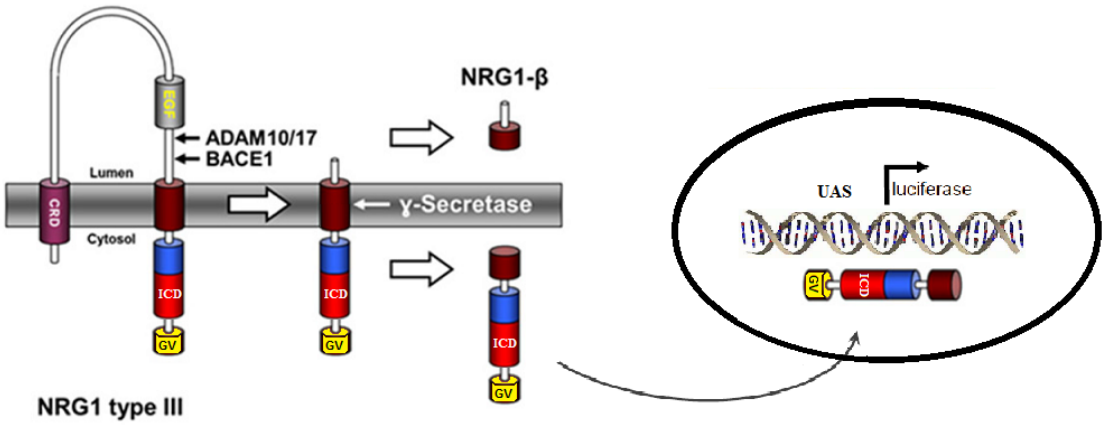

Figure 6: NRG1 cleavage assay. An example of NRG1 Type III construct. The cytoplasmic tail is fused with GV. After the γ-secretase cleavage, NRG1-ICD is released and translocate into the nucleus. GV binds specifically to UAS, eventually resulting in activation of the luciferase expression. The luciferase activity will be measured with LumiCycle or a microplate reader. CRD, cysteine-rich domain; EGF, epidermal growth factor; GV, Gal4-VP16; ICD, intracellular domain; UAS, upstream activating sequence. Modified from (Fleck et al. 2016).

3 Aim of the study

Schizophrenia is a psychiatric disorder that seriously affects the lives of the patients. Medication that can alleviate positive symptoms and cognitive deficits has yet to be found. Genetic studies contribute towards researching the pathophysiological mechanisms of schizophrenia. *NRG1* and *ERBB4* have been identified as susceptibility genes. Association of defective NRG1-ERBB4 signaling and neurological dysfunction implicated in schizophrenia has been confirmed by many studies. These findings signify the importance of this mechanism, which might eventually lead to new drug developments and therapeutics for patients with de-regulated NRG1-ERBB4 signaling.

The aim of this study was to establish a robust and sensitive NRG1 intracellular cleavage assay in a plate format suitable for high-throughput screening (HTS). The assay was first tested with different constructs and compounds to find precise conditions in which the cleavage is stimulated or inhibited. The protocols were adapted to both 96-well and 384-well format, and a small molecule screen was performed including previously identified positive and negative controls.

As compound library, the National Institutes of Health (NIH) clinical collection was used, which consists of 727 small molecules. The screen was performed to find potential modulators of NRG1 processing from the compound library, both as activators and inhibitors. Once potential hits have been identified, they will undergo several secondary assays for validation. Since the Food and Drug Administration (FDA)-approved drugs from the compound library have already passed the preclinical and clinical trials, the validated compounds can be quickly tested on animal models and eventually on human. Moreover, these compounds might have a new potential that could be used for a treatment in new disease areas (drug repurposing).

4 Materials and Methods

4.1 Materials

4.1.1 Chemicals and reagents

Table 2. List of chemical and reagents used

Name	Manufacturer
$(MgCO_3)_4$*$Mg(OH)_2$*$5H_2O$	Merck
6x DNA Loading Dye	Thermo Scientific
Agarose	Bio-Rad
Ampicillin	Sigma
ATP	PJK
BSA	Sigma
Coelenterazin	PJK
Coenzyme A	PJK
DAPT	Enzo Life Sciences
Dialyzed FBS	Life Technologies
D-Luciferin	PJK
DMEM, 1 g/L Glucose, 500 ml	Lonza
DMSO	Merck
DNA Ladders (100 bp, 1kb)	Thermo Scientific
DTT	PJK
EDTA	Sigma
Ethanol	Sigma
EtOH	Merck
GlutaMAX	Life Technologies
HCl	Merck
Horse Serum	Life Technologies
Kanamycin	Sigma
KOH	Merck
$KxPO_4$	Merck
Lipofectamine 2000	Life Technologies
LY2811376	MedChem Express
$MgSO_4$*$7H_2O$	Merck
Na_2-EDTA	Merck
NaN_3	Merck
NaOH	Merck
Opti-MEM	Life Technologies
Passive Lysis Buffer (PLB)	Promega
PBS	Biochrom
Penicillin	Sigma
Poly-L-lysine Hydrobromide	Sigma-Aldrich
Streptomycin	Sigma
TAPI-1	Sigma Aldrich
Tricine	Merck
Trypan blue	Sigma
Trypsin 2,5 % (10X)	Life Technologies
Zeocin	Thermo Scientific

4.1.2 Laboratory equipment

Table 3. List of laboratory equipment used

Name	Manufacturer
Centrifuge 5810 R	Eppendorf
Delfia Plate shake	Wallac
Microscope Primovert	Zeiss
Finnpipette F1 Multichannel-pipette	Thermo Scientific
Heraeus Megafuge 16 Centrifuge	Thermo Scientific
Heracell 240i CO_2 incubator	Thermo Scientific
LumiCycle 32-channel Luminometer	Actimetrics
BioPhotometer 6131	Eppendorf
Safe 2020 Class II Biological Safety Cabinets	Thermo Scientific
Picodrop	Picodrop
Automated liquid handling system Microlab STAR Line	Hamilton Robotics
Luminometer	Berthold Technologies

4.1.3 Plasmids

Table 4. List of plasmids used

Constructs	Short name
pTag4C_mNrg1-typeI-beta1a-GV-2HA	N1-I-GV
pTag4C_mNrg1-typeIII-beta1a-GV-2HA	N1-III-GV
pTag4C_mErbb4-JMa-Cyt1-Flag	ERBB4-F
pcDNA3.1_BACE1 (V1662)	BACE1
pG5_Fluc	G5-luc
pGL4.20_10xUAS-MLP-luc2/Puro	G10-MLP-luc
pGL4.16_10xUAS_CMVmin_EXT-luc2	G10-CMV-luc
pcDNA3.1	pcDNA
TK Renilla TC35	TK Renilla

4.1.4 Restriction enzymes

Table 5. List of restriction enzymes used

Enzyme	Sequence	Supplied NEBuffer
BsrGI	T/GTACA	NEBuffer 2.1
HindIII-HF	A/AGCTT	CutSmart Buffer
HpaI	GTT/AAC	CutSmart Buffer
NcoI	C/CATGG	NEBuffer 3.1
PstI-HF	CTGCA/G	CutSmart Buffer
XbaI	T/CTAGA	CutSmart Buffer

4.1.5 Ready-made systems (Kits)

Table 6. List of kits used

Name	Manufacturer
NucleoBond Xtra Midi EF	Macherey-Nagel
NucleoSpin Plasmid Miniprep kit	Macherey-Nagel

4.1.6 Mammalian cell lines

PC12 rat adrenal medulla pheochromocytoma cell line

4.1.7 Solutions and buffers

4.1.7.1 Solutions for cell culture

PC12 growth medium

DMEM (1g/L Glucose)	500 ml
FBS	10 %
Horse Serum	5 %
GlutaMAX	1 %
Penicillin/Streptomycin	1 %

PC12 antibiotic free medium

DMEM (1g/L Glucose)	500 ml
FBS	10 %
GlutaMAX	1 %

PBS (1x)

PBS powder	9.55 g
ddH2O	1 L

Adjust to pH 7.2 with 1 M NaOH

PLL (0.02 mg/ml)

Dilute PLL with ddH2O to a concentration of 5 mg/ml

Aliquot in 1 ml volumes and freeze at -20°C

To create working solutions (0.02 mg/ml) dilute the aliquots 1:250 with ddH2O

Store in 50 ml falcons at -20°C

Trypsin-EDTA (10x, 100 ml)

Trypsin	5 g
EDTA	6.85 mM

Dilute Trypsin-EDTA 1:10 with PBS

Store in 50 ml aliquots at -20°C

4.1.7.2 Solutions for luciferase assay

Firefly Luciferase Assay buffer (according to (Gaunitz and Papke 1998))

		for 500 ml	for 1500 ml
Tricine	20 mM	1792 mg	5376 mg
$(MgCO_3)_4$*$Mg(OH)_2$*$5H_2O$ (Magnesiumcarbonate-Hydroxide-Pentahydrate)	1.07 mM	260 mg	780 mg
$MgSO_4$	2.67 mM	329 mg	987 mg
EDTA	0.1 mM	100 µl (0.5 M)	300 µl
DTT	33.3 mM	2570 mg	7710 mg
Coenzyme A	270 µM	105 mg	315 mg
D-Luciferin, free acid	470 µM	66 mg	198 mg
ATP	530 µM	146 mg	438 mg

To solve $(MgCO_3)_4$*$Mg(OH)_2$*$5H_2O$, adjust the pH value using HCl (37%, ~1.5 ml for 1.5 l) until the solution becomes clear. Afterwards, adjust the pH value to 7.8 using 5M NaOH (~10 ml for 1.5 l).
Add D-Luciferin and Coenzyme A afterwards, and then check pH.
Store buffers at -20°C without light, thaw at room temperature for use.

Renilla Luciferase Assay buffer

		for 500 ml	for 1500 ml
NaCl	1.1 M	32.15 g	96.45 g
Na2-EDTA	2.2 mM	2.2 ml (0.5 M)	6.6 ml
$KxPO_4$ (pH 5.1)	0.22 M	110 ml 1 M KH_2PO_4	330ml
BSA	0.44 mg/ml	220 mg	660 mg
NaN_3	1.3 mM	42.25 mg	126.75 mg
Coelenterazin (solved in EtOH)	1.43 mM	300 µl Stock	900 µl

$KxPO_4$ (pH 5.1): 1M KH_2PO_4, adjust the pH value to 5.1 using 2 M KOH (~20 ml for 1.5 l).
Adjust the pH value to 5.0.
Add Coelenterazin afterwards.
Coelenterazin stock: 1mg/ml in EtOH, store at -20°C.
Store buffers at -20°C without light, thaw at room temperature for use.

Lysis Buffer

Passive lysis buffer (5x)
Dilute 30 ml of 5x Buffer in 120 ml ddH_2O

4.2 Methods

4.2.1 Molecular biological techniques

4.2.1.1 Transformation of bacteria

Chemically competent bacteria (i.e. XL1 blue or DH5α) are stored in a -80°C laboratory freezer. The aliquot was thawed on ice and the ThermoMixer was set to 42°C. The LB medium (without antibiotic) was warmed up to 37°C in a water bath. The LB plates containing the appropriate antibiotic were placed in the incubator. 100-200 ng plasmid DNA was pipetted into an Eppendorf tube and was placed on ice. 15 µl of competent bacteria was added to plasmid tube and the mixture was incubated on ice for 15 minutes. The tube was heat shocked for 30-35 seconds at 42°C in the ThermoMixer and incubated for 2 minutes on ice directly afterwards. 600 µl of pre-warmed LB medium was added into the tube and mixed. The mixture was incubated at 37°C while gently shaking it (450 rpm) for 20 minutes in the ThermoMixer. Using a plate spreader, 200 µl of the bacteria was evenly distributed onto agar plates containing appropriate antibiotics. The plate was wrapped with parafilm and incubated overnight at 32°C.

4.2.1.2 Preparation of plasmids

4 ml of LB medium containing the appropriate antibiotics was prepared in a 15 ml round bottom tube. A single round large colony of bacteria was transferred using a micropipette into the tube with LB medium. The tube was capped and placed into the shaking incubator that was set at 37°C and 200 rpm for 6 hours. For Midi preparation, an Erlenmeyer flask was prepared with 150 ml of LB medium with appropriate antibiotics. After 6 hours of incubation, 500 µl of bacterial culture was transferred into the flask and incubated overnight in a shaking incubator (200 rpm, 37°C).

4.2.1.3 Plasmid purification

Plasmid DNA was done according to the manufacturer’s instructions using NucleoSpin Plasmid Kit (Macherey-Nagel) or NucleoBond Xtra Midi EF Kit (Macherey-Nagel), depending on the amounts of plasmid DNA needed. The basic principle is based on (Birnboim and Doly 1979).

4.2.1.3.1 Plasmid DNA Mini

The bacterial culture was transferred into a 2 ml Eppendorf tube and centrifuged for 30 seconds at 11,000 x g. Supernatant was discarded and the cell pellet was resuspended completely with 250 µl Buffer A1. 250 µl Buffer A2 was mixed into the tube for lysis and incubated for 5 minutes at room temperature. For neutralizing the lysate, 300 µl Buffer A3 was added and mixed to create the appropriate conditions for binding the plasmid DNA to the silica membrane of the NucleoSpin Plasmid. The tube was centrifuged for 5 minutes at 11,000 x g at room temperature. A NucleoSpin Plasmid Column was placed inside a 2 ml Collection Tube. The supernatant was loaded onto the column. The tube, along with the column, was centrifuged for 1 minute at 11,000 x g, so that the DNA bound to the silica membrane. The membrane was washed with 600 µl Buffer A4 and centrifuged for 1 minute at 11,000 x g. The membrane was dehydrated by centrifuge for 2 minutes at 11,000 x g. The column with the membrane was placed in a 1.5 ml Eppendorf tube. 50 µl Buffer AE was then added to elute the DNA. The 50 µl Buffer AE was incubated at room temperature for a minute afterwards. Finally, the tube was centrifuged for 1 minute at 11,000 x g.

4.2.1.3.2 Plasmid DNA Midi

First, the bacterial culture from the flask was transferred into a 50 ml Falcon tube. Using centrifuge at 2500 rpm for 15 minutes at 4°C, the bacterial cells were cultivated and harvested. The bacterial cells were resuspended using 8 ml RES-EF until no clumps were visible. 8 ml LYS-EF was then added into the mixture to lyse the cells. The tube was inverted 5 times and incubated at room temperature for 5 minutes. The NucleoBond Xtra Column and Filter was prepared with 15 ml EQU-EF for equilibration. For neutralization, 8 ml NEU-EF was added into the tube and then mixed by inverting the tube 10-15 times. The lysate was loaded on the filter by gravity flow and then was cleansed with 5 ml FIL-EF buffer. The plasmid DNA is bound to the NucleoBond Xtra Silica Resin. The filter was then discarded from the column and the column was washed 2 times with 35 ml ENDO-EF and 15 ml WASH-EF buffer to remove endotoxins completely. The plasmid DNA was eluted with 5 ml ELU-EF and the eluate was collected in a 50 ml Falcon tube. 3.5 ml Isopropanol was added for precipitation of the eluted plasmid DNA. The tube was centrifuged at 4°C, 11,000 rpm for 30 minutes. The supernatant was carefully discarded. The pellet was washed with 2 ml of 70% ethanol and transferred into a 2 ml Eppendorf tube. The tube was once again centrifuged at

room temperature with 11,000 rpm for 20 minutes. Ethanol was carefully removed and the pellet was left to dry at room temperature. The DNA pellet was finally dissolved using 200 µl TE-EF. Therefore, the concentration of plasmid DNA can be measured with a photometer.

4.2.1.4 Restriction Digest

The plasmid DNA produced by the purification from the previous process can be analyzed by DNA restriction digests using Type II restriction endonucleases. The Type II enzymes cut the DNA at a certain position within their recognition sequences. The total volume of the DNA sample was 25 µl, containing 1 µl of plasmid DNA, 0.5 µl of its respective enzyme, 2.5 µl of its respective buffer, and 21 µl ddH_2O. The sample was incubated at room temperature for 30-45 minutes. The agarose gel electrophoresis was then prepared to separate the DNA fragments. 3 µl 6x Loading Dye was added into the sample. A marker was prepared with 9 µl of GeneRuler 1kb DNA ladders and 1 µl 6x Loading Dye. 12 µl of the sample and 10µl of marker were added into the agarose gel. The electrophoresis of the agarose gel was set at 70 Volt for 40 minutes. The separated DNA fragments can be visualized under ultraviolet light by using a gel documentation system.

4.2.2 Cell Culture

4.2.2.1 Basics

4.2.2.1.1 Coating

Coating the culture dishes is necessary for the differentiation of certain cell line, such as PC12 cell line. The cultivated cells will adhere to the dishes, so that the higher chance of survival (Reid and Rojkind 1979; Balda and Matter 2003). For coating of 15 cm petri dish, 10 ml of poly-L-lysine (PLL) solution with a concentration of 0.02 mg/ml was placed and the dish was gently swirled until the solution was equally distributed throughout the surface of the petri dish. The dish was incubated at 37 °C for 30 minutes. Afterwards the solution was removed. The dish was washed twice, each with 10 ml ddH_2O. The dish was left to dry in the safety cabinet. As soon as the dish dried out, it can be kept in a refrigerator. The same procedure was also done for the coating of 3.5 cm petri dish, however 1 ml PLL 0.02 mg/ml and 2 ml ddH_2O were used instead.

Coating of 96-well plates was done manually with multichannel-pipette using 50 µl PLL 0.02 mg/ml per well. The plate was also incubated at 37°C for 30 minutes and washed twice as well, each with 100 µl ddH_2O per well. The coating can also be achieved with the assistance of a liquid handling system (Hamilton Robotics). 30 µl PLL 0.02 mg/ml was pipetted into each well and incubated for at least 20 minutes. The wells were washed twice with ddH_2O and dried out thereafter.

For the coating of 384-well plates, PLL solution (0.02 mg/ml) was diluted 1: 3 with ddH_2O. 20 µl of this solution was used to coat each well using multichannel-pipette. The plate was left to dry overnight inside the safety cabinet with UV light.

4.2.2.1.2 Passaging

A new PC12 cell line in a vial was thawed and collected from a nitrogen tank. The cells were centrifuged together with 30 ml of PC12 complete medium in a 50 ml Falcon tube for 5 minutes at room temperature (1200 rpm). The pellet was resuspended with 1 ml of growth medium and then plated in a coated 15 cm petri dish with 15 ml growth medium. The cells were passaged every 3-5 days depending on the dilution.

Passaging is used to dilute the cells in a new culture dish after reaching maximum density. If the cell density is too high, the proliferation rate will drop significantly and the culture will deteriorate. With passaging, the amount of subculture and passage number increases by one.

If the culture was already 80-90% confluent, they should be subcultured. The medium was removed from the petri dish. The cells were washed with 10 ml PBS to remove the serum residue, which contains Trypsin-Inhibitor. 5 ml Trypsin was gradually added dropwise. 3 ml Trypsin was then removed immediately. Trypsin detached the cells from the cultured surface. After waiting for 2-3 minutes, 10 ml of growth medium was added to petri dish. The cells that are mixed with the medium were carefully pipetted into a new 50 ml Falcon tube. Afterwards, the appropriate amount of cell suspension was added into a new petri dish. For example, a dilution 1:3 would mean 10 ml of growth medium and 5 ml of the suspension. The new cell culture was labelled and incubated at 37°C.

4.2.2.1.3 Transfection

Cells were transfected by means of lipofection using Lipofectamine 2000 (L2000). Lipofectamine reagents encase the DNA plasmid in a synthetic cationic liposome. This complex can fuse with the anionic cell membrane by endocytosis. The DNA plasmids will be released into the cytoplasm to reach the nucleus and begin their transcription.

4.2.2.2 Assays

4.2.2.2.1 Assay with LumiCycle

On the first day, the cells were transfected with the plasmid DNA and then seeded into the wells of a coated 3.5 cm culture dish. There were two replicates (two dishes) for each condition. In this experiment, 1.4 x 10^6 cells per dish in 1.5 ml growth medium is needed for the assay to work. The materials, such as the growth medium, antibiotic free medium, OptiMEM, PBS, and Trypsin were placed in a 37° C water bath. The DNA for each condition was prepared in their respective 1.5 ml Eppendorf tube (Table 7). Afterwards, 100 µl OptiMEM and 4.2 µl L2000 (3 x DNA amount) were added into a different empty tubes, vortexed for 5 seconds, and incubated for 5 minutes. Meanwhile, 100 µl of OptiMEM was added into each DNA tube and incubated for another 5 minutes. After 5 minutes, the DNA/OMEM-tube was mixed into its corresponding OMEM/L2000-tube, and incubated for 20 minutes at room temperature. In the meantime, the cells were split using antibiotic free medium and counted using Neubauer hemocytometer. The formula is: cell number on 4 corner squares / 4 * 10 (dilution factor from Trypan blue) * 10^4 ml^{-1} (volume factor in one corner square). Assuming the amount of cells was 3,000,000 per ml and approximately 2,800,000 cells were needed for each condition, about 1 ml of cells (the amount of cells per ml divided by the amount of cells needed per condition) was pipetted each into another four empty falcon tubes. After 20 minutes, each DNA/L2000 mixture was added into its respective falcon tubes filled with cells. The tubes were inverted three times and incubated for 5 hours at 37°C lying flat. After 5 hours, each tube was resuspended with the appropriate amount of growth medium until the desired volume was achieved (Final volume – cells volume – total volume of DNA/L2000/OMEM). The substrate luciferin (3 µl) was then added into each falcon tube (1: 1000) and 3 µl of DAPT (1:1000) was added into the second tube for the negative control. 1.5 ml of the transfected cells were transferred into 3.5 cm

dish. The dishes were wrapped with parafilm to prevent the evaporation of the liquid and were eventually placed into the LumiCycle for about two days. The progress of the induction kinetics can be observed through software program connected with the machine. The evaluation was performed using a LumiCycle Analysis program and Microsoft Excel.

Table 7. Protocol for NRG1 cleavage assay using LumiCycle

1 dish =1,400,000 cells=1.5 ml		2 dishes=2,800,000 cells=3 ml			
Transfection tube		**1 baseline**	**2 Negative control**	**3 Positive control 1**	**4 Positive control 2**
DNA	**N1-III-GV**	300 ng * 2 dishes = 600 ng	300 ng * 2 dishes = 600 ng	300 ng * 2 dishes = 600 ng	300 ng * 2 dishes = 600 ng
	G10-MLP-luc	300 ng * 2 dishes = 600 ng	300 ng * 2 dishes = 600 ng	300 ng * 2 dishes = 600 ng	300 ng * 2 dishes = 600 ng
	pcDNA	100 ng * 2 dishes = 200 ng	100 ng * 2 dishes = 200 ng		
	mERBB4-F			100 ng * 2 dishes = 200 ng	
	BACE1				100 ng * 2 dishes = 200 ng
Total amount		1400 ng	1400 ng	1400 ng	1400 ng
OptiMEM x2		100 µl	100 µl	100 µl	100 µl
L2000		4.2 µl	4.2 µl	4.2 µl	4.2 µl
Split cells					
Add cells					
5h incubator					
Resuspend		Until 3 ml	Until 3 ml	Until 3 ml	Until 3 ml
Luciferin		3 µl	3 µl	3 µl	3 µl
Additional compound			DAPT : 3µl		

4.2.2.2.2 Assay with 96-well plate

On the first day, the growth medium, transfection, OptiMEM, PBS, and Trypsin were first warmed up in a water bath. The DNAs for each condition were prepared according to the Table 8. In 96-well plate, one well contained 70,000 cells with a volume of 200 µl. In the example above, there were 4 conditions. Each condition had 24 replicates (wells), but 30 replicates were made in case there was a need of more. The ideal amount for OptiMEM is 250 µl for 6,000 ng DNA. Using rule of three: 250 µl x 1,050 ng / 6,000 ng = 43.75 µl OptiMEM. The amount of L2000 was roughly estimated: 3 x 1.05 µg DNA amount = 3.15 µl. About 43.75 µl OptiMEM and 3.15 µl L2000 were mixed in another Eppendorf tube. Another 43.75 µl OptiMEM was added in the DNA tube. After 5 minutes, both tubes were mixed together on a vortex mixer for 5 seconds. The tubes were incubated at room temperature for 20 minutes. Meanwhile, the cells were subcultured using antibiotic free medium. About 2,100,000 cells were needed for each condition. Assuming the amount of cells was 3,000,000 per ml, 1.43 ml cells had to be transferred into a new 50 ml falcon tube for each condition. After 20 minutes, the contents from DNA tube were pipetted into the corresponding falcon tube with the cells. The mixture was inverted three times. The falcon tubes were incubated for 2-3 hours lying flat. After 2-3 hours, each falcon tube was resuspended with the growth medium until 6 ml. For the second tube, 6 µl of DAPT (1:1000) was added. After mixing it well, the cells were placed in a labelled reservoir for each condition. The cells were pipetted into their respective wells through the usage of a multichannel-pipette (200 µl) (Figure 7). The 96-well plate was incubated for 24 hours at 37°C.

On the second day, the growth medium was removed from the plate. 35 µl Passive Lysis Buffer (PLB) was applied into each well. The plate was incubated on a plate shaker for about 20 minutes at room temperature. If the clear plate was used, the cells had to be transferred into the white plate for measurement. After 20 minutes, the plate was measured using a microplate reader (Berthold). The MicroWin 2000 software program was opened on PC:

- Open data : DLR_disp_FF15min_1sec_RN_2sec-byWell
- Click the ‘Plate picture’ → mark the wells which are used in the assay → pay attention to Inject 2 (blue = RN) and Inject 3 (red=FF)
- Next → OK

- Put the RN and FF hose into the falcon tube with ddH_2O
- Instrument → Wash 50 Cycle → Injector 2 and 3 in ddH_2O → OK
- Put the hose now into each respective substrates (Renilla Luciferase and Firefly Luciferase)
- Instrument → Prime → Injector 2 and 3 → Next (Repeat one more time)
- Instrument → Unload Plate → Put the white plate inside
- Write the data name and click 'Start'
- The results are automatically transferred on the desktop within the folder: 'Verknupfung mit Transfer'
- Termination: Instrument → Wash → Injector 2 and 3 in ddH_2O → 50 Cycle → Next
- Instrument → Wash → Injector 2 and 3 in Ethanol → 50 Cycle → Next
- Instrument → Wash → Injector 2 and 3 in ddH_2O → 50 Cycle → Next
- Instrument → Unload Injector → 15 Cycle → Next
- Close the program

The data obtained from the program was processed and analyzed with Microsoft Excel.

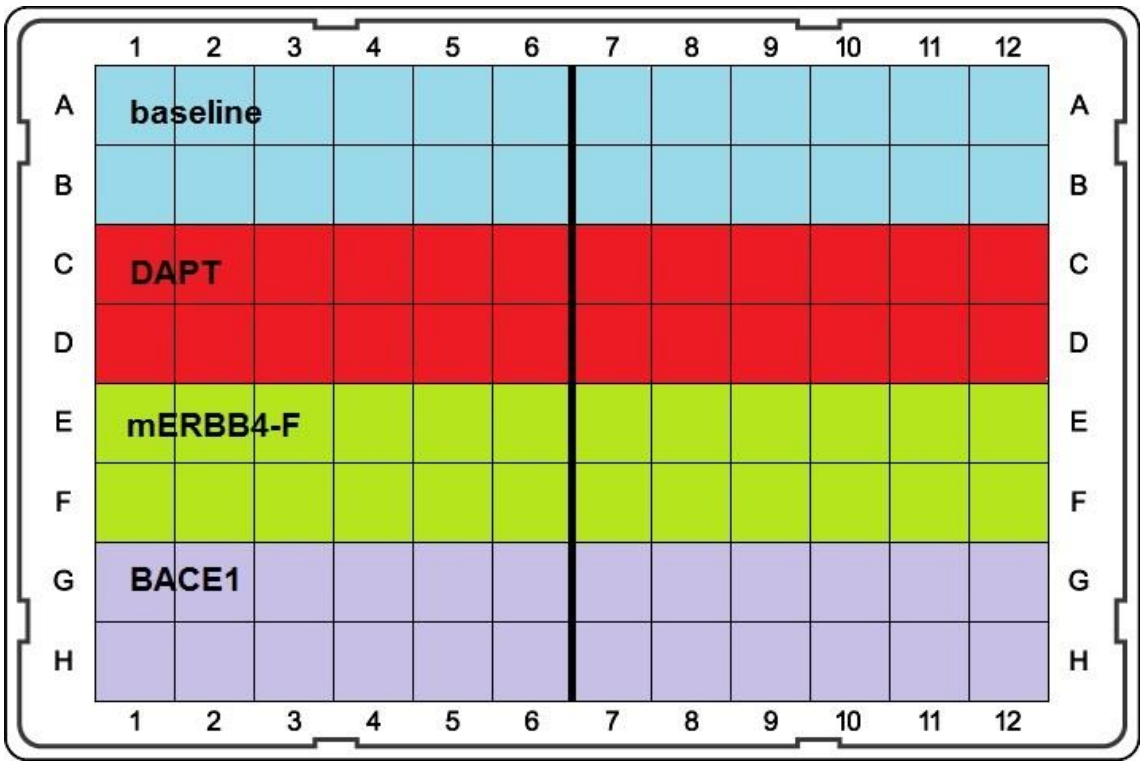

Figure 7: Plate layout of an assay in 96-well plate.

Table 8. Protocol for NRG1 cleavage assay using 96-well plate

1 well = 70,000 cells = 200 µl		30 wells = 2,100,000 cells = 6 ml			
Transfection tube		**1 baseline**	**2 Negative control**	**3 Positive control 1**	**4 Positive control 2**
DNA	**N1-III-GV**	15 ng * ~30 wells = 450 ng	15 ng * ~30 wells = 450 ng	15 ng * ~30 wells = 450 ng	15 ng * ~30 wells = 450 ng
	G10-MLP-luc	15 ng * ~30 wells = 450 ng	15 ng * ~30 wells = 450 ng	15 ng * ~30 wells = 450 ng	15 ng * ~30 wells = 450 ng
	pcDNA	5 ng * ~30 wells = 150 ng	5 ng * ~30 wells = 150 ng		
	mERBB4-F			5 ng * ~30 wells = 150 ng	
	BACE1				5 ng * ~30 wells = 150 ng
Total amount		1050 ng	1050 ng	1050 ng	1050 ng
OptiMEM x2		43.75 µl	43.75 µl	43.75 µl	43.75 µl
L2000		3.15 µl	3.15 µl	3.15 µl	3.15 µl
Split cells					
Add cells					
2h incubator					
Resuspend		Until 6 ml	Until 6 ml	Until 6 ml	Until 6 ml
Additional compound			DAPT : 6 µl		

4.2.2.2.3 Assay with 384-well plate

Once the assay has been successfully tested in 96-well plate, the same method was applied in 384-well plate with a few adjustments. The suitable amount of cells per well is 20,000 cells with a volume of 90 µl. Each condition had 96 replicates/wells (rounded up to 100 wells). The DNA plasmids were prepared in 1.5 ml Eppendorf tubes according to the Table 9. 105 µl of OptiMEM and 7.5 µl of L2000 were mixed in other tubes and incubated for 5 minutes. Another 105 µl of OptiMEM was inserted into each DNA tube and incubated for 5 minutes. Both tubes were mixed together and incubated for 20 minutes at room temperature. Meanwhile, the cells were split using antibiotic free medium and counted with the hemocytometer. Assuming the cell number was 2,000,000 cells per ml, 1 ml cell was pipetted into a new falcon tube for each condition. After 20 minutes, the DNA was transferred into the respective falcon tubes that contain the cells. The tubes were incubated lying flat at 37°C for 2-3 hours. After 2-3 hours, each tube was resuspended with growth medium until its volume reached 9 ml. The cells were applied into corresponding wells in 384-well plate using multichannel-pipette (90 µl) (Figure 8). The plate was incubated at 37°C for 24 hours.

On the following day, the medium was completely removed from the plate using a centrifuge (700 g for one minute). The plate was placed upside down with a reservoir under it to catch the medium. 15 µl PLB was applied into each well for lysis. The plate was placed on a plate shaker for 20 minutes at room temperature. The cells needed to be transferred into a white plate if a clear plate was used beforehand. Afterwards the plate was measured using microplate reader (Berthold).

- Open program MicroWin 2000 → Data : DLR_disp_FF15min_1sec_RN_2sec-384 byWell
- Click the ‘Plate picture’ → mark the wells which are used in the assay → pay attention to Inject 2 (blue = RN) and Inject 3 (red=FF)
- Next → OK
- Put the RN and FF hose into the falcon tube with ddH_2O
- Instrument → Wash 50 Cycle → Injector 2 and 3 in ddH_2O → OK
- Put the hose now into each respective substrates (Renilla Luciferase and Firefly Luciferase)
- Instrument → Prime → Injector 2 and 3 → Next (Repeat one more time)
- Instrument → Unload Plate → Put the white plate inside
- Write the data name and click ‘Start’

- The results are automatically transferred on the desktop within the folder: 'Verknupfung mit Transfer'
- Termination: Instrument → Wash → Injector 2 and 3 in ddH_2O → 50 Cycle → Next
- Instrument → Wash → Injector 2 and 3 in Ethanol → 50 Cycle → Next
- Instrument → Wash → Injector 2 and 3 in ddH_2O → 50 Cycle → Next
- Instrument → Unload Injector → 15 Cycle → Next
- Close the program

The data obtained from the program was processed and analyzed with Microsoft Excel.

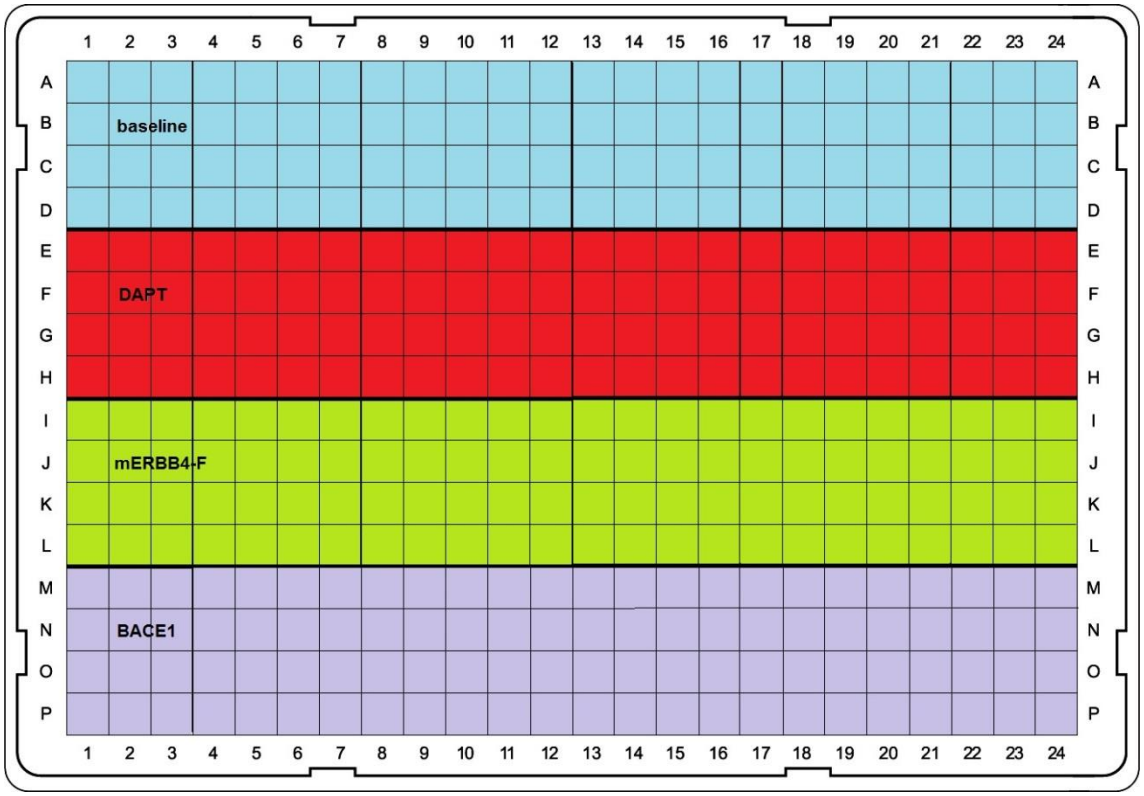

Figure 8: Plate layout of an assay in 384-well plate.

Table 9. Protocol for NRG1 cleavage assay using 384-well plate

1 well = 20,000 cells = 90 µl		100 wells = 2,000,000 cells = 9 ml			
Transfection tube		**1 Baseline**	**2 Negative control**	**3 Positive control 1**	**4 Positive control 2**
DNA	**N1-III-GV**	10 ng * ~100 wells = 1000 ng	10 ng * ~100 wells = 1000 ng	10 ng * ~100 wells = 1000 ng	10 ng * ~100 wells = 1000 ng
	G10-MLP-luc	10 ng * ~100 wells = 1000 ng	10 ng * ~100 wells = 1000 ng	10 ng * ~100 wells = 1000 ng	10 ng * ~100 wells = 1000 ng
	pcDNA	5 ng * ~100 wells = 500 ng	5 ng * ~100 wells = 500 ng		
	mERBB4-F			5 ng * ~100 wells = 500 ng	
	BACE1				5 ng * ~100 wells = 500 ng
Total amount		2500 ng	2500 ng	2500 ng	2500 ng
OptiMEM x2		105 µl	105 µl	105 µl	105 µl
L2000		7.5 µl	7.5 µl	7.5 µl	7.5 µl
Split cells					
Add cells					
2h incubator					
Resuspend		Until 9 ml	Until 9 ml	Until 9 ml	Until 9 ml
Additional compound			DAPT : 9 µl		

4.2.2.2.4 NRG1 cleavage screening

4.2.2.2.4.1 NIH Clinical Collection

The NIH Clinical Collection (NCC) is a plated array of 281 (NCC 201) and 445 (NCC 003) small molecules. The National Institutes of Health (NIH) assembled the collection through the Molecular Libraries Roadmap Initiative so that the compounds could be used in high throughput screening in biomedical research. These clinically tested compounds with already known safety profiles may offer an excellent starting point for therapeutic potential discovery and may be suitable for direct application to human.

4.2.2.2.4.2 Plate layout

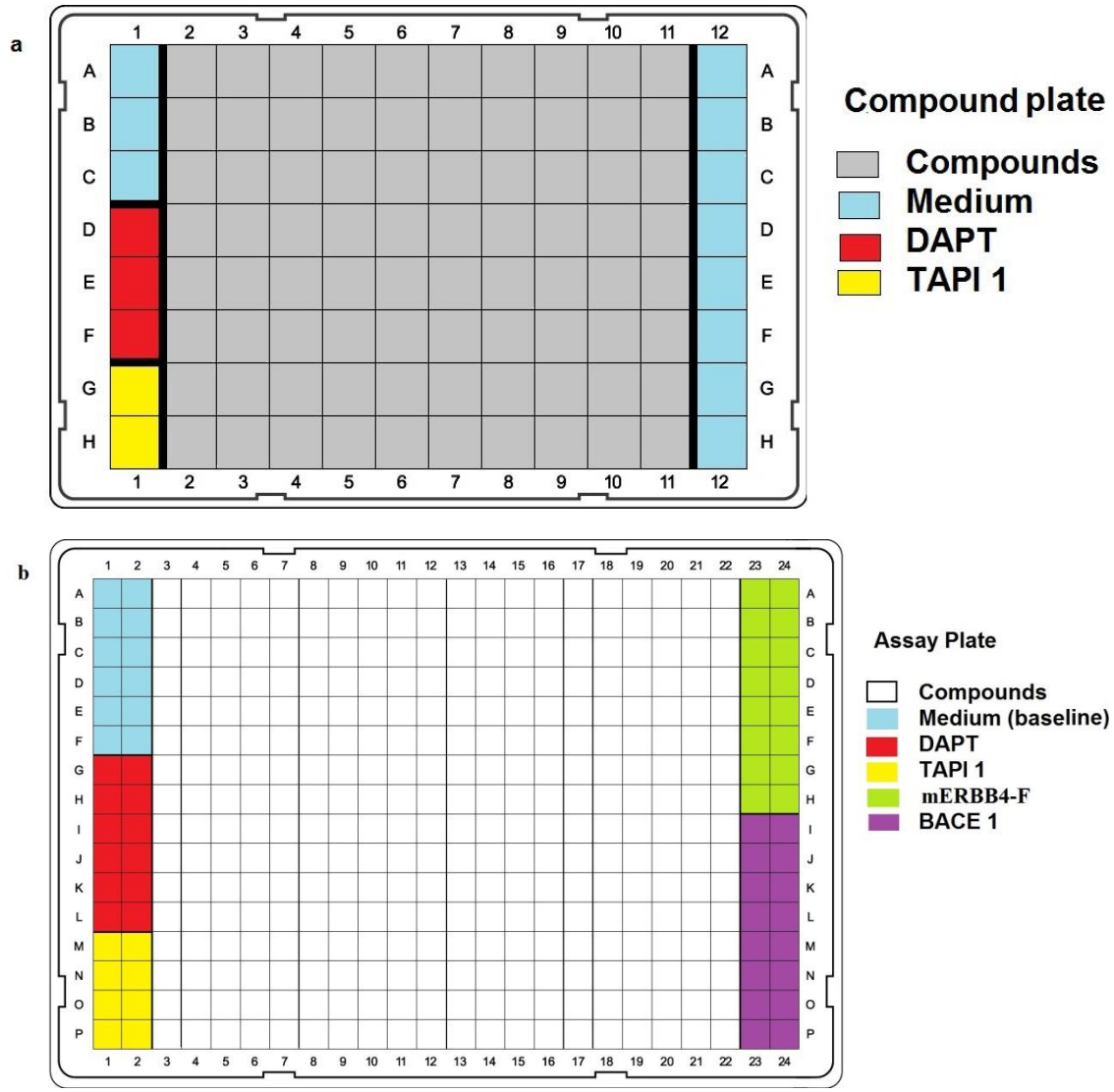

Figure 9: Plate layout for NRG1 cleavage assay screen. (a) Compound plate in 96-well plate. (b) Assay plate in 384-well plate. A compound in 1 well in compound plate was transferred to 4 wells in assay plate to create 4 replicates of said compound.

There were 727 compounds to be tested. A compound plate (96-well plate) consisted of 80 compounds from NIH clinical collection diluted in DMSO in the middle, two negative controls (DAPT and TAPI-1) at column 1 which also have been diluted in DMSO, and growth medium for the baseline at column 1 and positive controls at column 12. Each of these wells will be transferred equally into 4 wells of a 384-well plate (assay plate). For example, compound X, located at 2A in compound plate, was transferred into 3A, 4A, 3B, and 4B in the assay plate.

4.2.2.2.4.3 Preparation of the compound plates

A working stock contained all 727 compounds from NIH was already prepared in a sum of 10 of 96-well plates. Each compound had originally 5 µl as its volume and 10 mM as its concentration. For the screening, the final concentration of the compounds should be 10 µM. The compounds were first diluted 1:10 with DMSO (addition of 45 µl DMSO) to make a concentration of 1 mM. Ten other 96-well plates were prepared with 54 µl growth medium per well. 6 µl of the compounds were transferred onto the plate with the medium. The compounds were diluted 1:10 with the medium and had a total of 60 µl and 100 µM. Each well in 96-well plate that contained one compound was transferred onto four wells of 384-well plate (assay plate) to create four replicates of the corresponding compounds. 90 µl of PC12 cells were prepared in each well of 384-plate. 10 µl of the same compound (100 µM) were transferred into the four respective wells of 384-well plate containing 90 µl cells. Eventually, each well of 384-well plate had a volume of 100 µl and 10 µM compound. All transferring were done with the assistance of liquid handling system (Hamilton Robotics).

4.2.2.2.4.4 Screen protocol

A sum of ten 96-well plates for compound plate ten 384-well plates for assay plate was prepared. There were 12 transfection tubes in total. The first ten were used for the baseline, the negative controls (DAPT and TAPI-1), and the compounds. The transfection was prepared about 5,000 wells, which was then divided into 10 tubes. The first positive control was a co-transfection with mERBB4-F and the second was a co-transfection with BACE1. The DNAs were put into each respective tube and mixed with the appropriate number of OMEM shown in Table 10. Another amount of OMEM and L2000 was placed into different Eppendorf tubes, vortex and wait for 5 minutes. Put the OMEM/L2000 mix into DNA tubes, vortex and wait for 20 minutes. During the 20 minutes of anticipation, the PC12 cells were subcultured using antibiotic free medium. Assuming the cell number was 3,000,000 per ml, put 3.33 ml cells in the first ten tubes and insert 2 ml cells in the both tubes for positive controls. After 20 minutes, the DNA/L2000 was mixed into the cell tubes and incubated for 2 hours at 37°C. After 2 hours, each tube was resuspended with growth medium until the desired volume had been achieved. All ten tubes containing the same DNA were mixed together into a 500 ml flask. With the assistance of the liquid handling system (Hamilton Robotics), 90 µl

cells from the bottle were transferred into the column 1-22 in assay plate. 90 µl cells from the first positive control (mERBB4-F) were transferred into wells 23/24 A-H and 90 µl cells from the second positive control (BACE1) were transferred into wells 23/24 I-P (Figure 9). 10 µl of compounds or medium from the compound plate were then transferred into the assay plate according to the explanation above. After having a total of 100 µl and 10 µM for each well, the assay plates were incubated for 24 hours at 37°C.

The robot has been programmed to do the assay systematically. After 24 hours, the medium was removed from the plates completely using a centrifuge (700g, 1 minute). 20 µl PLB was put and the plate was shaken for about 20 minutes. Then, 30 µl of Firefly substrate was added into each well and the plate was incubated for 10 minutes. Afterwards, the plate was measured in microplate reader (Berthold). The analysis was done using cellHTS2 software program.

Table 10. Protocol for NRG1 cleavage assay screen in 384-well plate

1 well = 20,000 cells = 90 µl		500 wells = 10,000,000 cells = 45 ml			
Transfection tube		**1 Baseline, neg controls, compounds**	**1-10 (x10 plates)**	**11 Positive control 1**	**12 Positive control 2**
DNA	**N1-III-GV**	10 ng * 500 wells = 5000 ng	50,000 ng	10 ng * 300 wells = 3000 ng	10 ng * 300 wells = 3000 ng
	G10-MLP-luc	10 ng * 500 wells = 5000 ng	50,000 ng	10 ng * 300 wells = 3000 ng	10 ng * 300 wells = 3000 ng
	pcDNA	5 ng * 500 wells = 2500 ng	25,000 ng		
	mERBB4-F			5 ng * 300 wells = 1500 ng	
	BACE1				5 ng * 300 wells = 1500 ng
Total amount		12,500 ng		7500 ng	7500 ng
OptiMEM x2		520.83 µl		312.5 µl	312.5 µl
L2000		37.5 µl		22.5 µl	22.5 µl
Split cells					
Add cells					
2h incubator					
Resuspend		Until 45 ml		Until 27 ml	Until 27 ml
Additional compound					

5 Results

5.1 Promoter tests

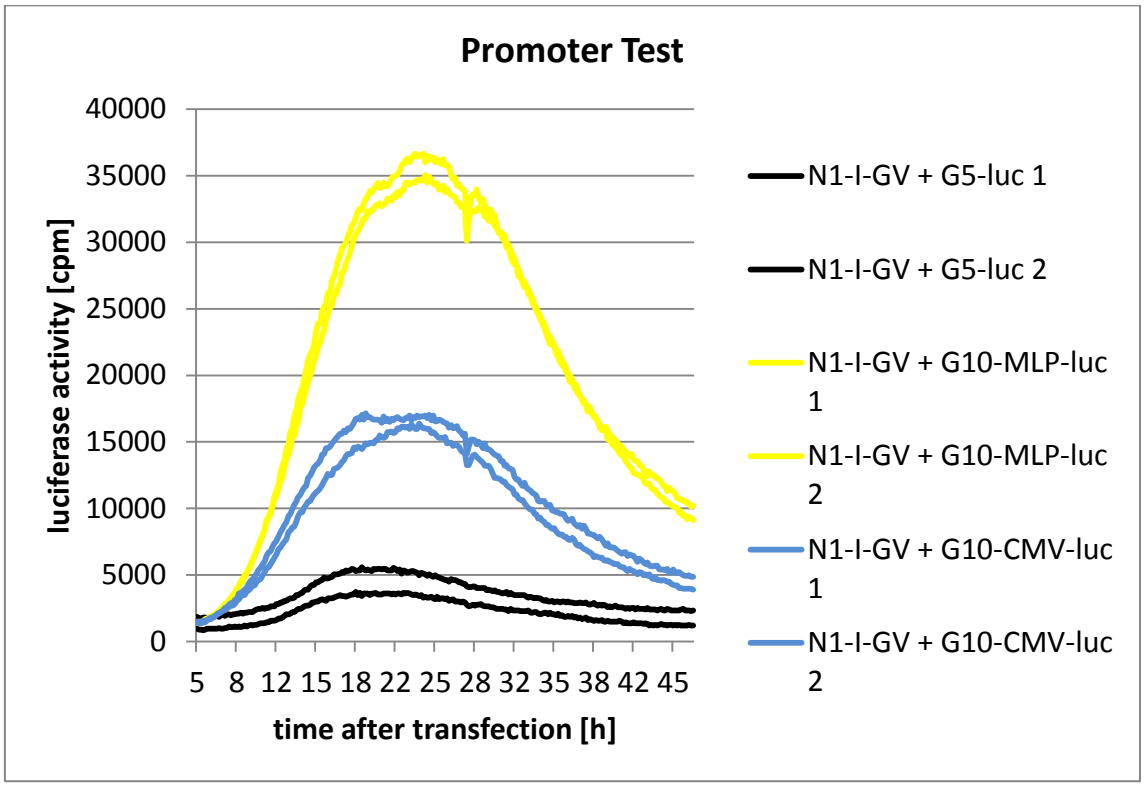

Figure 10: Result of LumiCycle measurement comparing G5-luc, G10-MLP-luc, and G10-CMV-luc. X-Axis: time after transfection [h]. Y-Axis: luciferase activity [cpm].

This assay was performed using LumiCycle method as explained above. The assay used three conditions with two replicas for each condition. Five hours after on plate transfection, the dishes were put into the LumiCycle and the activity was monitored. This line chart shows the comparison of using different available reporter genes. The black lines represent the use of G5-luc, the yellow lines show the G10-MLP-luc, and the blue lines are for the G10-CMV-luc. In the first hours, each condition increased steadily at its own pace and achieved its highest activity approximately 24 hours after the transfection. After reaching the peak, the luciferase activities fell down on the following hours. G5-luc has the least luciferase activity in comparison to other reporter genes. G10-CMV-luc shows a 3-fold increase in activity from G5-luc. G10-MLP-luc appeared to be the most optimal reporter gene in this experiment, resulting in almost 7-fold higher activity from G5-luc.

5.2 ERBB4 amount optimization

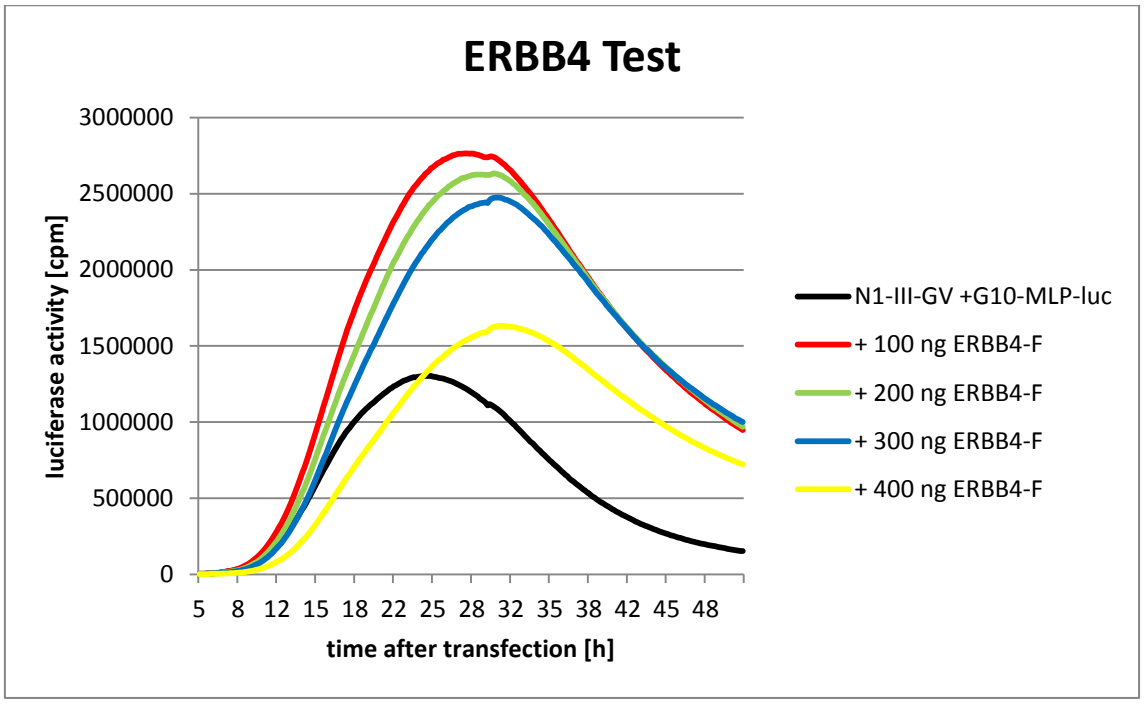

Figure 11: Result of LumiCycle measurement comparing different amount of ERBB4-F stimulation. X-Axis: time after transfection [h]. Y-Axis: luciferase activity [cpm].

This assay was made to define which amount of the positive control, ERBB4-F, would be optimal for the screening. The amount of N1-III-GV and G10-MLP-luc in this assay was always constant, which was 300 ng per dish. The amount of ERBB4-F being tested were 100 ng, 200 ng, 300 ng, and 400 ng. Therefore, five conditions were prepared, each with two replicas. Each line represents the average activity of two respective dishes. The black line is the condition for the cells transfected with only N1-III-GV and G10-MLP-luc, without any stimulation. The red line shows the activity with 100 ng ERBB4-F transfection, the green one is for 200 ng ERBB4-F, the blue one is for 300 ng ERBB4-F, and the yellow one is for 400 ng ERBB4-F. The baseline rose up and reached its peak at 1250000 cpm around 25 hours post transfection. All the conditions with the ERBB4-F stimulation grew up higher than the baseline with its peak around 28 hours post transfection. Relatively small difference can be seen between the red, green, and blue line, whose activities are twice as much as the baseline. The red line with 100 ng ERBB4-F has the highest level of activity, followed by 200 ng ERBB4-F, and lastly the 300 ng ERBB4-F. The yellow line with 400 ng ERBB4-F has the smallest level of stimulation compared to the other three.

5.3 Control tests

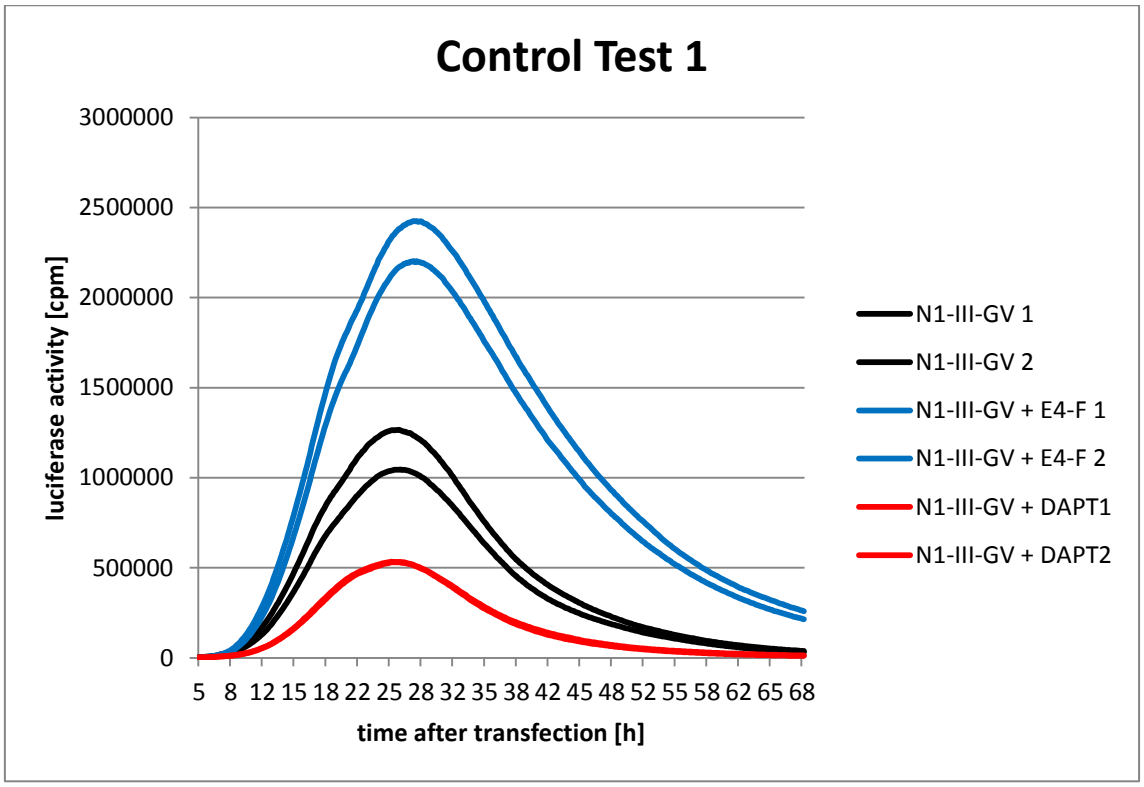

Figure 12: Result of LumiCycle measurement showing an assay with a positive and a negative control. X-Axis: time after transfection [h]. Y-Axis: luciferase activity [cpm].

This chart shows NRG1 cleavage assay with a positive and a negative control. Three conditions were prepared, each with two replicas. The black lines are the baseline, using only NRG1-III-GV and G10-MLP-luc. The blue lines represent the positive control, using co-transfection of ERBB4-F as stimulation for the assay. The red lines are the negative control, using 10 µM DAPT, a γ-secretase inhibitor, to inhibit the cleavage activity. They reached their own highest level of activity at approximately 25 hours post transfection. The positive control is twice as high compared to the baseline. Meanwhile, the negative control is around half the level of activity of the baseline.

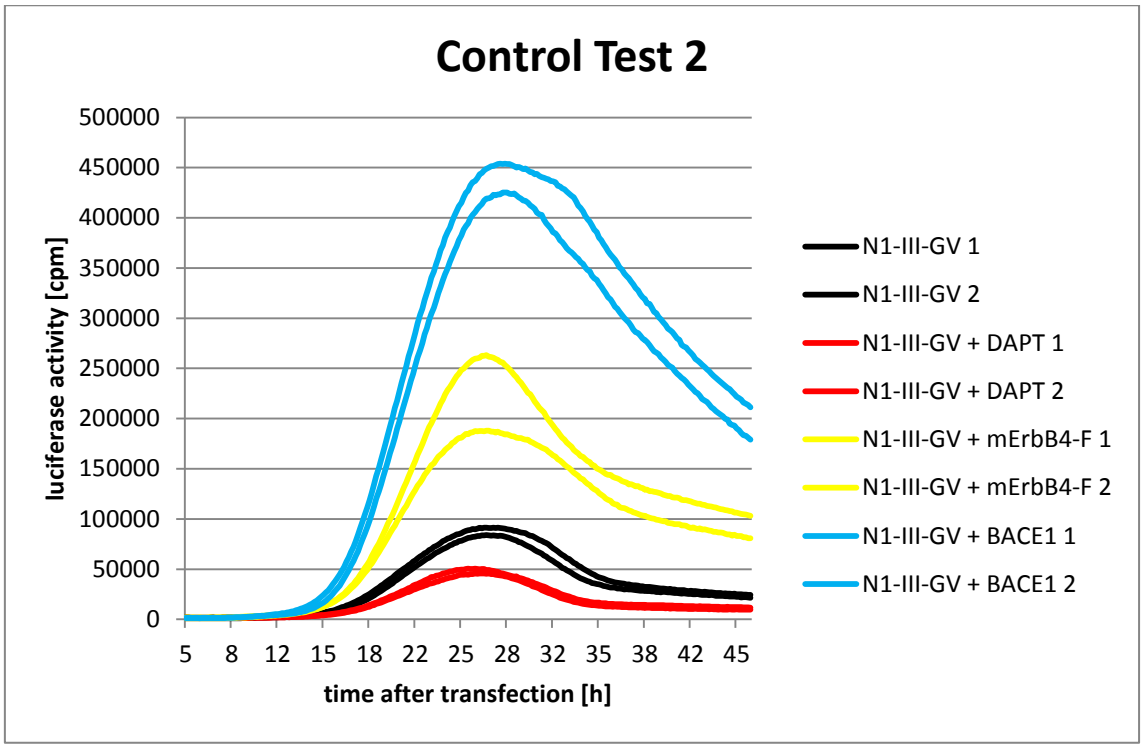

Figure 13: Result of LumiCycle measurement with two positive controls, ERBB4-F and BACE1, and a negative control, DAPT. X-Axis: time after transfection [h]. Y-Axis: luciferase activity [cpm].

This chart shows an assay with two positive controls and a negative control. The baseline is the black lines, which represent transfection of N1-III-GV and G10-MLP-luc. The first positive control is the yellow line, a co-transfection with ERBB4-F. The second positive control is also a co-transfection with BACE1, which illustrated by the blue lines. Lastly, the red lines show the negative control, which is DAPT. All the conditions have their peak at approximately 25 hours post transfection. As seen above, co-transfection with BACE1 stimulates the luciferase activity much better than with ERBB4-F. It is over four times higher than the baseline, whereas the co-transfection with ERBB4-F only twice as much. The negative control showed half of the activity compared to the baseline.

5.4 Assay results in 96-well plate

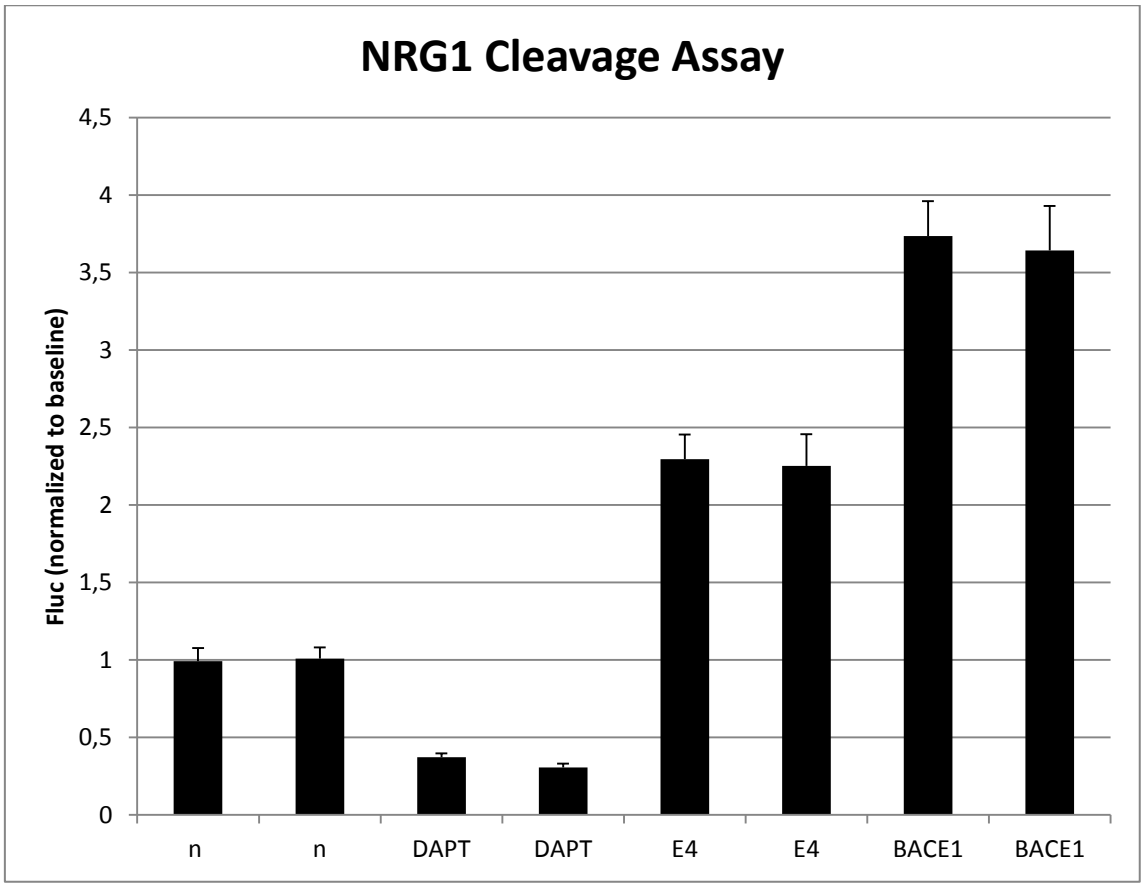

Figure 14: Chart representation of assay result in 96-well plate. ERBB4-F and BACE1 were positive controls and DAPT was negative control. n: baseline. Y-Axis: luciferase activity (normalized to baseline).

This chart represents the assay with the positive and negative controls, applied in 96-well plate format. The assay was measured around 25 hours after transfection using a microplate reader (Berthold Technologies). The result is nearly the same as the LumiCycle. DAPT, as a γ-secretase inhibitor, shows less than half as much activity as the baseline. The first positive control, ERBB4-F, had roughly 2.3 times higher activity than the baseline. The second positive control, BACE1, had almost 4 times increase in activity from the baseline.

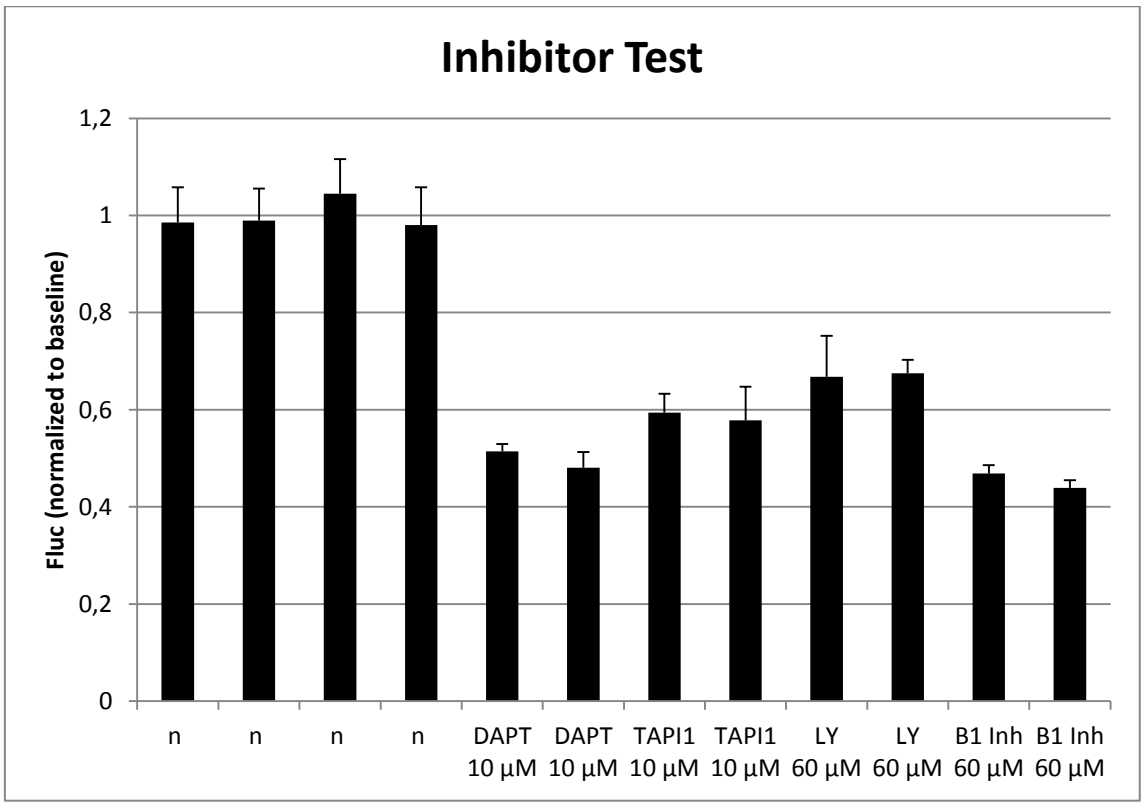

Figure 15: Inhibitor test result in 96-well plate. DAPT and TAPI-1 were tested in final concentration of 10 µM. LY2811376 and BACE1 Inhibitor IV were tested in final concentration of 60 µM. n: baseline. Y-Axis: luciferase activity (normalized to baseline).

This chart shows an assay comparing different negative controls. The negative controls being tested were DAPT, a γ-secretase inhibitor, TAPI-1, a TACE inhibitor, LY2811376, a BACE1 inhibitor, and β-secretase Inhibitor IV, also another BACE1 inhibitor. Using dose-response assay (not shown), the optimal amount of each negative control was determined. The final concentration of DAPT and TAPI-1 is 10 µM and the final concentration of LY2811376 and β-secretase Inhibitor IV is 60 µM. The assay was done in 96-well plate format and measured around 25 hours after the transfection using a microplate reader (Berthold Technologies). Each column represents 6 replicas. The luciferase activity of the cells with DAPT showed a decrease of about 50 per cent compared to the baseline (n). TAPI-1 had a 40 per cent activity reduction from baseline. LY2811376 inhibited the cleavage activity by 40 per cent and β-secretase Inhibitor IV blocked at the same level as DAPT. Considering the amount of compounds and its effects, DAPT appeared to be the best negative control for the screen, followed closely by TAPI-1.

5.5 Assay results in 384-well plate

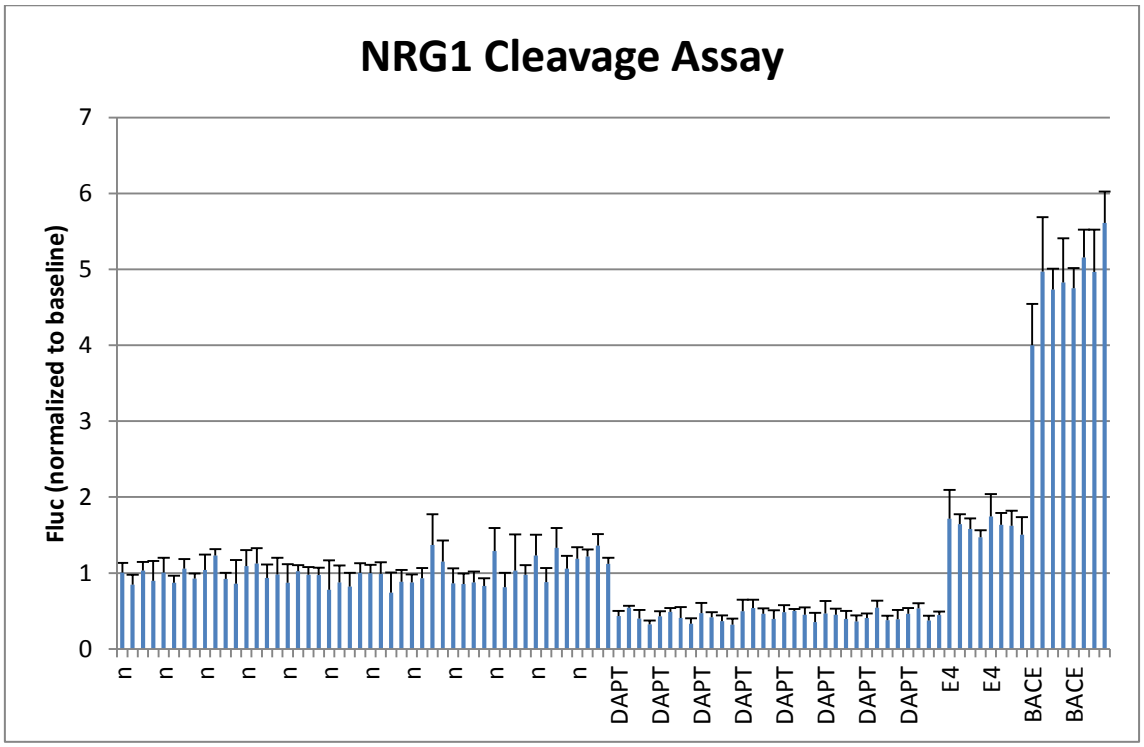

Figure 16: Chart representation of assay result in 384-well plate. ERBB4-F and BACE1 were positive controls and DAPT was negative control. n: baseline. Y-Axis: luciferase activity (normalized to baseline).

This assay was performed in 384-well format using a liquid handling system (Hamilton Robotics) and measured with microplate reader (Berthold Technologies). Each column represents 4 replicas of representative condition. As seen from the chart above, ERBB4-F stimulation showed only a 1.5-fold increase from baseline (n) compared to previous experiment with 96-well format. On the other hand, BACE1 stimulation still showed about four to five-fold increase in the level of activity from the baseline. DAPT inhibited the cleavage activity by ~50 per cent from baseline.

5.6 Screen report

5.6.1 Plate configuration

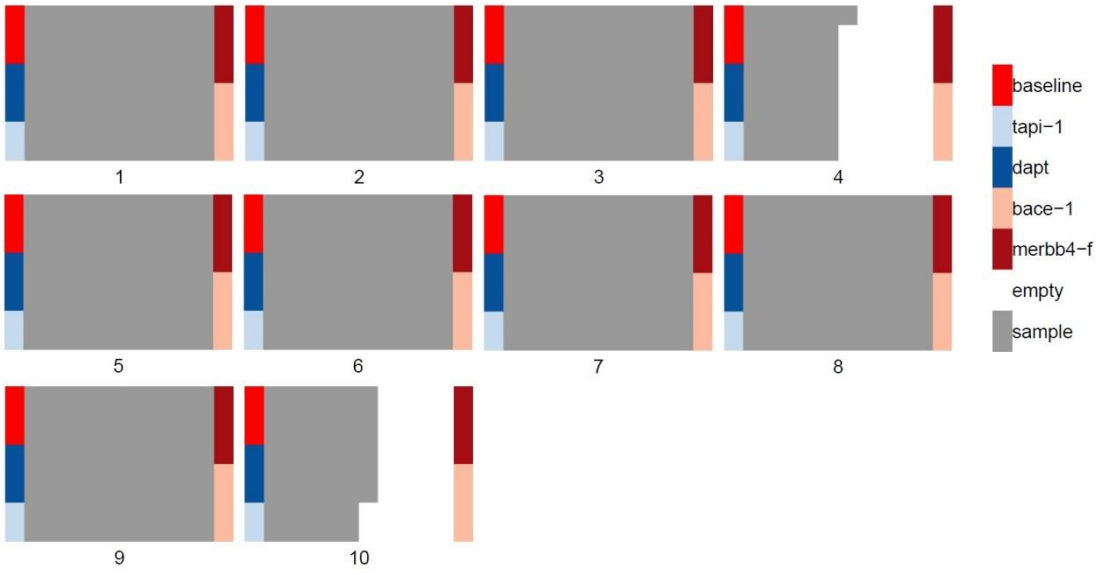

Figure 17: A graphical representation of plate configurations. The location of baseline, positive and negative controls, as well as the samples are indicated by the colour coding.

The samples from NCC 201, which consists of 281 compounds, were placed into Plate 1 – 4. The 446 samples from NCC 003 were placed into Plate 5 – 10.

5.6.2 Plate summary

Figure 18 and Figure 19 show a set of diagnostic plots which provide an overview of the general screen quality. Figure 18 represents each boxplots of raw and normalized values of each replicated samples for every plate. The normalization of the data was intended to adjust the values for some inevitable technical abnormalities in the signal. As can be seen in Figure 18, the signal intensities between plates could vary between one another due to different time points of measurement. Thus, a more significant measure of the effect is the signal relative to a typical value per plate, such as the plate median. CellHTS2 calculated the plate median normalization by dividing the raw intensity with its corresponding plate median only from the sample of each replicate to produce the normalized intensity (Boutros, Brás, and Huber 2006).

$$y(a)_{PR} = \frac{x(a)_{PR}}{median(s)_{PR}}$$

$x(a)_{PR}$ is the raw intensity of compound a in plate P and replicate R. The median is calculated among the wells annotated as sample in plate P and replicate R. $y(a)_{PR}$ is its normalized intensity.

Standard scores or z-scores were also calculated by cellHTS2 software. This scoring is important as a measure of evidence for a generated phenotype. Compounds with top z-scores will be identified as candidate modifiers and selected as 'hits' of the screen. First, the normalized value of each replicate of compound a has to be calculated.

$$z(a)_{PR} = \frac{y(a)_{PR} - \mu(s)_{PR}}{\sigma(s)_{PR}}$$

$y(a)_{PR}$ is the normalized intensity of compound a from plate P and replicate R, $\mu(s)_{PR}$ is the mean value of sample wells from replicate R in plate P, $\sigma(s)_{PR}$ is the standard deviation of sample wells from replicate R in plate P, and $z(a)_{PR}$ is the normalized value of compound a from replicate R in plate P. Instead of using σ and μ, more robust estimators were applied in the cellHTS2 software, like median absolute deviation (MAD) and median, respectively (Leys et al. 2013). Finally, the z-score of compound a is calculated by the median of the normalized values of all its 4 replicates. The raw values and the normalized values of all controls and samples, as well as the z-scores, can be found in Table 14 and 15. Compounds with a positive z-score indicate a strong increase in signal and can be identified as activators, whereas compounds with a negative z-score indicate a decrease in signal and thus, inhibitors. Several compounds with highest or lowest z-score were chosen to be further validated. Figure 19 represents the distribution of the normalized values of the positive and negative controls in the form of dot plots and density plots.

5.6.2.1 Boxplot

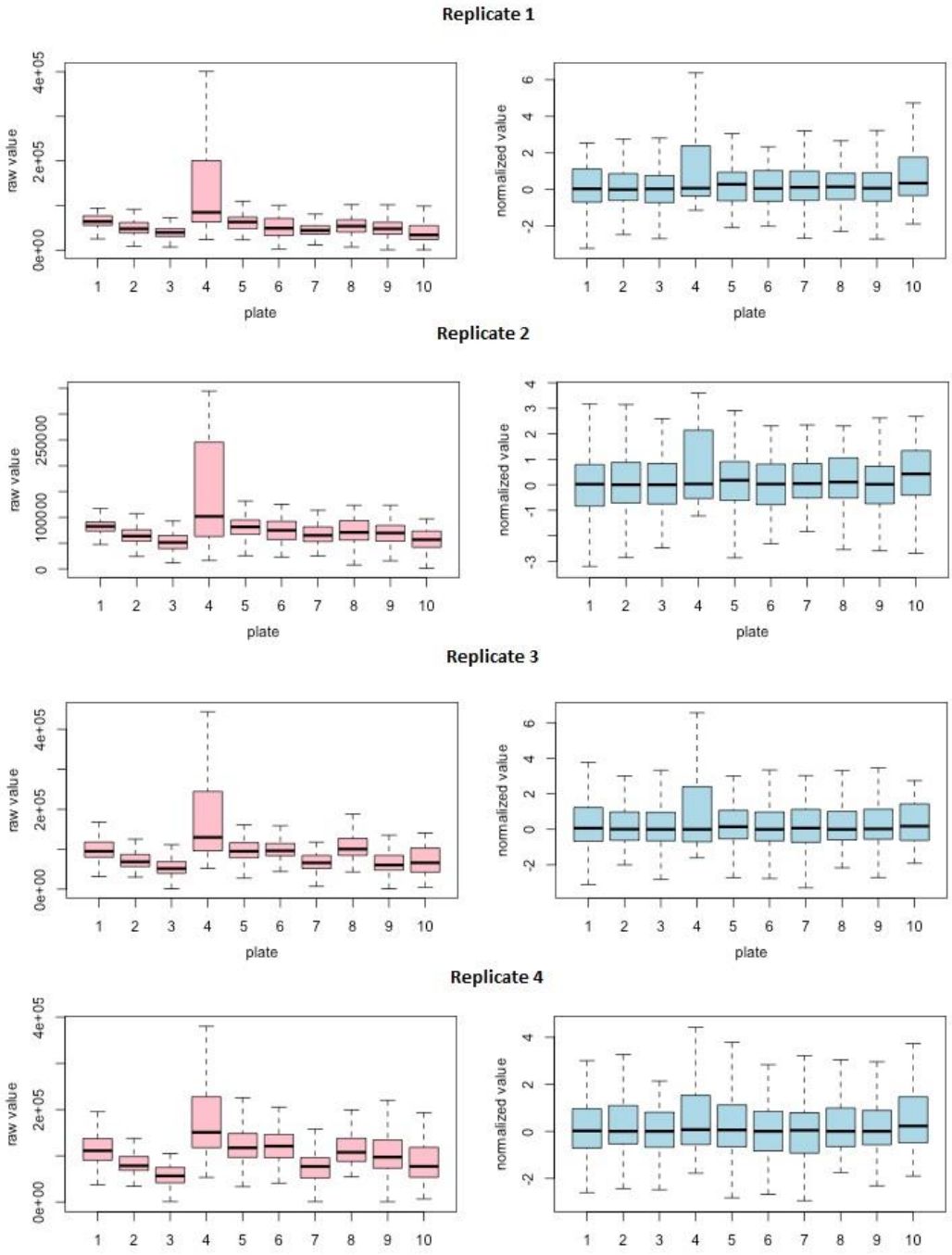

Figure 18: Plate normalization. Left: Box plots of raw values of samples only, grouped by plate for each replicate. Right: Box plots of normalized values of samples only, grouped by plate for each replicate. X-Axis: plate number. Y-Axis: raw/normalized value.

5.6.2.2 Control plot

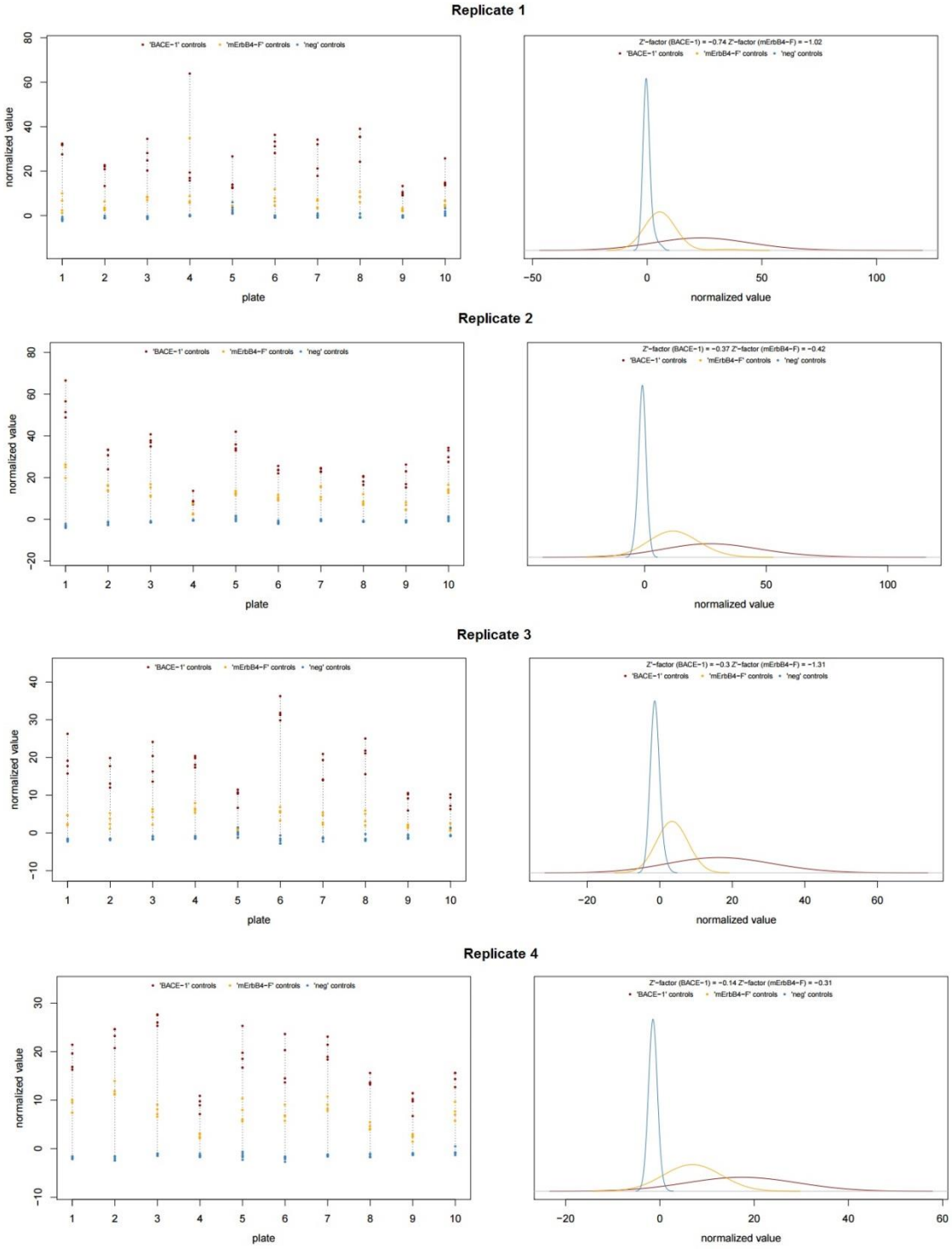

Figure 19: Controls plot. Left: Normalized values of positive and negative controls plotted against the plate number for each replicate. Red dot: BACE1. Yellow dot: ERBB4-F. Blue dot: DAPT and TAPI-1. X-Axis: plate number. Y-Axis: normalized values. Right: Distribution of positive and negative controls across the plates, represented by density estimates. Red line: BACE1. Yellow line: ERBB4-F. Blue line: DAPT and TAPI-1.

5.6.2.3 Z´-factor

CellHTS2 calculated Z´-factor, which is a parameter to evaluate the quality or performance of the assay itself, without any intervention of test compounds (Zhang, Chung, and Oldenburg 1999). The Z´-factor also plays a significant role for the assay development and optimization.

$$Z' = 1 - 3\frac{\sigma_{pos} + \sigma_{neg}}{|\mu_{pos} - \mu_{neg}|}$$

σ_{pos} and σ_{neg} are the standard deviations of the positive and negative controls, whereas μ_{pos} and μ_{neg} are their mean values. Z´-factor value is classified as following: Z´ = 1, an ideal assay; 1 > Z´ ≥ 0.5, an excellent assay; 0.5 > Z´ > 0, a double assay; Z´= 0, a "yes/no" type assay; Z´< 0 indicates that the assay conditions still need to be optimized. In the cellHTS2 software, robust estimators median and MAD were used instead μ and σ, respectively. Because there were 2 positive controls, cellHTS2 calculated the Z´-factor between each of the positive controls and the negative control.

Table 11. Z´-factor of screen. The Z´-factor was calculated between negative controls and each of the positive control.

Replicate	Z´-factor (BACE1)	Z´-factor (mERBB4-F)
1	-0.74	-1.02
2	-0.37	-0.42
3	-0.3	-1.31
4	-0.14	-0.31

5.6.3 Screen summary

5.6.3.1 Screen-wide image plot of the scored values

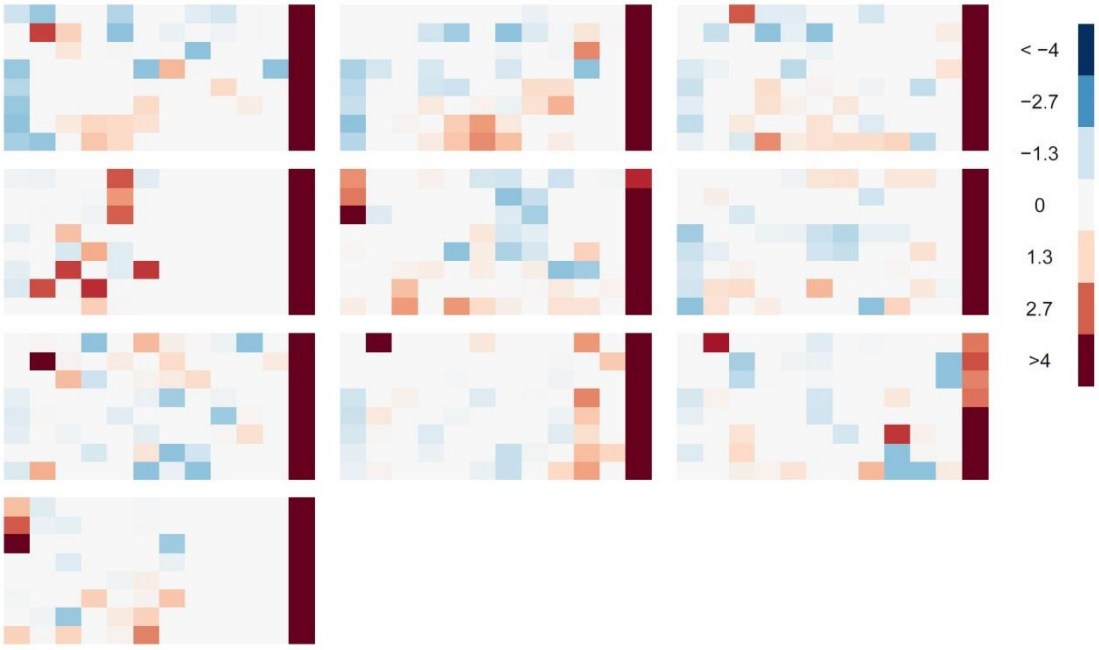

Figure 20: Image plot of assay scores (z-scores), stratified by plates. The scored values for each plate are indicated by colour coding on a rectangular grid.

The plates were arranged consecutively from left to right. The controls and the samples were organized as in Figure 17. The baseline and the negative controls were placed at the left side of each plate. The positive controls were placed at the right side of each plate. The blue colors indicate negative z-score. Therefore, compounds that have a blue color act as inhibitors of the cleavage. On the other hand, compounds that have a red color induce stimulation of the cleavage activity.

5.6.3.2 Q-Q Plot

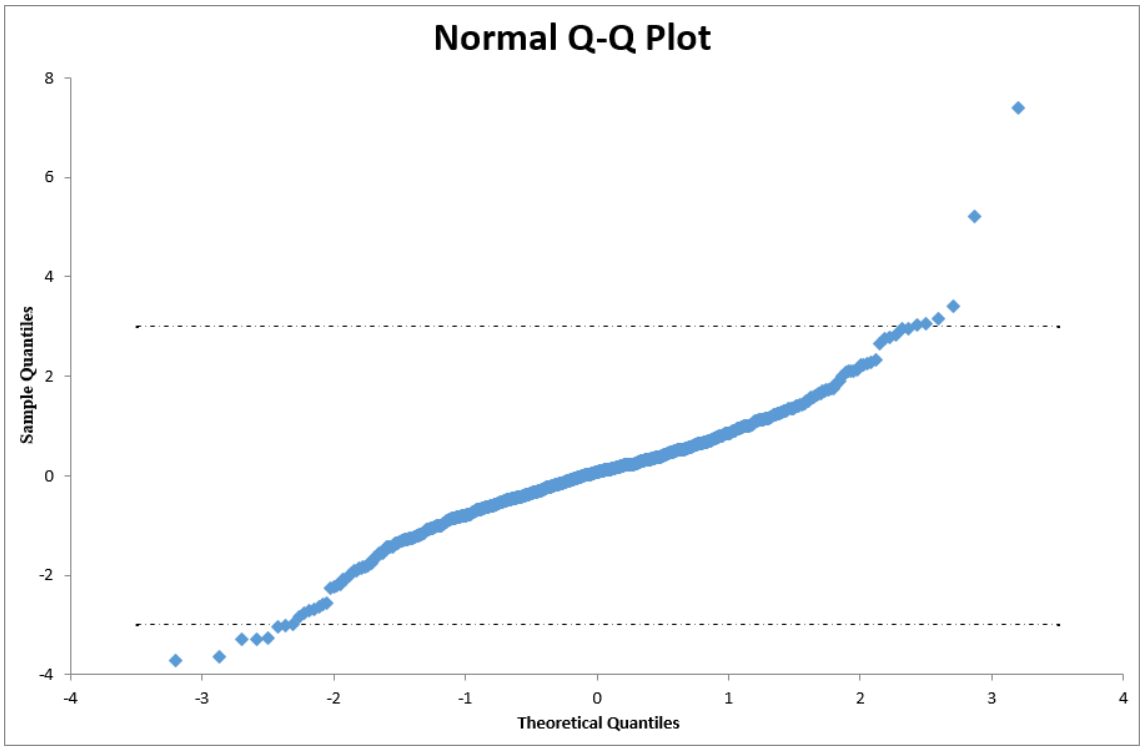

Figure 21: Normal Q-Q plot of assay scores. X-Axis: Theoretical quantiles. Y-Axis: Sample quantiles.

The samples were distributed based on their quantiles, increasing from left to right. Sample quantiles refer to assay scores. The samples that are above the first or below the second dashed lines are the outliers, which might be the potential activators or inhibitors of the assay. The highest score of the samples was 7.4, illustrated by Irsogladine maleate (top right) and Ifenprodil held the lowest score, which was -3.72 (bottom left).

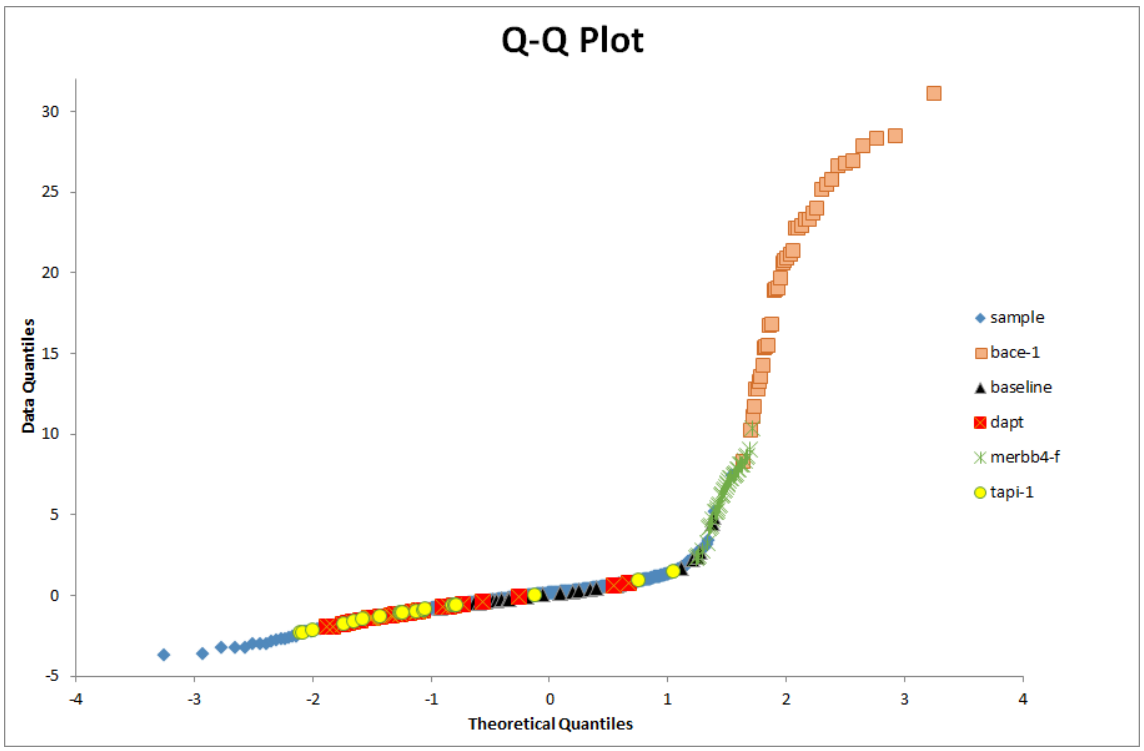

Figure 22: Q-Q plot with all samples and controls. X-Axis: Theoretical quantiles. Y-Axis: Data quantiles.

The data were distributed based on their quantiles, increasing from left to right. Data quantiles refer to the assay scores of all 10 plates. 727 samples are represented by the blue diamonds. 30 baselines are represented by black triangles. 30 DAPT are represented by red squares. 20 TAPI-1 are represented by yellow circles. 40 mERBB4-F are represented by green crosses. 40 BACE1 are represented by orange squares.

5.6.4 Screen results (see appendices)

5.6.5 Potential hits

5.6.5.1 Activators

Table 12. List of potential activators

plate	well	score	GeneID	Uses & Effects
7	B02	7.4	IRSOGLADINE MALEATE	Phosphodiesterase inhibitor, gastroprotective
8	A02	5.21	NITRENDIPINE	Dihydropyridine calcium channel blocker, antihypertensive
9	A02	3.41	BESTATIN	Reversible protease inhibitor, adjuvant therapy for AML, CML, and lung cancer
4	G04	3.15	4-(AMINOMETHYL) BENZENESULFONAMIDE ACETATE	Sulfonamide, topical anti-infective, burn therapy
4	F06	3.05	Allegra	Antihistamine drug, for seasonal allergic rhinitis
9	F09	3.04	Zolmitriptan	Selective serotonin receptor agonist of the 1B and 1D subtypes, anti-migraine
1	B02	2.96	DIPYRIDAMOLE	Phosphodiesterase inhibitor, platelet aggregation inhibitor
4	F03	2.96	RIFAPENTINE	Antimycobacterial, for pulmonary tuberculosis
4	G02	2.84	NALTREXONE HYDROCHLORIDE	Antagonists at the μ-opioid receptor (MOR), the κ-opioid receptor (KOR), management of opiate cessation
4	A05	2.78	Citalopram	Antidepressant drug of the selective serotonin reuptake inhibitor (SSRI)
3	A03	2.75	Albendazole	Anthelminthic, inhibiting the formation of microtubules from tubulin

5.6.5.2 Inhibitors

Table 13. List of potential inhibitors

plate	well	score	GeneID	Uses & Effects
5	F09	-3.72	Ifenprodil	NMDA receptor inhibitor
6	H08	-3.64	IDARUBICIN_HCl	Anthracycline, flagged
7	A10	-3.29	Doxorubicin_Hydrochloride	Anthracycline, flagged
7	H06	-3.29	EPIRUBICIN_HYDROCHLORIDE	Anthracycline, flagged
7	G07	-3.27	TRIPTOLIDE	Antineoplastic agent, flagged
5	E05	-3.05	diphenylcyclopropenone	Allergic contact synthetizer, flagged
1	D06	-3.01	Spironolactone	Diuretic/antihypertensive, Mineralocorticoid receptor (MR) antagonist
3	B04	-3	MITOXANTRONE	Topoisomerase Inhibitor, flagged
1	B05	-2.84	PERPHENAZINE	Dopamine antagonist with antiemetic and antipsychotic properties
7	A04	-2.76	TOPOTECAN_HCL	Topoisomerase inhibitor, treatment of ovarian cancer
9	H09	-2.71	DACTINOMYCIN	Actinomycin D, inhibits transcription, treatment of Wilms' tumour, Ewing's sarcoma, metastatic nonseminomatous testicular cancer
9	C11	-2.7	HOMOHARRINGTONINE	Antineoplastic, flagged
1	D11	-2.65	Pamelor	second-generation tricyclic antidepressant (TCA)
1	C08	-2.6	Annoyltin	Amitriptyline, antidepressant
7	H08	-2.57	MOSAPRIDE_CITRATE	Serotonin receptor agonist, gastroprokinetic agent
9	B11	-2.27	Cyproheptadine-Hydrochloride	Serotonin antagonist, Histamine H1 Antagonist, anti-allergic, antipruritic
2	D10	-2.23	Thioridazine_Hydrochloride	Antipsychotic, flagged
5	B07	-2.22	CGS_15943	antagonist for the adenosine receptors A_1 and A_{2A}

5.6.5.3 Potential hits from NCC 201

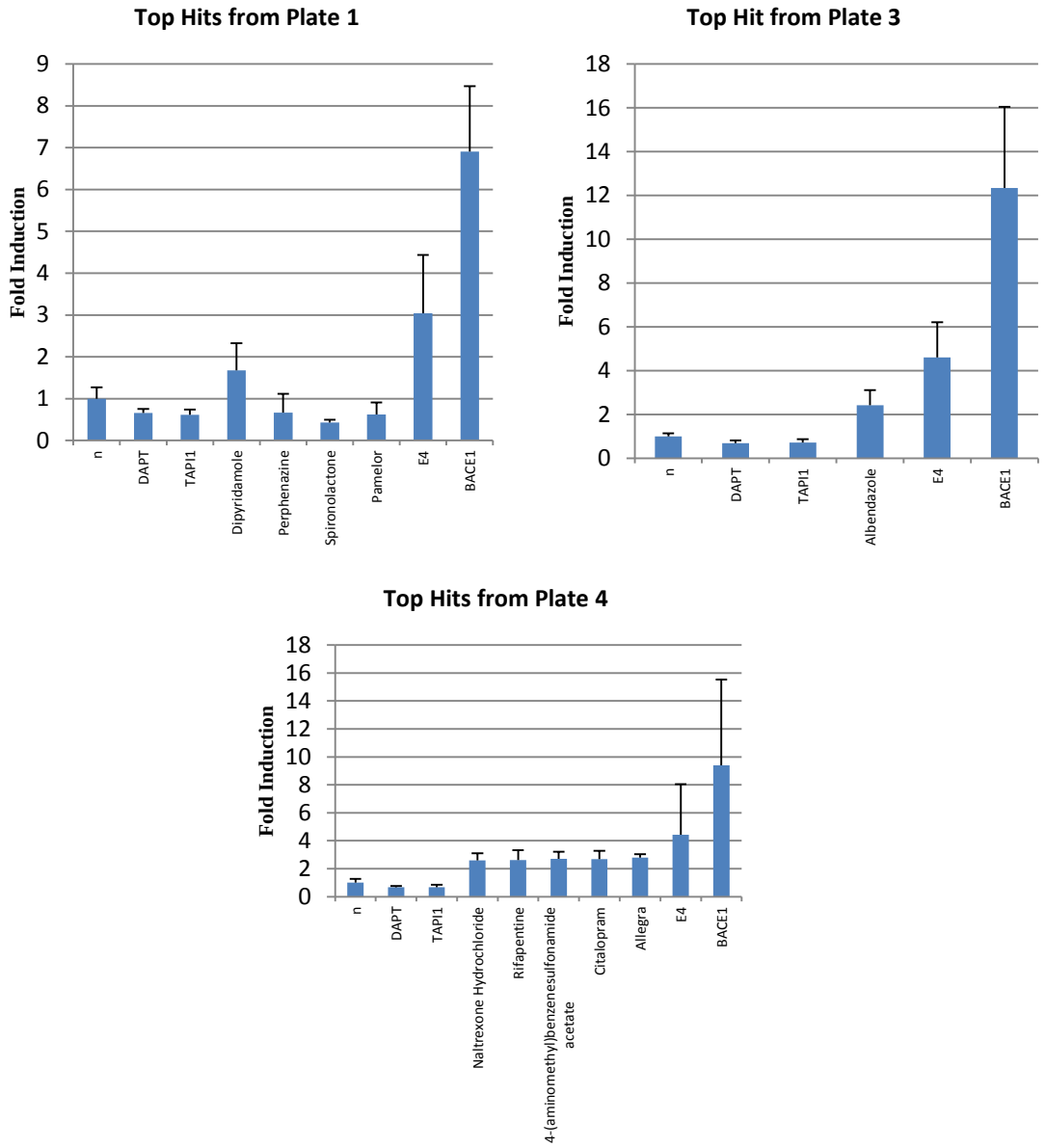

Figure 23: Graphical representation of top hits from NCC 201. n: baseline. Negative controls: DAPT and TAPI1. Positive controls: E4 (ERBB4-F) and BACE1.

The selected hits were represented in a column chart with the baseline, positive, and negative controls from the corresponding plates. Plate 2 was excluded from the selection because none of its compound carried any significant score. Top activators from NCC 201 were Dipyridamole, Albendazole, Naltrexone, Rifapentine, 4-(aminomethyl) benzensulfonamide acetate, Citalopram, and Allegra. Top inhibitors from NCC 201 were Perphenazine, Spironolactone, and Pamelor.

5.6.5.4 Potential hits from NCC 003

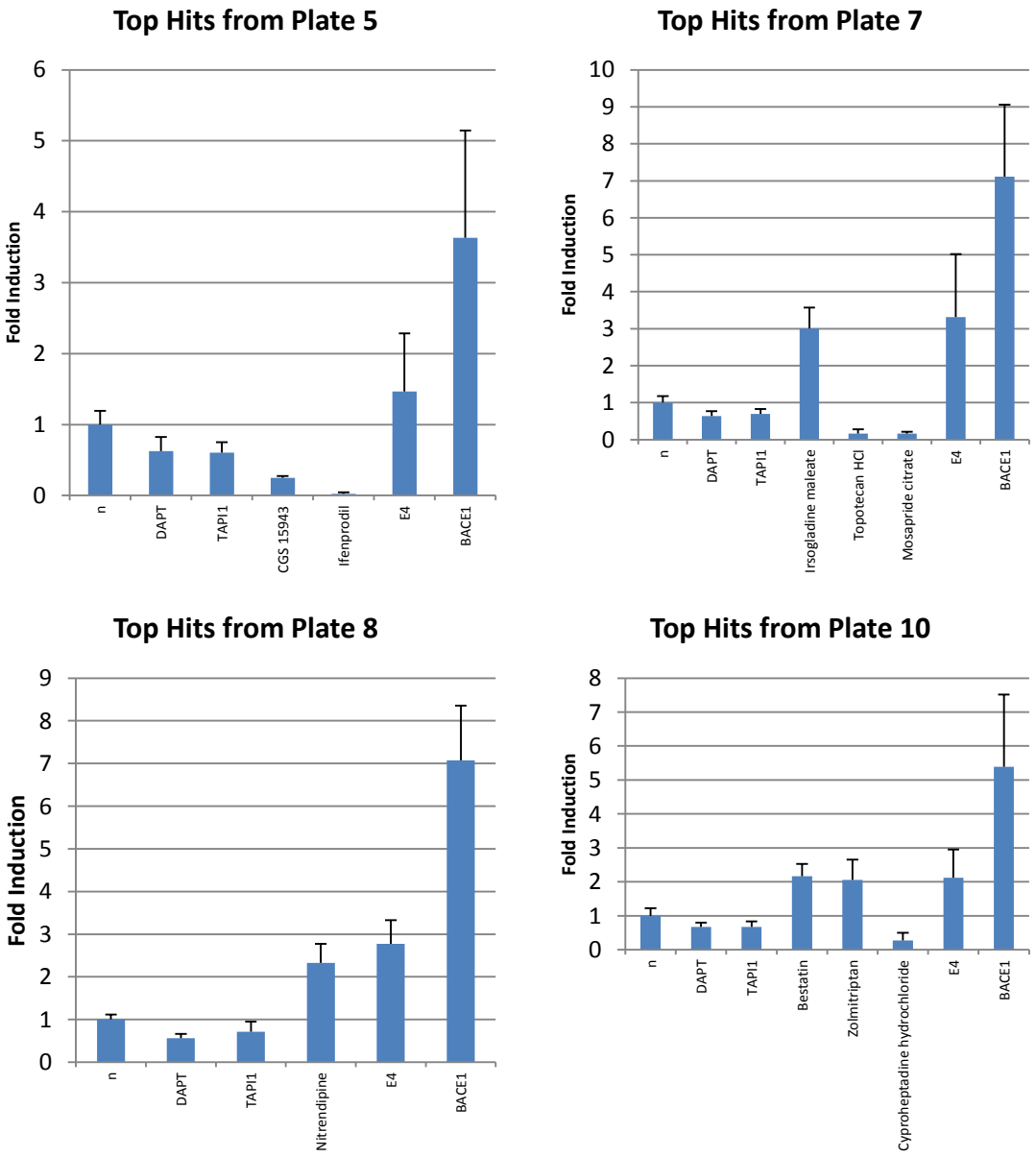

Figure 24: Graphical representation of top hits from NCC 003. n: baseline. Negative controls: DAPT and TAPI1. Positive controls: E4 (ERBB4-F) and BACE1.

The selected hits were represented in a column chart with the baseline, positive, and negative controls from the corresponding plates. Plate 6 and 10 were excluded from the selection because none of its compound carried any significant score. Top activators from NCC 003 were Irsogladine maleate, Nitrendipine, Bestatin, and Zolmitriptan. Top inhibitors from NCC 003 were CGS 15943, Ifenprodil, Topotecan, Mosapride citrate, and Cyproheptadine hydrochloride.

6 Discussion

6.1 Establishment of the assay and controls

The use of bioluminescent assays has been commonly used for drug discovery by means of HTS. In HTS applications, luciferase has been well known as a reporter gene, which can be used to detect changes in assay regulation. This knowledge was implemented in the cleavage assay. The cleaved NRG1-ICD-GV binds to the UAS, which drives the reporter gene luciferase. The luciferase induction kinetics, which can be measured with a luminometer, will also determine whether the intracellular cleavage of NRG1 was stimulated or inhibited by the compounds.

NRG1 cleavage assay was first performed in living cells, using 3.5 cm culture dishes in a 32-channel luminometer (LumiCycle). The advantage of using LumiCycle is the 'online' measurement function, which helps in monitoring the progress of the assay in real time. The peak of luciferase activity was crucial for the measurement in 96- and 384-well plates, which is around 24-26 hour after transfection. The adaptation to 96-well plate was in accordance with the existing protocol. The amount of cells was optimized to 70,000 cells per well for better results.

The protocol for 384-well plate had to be established from the beginning. After many experiments and consultations with other colleagues, 20,000 PC12 cells in a well were found to be the most favorable amount. All other amounts have also been adjusted for robust results.

At first, all the pipetting had to be done manually using a multi-channel pipette. When transferring the assay to the robot (Hamilton Robotics), some issues arose that needed better adjustments, for instance the correct tips, dispense velocity, etc. Other issues such as coating or medium removal have all been resolved.

A new transfection method was being developed by other colleagues. It was called 'in solution transfection'. Until then, 'on plate transfection' was always the method of transfection. The idea of on plate transfection is to seed the cells on plates/dishes beforehand on the first day and further continue the DNA transfection into the seeded cells on plate on the next day. The concept of 'in solution transfection' is to directly transfect the DNA into the cells first, and then seed the DNA-transfected cells into the plate on the same day. The benefit of using 'in solution transfection' is that it is efficient as a large number of cells can be transfected at once. After going through many

experiments to compare the results adequately, it was concluded that the results from ‘in solution transfection’ were slightly better. Moreover, ‘on plate transfection’ can be hardly done on 384-well plate. Therefore, ‘in solution transfection’ was used for the screen.

As can be seen from Figure 10, it was shown that both G10-MLP-luc and G10-CMV-luc had better results than G5-luc. The existence of promoters influences the luciferase expression. A test conducted by colleagues also displayed a better induction using 10xUAS instead of 5xUAS. However, a report indicated that a high methylation status inhibits the binding of Gal4 to the UAS. It was suggested, that by reducing the number of repeated UAS sequences, the tendency of them being methylated might be reduced (Gälweiler et al. 2000; Engineer et al. 2005). Another publication has also studied the comparison of activity between MLP and CMV promoter (Goossens et al. 2000). In contrast to this study, the results showed that MLP promoter induces higher gene expression than CMV promoter.

NRG1 type III used in the screen due to its relevance to schizophrenia. A lot of studies have explained its significance in neuroconnectivity, myelination, and cognitive functions. Furthermore, the mutation and cleavage processing of NRG1 type III have also been well examined. Nevertheless, validation assays with NRG1 type I should also be performed.

The search for positive control compounds that stimulate NRG1 cleavage was not successful. Therefore, ERBB4 and BACE1 overexpression were chosen as the positive controls of the screen.

The interaction with ERBB4 can activate the translocation of NRG1-ICD into the nucleus (Mei and Xiong 2008). Regardless, it is not known exactly how the mechanism of this stimulation works as it is still unexplained, whether the binding with ERBB4 could also stimulate the extracellular cleavage, or just the intracellular cleavage. According to the result, a lower amount of ERBB4-F proved to have a better outcome than a higher amount (Figure 11). The secretory system of the cells might be plugged due to the artificially overexpression of ERBB4, so that less ERBB4 could reach the plasma membrane. Additional experiments, such as two-cell assay or immunofluorescence still need to be done. As stated above, an optimal amount of ERBB4 can increase the cleavage activity by ~2.5-fold from the baseline. Hence, less amount of ERBB4 was used for cleavage stimulation control in the screen.

NRG1 belongs to the physiological substrates of β-secretase (BACE1) (Fleck et al. 2013, 2016). BACE1 is an enzyme that can cleave NRG1 extracellularly, leaving a membrane-bound NRG1-CTF (Figure 4 and 5). NRG1-CTF will be further intracellularly cleaved by γ-secretase to release NRG1-ICD. Experiments with BACE1 showed an outstanding result, with an increase of ~400 per cent from baseline (Figure 13). The extracellular cleavage of NRG1 could be essential for the processing of intracellular cleavage. With the additional source of BACE1, NRG1 could be cleaved extracellularly much faster and γ-secretase could do its work more effectively. A comparison with overexpressed γ-secretase would provide an interesting result.

As explained above, pro-NRG1 can be cleaved by these known enzymes: γ-secretase, TACE, and BACE1. The controls for successful inhibition of cleavage for the screen were inhibitors of these enzymes.

DAPT is a γ-secretase inhibitor and it can indirectly inhibit Notch signaling that is involved in many diseases, such as cancers or atherosclerosis (Micchelli et al. 2003; L.-C. Li et al. 2014; Qin et al. 2016). It can also reduce the Aβ peptides levels in the transgenic mouse which might be useful for Alzheimer's disease study (Dovey et al. 2001; Lanz et al. 2003). DAPT targets the C-terminal fragment of presenilin, a catalytic subunit of the γ-secretase intramembrane protease complex, by synthesizing a photoactivable DAPT derivative (Morohashi et al. 2006). By inhibiting the γ-secretase, the intracellular cleavage of NRG1 will be reduced. Thus, less NRG1-ICD will be released. In the assay, DAPT inhibited the activity as expected by ~50 per cent from baseline (Figure 14). Therefore, it is suitable for the screen.

TAPI-1 is a potent inhibitor of matrix metalloproteinases and TACE (Sun et al. 2015). TAPI-1 also reduced muscarinic receptor-stimulated sAPP α (soluble N-terminal fragment of APP) release in HEK-293 cells (Slack, Ma, and Seah 2001; Doedens, Mahimkar, and Black 2003). Like BACE1, TACE can also cleave NRG1 extracellularly, but on different positions (Figure 5). At a final concentration of 10 µM, TAPI-1 showed a satisfying result, with a reduction of activity by ~50 per cent from the baseline (Figure 15).

As mentioned above, BACE1 is responsible for the extracellular cleavage. Two BACE1 inhibitors were tested, namely LY2811376 and β-secretase Inhibitor IV. LY2811376 is a BACE1 Inhibitor with marked Aβ-lowering effects in animal models (May et al. 2010, 2011; Portelius et al. 2014). β-secretase Inhibitor IV is a cell-permeable inhibitor that binds to BACE-1 active site and blocks its proteolytic activity (Potter and Dressler 2000; Stachel et al. 2004). After some dose response assays, both LY2811376 and β-secretase IV did not make any convincing inhibition unless used at high concentrations (60-80 μM). Both of them only showed a 20 per cent reduction from baseline at a final concentration of 10 μM (Figure 15), thus BACE1 inhibitors were not used as controls.

DAPT worked presumably the best because it inhibits directly the intracellular cleavage of NRG1-ICD from the rest of NRG1. Because the intracellular cleavage is blocked by DAPT, NRG1-ICD cannot be released, despite the event of the extracellular cleavage. Even though the extracellular cleavage is blocked (by TAPI-1, LY2811376, or β-secretase Inhibitor IV), NRG1-ICD can still be intracellularly cleaved by γ-secretase. This could still result in the occurrence of intracellular cleavage despite the extracellular cleavage being blocked.

Both BACE1 inhibitors need to have a higher concentration to be at the same level as TACE inhibitor. Unfortunately, it is still unknown, exactly how the mechanism of action of these inhibitors works. LY2811376 and β-secretase Inhibitor IV at the final concentration of 60 μM have the same result as TAPI-1 at 10 μM. DAPT and TAPI-1 were then chosen to be the negative controls for the screen.

6.2 Screen and hits

The compounds used for the screen were supplied from NIH Clinical Collection. Additionally, all compounds have already been clinically tested and approved by FDA with its known safety profile. Therefore, the upcoming hits could be directly tested on animals and in clinical trials. Many drugs still have undiscovered therapeutic efficacy aside from its original indication. This screen can help to find drugs that could provide some insight in relevance to NRG1 back signaling and perhaps schizophrenia.

The screen was planned to be done in 384-well plate instead of 96-well plate after considering time and cost efficiency, and also the high amount of plates for replicates. The tests that were carried out between 4 replicates and 8 replicates beforehand in order to correlate the results appear to be nearly identical. Four replicates were then selected for each compound. In order to create 4 replicates of all 727 compounds, only 10 384-well plates were needed instead of 40 96-well plates. Renilla luciferase was not used for the screen due to its inconstant value in the 384-well plate. The discovered hits should be then individually validated with Renilla luciferase or other secondary assays to investigate their toxicity.

After the assay was robust enough to be done in 384-well plates, the screen was ready to be performed as well. The protocol was carefully planned and all the materials needed for the screen were prepared beforehand. During the course of the screen, there were some new ideas for improvements. Two hours after transfection, ten 50 ml Falcon tubes containing the cells with the same transfected DNA should be first mixed together as one before pouring them into the reservoir, instead of pouring them one by one. The chance for every well to have the same amount of cells might be better. The method of using a centrifuge to remove the medium from the plates could also be improved.

However, there were also some problems throughout the screen. The cells were not evenly distributed for each well on some plates. There were wells that had more volume than the others. There was only one microplate reader (Berthold), so the other following plates had to wait in a queue. Consequently, the exact time of measurement for the other plates would also be delayed. The robot also unknowingly stopped functioning as it pipetted the Firefly buffer onto plate 7, so the application had to be done manually.

Some problems in replicate performances were also observed. After looking at the raw data in Microsoft Excel, every 2 rows in some plates had somehow bigger values than

the rows before, especially the positive controls. That suggests, either the first and the second replicates had lower values, or the third and fourth replicates' value was too high. There could be many reasons behind this, such as volume differences in some wells due to pipetting error. Due to volume differences, the wells that had more volume might also have a higher value. Such event was also observed during the measurement. As the pipette tips passed into certain wells, the tip volume would also be added up into the mixture volume of PLB and Firefly buffer, so that the total volume would exceed the maximal well volume, which can result in the possible contamination to the nearby wells. Another explanation is the position of the wells on the screening plate, specifically on the outer rows and columns. The value on said rows and column would differ from wells in the interior plate. This phenomenon is called edge effects, which are caused by rapid evaporation at the corners and edges during the screen (Carralot et al. 2012).

The analysis of the screen was done by cellHTS2 software (Boutros, Brás, and Huber 2006). CellHTS2 is a software program designed to analyze cell-based high-throughput screens. Raw data files need to be imported and the experimental outliers will be identified. It is also capable of normalizing data for systematic technical variations. After the phenotypes scoring and statistical summary, the complete analysis can be documented as a set of HTML pages.

As shown in Figure 19, the normalized values of the negative controls have an average of about -1 and have a defined peak for each replicate. In contrast, the normalized values of the positive controls, especially BACE1, scatter overall. They are not dense enough to make a defined peak equivalent to the negative controls. Some technical errors, as explained above, are to be considered. Z´-factor is also the ratio of the separation between these two peaks to the assay dynamic range. Based on Table 11, all Z´-factors were smaller than 0. The activation from the positive controls was very strong but the replicate performance was not robust enough. The positive controls have to be optimized again, perhaps using a compound rather than a co-transfection. As an alternative, the position of the wells could be changed considering the edge effects.

The following were the top activators of the screen: Irsogladine maleate, Nitrendipine, Bestatin, 4-(aminomethyl) benzensulfonamide acetate, Allegra, Zolmitriptan, Dipyridamole, Rifapentine, Naltrexone hydrochloride, Citalopram, and Albendazole. Brief information about its effects and uses can be seen on Table 12. Among all of them,

only Citalopram is known for its practice in psychiatric diseases.

Citalopram belongs to selective serotonin reuptake inhibitor (SSRI), a class of antidepressant agents. SSRI increases the neurotransmitter serotonin-level in the blood by inhibiting its resorption into the presynaptic cell. Contrary to antipsychotics, they have little affinity for the dopamine D2 receptor (Baumann 1996; Mandrioli et al. 2012). The increase in serotonin-level may have an invigorating effect that might help relieve the negative symptoms of schizophrenia patients. Citalopram is indicated for depression, anxiety disorder, and obsessive-compulsive disorder. There was a recent clinical trial conducted to treat schizophrenia patients with antidepressant Citalopram. The result suggested that Citalopram had some effect on one of the negative symptoms, avolition/amotivation, although further investigation still needs to be done (Barnes et al. 2016).

The top inhibitors included: Ifenprodil, Idarubicin, Doxorubicin, Epirubicin, Triptolide, Diphenylcyclopropenone, Spironolactone, Mitoxantrone, Perphenazine, Topotecan, Dactinomycin, Homoharringtonine, Pamelor, Annoyltin, Mosapride citrate, Cyproheptadine hydrochloride, Thioridazine hydrochloride, and CGS 15943 (Table 13). Some of the inhibitors that were flagged (Idarubicin, Doxorubicin, Epirubicin, Triptolide, Diphenylcyclopropenone, Mitoxantrone, Homoharringtonine, and Thioridazine hydrochloride) are considered toxic because of the low level of Renilla luciferase during a previous screen performed by colleagues. Among the rest, there are some interesting compounds that have also been used for psychiatric disease treatments, like Ifenprodil, Perphenazine, Pamelor, and Annoyltin.

Ifenprodil is an N-methyl-D-aspartate receptor (NMDAR) antagonist (Reynolds and Miller 1989). NMDA receptors are ionotropic glutamate receptors that play a critical role in synaptic plasticity and memory functions (F. Li and Tsien 2009). However, NMDA receptors are also involved in excitotoxicity, a condition by which the nerve cells are damaged due to overstimulation by neurotransmitters such as glutamate (Choi, Koh, and Peters 1988). Excitotoxicity might lead to some neurodegenerative diseases, such as Alzheimer’s disease or Huntington’s disease (Parsons and Raymond 2014). Preventing excessive excitotoxicity by using an NMDAR antagonist such as Ifenprodil might provide some therapeutic benefits. Furthermore, glutamate hypothesis of schizophrenia suggests that hypofunction of NMDA receptor signaling may contribute to the symptoms of schizophrenia (Lisman et al. 2008). This statement can be related to

the side effects of NMDAR antagonist, which reproduce similar symptoms resembling psychosis, such as hallucinations, delusions, catatonia, and thought disorders (Aarts and Tymianski 2003; Erhardt et al. 2007).

Perphenazine is classified as a typical antipsychotic drug. It is a dopamine (D2) antagonist with antiemetic properties and extrapyramidal side-effects (Sweet et al. 2000). It blocks postsynaptic D2 receptors, which prevent dopamine excess in brain. Hyperactivity in brain dopamine systems was hypothesized to be the cause of schizophrenia (Howes and Kapur 2009). D2 receptor antagonist is used to reduce psychotic symptoms of schizophrenia, such as aggressions, hallucinations or delusions (Hartung, Sampson, and Leucht 2015). It was also suggested, that D2 receptor antagonist may hold promises against tauopathies and neurotoxicity (McCormick et al. 2013).

Pamelor and Annoyltin are the hydrochloride salt forms of Nortriptyline and Amitriptyline, respectively. Both of them are tricyclic antidepressant (TCA) drugs. Amitriptyline blocks norepinephrine and serotonin presynaptic receptors, thus inhibiting the reuptake and increasing norepinephrine and serotonin levels in synaptic clefts in the CNS, whereas Nortriptyline blocks selectively the norepinephrine reuptake (Tatsumi et al. 1997). They are used to treat psychological disorders, such as major depression or personality disorder, and are also used in pain management (Kevin Hicks et al. 2016). However, due to its anticholinergic side effects, the indication has to be considered carefully (Gray et al. 2015). A combination of antipsychotic Perphenazine and antidepressant Amitriptyline was more effective against depression form of schizophrenia than a monotherapy (Prusoff et al. 1979).

It was interesting to see that drugs from antidepressant class can function both as activator (Citalopram) and also inhibitor (Pamelor, Annoyltin) of the assay. Both Citalopram and Annoyltin have the same mechanism of action, which resulting in a high level of serotonin in synaptic clefts in the CNS. There could be a connection between serotonin and NRG1 cleavage activity, as experiments with serotonin receptor can also be done in PC12 cells (Homma et al. 2006; Koizumi and Nakajima 2014). Animal studies proposed the involvement of NRG1 gene expression in the development of the serotonergic system (Dean et al. 2008). There might be another different mechanism of action of the drugs, which is still yet to be discovered. Other intriguing results from the screen are Ifenprodil and Perphenazine. Ifenprodil, as an NMDA receptor antagonist,

might cause irregularity in glutamatergic signaling, as stated in the glutamate hypothesis of schizophrenia. Research done on animal models showed that defect on glutamatergic synapses might lead to behavioral abnormalities in ErbB2/B4-deficient mice (Barros et al. 2009). On the other hand, dopamine hypothesis of schizophrenia suggests hyperactivity of dopamine in brain might also lead to schizophrenia, which can be treated by Perphenazine, a dopamine receptor antagonist. Both hypotheses might complement each other, which result into both dopaminergic and glutamatergic abnormalities in CNS and eventually the symptom complex of schizophrenia. Nevertheless, all selected hits will go through further validation.

6.3 Outlook

Following the flow of drug discovery process, the identified hits will be further validated. The selected compounds will be individually tested again along with the positive and negative controls with LumiCycle live cell measurements and in 96-well plates for confirmation. Afterwards, the compounds will undergo 96-well plate methods with Renilla luciferase for toxicity tests. Technical control assays using CMV-GV also need to be done to check whether the compounds actually modify the NRG1 cleavage activity or rather they have more effects on the GV, the UAS promoter, or the Firefly luciferase. Dose response assays of the compounds will be established with the robot. After the qualified compounds passed the control tests, secondary assays and secondary targets using the compounds will be planned. To test whether the compounds also have an effect to ERBB4, ERBB4-PI3K split TEV assay will be done (Wehr et al. 2006). Another example would be a NRG1-LIMK1 split TEV assay to test whether the compounds have any effects on its interaction. Alternative targets for the compounds, such as APP or Notch which are similarly processed by BACE1, TACE, and γ-secretase (see above), could also be tested to see, whether the compounds inhibit/activate specifically the NRG1 cleavage or generally also the other targets which could cause some side effects. Additional testing, like orthogonal validation includes western blot, e.g. NRG1 cleavage products. The following step would be preclinical studies using mouse models to collect information regarding in vivo processing of NRG1 in the mouse brain and the first human dose. After being approved by the regulatory authorities, the drugs may advance to the clinical phase, where the drugs could be tested on human subjects suffering from schizophrenia.

7 Conclusions

Schizophrenia is a developmental, long-term mental disorder that causes a number of different psychological symptoms, such as positive symptoms, negative symptoms, and cognitive deficits. The exact causes of schizophrenia are unknown, but research suggests that a combination of physical, genetic, psychological, and environmental factors might contribute to the development of schizophrenia. In recent years, genome-wide association studies (GWAS) provided insights into possible etiological mechanisms of this symptom complex. Several genes have been associated with the disease, such as Neuregulin-1 (*NRG1*). This gene was identified as risk factor for schizophrenia in a certain population. Therefore, an improved understanding of NRG1 signaling could eventually lead to the development of more effective therapies for certain forms of schizophrenia.

A mutation within the transmembrane domain of NRG1 (V321L) was found to be associated with schizophrenia. It interferes with the intracellular cleavage of NRG1, which consecutively impairs the physiological NRG1 signaling, for instance in the development of cortical dendrite formation and neuroconnectivity. This so-called backward signaling of NRG1 is our main focus, in which the intracellular domain of NRG1 is crucial. Although there were already some hypotheses regarding its function as explained above, it is still unclear which signaling pathways it affects. Therefore, NRG1 cleavage assay was established and then a high-throughput screening (HTS) approach was conducted to find potential modulators of NRG1 cleavage.

The assay was first carried out in living cells to observe the NRG1 cleavage kinetics. After an ideal time point of the cleavage was determined, the assay was adapted to a 96-well format and then to a 384-well format for robotics. Inhibitors for the enzymes that play important roles for the NRG1 processing (BACE1, TACE, and γ-secretase) were included in the screen as control for down-modulation. Controls for activation were co-transfection with ERBB4 or BACE1 constructs, which stimulate the intracellular cleavage of NRG1.

After the assay was robust enough in 384-well plates, the screen was performed using an automated liquid handling system assembled with a microplate reader. The NIH Clinical Collection (NCC) compound library consists of more than 700 drugs that have been FDA-approved. All these drugs have been tested on humans and their safety profiles are already known. Via this drug repurposing strategy, the costs and time for application of identified hits can be reduced significantly, so that validated hits could be tested in mouse models and clinical trials rapidly.

Screen results revealed some promising modulators of NRG1 cleavage, mainly because of their history of use in psychiatric diseases. Citalopram, which has been used as antidepressant, was identified as a potential activator of NRG1 intracellular cleavage. Moreover, Pamelor and Annoyltin, which also belong to antidepressant class, were found to be potential cleavage inhibitors. Another interesting hit was Perphenazine, a dopamine receptor antagonist that has already been used for treatment of schizophrenia. The most potent inhibitor from the screen was Ifenprodil, an NMDA receptor antagonist, which has been linked with the glutamate hypothesis of schizophrenia. These substances should be further validated, e.g. in cellular toxicity tests, so-called counter assays to asses specificity, and dose response assays. After the selected compounds pass all the necessary criteria, the compounds would be evaluated in mouse models of schizophrenia and finally in clinical trials.

8 Zusammenfassung

Schizophrenie ist eine entwicklungsbedingte, langfristige psychische Störung, die eine Reihe von verschiedenen psychologischen Symptomen verursacht, wie zum Beispiel positive Symptome, negative Symptome, und kognitive Defizite. Die genauen Ursachen der Schizophrenie sind unbekannt, aber die Forschung lässt darauf schließen, dass eine Kombination von physischen, genetischen, psychologischen und Umweltfaktoren zur Entwicklung der Schizophrenie beiträgt. Sogenannte *genome-wide association studies* (GWAS) lieferten insbesondere einen Einblick in die möglichen ätiologischen Mechanismen dieser Erkrankung. Mehrere Gene wurden mit der Krankheit assoziiert, darunter Neuregulin-1 (*NRG1*). Dieses Gen wurde als Risikofaktor für Schizophrenie in einer bestimmten Population identifiziert. Daher könnte ein verbessertes Verständnis der NRG1-Signaltransduktion zur Entwicklung von wirksameren Therapien für bestimmte Formen der Schizophrenie führen.

Es wurde ein Zusammenhang zwischen einer Mutation, die innerhalb der Transmembrandomäne von NRG1 lokalisiert (V321L), und Schizophrenie identifiziert. Diese Mutation interferiert mit der intrazellulären Spaltung von NRG1, was folglich die physiologische Funktion von NRG1 beeinträchtigt, zum Beispiel bei der Entwicklung der kortikalen Dendritbildung und der Neurokonnektivität. Diese „Rückwärtssignalisierung“ von NRG1 ist unser Schwerpunkt, bei der die intrazelluläre Domäne von NRG1 eine kritische Rolle übernimmt. Zwar gab es bereits einige Hypothesen über ihre Funktion, wie oben erläutert, aber es ist noch unklar, welche Signalwege sie im Detail beeinflusst. Daher wurde der NRG1 Spaltungs-Assay etabliert und anschließend ein Screening durchgeführt, um potentielle Modulatoren der NRG1-Prozessierung zu finden.

Der Assay wurde zuerst in lebenden Zellen durchgeführt, um die NRG1-Spaltkinetik zu untersuchen. Nachdem ein idealer Zeitpunkt der Spaltung bestimmt wurde, wurde der Assay in 96-Well-Platten und schließlich in 384-Well-Platten für das automatisierte Screening angepasst. Inhibitoren für Enzyme, die bei der NRG1-Verarbeitung (BACE1, TACE und γ-Sekretase) wichtige Rollen spielen, wurden als Kontrollen einbezogen. Kontrollen für Aktivatoren der NRG1-Spaltung waren die Co-Transfektion mit ERBB4- oder BACE1-Konstrukten, die die intrazelluläre Spaltung von NRG1 stimulieren.

Nachdem der Assay in 384-Well-Platten robust etabliert wurde, konnte der Screen unter Verwendung eines Roboters durchgeführt werden. Die hier verwendete NIH Clinical Collection (NCC) Compound Bibliothek besteht aus mehr als 700 Medikamenten, die bereits FDA-zugelassen sind. Alle diese Medikamente wurden am Menschen getestet und ihre Sicherheitsprofile sind bereits bekannt. Durch diese „drug repurposing"-Strategie können die Kosten und die Zeit für die weitere Entwicklung der identifizierten Hits deutlich verringert werden, so dass validierte Medikament-Kandidaten schnell in klinischen Studien eingesetzt werden können.

Im Screen wurden einige vielversprechende Modulatoren der NRG1-Spaltung identifiziert, welche vor allem aufgrund ihrer Verwendung bei psychiatrischen Erkrankungen interessant sind. Citalopram, das als Antidepressivum verwendet wird, ist ein potentieller Aktivator der intrazellulären NRG1-Spaltung. Darüber hinaus wurden Pamelor und Annoyltin, ebenfalls Antidepressiva, als potenzielle Inhibitoren identifiziert. Ein weiterer interessanter Kandidat ist Perphenazin, ein Dopamin-Rezeptor-Antagonist, der bereits zur Behandlung von Schizophrenie verwendet wird. Der potenteste Inhibitor aus dem Screen war Ifenprodil, ein NMDA-Rezeptor-Antagonist, der mit der Glutamat-Hypothese der Schizophrenie assoziiert wird. Alle diese Substanzen müssen weiter validiert werden, z.B. in Toxizitätstests, sog. Counter-Assays als Spezifitätskontrollen, und Dose-Response-Assays. Nachdem die ausgewählten Compounds alle notwendigen Kontrollen durchlaufen haben, können sie in Mausmodellen der Schizophrenie und schließlich in klinischen Studien evaluiert werden.

9 Abbreviations

µ	micro
ADAM	a disintegrin and metalloprotease
APP	amyloid precursor protein
ATP	Adenosine triphosphate
BACE	β-site amyloid precursor protein cleaving enzyme
BSA	Bovine Serum Albumin
CNS	Central nervous system
cpm	Counts per minute
CRD	Cysteine-rich domain
CTF	C-terminal fragment
CYT	Cytochrome
DAPT	N-[N-(3,5-Difluorophenacetyl-L-alanyl)]-S-phenylglycine t-butyl ester
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTT	1,4-dithiothreitol
e.g.	For example/exempli gratia
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ERBB4/E4	Receptor tyrosine kinase family
FBS	Fetal bovine serum
FDA	Food and Drug Administration
Fluc	Firefly luciferase
g	gram, gradians
GV	Gal4-VP16
GWAS	Genome-wide association study
h	hour
HER	Human epidermal growth factor receptor
HS	Horse serum
HTML	Hypertext Markup Language
HTS	High-throughput screening
ICD	Intracellular domain
Ig	Immunoglobulin
IL-1R2	Interleukin-1 Receptor II
JMa	Juxtamembrane region a
JMb	Juxtamembrane region b
l	liter
L2000	Lipofectamine2000
LB medium	Lysogeny broth medium
LIMK1	Lin11, Isl-1, & Mec-3 kinase 1
M	Molar
MAD	Median Absolute Deviation
MAPK	mitogen-activated protein kinase
MLSMR	Molecular Libraries Small Molecule Repository
n	Nano

NCC	NIH Clinical Collection
NIH	National Institutes of Health
NMDAR	N-Methyl-D-aspartate Receptor
NRG	Neuregulin
NTF	N-terminal fragment
OMEM	OptiMEM
PBS	Phosphate buffered saline
PC12	Rat pheochromocytoma cell line
PI3K	phosphoinositide 3-kinase
PKB	Protein Kinase B
PKC	Protein Kinase C
PLB	Passive Lysis Buffer
PLL	Poly-L-lysine
RLU	Relative luciferase units
rpm	Revolution per minute
RTK	Receptor tyrosine kinase
SNP	Single nucleotide polymorphism
SSRI	Selective serotonin reuptake inhibitor
TACE	TNF-α converting enzyme
TAPI-1	TNF-α protease inhibitor 1
TCA	Tricyclic Antidepressant
TEV	Tobacco etch virus
TK	Tyrosine kinase
TMD	Transmembrane domain
TNF-α	Tumor necrosis factor-α
UAS	Upstream activating sequence
UV	Ultraviolet
VGSC	voltage-gated sodium channel

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12.1 NCC 201 screen result

Table 14. Screen result from NCC 201

plate	position	score	well	wellAnno	raw_r1_ch1	raw_r2_ch1	raw_r3_ch1	raw_r4_ch1	normalized_r1_ch1	normalized_r2_ch1	normalized_r3_ch1	normalized_r4_ch1	GeneID
1	1	-1.35	A01	baseline	55360	48340	59570	82130	-0.696	-3.128	-1.711	-0.999	control
1	13	0.24	B01	baseline	88180	89060	90840	81810	2.052	0.612	-0.131	-1.01	control
1	25	0.65	C01	baseline	77120	66410	118400	115230	1.126	-1.468	1.262	0.173	control
1	37	-1.91	D01	dapt	40980	47560	63140	56070	-1.9	-3.199	-1.53	-1.922	control
1	49	-1.62	E01	dapt	55460	48400	61420	64710	-0.687	-3.122	-1.617	-1.616	control
1	61	-1.92	F01	dapt	46940	41680	56170	55310	-1.401	-3.739	-1.883	-1.949	control
1	73	-2.33	G01	tapi-1	35000	37980	48840	48840	-2.401	-4.079	-2.253	-2.178	control
1	85	-1.76	H01	tapi-1	44150	60150	56010	65240	-1.635	-2.043	-1.891	-1.597	control
1	2	-1.91	A02	sample	37820	53340	60810	79150	-2.165	-2.669	-1.648	-1.105	SAM002264608_DOXEPIN_HYDROCHLORIDE
1	14	2.96	B02	sample	70570	116860	167870	187970	0.578	3.164	3.761	2.748	SAM002264609_DIPYRIDAMOLE
1	26	0.4	C02	sample	84550	88120	90070	118330	1.748	0.525	-0.17	0.283	SAM002264610_Propofol
1	38	-0.61	D02	sample	58540	82290	65400	87760	-0.43	-0.01	-1.416	-0.8	SAM002264611_ETHACRYNIC_ACID
1	50	-0.02	E02	sample	68600	96010	84650	81550	0.413	1.25	-0.444	-1.02	SAM002264612_FLUTAMIDE
1	62	0.35	F02	sample	82090	88700	95860	103630	1.542	0.579	0.123	-0.238	SAM002264613_FENOFIBRATE
1	74	0.49	G02	sample	88670	100290	79720	91610	2.093	1.643	-0.693	-0.664	SAM002264614_FUROSEMIDE
1	86	-1.96	H02	sample	31620	54510	66500	80110	-2.684	-2.561	-1.361	-1.071	SAM002264615_5-FLUOROURACIL
1	3	0.19	A03	sample	65460	105830	98170	86510	0.15	2.152	0.239	-0.844	SAM002264616_Folic_acid
1	15	1.43	B03	sample	72780	101050	125970	144770	0.763	1.713	1.644	1.219	SAM002264617_HYDROCORTISONE
1	27	1.06	C03	sample	86360	103710	88320	116490	1.9	1.957	-0.258	0.217	SAM002264618_Cortell
1	39	-0.37	D03	sample	55840	93500	67800	107780	-0.656	1.019	-1.295	-0.091	SAM002264619_IBUPROFEN

1	51	0.22	E03	sample	85460	91290	77780	99490	1.825	0.816	-0.791	-0.385	SAM002264620_KETOPROFEN
1	63	0.58	F03	sample	72020	87490	107400	123230	0.699	0.467	0.706	0.456	SAM002264621_MINOCYCLINE_HYDROCHLORIDE
1	75	0.99	G03	sample	79180	85350	122190	129700	1.299	0.271	1.453	0.685	SAM002264623_MICONAZOLE_NITRATE
1	87	0.68	H03	sample	80970	87800	110370	113820	1.449	0.496	0.856	0.123	SAM002297829_METYRAPONE
1	4	0.48	A04	sample	82780	80880	1e+05	127840	1.6	-0.14	0.332	0.619	SAM002264625_NORFLOXACIN
1	16	0.31	B04	sample	68130	85170	113610	111790	0.373	0.254	1.02	0.051	SAM002264627_NADOLOL
1	28	-0.24	C04	sample	53550	86470	76530	141280	-0.847	0.374	-0.854	1.095	SAM002264628_Disipal
1	40	0.09	D04	sample	63720	86350	97010	100900	0.004	0.363	0.181	-0.335	SAM002264629_OFLOXACIN
1	52	0.12	E04	sample	67250	82210	98450	110070	0.3	-0.017	0.254	-0.01	SAM002264631_PINDOLOL
1	64	0.21	F04	sample	65290	83730	99170	136840	0.136	0.122	0.29	0.938	SAM002264632_PRAZIQUANTEL
1	76	1.36	G04	sample	70470	111910	135820	119550	0.569	2.71	2.142	0.326	SAM002264634_Benzenebutanoic_Acid
1	88	1.59	H04	sample	82300	86390	133760	156230	1.56	0.366	2.038	1.624	SAM002264635_PREDNISOLONE_ACETATE
1	5	-1.64	A05	sample	37550	59440	72050	77120	-2.187	-2.108	-1.08	-1.177	SAM002264636_Phenergan
1	17	-2.84	B05	sample	15870	39860	58310	102090	-4.003	-3.906	-1.774	-0.292	SAM002264637_PERPHENAZINE
1	29	0.71	C05	sample	60500	101130	100860	139720	-0.265	1.72	0.375	1.04	SAM002264639_Prednisolone
1	41	0.11	D05	sample	67090	81620	100800	101760	0.286	-0.072	0.372	-0.304	SAM002264640_PRILOCAINE_HYDROCHLORIDE
1	53	-0.15	E05	sample	80500	79330	80210	109660	1.409	-0.282	-0.668	-0.024	SAM002264641_Prednisone
1	65	-0.37	F05	sample	56550	78530	85890	103650	-0.596	-0.355	-0.381	-0.237	SAM002264642_DL-PENICILLAMINE
1	77	1.33	G05	sample	94150	81930	116900	151990	2.552	-0.043	1.186	1.474	SAM002264643_PIPERACILLIN_SODIUM_SALT
1	89	1.3	H05	sample	76700	95010	121860	160910	1.091	1.158	1.436	1.79	SAM002264644_Quinidine_hydrochloride_monohydrate
1	6	0.24	A06	sample	53900	81210	105040	133280	-0.818	-0.109	0.587	0.812	SAM002264645_Ranitidine_hydrochloride
1	18	-0.39	B06	sample	63210	78900	76770	97260	-0.039	-0.321	-0.842	-0.463	SAM002264646_RIFAMPICIN
1	30	-0.59	C06	sample	59790	75410	82920	82980	-0.325	-0.642	-0.531	-0.969	SAM002264647_Retinoic_acid
1	42	-3.01	D06	sample	28950	41120	31850	36790	-2.907	-3.791	-3.111	-2.604	SAM002264648_Spironolactone
1	54	0.68	E06	sample	70790	90790	125210	108550	0.596	0.77	1.606	-0.064	SAM002264649_Trimethoprim

1	66	1.3	F06	sample	80380	79610	117090	149780	1.399	-0.256	1.195	1.396	SAM002264650_Tyzine
1	78	1.13	G06	sample	81490	90810	123150	115250	1.492	0.772	1.502	0.173	SAM002264651_L-THYROXINE
1	90	0.03	H06	sample	55440	67650	158440	131410	-0.689	-1.354	3.285	0.746	SAM002264652_Artane
1	7	0.1	A07	sample	64640	83760	79400	129970	0.081	0.125	-0.709	0.695	SAM002264653_URSODEOXYCHOLIC_ACID
1	19	-0.86	B07	sample	44500	74930	72810	122970	-1.605	-0.686	-1.042	0.447	SAM002554879_Dapsone
1	31	-0.11	C07	sample	70490	80410	88860	109140	0.571	-0.183	-0.231	-0.043	SAM002554881_Symmetrel
1	43	1.75	D07	sample	63500	87780	166290	195300	-0.014	0.494	3.681	3.008	SAM002554882_WARFARIN_SODIUM
1	55	0.05	E07	sample	63730	82090	95480	139020	0.005	-0.028	0.104	1.015	SAM002554883_Acetazolamide
1	67	-0.46	F07	sample	59050	83840	82830	67590	-0.387	0.132	-0.536	-1.514	SAM002554884_Allopurinol
1	79	0.07	G07	sample	63620	83360	100810	111900	-0.004	0.088	0.373	0.055	SAM002554885_ATROPINE
1	91	0.47	H07	sample	83540	82510	111960	102620	1.664	0.01	0.936	-0.274	SAM002554914_Nalidixic_Acid
1	8	-1.01	A08	sample	47930	73150	70320	100950	-1.318	-0.849	-1.168	-0.333	SAM003107539_3,5,3'-TRIIODOTHYRONINE
1	20	-0.31	B08	sample	59680	65140	87790	108190	-0.334	-1.585	-0.285	-0.076	SAM002703134_HYDROFLUMETHIAZIDE
1	32	-2.6	C08	sample	27390	49940	49510	87450	-3.038	-2.981	-2.219	-0.811	SAM002554886_Annoyltin
1	44	-0.66	D08	sample	50510	71580	88300	100850	-1.102	-0.994	-0.259	-0.336	SAM002554887_Busulfan
1	56	0.48	E08	sample	56200	83700	116410	134220	-0.626	0.119	1.161	0.845	SAM002554888_Chlorzoxazone
1	68	-0.45	F08	sample	61430	79940	79950	88470	-0.188	-0.226	-0.681	-0.775	SAM002554891_Chlorothiazide
1	80	-0.11	G08	sample	61000	82820	87760	110630	-0.224	0.039	-0.286	0.01	SAM002554892_Cimetidine
1	92	-0.28	H08	sample	74710	78480	89490	89110	0.924	-0.36	-0.199	-0.752	SAM002554889_Carisoprodol
1	9	-1.28	A09	sample	45640	65870	73100	80940	-1.51	-1.518	-1.027	-1.041	SAM002554890_Chlorpropamide
1	21	-0.81	B09	sample	48920	65950	85750	167640	-1.235	-1.511	-0.388	2.028	SAM002554894_Bentyl
1	33	-0.44	C09	sample	37980	73330	92480	117930	-2.151	-0.833	-0.048	0.268	SAM002554895_Chloroxine
1	45	0.54	D09	sample	59610	77620	127800	150180	-0.34	-0.439	1.736	1.41	SAM002554896_Diflunisal
1	57	1.3	E09	sample	49150	100160	130980	137990	-1.216	1.631	1.897	0.979	SAM002554898_Econazole_Nitrate
1	69	-0.64	F09	sample	1780	73320	91000	97860	-5.182	-0.834	-0.123	-0.442	SAM002554899_Ethionamide
1	81	0.28	G09	sample	74920	86380	91720	115940	0.942	0.365	-0.086	0.198	SAM002554900_Methocarbamol

1	93	0.45	H09	sample	62100	90590	96310	153260	-0.131	0.752	0.146	1.519	SAM002554901_Hydrochlorothiazide
1	10	-0.67	A10	sample	64470	74820	80600	52600	0.067	-0.696	-0.648	-2.045	SAM002554902_Vistaril_Pamoate
1	22	0.67	B10	sample	12490	88450	130680	132270	-4.286	0.556	1.882	0.776	SAM002554903_Hexachlorophene
1	34	0.02	C10	sample	60680	76000	99300	122730	-0.25	-0.588	0.297	0.438	SAM002554904_Isoniazid
1	46	0.52	D10	sample	55810	101480	94350	138620	-0.658	1.752	0.046	1.001	SAM002554905_Duvadilan
1	58	-0.34	E10	sample	59580	77900	86890	124280	-0.342	-0.413	-0.33	0.493	SAM002554906_Isuprel
1	70	0.87	F10	sample	67160	96150	102970	147960	0.292	1.263	0.482	1.332	SAM002554907_Triclosan
1	82	0.23	G10	sample	67830	83580	120950	80720	0.348	0.108	1.39	-1.049	SAM002554908_Mefenamic_Acid
1	94	0.51	H10	sample	88780	91680	96790	111330	2.103	0.852	0.17	0.035	SAM002554910_Cantil
1	11	0.31	A11	sample	54340	92020	126820	102980	-0.781	0.883	1.687	-0.261	SAM002554911_Maxolon
1	23	-0.09	B11	sample	65590	76360	92510	106830	0.161	-0.555	-0.046	-0.125	SAM002554912_Methyldopa
1	35	-0.07	C11	sample	64740	75180	89260	112500	0.09	-0.663	-0.211	0.076	SAM002554913_NITROFURANTOIN
1	47	-2.65	D11	sample	25230	44240	52400	80800	-3.219	-3.504	-2.073	-1.046	SAM002554915_Pamelor
1	59	-0.41	E11	sample	58300	66190	87060	100080	-0.45	-1.489	-0.322	-0.364	SAM002554916_Albalon
1	71	-0.35	F11	sample	67910	63340	81030	108280	0.355	-1.75	-0.626	-0.073	SAM002554917_Nicotinic_Acid
1	83	-0.48	G11	sample	60190	58980	96270	91600	-0.291	-2.151	0.143	-0.664	SAM002554918_Norflex
1	95	0.37	H11	sample	71550	90730	94900	78970	0.66	0.765	0.074	-1.111	SAM002554919_Oxytetracycline_hydrochloride
1	12	4.73	A12	merbb4-f	76960	366940	133820	319790	1.113	26.129	2.041	7.416	control
1	24	8.65	B12	merbb4-f	181620	353070	185390	319840	9.877	24.856	4.646	7.417	control
1	36	6.29	C12	merbb4-f	93400	298120	139440	395450	2.489	19.81	2.325	10.094	control
1	48	8.08	D12	merbb4-f	144040	369190	184830	376920	6.73	26.336	4.618	9.438	control
1	60	26.56	E12	bace-1	442120	808230	442320	715700	31.69	66.653	17.627	21.433	control
1	72	26.9	F12	bace-1	392690	612310	612870	662860	27.551	48.662	26.244	19.562	control
1	84	25.78	G12	bace-1	451190	697410	471590	584970	32.449	56.476	19.106	16.805	control
1	96	23.98	H12	bace-1	441630	641770	406620	571210	31.649	51.367	15.824	16.317	control

2	1	-0.32	A01	baseline	43170	50210	61700	74040	-0.299	-0.984	-0.348	-0.24	control
2	13	-0.41	B01	baseline	46540	53600	52220	77330	-0.088	-0.74	-0.847	-0.06	control
2	25	-0.21	C01	baseline	42700	62460	70920	72730	-0.328	-0.099	0.137	-0.312	control
2	37	-1.75	D01	dapt	32670	30830	39550	42370	-0.957	-2.385	-1.514	-1.978	control
2	49	-1.55	E01	dapt	31840	41350	38950	50060	-1.009	-1.625	-1.545	-1.556	control
2	61	-1.41	F01	dapt	45370	47670	36890	34100	-0.161	-1.168	-1.654	-2.432	control
2	73	-2.18	G01	tapi-1	26140	24280	31390	34370	-1.367	-2.858	-1.943	-2.418	control
2	85	-1.61	H01	tapi-1	32000	41310	37640	49100	-0.999	-1.628	-1.614	-1.609	control
2	2	-0.5	A02	sample	46780	49490	55980	72060	-0.073	-1.036	-0.649	-0.349	SAM002554920_Novocain
2	14	-0.22	B02	sample	49100	63820	59850	60980	0.073	-0.001	-0.445	-0.957	SAM002554921_Pyrimethamine
2	26	0.42	C02	sample	48020	86210	72090	90010	0.005	1.617	0.199	0.636	SAM002554922_Pro-Banthine
2	38	-1.22	D02	sample	34810	55450	35790	48790	-0.823	-0.606	-1.712	-1.626	SAM002554923_Probenecid
2	50	-0.66	E02	sample	42410	58330	50700	49210	-0.347	-0.398	-0.927	-1.603	SAM002554924_PYRIDINE-2-ALDOXIME_METHOCHLORIDE
2	62	-0.4	F02	sample	40310	70670	51610	72470	-0.478	0.494	-0.879	-0.327	SAM002554925_Primidone
2	74	-0.21	G02	sample	43600	67690	57670	75690	-0.272	0.279	-0.56	-0.15	SAM002554926_Propylthiouracil
2	86	-0.18	H02	sample	54270	65530	59320	68410	0.397	0.122	-0.473	-0.549	SAM002554927_Pyrazinamide
2	3	-0.2	A03	sample	47860	54940	84120	71380	-0.005	-0.643	0.832	-0.386	SAM002554928_Pronestyl
2	15	-0.06	B03	sample	50080	76520	63550	67260	0.134	0.917	-0.251	-0.612	SAM002554929_Sulfisoxazole
2	27	-0.43	C03	sample	38950	58430	60220	70510	-0.564	-0.391	-0.426	-0.434	SAM002554930_Sulfamethoxazole
2	39	-0.39	D03	sample	43340	55330	68970	69610	-0.288	-0.615	0.035	-0.484	SAM002554931_Sulfacetamide
2	51	0.02	E03	sample	49210	70940	67660	70720	0.08	0.513	-0.034	-0.423	SAM002554932_Sulfinpyrazone
2	63	-0.41	F03	sample	38490	60690	56100	74100	-0.592	-0.227	-0.643	-0.237	SAM002554933_Sulindac
2	75	0.76	G03	sample	55760	63850	96950	97080	0.49	0.001	1.507	1.024	SAM002554934_TETRACYCLINE
2	87	-0.05	H03	sample	51800	58820	74690	72320	0.242	-0.362	0.336	-0.335	SAM002554935_Theophylline
2	4	0.22	A04	sample	47130	57580	78120	87400	-0.051	-0.452	0.516	0.493	SAM002554936_Tolbutamide

2	16	-1.33	B04	sample	36920	46490	41230	52640	-0.691	-1.253	-1.425	-1.415	SAM002554937_Triamterene
2	28	-0.49	C04	sample	46010	72190	51920	59610	-0.121	0.604	-0.863	-1.032	SAM002554938_Intropin
2	40	-1.22	D04	sample	26940	49060	47050	51560	-1.316	-1.068	-1.119	-1.474	SAM002564189_AMOXAPINE
2	52	-1.26	E04	sample	33490	30940	41720	58010	-0.906	-2.377	-1.4	-1.12	SAM002564191_Adenine_9-beta
2	64	0.99	F04	sample	57530	87580	82330	100900	0.601	1.716	0.738	1.234	SAM002564193_ATENOLOL
2	76	-0.2	G04	sample	31810	72240	61140	77870	-1.011	0.607	-0.377	-0.03	SAM002703133_Tamoxifen
2	88	0.96	H04	sample	53720	74390	90120	123410	0.362	0.763	1.148	2.469	SAM002564195_BUMETANIDE
2	5	0.52	A05	sample	36880	50750	101080	122370	-0.693	-0.945	1.725	2.412	SAM002564250_CLOBETASOL_PROPIONATE
2	17	-1.83	B05	sample	10060	32130	42420	70550	-2.375	-2.291	-1.363	-0.432	SAM002564196_Sonazine
2	29	0.65	C05	sample	61680	69840	57400	94260	0.861	0.434	-0.574	0.869	SAM002564200_CEFAZOLIN_SODIUM_SALT
2	41	-0.19	D05	sample	45110	60900	59590	91910	-0.177	-0.212	-0.459	0.74	SAM002564201_CAPTOPRIL
2	53	-1.37	E05	sample	24720	43680	45990	54940	-1.456	-1.456	-1.175	-1.289	SAM002564202_CHLORAMBUCIL
2	65	0.21	F05	sample	43440	54580	89570	91350	-0.282	-0.669	1.119	0.71	SAM002564251_CEFOXITIN_SODIUM_SALT
2	77	1.49	G05	sample	69860	85990	125180	88400	1.374	1.601	2.993	0.548	SAM002564203_DANAZOL
2	89	1.57	H05	sample	72150	86180	102340	96810	1.518	1.615	1.791	1.009	SAM002564204_(+)-CIS-DILTIAZEM_HYDROCHLORIDE
2	6	-0.11	A06	sample	43910	70810	68750	72600	-0.253	0.504	0.023	-0.319	SAM002564205_DIGOXIN
2	18	-0.08	B06	sample	43740	61410	68410	82300	-0.263	-0.175	0.005	0.213	SAM002564206_17-BETA-ESTRADIOL_17-VALERATE
2	30	-0.22	C06	sample	42450	59260	66330	93390	-0.344	-0.331	-0.104	0.822	SAM002564207_EDROPHONIUM_CHLORIDE
2	42	0.37	D06	sample	48660	80880	81610	70850	0.045	1.232	0.7	-0.415	SAM002564208_FLUOCINOLONE_ACETONIDE
2	54	0.56	E06	sample	59310	69560	68570	117380	0.713	0.414	0.014	2.138	SAM002564209_Flurbiprofen
2	66	0.79	F06	sample	65880	66990	77020	99720	1.125	0.228	0.458	1.169	SAM002564210_GLIPIZIDE
2	78	2.08	G06	sample	87670	92480	107310	116550	2.49	2.07	2.053	2.093	SAM002564211_GEMFIBROZIL
2	90	2.24	H06	sample	84520	88500	109850	137840	2.293	1.782	2.186	3.261	SAM002564212_Glyburide
2	7	0.2	A07	sample	43970	52410	105500	90290	-0.249	-0.826	1.957	0.651	SAM002564213_HYDROCORTISONE_HEMISUCCINATE
2	19	-2.18	B07	sample	10340	29040	30230	45650	-2.357	-2.514	-2.004	-1.798	SAM002564214_INDAPAMIDE

2	31	-0.79	C07	sample	35460	51120	53070	81740	-0.782	-0.919	-0.802	0.182	SAM002564215_IPRATROPIUM_BROMIDE_MONOHYDRATE
2	43	-1.19	D07	sample	24590	51220	34010	82590	-1.464	-0.911	-1.805	0.229	SAM002564216_Tofranil
2	55	0.45	E07	sample	44710	75520	69190	119920	-0.202	0.844	0.046	2.278	SAM002564217_LABETALOL_HYDROCHLORIDE
2	67	-0.82	F07	sample	25460	46200	67000	71740	-1.409	-1.274	-0.069	-0.367	SAM002564218_Imodium
2	79	0.99	G07	sample	68070	73740	74950	120790	1.262	0.716	0.349	2.325	SAM002564219_Pro-Amatine
2	91	1.69	H07	sample	68760	98880	104890	104780	1.305	2.532	1.925	1.447	SAM002564220_Medroxyprogesterone_17-acetate
2	8	-0.86	A08	sample	23850	54420	49000	65710	-1.51	-0.68	-1.016	-0.698	SAM002564222_19-NORETHINDRONE_ACETATE
2	20	-1.11	B08	sample	21250	45710	68210	61770	-1.673	-1.31	-0.005	-0.914	SAM002564223_19-Norethindrone
2	32	-0.55	C08	sample	39110	52530	71560	68390	-0.554	-0.817	0.171	-0.55	SAM002564224_NICOTINE
2	44	-0.16	D08	sample	29950	62200	83360	74890	-1.128	-0.118	0.792	-0.194	SAM002564254_Cardene
2	56	1.24	E08	sample	61110	119480	89870	103000	0.826	4.021	1.135	1.349	SAM002564225_NABUMETONE
2	68	1.13	F08	sample	61100	110440	95410	73000	0.825	3.367	1.426	-0.297	SAM002564226_OXYBUTYNIN_CHLORIDE
2	80	-0.45	G08	sample	59200	62750	51670	63380	0.706	-0.078	-0.876	-0.825	SAM002564227_Mestinon
2	92	0.42	H08	sample	55190	73470	62110	85510	0.454	0.696	-0.326	0.389	SAM002564228_Rythmol
2	9	0.21	A09	sample	56010	62520	66450	92060	0.506	-0.095	-0.098	0.749	SAM002564229_Pfizerpen
2	21	-0.69	B09	sample	35730	55750	56720	60990	-0.765	-0.584	-0.61	-0.957	SAM002564230_Valproic_Acid
2	33	-0.72	C09	sample	30090	52070	57070	96560	-1.119	-0.85	-0.592	0.996	SAM002564231_Kemadrin
2	45	0.54	D09	sample	51850	75390	83990	77280	0.245	0.835	0.825	-0.063	SAM002564232_Proxymetacaine
2	57	1.22	E09	sample	66520	81410	102380	79020	1.165	1.27	1.793	0.033	SAM002703137_NALOXONE_HYDROCHLORIDE
2	69	1.82	F09	sample	91760	84900	83810	117020	2.747	1.522	0.816	2.118	SAM002564233_SPECTINOMYCIN_DIHYDROCHLORIDE_PENTAHYDRATE
2	81	0.57	G09	sample	62320	107460	67080	82940	0.901	3.152	-0.065	0.248	SAM002564235_TROPICAMIDE
2	93	0.93	H09	sample	61880	94030	83420	96440	0.874	2.182	0.795	0.989	SAM002564236_TOLAZAMIDE
2	10	0.48	A10	sample	43730	53170	99930	100800	-0.264	-0.771	1.664	1.228	SAM002564237_TRIAMCINOLONE_ACETONIDE
2	22	1.07	B10	sample	54830	64110	100690	109970	0.432	0.02	1.704	1.732	SAM002564238_S(-)-Timolol_maleate
2	34	2.26	C10	sample	62620	110470	113300	117610	0.92	3.37	2.368	2.151	SAM002564239_THIABENDAZOLE

2	46	-2.23	D10	sample	8550	15890	30330	51600	-2.469	-3.464	-1.999	-1.472	SAM002564240_THIORIDAZINE_HYDROCHLORIDE
2	58	-0.02	E10	sample	43010	67760	67950	78070	-0.309	0.284	-0.019	-0.019	SAM002564241_Altretamine
2	70	0.25	F10	sample	48330	73370	56320	87080	0.024	0.689	-0.631	0.475	SAM002564257_Phylloquinone
2	82	-0.28	G10	sample	38420	68910	69100	67100	-0.597	0.367	0.042	-0.621	SAM002564242_Eryped
2	94	-0.26	H10	sample	62300	62130	60740	70390	0.9	-0.123	-0.398	-0.441	SAM002564244_Dibenzyline
2	11	0.06	A11	sample	33130	67300	65950	84030	-0.928	0.25	-0.124	0.308	SAM002564258_6ALPHA-METHYL-11BETA-HYDROXYPROGESTERONE
2	23	0.68	B11	sample	54190	70710	84690	101000	0.392	0.497	0.862	1.239	SAM002564245_Thalidomide
2	35	0.29	C11	sample	62400	59250	85490	72040	0.906	-0.331	0.904	-0.35	SAM002589919_Aminolevulinic_Acid
2	47	0.49	D11	sample	63870	66840	76970	87970	0.999	0.217	0.456	0.524	SAM002589920_Carbinoxamine_Maleate
2	59	0.06	E11	sample	58440	55520	61230	87280	0.658	-0.601	-0.373	0.486	SAM002589921_Demeclocycline
2	71	0.47	F11	sample	65900	68700	79590	78770	1.126	0.352	0.594	0.019	SAM002589925_Westcort
2	83	-0.16	G11	sample	56360	59230	68600	54640	0.528	-0.333	0.015	-1.305	SAM002589926_DEPRENALIN
2	95	-0.1	H11	sample	53640	62370	66580	68970	0.357	-0.106	-0.091	-0.519	SAM002589927_6-[2-ETHOXY-1-NAPHTHAMIDO]-PENICILLIN_SODIUM_SALT
2	12	8.37	A12	merbb4-f	97820	252190	88100	331370	3.127	13.609	1.042	13.882	control
2	24	9.02	B12	merbb4-f	146840	287620	139360	294170	6.2	16.169	3.74	11.841	control
2	36	6.92	C12	merbb4-f	88660	289980	114250	284040	2.553	16.34	2.418	11.285	control
2	48	8.13	D12	merbb4-f	103850	257210	166540	280570	3.505	13.972	5.17	11.094	control
2	60	19.02	E12	bace-1	262130	490570	296890	526930	13.427	30.833	12.031	24.615	control
2	72	20.88	F12	bace-1	383110	397110	406010	456680	21.01	24.08	17.774	20.759	control
2	84	23.3	G12	bace-1	398190	523670	444880	527340	21.955	33.225	19.82	24.637	control
2	96	22.9	H12	bace-1	408230	525000	316740	501330	22.585	33.321	13.076	23.21	control
3	1	-0.47	A01	baseline	34940	45170	40590	44260	-0.354	-0.386	-0.615	-0.549	control
3	13	-0.33	B01	baseline	45290	46750	45040	47850	0.511	-0.287	-0.368	-0.389	control
3	25	-0.63	C01	baseline	30590	43560	31620	44250	-0.718	-0.486	-1.114	-0.549	control

3	37	-1.15	D01	dapt	34200	38050	25130	23690	-0.416	-0.83	-1.474	-1.465	control
3	49	-1.38	E01	dapt	27430	29710	20950	24730	-0.982	-1.351	-1.707	-1.418	control
3	61	-1.02	F01	dapt	28970	27230	34610	31840	-0.853	-1.505	-0.947	-1.102	control
3	73	-1.49	G01	tapi-1	21200	27750	20380	32650	-1.502	-1.473	-1.738	-1.066	control
3	85	-0.9	H01	tapi-1	36110	30520	37040	34550	-0.256	-1.3	-0.812	-0.981	control
3	2	-0.36	A02	sample	38030	42240	43590	50590	-0.096	-0.569	-0.448	-0.267	SAM002589929_Primaquine_Diphosphate
3	14	-1.43	B02	sample	13660	20590	39600	35560	-2.133	-1.92	-0.67	-0.936	SAM002589930_Micropenin
3	26	-0.5	C02	sample	33110	44270	42690	44280	-0.507	-0.442	-0.498	-0.548	SAM002589932_DOXYCYCLINE
3	38	-0.83	D02	sample	29040	35920	48840	38420	-0.847	-0.963	-0.156	-0.809	SAM002699895_Beclomethasone_dipropionate
3	50	-0.53	E02	sample	35610	36440	50200	39470	-0.298	-0.931	-0.081	-0.762	SAM002589934_Cromolyn_Sodium
3	62	-0.65	F02	sample	40620	42830	37730	38290	0.121	-0.532	-0.774	-0.815	SAM002589935_Priscoline
3	74	-0.45	G02	sample	32070	54650	37710	49850	-0.594	0.206	-0.775	-0.3	SAM002589937_Mercaptopurine
3	86	-0.17	H02	sample	47700	51170	45740	46760	0.713	-0.012	-0.329	-0.437	SAM002589938_Azathioprine
3	3	2.75	A03	sample	70590	90950	103320	138400	2.626	2.471	2.872	3.642	SAM002589939_Albendazole
3	15	0.13	B03	sample	53860	43940	57410	55160	1.227	-0.463	0.32	-0.063	SAM002589940_Griseofulvin
3	27	-0.35	C03	sample	38300	46900	44010	38100	-0.073	-0.278	-0.425	-0.823	SAM002589936_Lincomycin_hydrochloride
3	39	0.1	D03	sample	40680	52630	56440	54450	0.126	0.08	0.266	-0.095	SAM002589943_Methazolamide
3	51	0.26	E03	sample	42070	65930	56510	53390	0.242	0.909	0.27	-0.142	SAM002589944_Terbutaline_Sulfate
3	63	0.07	F03	sample	39540	50770	60700	59170	0.031	-0.037	0.503	0.115	SAM002589979_Mupirocin
3	75	0.97	G03	sample	50880	71200	68550	78340	0.978	1.238	0.939	0.969	SAM002589945_FLUOCINOLONE_ACETONIDE_21-ACETATE
3	87	-1.15	H03	sample	42510	28530	24730	36740	0.279	-1.424	-1.497	-0.884	SAM002699894_Mefloquine_hydrochloride
3	4	-1.02	A04	sample	27470	31760	32550	45540	-0.978	-1.223	-1.062	-0.492	SAM002589947_Floxuridine
3	16	-3	B04	sample	530	580	790	810	-3.23	-3.168	-2.827	-2.483	SAM002699896_MITOXANTRONE
3	28	-0.64	C04	sample	30500	58820	41730	19100	-0.725	0.466	-0.552	-1.669	SAM002699897_ENALAPRIL_MALEATE
3	40	0.51	D04	sample	43100	62460	52050	73480	0.328	0.693	0.022	0.752	SAM002699898_BUDESONIDE

3	52	1.22	E04	sample	45880	50570	111530	98880	0.56	-0.049	3.328	1.883	SAM002699899_RAMIPRIL
3	64	1.18	F04	sample	52950	82770	73410	74920	1.151	1.96	1.209	0.816	SAM002699893_DEPO-MEDROL
3	76	0.29	G04	sample	60490	57690	47000	60740	1.782	0.395	-0.259	0.185	SAM002699901_(+/-)-NOREPINEPHRINE_HYDROCHLORIDE
3	88	2.23	H04	sample	58480	92880	93520	104690	1.614	2.591	2.327	2.142	SAM002699903_AMCINONIDE
3	5	-1.06	A05	sample	16660	27350	40420	46390	-1.882	-1.498	-0.624	-0.454	SAM002699904_Clomid
3	17	-0.95	B05	sample	26620	39430	27000	37430	-1.049	-0.744	-1.37	-0.853	SAM003107541_PHENTOLAMINE_HCL
3	29	-0.68	C05	sample	33700	46890	22270	36330	-0.458	-0.279	-1.633	-0.902	SAM002548956_FLUDARABINE
3	41	-1.55	D05	sample	14800	11590	37140	32550	-2.037	-2.481	-0.807	-1.07	SAM002548950_Testosterone
3	53	0.45	E05	sample	44110	37890	66100	67610	0.412	-0.84	0.803	0.491	SAM002548955_Isotretinoin
3	65	0.84	F05	sample	48730	65400	58860	77380	0.799	0.876	0.4	0.926	SAM002548951_Methimazole
3	77	0.25	G05	sample	42030	68250	53710	62410	0.239	1.054	0.114	0.259	SAM002548957_Zonisamide
3	89	0.95	H05	sample	52120	56770	90100	75070	1.082	0.338	2.137	0.823	SAM002548958_Brimonidine
3	6	-0.07	A06	sample	14410	36250	67860	74710	-2.07	-0.942	0.901	0.807	SAM002548959_Mebendazole
3	18	-2	B06	sample	7450	21070	13510	24460	-2.652	-1.89	-2.12	-1.43	SAM002548969_Duremesin
3	30	-0.84	C06	sample	7000	42200	31720	45540	-2.689	-0.571	-1.108	-0.492	SAM002548968_Flecainide_Acetate
3	42	0.67	D06	sample	28130	61260	64470	91980	-0.923	0.618	0.712	1.576	SAM002548966_Dilantin
3	54	0.76	E06	sample	46440	65950	68420	69320	0.607	0.911	0.932	0.567	SAM002548945_Miochol
3	66	0.09	F06	sample	33210	43710	77620	71470	-0.499	-0.477	1.443	0.663	SAM002703135_Dantrolene_sodium_salt
3	78	1.16	G06	sample	53290	69490	73210	75710	1.18	1.132	1.198	0.851	SAM002548948_Dexamethasone
3	90	1.13	H06	sample	50100	62480	83780	86710	0.913	0.694	1.786	1.341	SAM002548938_Cogentin_Mesylate
3	7	-0.1	A07	sample	35200	54150	49140	55160	-0.332	0.174	-0.14	-0.063	SAM002548936_Ganciclovir
3	19	-0.21	B07	sample	35100	50070	52560	44560	-0.341	-0.08	0.05	-0.535	SAM002548937_Mesna
3	31	-0.13	C07	sample	31060	40940	62970	65130	-0.678	-0.65	0.629	0.38	SAM002548942_Meclomen
3	43	-0.23	D07	sample	33130	44850	50650	79830	-0.505	-0.406	-0.056	1.035	SAM002589905_Fluconazole
3	55	0.15	E07	sample	46730	51740	56600	56160	0.631	0.024	0.275	-0.019	SAM002548965_Metaproterenol

3	67	1.01	F07	sample	50190	88480	48040	81230	0.921	2.316	-0.201	1.097	SAM002548974_Methoxsalen
3	79	0.31	G07	sample	29820	57600	55680	71160	-0.782	0.39	0.224	0.649	SAM002548963_Chloramphenicol
3	91	1.25	H07	sample	58480	88240	67520	69810	1.614	2.301	0.882	0.589	SAM002548961_Tizanidine_hydrochloride
3	8	-1.26	A08	sample	10760	33350	26480	53780	-2.375	-1.123	-1.399	-0.125	SAM002548935_Paroxetine
3	20	-0.3	B08	sample	34000	45540	53340	51050	-0.433	-0.363	0.094	-0.246	SAM002548934_mirtazapine
3	32	0.37	C08	sample	40970	61360	62120	59240	0.15	0.624	0.582	0.118	SAM002548930_Etomidate
3	44	0.15	D08	sample	39570	49740	76650	62430	0.033	-0.101	1.389	0.26	SAM002548933_Moban
3	56	-0.8	E08	sample	16790	36540	47540	41390	-1.871	-0.924	-0.229	-0.676	SAM002548940_fluvastatin
3	68	0.6	F08	sample	48390	53350	79120	66290	0.77	0.124	1.527	0.432	SAM002548931_Urecholine
3	80	-1.08	G08	sample	13660	30440	36310	41060	-2.133	-1.305	-0.853	-0.691	SAM002589901_Cefuroxime
3	92	1.24	H08	sample	72800	65490	69630	89830	2.81	0.882	0.999	1.48	SAM002548983_Cytoxan
3	9	0.33	A09	sample	42870	58070	47560	64290	0.309	0.419	-0.228	0.343	SAM002548982_Eszopiclone
3	21	0.07	B09	sample	40610	57500	43020	57010	0.12	0.383	-0.48	0.019	SAM002264598_Bendrofluazide
3	33	-0.52	C09	sample	30600	58480	39440	48360	-0.717	0.445	-0.679	-0.366	SAM002548975_Evista
3	45	0.01	D09	sample	44170	52690	44580	55190	0.417	0.083	-0.393	-0.062	SAM002548971_zidovudine
3	57	-0.38	E09	sample	21440	51870	37560	59050	-1.482	0.032	-0.783	0.11	SAM002548976_Clozapine
3	69	-0.52	F09	sample	35750	39190	45360	40870	-0.286	-0.759	-0.35	-0.7	SAM002264590_Ampicillin_Sodium
3	81	0.22	G09	sample	40650	61870	56210	60600	0.123	0.656	0.253	0.179	SAM002264591_ACEBUTOLOL_HYDROCHLORIDE
3	93	1.39	H09	sample	59910	76580	73410	72260	1.733	1.574	1.209	0.698	SAM002264592_AMOXICILLIN_CRYSTALLINE
3	10	-0.13	A10	sample	38810	47520	51260	51380	-0.031	-0.239	-0.022	-0.232	SAM002699891_(+/-)-Epinephrine_hydrochloride
3	22	-0.08	B10	sample	12210	35650	69070	75100	-2.254	-0.98	0.968	0.824	SAM002264595_5-Azacytidine
3	34	0.2	C10	sample	38320	51540	58510	70130	-0.071	0.012	0.381	0.603	SAM002264597_Buspar
3	46	-0.46	D10	sample	35160	40160	41030	72380	-0.336	-0.699	-0.591	0.703	SAM002264596_Flumadine
3	58	-1.5	E10	sample	20730	28130	26080	19770	-1.542	-1.449	-1.422	-1.639	SAM002548978_Podofilox
3	70	-0.33	F10	sample	48090	74410	16900	25140	0.745	1.439	-1.932	-1.4	SAM002264599_D-CYCLOSERINE

3	82	-0.48	G10	sample	35390	41060	56290	41480	-0.316	-0.642	0.258	-0.672	SAM002264600_CORTISONE_ACETATE
3	94	-1.55	H10	sample	29200	27500	16190	20460	-0.834	-1.488	-1.971	-1.608	SAM002264601_Anafranil
3	11	0.43	A11	sample	41460	43550	100030	71810	0.191	-0.487	2.689	0.678	SAM002264603_Carbamazepine
3	23	0.89	B11	sample	41660	64050	69470	80260	0.208	0.792	0.99	1.054	SAM002699890_Memantine_hydrochloride
3	35	-0.06	C11	sample	38470	51720	42660	54990	-0.059	0.023	-0.5	-0.071	SAM002264605_Norpramin
3	47	1.12	D11	sample	46850	76730	66310	88600	0.641	1.583	0.815	1.425	SAM002264606_Mexitil
3	59	0.34	E11	sample	56100	59270	54250	60870	1.415	0.494	0.144	0.191	SAM002264607_Norpace
3	71	0.27	F11	sample	42550	55400	69980	50050	0.282	0.252	1.019	-0.291	SAM002589948_STAVUDINE
3	83	0.52	G11	sample	47200	66220	50550	64710	0.671	0.928	-0.061	0.362	SAM002589981_Doxazosin
3	95	-0.68	H11	sample	35050	38360	40270	40180	-0.345	-0.811	-0.633	-0.73	SAM002589949_Minoxidil
3	12	8.58	A12	merbb4-f	136190	228830	152530	259920	8.109	11.074	5.607	9.053	control
3	24	7.5	B12	merbb4-f	140570	320320	125120	203330	8.475	16.782	4.083	6.533	control
3	36	7.77	C12	merbb4-f	139970	232140	90720	216590	8.424	11.28	2.171	7.124	control
3	48	7.47	D12	merbb4-f	120610	294220	164560	239250	6.806	15.154	6.276	8.132	control
3	60	27.83	E12	bace-1	376670	611320	344260	673350	28.208	34.939	16.264	27.459	control
3	72	25.42	F12	bace-1	337120	658520	486670	639210	24.902	37.884	24.179	25.939	control
3	84	31.11	G12	bace-1	452170	701880	417580	678750	34.518	40.59	20.339	27.699	control
3	96	22.75	H12	bace-1	280040	644090	296690	626310	20.132	36.984	13.62	25.365	control
4	1	-0.79	A01	baseline	63166	67322	71040	88450	-0.357	-0.472	-1.225	-1.109	control
4	13	0.29	B01	baseline	132015	133237	113450	151480	1.017	0.495	-0.34	0.085	control
4	25	-0.21	C01	baseline	114841	119150	96060	109250	0.674	0.288	-0.703	-0.715	control
4	37	-0.96	D01	dapt	73983	62982	63250	67920	-0.141	-0.536	-1.388	-1.499	control
4	49	-0.68	E01	dapt	90730	68574	86260	72400	0.193	-0.454	-0.907	-1.414	control
4	61	-0.99	F01	dapt	75053	51095	68810	68280	-0.12	-0.71	-1.272	-1.492	control
4	73	-1.1	G01	tapi-1	65396	58887	52710	53220	-0.313	-0.596	-1.608	-1.777	control

4	85	-0.62	H01	tapi-1	86421	75145	87410	95510	0.107	-0.357	-0.883	-0.976	control
4	2	-0.83	A02	sample	56809	62249	62640	87820	-0.484	-0.547	-1.401	-1.121	SAM002699889_Inderal
4	14	-0.55	B02	sample	65244	70194	97420	104110	-0.316	-0.43	-0.674	-0.812	SAM002589951_Ribavirin
4	26	-0.66	C02	sample	45961	57329	68270	117510	-0.701	-0.619	-1.283	-0.558	SAM002589982_Terazosin
4	38	-0.48	D02	sample	56840	53539	106920	129290	-0.484	-0.674	-0.476	-0.335	SAM002589983_Chlorthalidone
4	50	-0.23	E02	sample	64388	51278	123990	168370	-0.333	-0.708	-0.12	0.406	SAM002589984_METHYLPREDNISOLONE
4	62	0.35	F02	sample	83976	88957	160870	185820	0.058	-0.155	0.65	0.736	SAM002589985_Phenelzine
4	74	2.84	G02	sample	200589	265283	306460	318470	2.385	2.433	3.689	3.251	SAM002589986_NALTREXONE_HYDROCHLORIDE
4	86	0.2	H02	sample	69125	90027	163330	175740	-0.238	-0.139	0.701	0.545	SAM002589987_Glycopyrrolate
4	3	-0.02	A03	sample	125781	112274	118520	118630	0.892	0.187	-0.234	-0.537	SAM002589988_Ethambutol
4	15	-0.01	B03	sample	87613	99561	125220	145490	0.131	0.001	-0.094	-0.028	SAM002589989_Cetirizine
4	27	-0.46	C03	sample	68452	105826	97090	111390	-0.252	0.093	-0.681	-0.674	SAM002589990_DICLOXACILLIN_SODIUM
4	39	1.65	D03	sample	204440	177059	233540	185160	2.462	1.138	2.167	0.724	SAM002589991_Meloxicam
4	51	-1.18	E03	sample	24019	16410	59740	124900	-1.139	-1.219	-1.461	-0.418	SAM002589992_DAUNORUBICIN_HYDROCHLORIDE
4	63	2.96	F03	sample	231179	216694	269430	380150	2.996	1.72	2.916	4.42	SAM002589953_RIFAPENTINE
4	75	0.69	G03	sample	83579	154873	158950	188030	0.05	0.813	0.61	0.778	SAM002589954_Penicillin_V
4	87	0.22	H03	sample	75267	92533	155420	190210	-0.116	-0.102	0.536	0.82	SAM002589955_Gatifloxacin
4	4	-0.44	A04	sample	56565	68819	119060	124720	-0.489	-0.45	-0.223	-0.422	SAM002589956_clopidogrel
4	16	-0.7	B04	sample	36426	35479	104950	150980	-0.891	-0.94	-0.517	0.076	SAM002643511_CEFOTAXIME_SODIUM_SALT
4	28	-0.81	C04	sample	32790	44738	116770	103930	-0.964	-0.804	-0.271	-0.816	SAM002589994_LAMIVUDINE
4	40	-0.52	D04	sample	41713	45044	137850	133460	-0.785	-0.799	0.17	-0.256	SAM002589958_Ondansetron
4	52	1.89	E04	sample	192094	252051	175330	229280	2.216	2.239	0.952	1.56	SAM002589959_Betamethasone
4	64	0.16	F04	sample	84710	115941	117590	160840	0.073	0.241	-0.253	0.263	SAM002589995_Celecoxib
4	76	3.15	G04	sample	310327	344461	258950	220280	4.575	3.595	2.698	1.39	SAM002589996_4-(AMINOMETHYL)BENZENESULFONAMIDE_ACETATE
4	88	1.44	H04	sample	173850	261280	179140	188710	1.852	2.374	1.031	0.791	SAM002589997_THIOTHIXENE

4	5	2.78	A05	sample	359924	233410	301700	227810	5.565	1.965	3.59	1.532	SAM002589960_Citalopram
4	17	2.12	B05	sample	165508	245603	244660	257570	1.685	2.144	2.399	2.097	SAM002589961_Azithromycin
4	29	2.66	C05	sample	237994	248414	299000	242570	3.132	2.185	3.534	1.812	SAM002589963_Lovastatin
4	41	-0.18	D05	sample	62615	50056	129730	146970	-0.368	-0.726	0	0	SAM002589964_Aminoglutethimide
4	53	-1.01	E05	sample	27992	50667	84170	85120	-1.059	-0.717	-0.951	-1.172	SAM002589965_Prozac
4	65	-1.01	F05	sample	27503	60018	72180	96970	-1.069	-0.579	-1.201	-0.948	SAM002589966_FluniSOLIDe
4	77	-0.39	G05	sample	81073	75848	100620	124580	0	-0.347	-0.608	-0.424	SAM002589967_Acyclovir
4	89	0.68	H05	sample	155973	147478	161420	155150	1.495	0.704	0.662	0.155	SAM002589968_Etodolac
4	6	-1.01	A06	sample	35296	43699	76100	88970	-0.914	-0.819	-1.12	-1.1	SAM002589969_Simvastatin
4	18	-0.31	B06	sample	81409	90944	89500	120910	0.007	-0.126	-0.84	-0.494	SAM002589970_Rifabutin
4	30	0.01	C06	sample	75420	127553	136250	137020	-0.113	0.412	0.136	-0.189	SAM002589971_Felodipine
4	42	0.38	D06	sample	106132	101762	142460	184820	0.5	0.033	0.266	0.718	SAM002589972_Quinapril_hydrochloride
4	54	0.12	E06	sample	92105	125934	130510	137280	0.22	0.388	0.016	-0.184	SAM002589973_Acitretin
4	66	3.05	F06	sample	326004	266780	291080	290820	4.888	2.455	3.368	2.727	SAM002700173_Allegra
4	78	0.39	G06	sample	93297	125384	148420	172120	0.244	0.38	0.39	0.477	SAM002700174_Fluorometholone
4	90	-0.41	H06	sample	47275	99500	67460	139520	-0.674	0	-1.3	-0.141	SAM002700175_Sertraline
4	7	-0.3	A07	sample	59590	77406	116830	144040	-0.429	-0.324	-0.269	-0.056	SAM002703129_CARBIDOPA
4	19	NA	B07	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	31	NA	C07	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	43	NA	D07	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	55	NA	E07	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	67	NA	F07	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	79	NA	G07	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	91	NA	H07	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	8	NA	A08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	20	NA	B08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA

4	32	NA	C08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	44	NA	D08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	56	NA	E08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	68	NA	F08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	80	NA	G08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	92	NA	H08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	9	NA	A09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	21	NA	B09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	33	NA	C09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	45	NA	D09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	57	NA	E09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	69	NA	F09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	81	NA	G09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	93	NA	H09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	10	NA	A10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	22	NA	B10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	34	NA	C10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	46	NA	D10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	58	NA	E10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	70	NA	F10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	82	NA	G10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	94	NA	H10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	11	NA	A11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	23	NA	B11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	35	NA	C11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	47	NA	D11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA

4	59	NA	E11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	71	NA	F11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	83	NA	G11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	95	NA	H11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	12	4.16	A12	merbb4-f	368848	274820	424159	259030	5.743	2.573	6.146	2.124	control
4	24	5.21	B12	merbb4-f	514828	258300	511558	276370	8.656	2.33	7.971	2.453	control
4	36	7.09	C12	merbb4-f	1829967	619190	443931	301880	34.902	7.627	6.559	2.937	control
4	48	4.32	D12	merbb4-f	400690	259490	390972	314660	6.378	2.348	5.454	3.179	control
4	60	12.74	E12	bace-1	866471	692630	1106237	664150	15.674	8.704	20.385	9.804	control
4	72	13.51	F12	bace-1	1052086	586870	994483	620440	19.378	7.152	18.052	8.976	control
4	84	16.72	G12	bace-1	3285738	1021600	1083287	719600	63.954	13.532	19.906	10.855	control
4	96	12.74	H12	bace-1	927894	683600	963862	525720	16.9	8.572	17.413	7.18	control

12.2 NCC 003 Screen Result

Table 15. Screen result from NCC 003

plate	position	score	well	WellAnno	raw_r1_ch1	raw_r2_ch1	raw_r3_ch1	raw_r4_ch1	normalized_r1_ch1	normalized_r2_ch1	normalized_r3_ch1	normalized_r4_ch1	GeneID
5	1	2.21	A01	baseline	109790	131590	126410	124180	3.056	2.916	1.496	0.296	control
5	13	2.41	B01	baseline	117530	123790	146100	144740	3.514	2.489	2.335	1.002	control
5	25	4.86	C01	baseline	156570	196890	182760	174780	5.827	6.485	3.897	2.033	control
5	37	-0.58	D01	dapt	72700	80330	61390	49510	0.858	0.114	-1.274	-2.266	control
5	49	0.74	E01	dapt	118820	109040	86610	71800	3.591	1.683	-0.199	-1.501	control
5	61	0.59	F01	dapt	159130	96200	95980	84650	5.979	0.981	0.2	-1.06	control
5	73	-0.62	G01	tapi-1	81030	63050	81480	63860	1.352	-0.831	-0.418	-1.774	control
5	85	0.88	H01	tapi-1	101210	81520	128230	98220	2.547	0.179	1.574	-0.595	control
5	2	-0.65	A02	sample	74310	63690	71880	100680	0.954	-0.796	-0.827	-0.51	SAM001247063_Nalbuphine
5	14	-0.6	B02	sample	65210	59470	82450	91530	0.415	-1.026	-0.377	-0.824	SAM001247072_Raclopride
5	26	-1.08	C02	sample	46170	87640	57410	69630	-0.713	0.514	-1.444	-1.576	SAM001247069_Zacopride
5	38	0.11	D02	sample	57340	94340	97800	109000	-0.052	0.88	0.277	-0.225	SAM001247068_SKF_83566
5	50	0.53	E02	sample	41990	86430	133770	133570	-0.961	0.447	1.81	0.619	SAM001246964_3'-deoxydenosine
5	62	0.29	F02	sample	60950	110850	88880	127960	0.162	1.782	-0.103	0.426	SAM001246965_AM_404
5	74	0.35	G02	sample	62290	95200	102160	119040	0.242	0.927	0.463	0.12	SAM001246962_PILOCARPINE_HYDROCHLORIDE
5	86	0.53	H02	sample	69660	94620	100400	77860	0.678	0.895	0.388	-1.293	SAM001246963_NIFEDIPINE
5	3	0.19	A03	sample	72140	84010	78230	117410	0.825	0.315	-0.556	0.064	SAM001246961_Flurbiprofen
5	15	0.17	B03	sample	62800	97280	86990	117270	0.272	1.04	-0.183	0.059	SAM001247015_3-HYDROXY-1,2-DIMETHYL-4(1H)-PYRIDONE
5	27	-0.56	C03	sample	36290	76630	67040	123180	-1.299	-0.088	-1.033	0.262	SAM001247061_Loxapine-succinate

5	39	-0.32	D03	sample	58100	104810	67900	97040	-0.007	1.452	-0.997	-0.635	SAM001247062_d-3-Methoxy-N-methylmorphinan_hydrobromide
5	51	-0.33	E03	sample	40170	66430	100570	115210	-1.069	-0.646	0.395	-0.011	SAM001247059_Duloxetine
5	63	0.78	F03	sample	62720	85660	141370	149410	0.267	0.405	2.134	1.162	SAM001247060_Glycine,_N-[2-[(acetylthio)methyl]-1-oxo-3-phenylpropyl]-,phenylmethyl_ester_[CAS]
5	75	1.63	G03	sample	84700	94480	173520	165000	1.569	0.887	3.504	1.697	SAM001247057_Benzeneacetic_acid,_2-[(2,6-dichlorophenyl)amino]-,_monosodium_salt_[CAS]
5	87	2.09	H03	sample	51570	115110	142090	225790	-0.393	2.015	2.165	3.783	SAM001247039_PROGESTERONE
5	4	0.82	A04	sample	86230	79030	128910	102290	1.66	0.043	1.603	-0.455	SAM001247033_FAMOTIDINE
5	16	0.53	B04	sample	70080	89410	101960	120130	0.703	0.61	0.455	0.157	SAM001246999_SR_57,227A
5	28	-0.07	C04	sample	58320	81600	87850	105090	0.007	0.183	-0.147	-0.359	SAM001247003_Pancuronium
5	40	-0.51	D04	sample	57140	68580	79830	82860	-0.063	-0.528	-0.488	-1.122	SAM001247010_METRONIDAZOLE
5	52	-0.58	E04	sample	47660	67760	77650	115640	-0.625	-0.573	-0.581	0.003	SAM001246967_Benzeneacetic_acid,_Alpha-(hydroxymethyl)-,_9-methyl-3-oxa-9-azatricyclo[3.3.1.02,4]non-7-yl_ester,_[7(S)-(1Alpha,2ÃƒÂ¿,4ÃƒÂ¿,5Alpha,7ÃƒÂ¿)]-_[CAS]
5	64	0.88	F04	sample	50120	91280	116100	147240	-0.479	0.712	1.057	1.088	SAM001246968_Benzeneacetonitrile,_Alpha-[3-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]propyl]-3,4-dimethoxy-Alpha-(1-methylethyl)-,_(R)-_[CAS]
5	76	0.01	G04	sample	58100	79750	72490	116350	-0.007	0.082	-0.801	0.028	SAM001246969_Selegiline-hydrochloride
5	88	0.63	H04	sample	67460	91110	91640	150490	0.548	0.703	0.015	1.199	SAM001246970_Capsaicin
5	5	0.01	A05	sample	58390	94420	91510	107060	0.011	0.884	0.009	-0.291	SAM001246971_SALBUTAMOL_SULFATE
5	17	-0.31	B05	sample	66310	69470	72200	111480	0.48	-0.48	-0.813	-0.139	SAM001246972_(Â±)-Vesamicol_hydrochloride
5	29	0	C05	sample	86630	81690	87050	87600	1.684	0.188	-0.181	-0.959	SAM001246993_Picrotin_-_Picrotoxinin
5	41	-0.45	D05	sample	49580	72270	80020	103170	-0.511	-0.327	-0.48	-0.425	SAM001247000_Terazosin
5	53	-3.05	E05	sample	1310	3810	27440	98350	-3.371	-4.069	-2.721	-0.59	SAM001247027_diphenylcyclopropenone
5	65	0.29	F05	sample	58080	96400	98440	123840	-0.008	0.992	0.305	0.285	SAM001247016_4-Thiazolidinecarboxylic_acid,_2-oxo-,_(R)-_[CAS]

5	77	0.13	G05	sample	52080	59940	116080	133710	-0.363	-1.001	1.056	0.623	SAM001247051_Mesoridazine
5	89	2.1	H05	sample	64110	81340	181480	243950	0.35	0.169	3.843	4.406	SAM001247053_3(2H)-Pyridazinone,_6-[4-(difluoromethoxy)-3-methoxyphenyl]-_[CAS]
5	6	-1.24	A06	sample	28650	48540	71320	107910	-1.751	-1.624	-0.851	-0.262	SAM001247054_10H-Phenothiazine,_2-chloro-10-[3-(4-methyl-1-piperazinyl)propyl-_[CAS]
5	18	0.05	B06	sample	65860	75870	96870	107800	0.453	-0.13	0.238	-0.266	SAM001247055_1H-Cyclopenta[b]quinolin-9-amine,_2,3,5,6,7,8-hexahydro-,_monohydrochloride-_[CAS]
5	30	-0.44	C06	sample	48240	88100	84370	89390	-0.591	0.539	-0.295	-0.898	SAM001247056_CLOTRIMAZOLE
5	42	1.01	D06	sample	54510	87120	127510	172780	-0.219	0.485	1.543	1.964	SAM001246987_LORATADINE
5	54	0.89	E06	sample	73960	91770	111140	162660	0.933	0.739	0.846	1.617	SAM001247037_PHENELZINE_SULFATE_SALT
5	66	0.9	F06	sample	70410	102460	116460	132580	0.723	1.324	1.072	0.585	SAM001246997_Riluzole
5	78	0.12	G06	sample	47810	63800	111470	162110	-0.616	-0.79	0.86	1.598	SAM001247004_Naltrindole
5	90	1.27	H06	sample	63330	82630	161460	180950	0.303	0.24	2.99	2.244	SAM001247026_Nornicotine
5	7	-1.29	A07	sample	32590	45190	78130	84860	-1.518	-1.807	-0.561	-1.053	SAM001247052_Bifemelane
5	19	-2.22	B07	sample	34230	36300	40780	33130	-1.421	-2.293	-2.152	-2.828	SAM001246973_CGS_15943
5	31	-1.07	C07	sample	44970	70930	42790	75970	-0.784	-0.4	-2.067	-1.358	SAM001246974_Cinanserin
5	43	-1.2	D07	sample	48910	57200	60210	79110	-0.551	-1.15	-1.324	-1.25	SAM001246975_Cisapride
5	55	-1.69	E07	sample	5990	25810	79320	130210	-3.094	-2.866	-0.51	0.503	SAM001246981_Indatraline
5	67	-0.24	F07	sample	88280	75250	83610	106070	1.781	-0.164	-0.327	-0.325	SAM001247045_TRAZODONE_HYDROCHLORIDE
5	79	-0.57	G07	sample	22970	31620	113510	176180	-2.088	-2.548	0.947	2.081	SAM001246995_Prazosin
5	91	0.85	H07	sample	77110	82820	113900	136900	1.12	0.25	0.963	0.733	SAM001247001_URAPIDIL_HYDROCHLORIDE
5	8	-0.31	A08	sample	50380	53730	87720	135290	-0.464	-1.34	-0.152	0.678	SAM001247007_(-)-Cotinine
5	20	-1.43	B08	sample	38050	47740	68980	60600	-1.194	-1.667	-0.951	-1.886	SAM001247014_D-CYCLOSERINE
5	32	-1.76	C08	sample	41610	41070	41820	72440	-0.983	-2.032	-2.108	-1.479	SAM001246977_Fluvoxamine
5	44	-1	D0	sample	28640	46460	85270	124780	-1.752	-1.737	-0.257	0.317	SAM001246976_Doxepin

			8										
5	56	-1.24	E08	sample	29920	41250	74330	92210	-1.676	-2.022	-0.723	-0.801	SAM001247046_Fluoperazine
5	68	0.86	F08	sample	64840	76680	122450	160050	0.393	-0.086	1.328	1.527	SAM001247023_(+)-3-HYDROXY-N-METHYLMORPHINAN_D-TARTRATE
5	80	0.66	G08	sample	68740	81710	130100	135700	0.624	0.189	1.654	0.692	SAM001247049_L-Ornithine,_N5-[imino(methylamino)methyl]-[CAS]
5	92	0.14	H08	sample	61930	75470	100320	117280	0.22	-0.152	0.385	0.06	SAM001246989_Maprotilline_HCl
5	9	-1.37	A09	sample	26470	34470	76590	90560	-1.88	-2.393	-0.626	-0.857	SAM001247038_Pizotyline
5	21	0.04	B09	sample	70020	70790	91630	117230	0.7	-0.407	0.014	0.058	SAM001247032_BETA-ESTRADIOL
5	33	-0.51	C09	sample	47800	70780	58200	128900	-0.617	-0.408	-1.41	0.458	SAM001247019_N,N'-DIACETYL-1,6-DIAMINOHEXANE
5	45	0.18	D09	sample	68250	87970	87410	100580	0.595	0.532	-0.165	-0.514	SAM001247024_DIPHENHYDRAMINE-HYDROCHLORIDE
5	57	0.59	E09	sample	64950	73190	125780	138150	0.399	-0.276	1.47	0.776	SAM001246978_Galanthamine
5	69	-3.72	F09	sample	1320	2450	8020	2000	-3.37	-4.143	-3.548	-3.896	SAM001246980_Ifenprodil
5	81	0.94	G09	sample	2690	86760	124710	163180	-3.289	0.465	1.424	1.635	SAM001247028_TETRAETHYLTHIURAM_DISULFIDE
5	93	1.13	H09	sample	68980	74150	146550	162750	0.638	-0.224	2.355	1.62	SAM001246994_Piribedil
5	10	-0.62	A10	sample	43770	71700	82290	55730	-0.855	-0.358	-0.383	-2.053	SAM001246983_Ketoconazole
5	22	-0.25	B10	sample	67800	78460	79320	95980	0.568	0.012	-0.51	-0.671	SAM001247047_TRIPELENNAMINE_HYDROCHLORIDE
5	34	0.14	C10	sample	71310	78030	91070	124140	0.776	-0.012	-0.009	0.295	SAM001246996_Pyrazinecarboxamide,_3,5-diamino-N-(aminoiminomethyl)-6-chloro-_[CAS]
5	46	0.51	D10	sample	81320	97260	89560	115020	1.369	1.039	-0.074	-0.018	SAM001247002_9-AMINO-1,2,3,4-TETRAHYDROACRIDINE_HYDROCHLORIDE
5	58	1.43	E10	sample	94750	137730	102930	135820	2.165	3.251	0.496	0.696	SAM001247008_ETHYNYLESTRADIOL
5	70	-1.83	F10	sample	44250	34580	53290	56290	-0.827	-2.387	-1.619	-2.033	SAM001247012_2(1H)-Pyrimidinone,_4-amino-1-ÃfÂ¿-D-arabinofuranosyl-_[CAS]
5	82	-0.11	G10	sample	69270	81970	67150	103220	0.655	0.204	-1.029	-0.423	SAM001246985_L-Glutamic_acid,_N-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-_[CAS]
5	94	1.1	H10	sample	76360	98810	98290	185350	1.075	1.124	0.298	2.395	SAM001247043_TFMPP

5	11	-0.74	A11	sample	46260	64030	95960	89930	-0.708	-0.777	0.199	-0.879	SAM001247006_Pramipexole
5	23	-0.02	B11	sample	69530	80970	86700	99940	0.671	0.149	-0.196	-0.536	SAM001247018_LIDOCAINE
5	35	0.09	C11	sample	61820	77460	97640	99290	0.214	-0.043	0.271	-0.558	SAM001246982_Indomethacin
5	47	0.56	D11	sample	81890	87910	105000	97120	1.403	0.528	0.584	-0.632	SAM001246988_LY_171883
5	59	-0.57	E11	sample	51850	61030	103970	93270	-0.377	-0.941	0.54	-0.764	SAM001246991_Paroxetine
5	71	0.05	F11	sample	57680	83330	93490	115450	-0.031	0.278	0.094	-0.003	SAM001247031_Epigallocatechin_gallate
5	83	0.81	G11	sample	69920	116130	113100	132440	0.694	2.071	0.929	0.58	SAM001247020_5-Amino-2-hydroxy-benzoic_acid
5	95	0.15	H11	sample	46320	60960	114660	161000	-0.704	-0.945	0.996	1.56	SAM001247025_Oxiranecarboxylic_acid,_2-[6-(4-chlorophenoxy)hexyl]-,_ethyl_ester-_[CAS]
5	12	3.21	A12	merbb4-f	72390	329830	60260	278430	0.84	13.751	-1.322	5.59	control
5	24	7.43	B12	merbb4-f	134630	294470	118900	416900	4.527	11.819	1.176	10.341	control
5	36	5.37	C12	merbb4-f	103660	311310	120430	349950	2.693	12.739	1.242	8.044	control
5	48	4.25	D12	merbb4-f	99040	298690	109200	292730	2.419	12.049	0.763	6.08	control
5	60	18.9	E12	bace-1	270140	701430	246060	850960	12.556	34.063	6.594	25.237	control
5	72	15.46	F12	bace-1	267590	684300	345170	655290	12.404	33.126	10.817	18.522	control
5	84	23.26	G12	bace-1	508830	737740	337400	693260	26.696	36.047	10.486	19.825	control
5	96	15.38	H12	bace-1	295280	844460	358870	602880	14.045	41.88	11.401	16.724	control
6	1	-0.21	A01	baseline	80283	85935	78350	78900	1.436	0.527	-0.944	-1.407	control
6	13	0.63	B01	baseline	81529	108142	91640	84390	1.491	1.531	-0.238	-1.223	control
6	25	0.18	C01	baseline	82975	86936	78150	114370	1.554	0.572	-0.955	-0.217	control
6	37	-1.82	D01	dapt	28994	29283	65790	57540	-0.819	-2.035	-1.613	-2.123	control
6	49	-1.38	E01	dapt	43969	48842	44020	72920	-0.16	-1.15	-2.771	-1.607	control
6	61	-1.23	F01	dapt	47084	60769	57800	65880	-0.023	-0.611	-2.038	-1.844	control

6	73	-1.31	G01	tapi-1	24855	38384	84060	68550	-1.001	-1.623	-0.641	-1.754	control
6	85	-2.31	H01	tapi-1	40542	31374	45220	40860	-0.311	-1.94	-2.707	-2.683	control
6	2	-0.03	A02	sample	57119	73096	82100	120500	0.418	-0.054	-0.745	-0.011	SAM001246761_Cephalexin_monohydrate
6	14	0.86	B02	sample	88761	73274	98410	168740	1.809	-0.046	0.123	1.607	SAM001246762_PIDOTIMOD
6	26	0.5	C02	sample	50822	61392	117510	146730	0.141	-0.583	1.139	0.869	SAM001246757_RAMIPRIL
6	38	-0.32	D02	sample	40854	66576	85610	116380	-0.297	-0.349	-0.558	-0.149	SAM001246642_FENPIVERINIUM_BROMIDE
6	50	-0.94	E02	sample	49220	68022	60850	73060	0.07	-0.283	-1.875	-1.603	SAM001246751_nandrolone
6	62	0.49	F02	sample	90875	71115	114390	121050	1.902	-0.143	0.973	0.007	SAM001246752_NIZATIDINE
6	74	1.17	G02	sample	58121	75143	136620	176680	0.462	0.039	2.155	1.874	SAM001246753_5-FLUOROCYTOSINE
6	86	1.15	H02	sample	70003	71783	151700	160060	0.984	-0.113	2.957	1.316	SAM001246754_Trileptal
6	3	0.11	A03	sample	51289	75699	110340	120620	0.161	0.064	0.757	-0.007	SAM001246756_TROXIPIDE
6	15	0.37	B03	sample	74587	87092	98960	125530	1.186	0.579	0.152	0.158	SAM001246647_ACTARIT
6	27	-1.17	C03	sample	12772	29327	83600	96610	-1.532	-2.033	-0.665	-0.813	SAM001246645_AZELASTINE_HCl
6	39	0.55	D03	sample	49087	93256	115040	127990	0.065	0.858	1.007	0.24	SAM001246759_TOCAINIDE
6	51	0.31	E03	sample	54850	91097	86610	130020	0.318	0.76	-0.505	0.308	SAM001246760_TAXIFOLIN-(+/-)
6	63	0.8	F03	sample	52335	74253	124800	162480	0.207	-0.002	1.526	1.397	SAM001246758_LEVOFLOXACIN
6	75	1.23	G03	sample	46461	94991	146970	166300	-0.051	0.936	2.706	1.525	SAM001246643_CEFATRIZINE_PROPYLENE_GLYCOL
6	87	0.17	H03	sample	15910	107407	88650	142770	-1.394	1.497	-0.397	0.736	SAM001246700_IDEBENONE
6	4	0.53	A04	sample	65775	77635	114310	128710	0.798	0.151	0.968	0.264	SAM001246703_LEVOSULPIRIDE
6	16	0.23	B04	sample	75321	88471	92650	107080	1.218	0.641	-0.184	-0.461	SAM001246706_Pemoline
6	28	-0.35	C04	sample	40676	52803	88820	131620	-0.305	-0.971	-0.388	0.362	SAM001246649_LETROZOLE
6	40	-0.83	D04	sample	29060	48085	87370	95880	-0.816	-1.185	-0.465	-0.837	SAM001246650_MEROPENEM

6	52	-0.32	E04	sample	55473	57008	82120	124080	0.345	-0.781	-0.744	0.109	SAM001246637_ORLISTAT
6	64	-0.06	F04	sample	63617	82063	79030	106610	0.703	0.352	-0.908	-0.477	SAM001246631_ONDANSETRON-HYDROCHLORIDE
6	76	0.4	G04	sample	59300	107452	98200	129110	0.514	1.499	0.111	0.278	SAM001246694_LEVONORGESTREL
6	88	0.89	H04	sample	79304	116197	88360	132550	1.393	1.895	-0.412	0.393	SAM001246695_CETRAXATE_HCl
6	5	-0.81	A05	sample	26234	59389	120930	90120	-0.94	-0.673	1.321	-1.03	SAM001246696_Alprazolam
6	17	-0.01	B05	sample	31486	55428	109230	177750	-0.709	-0.853	0.698	1.909	SAM001246697_LAMOTRIGINE
6	29	0.01	C05	sample	47885	74320	94360	152840	0.012	0.002	-0.093	1.074	SAM001246702_483-63-6
6	41	-0.86	D05	sample	39096	44614	103250	81030	-0.375	-1.341	0.38	-1.335	SAM001246699_AMFEBUTAMONE_HCl
6	53	-0.24	E05	sample	46149	67755	87940	115060	-0.065	-0.295	-0.434	-0.194	SAM001246638_ALFUZOSIN
6	65	0.03	F05	sample	48953	75766	96070	115470	0.059	0.067	-0.002	-0.18	SAM001246639_Amisulpride
6	77	-0.6	G05	sample	61859	67021	79600	94190	0.626	-0.328	-0.878	-0.894	SAM001246632_LOFEPRAMINE
6	89	0.13	H05	sample	46350	195501	102130	89390	-0.056	5.48	0.32	-1.055	SAM001246688_PEROSPIRONE_HCl
6	6	1.09	A06	sample	32799	79994	151380	178340	-0.651	0.258	2.94	1.929	SAM001246689_DOCETAXEL
6	18	-0.46	B06	sample	19893	37872	101550	134950	-1.219	-1.646	0.29	0.474	SAM001246690_HONOKIOL
6	30	-0.43	C06	sample	27703	67310	106260	104810	-0.876	-0.315	0.54	-0.538	SAM001246691_TOLTERODINE_TARTRATE
6	42	-1.39	D06	sample	17490	23164	68710	105570	-1.325	-2.311	-1.457	-0.512	SAM001246692_CARMOFUR
6	54	-1.29	E06	sample	18291	34245	93830	82100	-1.289	-1.81	-0.121	-1.299	SAM001246633_PAROXETINE
6	66	0.25	F06	sample	64996	75009	72020	134870	0.764	0.033	-1.281	0.471	SAM001246634_OLMESARTAN_MEDOXOMIL
6	78	1.75	G06	sample	76700	123606	143110	133480	1.279	2.23	2.5	0.424	SAM001246635_LOSARTAN_Potassium
6	90	0.62	H06	sample	70181	79793	99660	152120	0.992	0.249	0.189	1.05	SAM001246636_TEMOZOLOMIDE
6	7	1.15	A07	sample	23497	90964	125280	171750	-1.06	0.754	1.552	1.708	SAM001246682_Methyltestosterone
6	19	0.32	B07	sample	47907	74854	107710	158490	0.013	0.026	0.617	1.263	SAM001246686_TOSUFLOXACIN_TOSYLATE

6	31	-0.62	C07	sample	36937	57208	78400	106590	-0.47	-0.772	-0.942	-0.478	SAM001246687_MECILLINAM
6	43	-1.6	D07	sample	12127	43168	59970	72150	-1.56	-1.407	-1.922	-1.633	SAM001246626_ATOMOXETINE_HCl
6	55	-1.44	E07	sample	19870	33600	64870	100810	-1.22	-1.839	-1.661	-0.672	SAM001246628_ARTESUNATE
6	67	-0.68	F07	sample	29661	41566	86680	103960	-0.789	-1.479	-0.501	-0.566	SAM001246679_ITRACONAZOLE
6	79	0.23	G07	sample	72651	81485	98680	95850	1.101	0.325	0.137	-0.838	SAM001246674_CEFPODOXIME_PROXETIL
6	91	0.29	H07	sample	62949	82352	100300	74460	0.674	0.365	0.223	-1.556	SAM001246680_Buflomedil_HCl
6	8	0.16	A08	sample	26724	42166	132110	157860	-0.919	-1.452	1.915	1.242	SAM001246614_4-Chloro-N-(2-morpholin-4-yl-ethyl)-benzamide
6	20	-1.32	B08	sample	18090	44792	70500	93370	-1.298	-1.333	-1.362	-0.921	SAM001246616_HALOMETASONE_MONOHYDRATE
6	32	-0.49	C08	sample	41721	61147	88750	84970	-0.259	-0.594	-0.391	-1.203	SAM001246681_TRICLABENDAZOLE
6	44	-0.94	D08	sample	27547	45059	77450	96300	-0.882	-1.321	-0.992	-0.823	SAM001246617_ROFECOXIB
6	56	-0.28	E08	sample	45905	71516	87860	88820	-0.075	-0.125	-0.439	-1.074	SAM001246618_BISOPROLOL_FUMARATE
6	68	0.42	F08	sample	61036	183507	100990	124630	0.59	4.938	0.26	0.127	SAM001246623_EZETIMIBE
6	80	0.1	G08	sample	81151	80038	95070	99470	1.474	0.26	-0.055	-0.717	SAM001246625_TIAGABINE_HCl
6	92	-3.64	H08	sample	1847	2092	740	970	-2.012	-3.264	-5.073	-4.021	SAM001246676_IDARUBICIN_HCl
6	9	1	A09	sample	25856	55784	168210	205390	-0.957	-0.836	3.836	2.837	SAM001246685_FLUBENDAZOLE
6	21	0.33	B09	sample	47351	46639	108990	141020	-0.012	-1.25	0.685	0.677	SAM001246677_TACROLIMUS
6	33	-0.48	C09	sample	46394	54672	83250	112340	-0.054	-0.887	-0.684	-0.285	SAM001246749_VALACICLOVIR_HYDROCHLORIDE
6	45	-0.91	D09	sample	19804	61236	68890	124990	-1.223	-0.59	-1.448	0.139	SAM001246748_CLARITHROMYCIN
6	57	-0.5	E09	sample	22496	76634	111220	80330	-1.104	0.106	0.804	-1.359	SAM001246750_ARIPIPRAZOLE
6	69	0.72	F09	sample	90341	108609	94190	93360	1.878	1.552	-0.102	-0.922	SAM001246747_TRIMEBUTINE_MALEATE
6	81	0.04	G09	sample	41922	124096	96140	123340	-0.25	2.252	0.002	0.084	SAM001246746_Mestanolone
6	93	1.38	H09	sample	100799	123962	105940	109770	2.338	2.246	0.523	-0.371	SAM001246719_NISOLDIPINE

6	10	1.03	A10	sample	75543	106339	111610	125820	1.228	1.449	0.825	0.167	SAM001246720_PICEID
6	22	0.16	B10	sample	40208	88649	73050	144780	-0.326	0.649	-1.226	0.803	SAM001246716_1-(2-Methyl-5-nitro-imidazol-1-yl)-propan-2-ol
6	34	-0.19	C10	sample	33155	64551	97090	130250	-0.636	-0.44	0.052	0.316	SAM001246717_NIFEKALANT_HCl
6	46	-0.31	D10	sample	51089	67956	89680	105390	0.153	-0.286	-0.342	-0.518	SAM001246721_NATEGLINIDE
6	58	1.16	E10	sample	36158	100042	129170	155310	-0.504	1.164	1.759	1.157	SAM001246722_MEGESTROL_ACETATE
6	70	0.27	F10	sample	46083	82152	100640	129710	-0.067	0.356	0.241	0.298	SAM001246728_ORMETOPRIM
6	82	1.03	G10	sample	44035	64752	149010	186840	-0.158	-0.431	2.814	2.214	SAM001246738_ZILEUTON
6	94	0.42	H10	sample	40319	74409	117280	145550	-0.321	0.006	1.126	0.829	SAM001246729_STAVUDINE
6	11	0.22	A11	sample	54583	61458	112210	124610	0.306	-0.58	0.857	0.127	SAM001246730_Gabexate-mesylate
6	23	0.62	B11	sample	72161	125342	99100	114770	1.079	2.308	0.159	-0.203	SAM001246724_OXICONAZOLE_NITRATE
6	35	0	C11	sample	50221	90719	78560	117450	0.114	0.743	-0.933	-0.114	SAM001246731_KITASAMYCIN
6	47	0.05	D11	sample	61347	97550	86660	93020	0.604	1.052	-0.502	-0.933	SAM001246732_FAMCICLOVIR
6	59	0.18	E11	sample	62971	76789	86030	128180	0.675	0.113	-0.536	0.246	SAM001246725_Sotalol hydrochloride
6	71	0.23	F11	sample	58833	89228	95650	102750	0.493	0.675	-0.024	-0.607	SAM001246726_RUFLOXACIN_HCl
6	83	-0.76	G11	sample	21094	24120	89480	110470	-1.166	-2.268	-0.352	-0.348	SAM001246778_TAXIFOLIN-(+)
6	95	0.08	H11	sample	73652	84867	84640	111180	1.145	0.478	-0.61	-0.324	SAM001246782_ALOSETRON_HCl
6	12	5.19	A12	merbb4-f	153223	301100	158875	291780	4.643	10.254	3.339	5.735	control
6	24	10.32	B12	merbb4-f	313233	329860	199128	391880	11.677	11.554	5.48	9.093	control
6	36	6.87	C12	merbb4-f	191718	280360	225362	325230	6.335	9.316	6.876	6.857	control
6	48	7.27	D12	merbb4-f	226207	279200	204624	320350	7.851	9.264	5.772	6.693	control
6	60	26.73	E12	bace-1	872543	597870	656015	552230	36.267	23.671	29.783	14.473	control
6	72	25.14	F12	bace-1	689770	561860	694465	726560	28.231	22.043	31.829	20.321	control

6	84	28.43	G12	bace-1	806902	593670	778620	528010	33.381	23.481	36.305	13.66	control
6	96	28.33	H12	bace-1	759595	637420	682672	825390	31.301	25.459	31.201	23.637	control
7	1	0.06	A01	baseline	43130	67590	66600	70910	0.011	0.167	0.105	-0.191	control
7	13	0.18	B01	baseline	54040	54040	79450	60010	0.94	-0.477	0.838	-0.619	control
7	25	0.37	C01	baseline	73610	72150	64950	84880	2.605	0.383	0.011	0.358	control
7	37	-0.94	D01	dapt	43140	57370	37440	35240	0.012	-0.319	-1.559	-1.591	control
7	49	-1.06	E01	dapt	31670	51100	26280	46130	-0.964	-0.617	-2.195	-1.164	control
7	61	-1	F01	dapt	42390	49550	40210	42630	-0.052	-0.691	-1.401	-1.301	control
7	73	-0.64	G01	tapi-1	54880	62940	43140	43340	1.011	-0.054	-1.233	-1.273	control
7	85	-1.13	H01	tapi-1	42860	45320	40920	34820	-0.012	-0.892	-1.36	-1.608	control
7	2	0.72	A02	sample	63600	70530	58070	104800	1.753	0.306	-0.382	1.14	SAM001246723_BUPROPION_HYDROCHLORIDE
7	14	7.4	B02	sample	218380	239530	178140	157850	14.926	8.336	6.467	3.222	SAM001246718_IRSOGLADINE_MALEATE
7	26	0.23	C02	sample	45170	83410	62030	82900	0.185	0.918	-0.156	0.28	SAM001246733_ACARBOSE
7	38	-0.34	D02	sample	43370	46540	69550	57740	0.031	-0.834	0.273	-0.708	SAM001246739_BENPROPERINE_PHOSPHATE
7	50	0.7	E02	sample	61400	73960	62450	99660	1.566	0.469	-0.132	0.938	SAM001246740_PHENPROBAMATE
7	62	0.43	F02	sample	50540	106730	65770	81260	0.642	2.026	0.057	0.216	SAM001246743_MEMANTINE_HYDROCHLORIDE
7	74	0.61	G02	sample	54970	82690	70720	79460	1.019	0.884	0.34	0.145	SAM001246736_Carvedilol
7	86	1.87	H02	sample	62260	108180	103580	104140	1.639	2.095	2.214	1.114	SAM001246741_LOMIFYLLINE
7	3	0.22	A03	sample	64980	63540	69200	80590	1.871	-0.026	0.253	0.189	SAM001246742_PAZUFLOXACIN
7	15	0.77	B03	sample	52850	78780	82440	87970	0.838	0.698	1.008	0.479	SAM001246745_MIGLITOL
7	27	1.71	C03	sample	69440	113540	85330	101800	2.25	2.35	1.173	1.022	SAM001246737_TRANILAST
7	39	0.3	D03	sample	45370	80800	57760	85700	0.202	0.794	-0.4	0.39	SAM001246652_OLANZAPINE

7	51	0.32	E03	sample	42840	64890	76750	91070	-0.014	0.038	0.684	0.601	SAM001246653_Nefazodone
7	63	-0.81	F03	sample	34620	45630	41170	56820	-0.713	-0.877	-1.346	-0.744	SAM001246654_MOXIFLOXACIN_HCl
7	75	-0.48	G03	sample	53520	54520	55940	60420	0.895	-0.454	-0.503	-0.603	SAM001246655_NELFINAVIR_MESYLATE
7	87	0.34	H03	sample	47790	69820	53130	86210	0.408	0.273	-0.664	0.41	SAM001246656_PRAVASTATIN_Sodium
7	4	-2.76	A04	sample	2960	4690	17570	16990	-3.408	-2.822	-2.692	-2.308	SAM001246651_TOPOTECAN_HCL
7	16	0.28	B04	sample	66850	84500	49370	65190	2.03	0.97	-0.878	-0.415	SAM001246539_LEVETIRACETAM
7	28	-1.36	C04	sample	12120	30930	49000	46620	-2.628	-1.575	-0.899	-1.144	SAM001246540_PRAMIPEXOLE_HCl
7	40	-0.1	D04	sample	42960	80860	61160	53670	-0.003	0.797	-0.206	-0.868	SAM001246595_RISPERIDONE
7	52	-0.05	E04	sample	43940	61390	54900	76720	0.08	-0.128	-0.563	0.037	SAM001246600_PIOGLITAZONE_HCl
7	64	-0.62	F04	sample	34580	62570	55630	45810	-0.717	-0.072	-0.521	-1.176	SAM001246552_CILASTATIN_Na
7	76	-1.18	G04	sample	28850	36180	59480	46240	-1.204	-1.326	-0.301	-1.159	SAM001246661_ARGATROBAN
7	88	-0.15	H04	sample	39380	60530	69870	72370	-0.308	-0.169	0.291	-0.133	SAM001246603_VALDECOXIB
7	5	0.6	A05	sample	31700	55400	117790	116960	-0.962	-0.413	3.025	1.617	SAM001246658_NAFTOPIDIL
7	17	0.91	B05	sample	31970	68700	108430	116530	-0.939	0.219	2.491	1.6	SAM001246662_Nobiletin
7	29	0.2	C05	sample	35400	86170	69460	79320	-0.647	1.049	0.268	0.139	SAM001246541_FINASTERIDE
7	41	0.09	D05	sample	49460	65250	66040	78440	0.55	0.055	0.073	0.105	SAM001246549_ZOLPIDEM_TARTRATE
7	53	-1.02	E05	sample	35470	46650	41740	44850	-0.641	-0.828	-1.313	-1.214	SAM001246551_Viramune
7	65	-0.55	F05	sample	38990	53430	54190	52080	-0.341	-0.506	-0.603	-0.93	SAM001246601_TOPIRAMATE
7	77	-0.47	G05	sample	40200	62610	52420	42430	-0.238	-0.07	-0.704	-1.309	SAM001246664_VORICONAZOLE
7	89	-0.01	H05	sample	51250	56170	71060	66310	0.702	-0.376	0.359	-0.371	SAM001246665_FENOLDOPAM_MESYLATE
7	6	1.73	A06	sample	36510	95110	99430	144250	-0.552	1.474	1.977	2.689	SAM001246610_ROSIGLITAZONE_MALEATE
7	18	0.5	B06	sample	34170	75130	73130	135550	-0.752	0.525	0.477	2.347	SAM001246668_ESCITALOPRAM_OXALATE

7	30	0.8	C06	sample	50100	85200	70980	114620	0.604	1.003	0.354	1.525	SAM001246609_ZERANOL
7	42	-0.86	D06	sample	33570	62120	35370	52320	-0.803	-0.093	-1.677	-0.921	SAM001246671_LATANOPROST
7	54	-0.32	E06	sample	37750	65430	49530	70710	-0.447	0.064	-0.869	-0.199	SAM001246673_2',3'-DIDEOXYINOSINE
7	66	-0.99	F06	sample	17350	43450	47350	73680	-2.183	-0.98	-0.993	-0.082	SAM001246666_Sertraline
7	78	1.09	G06	sample	80600	75710	77050	113490	3.2	0.552	0.701	1.481	SAM001246670_CALCIPOTRIOL
7	90	-3.29	H06	sample	910	830	1620	2790	-3.582	-3.006	-3.602	-2.865	SAM001246559_EPIRUBICIN_HYDROCHLORIDE
7	7	0.86	A07	sample	20960	73200	87480	115440	-1.876	0.433	1.296	1.557	SAM001246612_BICALUTAMIDE
7	19	1.26	B07	sample	46280	77780	97540	152560	0.279	0.651	1.869	3.015	SAM001246672_BENIDIPINE_HCl
7	31	0.95	C07	sample	39670	92380	87440	91430	-0.283	1.344	1.293	0.615	SAM001246669_AMLEXANOX
7	43	-1.82	D07	sample	21270	26600	34270	25200	-1.849	-1.781	-1.739	-1.985	SAM001246554_CERIVASTATIN_Na
7	55	-0.63	E07	sample	36740	48770	58260	51680	-0.533	-0.728	-0.371	-0.946	SAM001246560_ICARIIN
7	67	-0.43	F07	sample	38160	42660	68010	64550	-0.412	-1.018	0.185	-0.44	SAM001246562_METHYLANDROSTENEDIOL
7	79	-3.27	G07	sample	1540	700	950	700	-3.529	-3.012	-3.64	-2.947	SAM001246553_TRIPTOLIDE
7	91	-0.9	H07	sample	48360	74790	8010	18240	0.456	0.509	-3.237	-2.259	SAM001246608_ROSIGLITAZONE_HCl
7	8	0.68	A08	sample	46650	81650	83760	89110	0.311	0.835	1.083	0.524	SAM001246561_FTORAFUR
7	20	0.56	B08	sample	44870	84310	67510	126990	0.159	0.961	0.157	2.011	SAM001246769_OLIGOMYCIN_C
7	32	1.27	C08	sample	42830	92000	136920	106600	-0.014	1.326	4.116	1.21	SAM001246712_BENAZEPRIL_HCl
7	44	0.38	D08	sample	46030	70420	72920	90880	0.258	0.301	0.465	0.593	SAM001246765_Oxymetholone
7	56	-0.6	E08	sample	18380	48530	56590	63880	-2.095	-0.739	-0.466	-0.467	SAM001246714_IPRIFLAVONE
7	68	0.14	F08	sample	49430	62610	57480	84470	0.547	-0.07	-0.416	0.342	SAM001246770_OXAPROZIN
7	80	-1.3	G08	sample	24390	52580	20430	49840	-1.584	-0.547	-2.529	-1.018	SAM001246766_ROLIPRAM
7	92	-2.57	H08	sample	15390	10200	6980	10280	-2.35	-2.56	-3.296	-2.571	SAM001246713_MOSAPRIDE_CITRATE

7	9	-0.82	A09	sample	24780	35080	62660	69260	-1.551	-1.378	-0.12	-0.256	SAM001246767_Isoquercitrin
7	21	0.05	B09	sample	37730	55770	93170	88620	-0.449	-0.395	1.62	0.504	SAM001246763_FLUMAZENIL
7	33	0.65	C09	sample	40480	79380	109900	90170	-0.214	0.727	2.574	0.565	SAM001246593_Ozagrel-hydrochloride
7	45	-0.79	D09	sample	23980	39380	57490	66130	-1.619	-1.174	-0.415	-0.378	SAM001246776_HYPEROSIDE
7	57	-1.86	E09	sample	11540	25650	31740	51010	-2.678	-1.826	-1.884	-0.972	SAM001246589_RIFABUTIN
7	69	0.69	F09	sample	64620	81490	63820	89930	1.84	0.827	-0.054	0.556	SAM001246533_ESMOLOL_HYDROCHLORIDE
7	81	-0.57	G09	sample	41530	53160	53100	60000	-0.125	-0.519	-0.665	-0.619	SAM001246586_TADALAFIL
7	93	0.03	H09	sample	55230	39620	103880	50980	1.041	-1.162	2.231	-0.973	SAM001246587_Modafinil
7	10	-3.29	A10	sample	640	630	2340	2060	-3.605	-3.015	-3.561	-2.894	SAM001246768_DOXORUBICIN_HYDROCHLORIDE
7	22	-0.62	B10	sample	44730	57060	47710	52570	0.147	-0.334	-0.973	-0.911	SAM001246764_MOXONIDINE_HCl
7	34	0.18	C10	sample	43040	59520	109900	84980	0.003	-0.217	2.574	0.362	SAM001246711_Nitrazepam
7	46	0.34	D10	sample	51410	68190	73430	68400	0.716	0.195	0.494	-0.289	SAM001246571_PEFLOXACIN_MESYLATE
7	58	0.74	E10	sample	56130	62930	95440	85150	1.117	-0.055	1.75	0.368	SAM001246572_VENLAFAXINE_HCl
7	70	1.16	F10	sample	57520	86840	76050	107900	1.236	1.081	0.644	1.261	SAM001246591_PANTOPRAZOLE_SODIUM_SALT
7	82	0.21	G10	sample	45370	57070	68520	84090	0.202	-0.333	0.214	0.327	SAM001246583_FLUTICASONE_PROPIONATE
7	94	-0.37	H10	sample	38110	64630	58940	38440	-0.416	0.026	-0.332	-1.466	SAM001246588_INDINAVIR_SULPHATE
7	11	-0.1	A11	sample	39490	60710	70930	74820	-0.299	-0.16	0.352	-0.037	SAM001246585_MIDAZOLAM_HCl
7	23	0.94	B11	sample	54810	82490	90040	77040	1.005	0.875	1.442	0.05	SAM001246582_LAMIVUDINE
7	35	0.29	C11	sample	49250	75400	65710	63100	0.532	0.538	0.054	-0.497	SAM001246564_PROCARBAZINE_HYDROCHLORIDE
7	47	0.25	D11	sample	47260	66790	66160	92100	0.363	0.129	0.08	0.641	SAM001246538_ESOMEPRAZOLE_Mg
7	59	-0.32	E11	sample	38730	58260	69180	66610	-0.363	-0.277	0.252	-0.36	SAM001246530_SULFASALAZINE
7	71	-0.44	F11	sample	56710	61740	51350	44670	1.167	-0.111	-0.765	-1.221	SAM001246567_TORASEMIDE

7	83	0.01	G11	sample	45010	68730	49370	72140	0.171	0.221	-0.878	-0.143	SAM001246576_TROPISETRON_HCl
7	95	0.29	H11	sample	50390	73920	59820	78420	0.629	0.467	-0.282	0.104	SAM001246534_Ranolazine_dihydrochloride
7	12	6.59	A12	merbb4-f	86100	264180	111990	349000	3.668	9.508	2.694	10.727	control
7	24	6.5	B12	merbb4-f	80730	390430	148630	284930	3.211	15.507	4.784	8.212	control
7	36	7.78	C12	merbb4-f	119350	289000	104350	306660	6.498	10.687	2.258	9.065	control
7	48	7.6	D12	merbb4-f	129640	398380	159470	275290	7.374	15.884	5.402	7.833	control
7	60	20.56	E12	bace-1	420710	542280	311490	544220	32.147	22.722	14.073	18.391	control
7	72	21.11	F12	bace-1	293020	584000	431830	559610	21.279	24.704	20.937	18.996	control
7	84	23.65	G12	bace-1	446560	574720	404730	662540	34.347	24.263	19.391	23.037	control
7	96	19.66	H12	bace-1	253470	544990	308470	620970	17.913	22.851	13.901	21.405	control
8	1	-0.26	A01	baseline	95318	67520	87360	81270	2.315	-0.039	-0.488	-0.869	control
8	13	-0.06	B01	baseline	82491	78654	70150	91170	1.644	0.428	-1.14	-0.542	control
8	25	0.45	C01	baseline	82257	101100	88030	89360	1.632	1.37	-0.463	-0.602	control
8	37	-1.32	D01	dapt	31941	50694	51440	58190	-1.001	-0.745	-1.848	-1.632	control
8	49	-1.4	E01	dapt	33184	42498	43080	55990	-0.936	-1.089	-2.164	-1.705	control
8	61	-1.24	F01	dapt	41164	47884	57670	54240	-0.519	-0.863	-1.612	-1.763	control
8	73	-0.99	G01	tapi-1	67665	49902	53040	71180	0.868	-0.778	-1.787	-1.203	control
8	85	-0.89	H01	tapi-1	35922	36156	91410	77770	-0.793	-1.355	-0.335	-0.985	control
8	2	5.21	A02	sample	154947	187285	245690	199450	5.435	4.985	5.503	3.037	SAM001246531_NITRENDIPINE
8	14	0.54	B02	sample	68511	79014	90180	126550	0.912	0.443	-0.382	0.627	SAM001246578_SAQUINAVIR_MESYLATE
8	26	0.65	C02	sample	36012	92705	113670	131280	-0.788	1.018	0.507	0.784	SAM001246775_BIFONAZOLE
8	38	-0.02	D02	sample	51181	67286	103800	99910	0.006	-0.049	0.134	-0.253	SAM001246579_SUMATRIPTAN_SUCCINATE

8	50	0.99	E02	sample	101911	92219	126340	121420	2.66	0.997	0.987	0.458	SAM001246574_EXEMESTANE
8	62	0.72	F02	sample	75429	109658	96770	112370	1.274	1.729	-0.132	0.159	SAM001246708_NITAZOXANIDE
8	74	0.57	G02	sample	62152	91048	114880	113610	0.58	0.948	0.553	0.2	SAM001246536_Diazepam
8	86	0.75	H02	sample	57216	67755	151510	143330	0.321	-0.029	1.939	1.182	SAM001246777_QUETIAPINE_HEMIFUMARATE
8	3	-0.25	A03	sample	71214	60477	86790	102660	1.054	-0.334	-0.51	-0.162	SAM001246528_RUTIN
8	15	0.52	B03	sample	58387	61089	122090	127730	0.383	-0.309	0.826	0.666	SAM001246580_PENCICLOVIR
8	27	-0.6	C03	sample	46335	76222	69700	79030	-0.248	0.326	-1.157	-0.943	SAM001246772_CALCITRIOL
8	39	0.84	D03	sample	68799	123457	120230	127470	0.928	2.308	0.755	0.658	SAM001246532_DIPHENOXYLATE
8	51	0.21	E03	sample	72583	84455	93450	98490	1.126	0.671	-0.258	-0.3	SAM001247005_Felbamate
8	63	0.54	F03	sample	68385	84599	100640	119780	0.906	0.678	0.014	0.404	SAM001247013_DROPERIDOL
8	75	-0.15	G03	sample	56261	63359	75840	104830	0.271	-0.214	-0.924	-0.091	SAM001247011_Pentoxifylline
8	87	0.32	H03	sample	68421	96777	93090	93790	0.908	1.188	-0.271	-0.456	SAM001246596_Ketorolac-tromethamine
8	4	0.06	A04	sample	43921	47019	113290	125700	-0.374	-0.899	0.493	0.599	SAM001246783_RITONAVIR
8	16	0.39	B04	sample	59450	76708	112220	103670	0.438	0.346	0.452	-0.129	SAM001246780_VINORELBINE_BITATRATE
8	28	-0.05	C04	sample	57900	63863	97320	107620	0.357	-0.192	-0.111	0.002	SAM001246624_LINEZOLID
8	40	-0.4	D04	sample	52532	56477	92320	77650	0.076	-0.502	-0.301	-0.989	SAM001246727_LOMERIZINE_DiHCl
8	52	0.15	E04	sample	66818	75483	99450	107520	0.824	0.295	-0.031	-0.002	SAM001246667_EFAVIRENZ
8	64	-1.08	F04	sample	31202	57504	62290	73420	-1.04	-0.459	-1.437	-1.129	SAM001246548_IRBESARTAN
8	76	-0.85	G04	sample	42660	51505	62600	77850	-0.44	-0.711	-1.425	-0.982	SAM001246546_REPAGLINIDE
8	88	0.38	H04	sample	45992	104830	115220	113610	-0.266	1.526	0.566	0.2	SAM001246555_Ethylestrenol
8	5	0.44	A05	sample	34697	86887	114840	117210	-0.857	0.773	0.552	0.319	SAM001246605_PTEROSTILBENE
8	17	0	B05	sample	53847	65196	127510	100180	0.145	-0.136	1.031	-0.244	SAM001246547_ROXATIDINE_ACETATE_HCl

8	29	-0.71	C05	sample	43921	72763	64270	76140	-0.374	0.181	-1.362	-1.039	SAM001246556_DEXBROMPHENIRAMINE_MALEATE
8	41	0.31	D05	sample	73501	77231	106830	84860	1.174	0.368	0.248	-0.751	SAM001246604_ANAGRELIDE_HCl
8	53	-0.77	E05	sample	37615	42263	99890	82150	-0.704	-1.098	-0.014	-0.84	SAM001246606_TEGASEROD_MALEATE
8	65	-0.6	F05	sample	33544	56459	97370	86650	-0.917	-0.503	-0.11	-0.692	SAM001246611_MILRINONE
8	77	-0.53	G05	sample	40011	59432	84010	93270	-0.579	-0.378	-0.615	-0.473	SAM001246575_LEVOCETIRIZINE
8	89	-0.32	H05	sample	49181	72078	86040	86680	-0.099	0.152	-0.538	-0.691	SAM001246599_Citalopram-hydrobromide
8	6	1.01	A06	sample	66620	114756	109780	143870	0.814	1.943	0.36	1.2	SAM001246558_TICLOPIDINE_HCl
8	18	0.35	B06	sample	72619	100002	77080	94640	1.127	1.324	-0.877	-0.427	SAM001246594_LOXOPROFEN_SODIUM
8	30	0.02	C06	sample	65359	121458	77160	85930	0.748	2.224	-0.874	-0.715	SAM001246577_ZAFIRLUKAST
8	42	-1.04	D06	sample	28590	50028	73830	75200	-1.176	-0.773	-1	-1.07	SAM001246565_TERBINAFINE_HCl
8	54	-0.43	E06	sample	37922	69142	95960	79990	-0.688	0.029	-0.163	-0.912	SAM001246584_ISRADIPINE
8	66	0.09	F06	sample	64026	82221	89850	74890	0.678	0.578	-0.394	-1.08	SAM001246581_VALSARTAN
8	78	0.15	G06	sample	61828	112324	77630	99400	0.563	1.841	-0.856	-0.27	SAM001247048_Piroxicam
8	90	-0.88	H06	sample	22879	33580	92210	109440	-1.475	-1.463	-0.305	0.062	SAM001246629_Glycopyrrolate
8	7	-0.22	A07	sample	38858	67322	91450	104470	-0.639	-0.047	-0.334	-0.102	SAM001246992_Physostigmine
8	19	-0.28	B07	sample	33022	106883	101540	89150	-0.945	1.612	0.048	-0.609	SAM001247050_LOBELINE_HYDROCHLORIDE
8	31	-0.09	C07	sample	65575	61900	79330	110430	0.759	-0.275	-0.792	0.095	SAM001247030_Doxylamine_succinate_salt
8	43	-0.05	D07	sample	56693	58927	76610	134450	0.294	-0.399	-0.895	0.889	SAM001247035_Milnacipran
8	55	-0.03	E07	sample	50622	43290	124460	106480	-0.024	-1.055	0.916	-0.036	SAM001247017_5-fluoro-2-pyrimidone
8	67	-0.17	F07	sample	45866	55829	98460	128650	-0.272	-0.529	-0.068	0.697	SAM001247022_Chlorpheniramine
8	79	-1.44	G07	sample	11620	19006	105550	83140	-2.064	-2.074	0.2	-0.808	SAM001246621_DOFETILIDE
8	91	-1.43	H07	sample	7026	7819	85660	95200	-2.305	-2.543	-0.553	-0.409	SAM001246675_FORMOTEROL_FUMARATE_DIHYDRATE

8	8	-0.13	A08	sample	36715	85049	83130	119530	-0.751	0.696	-0.648	0.395	SAM001246615_RIZATRIPTAN_BENZOATE
8	20	0.58	B08	sample	67538	119890	86310	116680	0.862	2.158	-0.528	0.301	SAM001246620_RIFAPENTINE
8	32	0.36	C08	sample	60783	73628	125580	101710	0.508	0.217	0.958	-0.194	SAM001246630_LOTEPREDNOL_ETABONATE
8	44	0.66	D08	sample	63161	112918	107450	128220	0.633	1.865	0.272	0.683	SAM001246684_ENALAPRILAT
8	56	-0.21	E08	sample	59288	75825	80210	85750	0.43	0.309	-0.759	-0.721	SAM001246627_Donepezil
8	68	-0.35	F08	sample	41381	66115	95390	91880	-0.507	-0.098	-0.184	-0.519	SAM001246755_Nimetazepam
8	80	-0.77	G08	sample	20537	24825	101710	121770	-1.598	-1.83	0.055	0.469	SAM001246701_NICORANDIL
8	92	0.07	H08	sample	65052	30013	107450	103670	0.731	-1.612	0.272	-0.129	SAM001246602_TELMISARTAN
8	9	-0.15	A09	sample	44641	61954	99410	107390	-0.337	-0.272	-0.032	-0.006	SAM001246542_ITOPRIDE_HCl
8	21	-0.7	B09	sample	41687	78582	76450	75160	-0.491	0.425	-0.901	-1.071	SAM001246597_RIFAXIMIN
8	33	-0.79	C09	sample	35201	63881	73890	84740	-0.831	-0.192	-0.998	-0.755	SAM001246657_MONTELUKAST_Na
8	45	-0.15	D09	sample	49902	71646	85090	100230	-0.061	0.134	-0.574	-0.243	SAM001246779_2',3'-DIDEOXYCYTIDINE
8	57	-0.81	E09	sample	25095	56495	70910	93670	-1.359	-0.501	-1.111	-0.459	SAM001247073_1H-Imidazol-2-amine,_N-(2,6-dichlorophenyl)-4,5-dihydro-_[CAS]
8	69	-1.06	F09	sample	41236	34391	65060	83950	-0.515	-1.429	-1.332	-0.781	SAM001247077_6H-Pyrido[2,3-b][1,4]benzodiazepin-6-one,_11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-_[CAS]
8	81	0.15	G09	sample	48136	72439	118780	111800	-0.154	0.167	0.701	0.14	SAM001247075_1H-Indole-2-propanoic_acid,_1-[(4-chlorophenyl)methyl]-3-[(1,1-dimethylethyl)thio]-Alpha,Alpha-dimethyl-5-(1-methylethyl)-_[CAS]
8	93	1.34	H09	sample	93949	94363	142140	139860	2.244	1.087	1.585	1.067	SAM001247074_1H-Imidazole-5-carboxylic_acid,_1-(1-phenylethyl)-,_ethyl_ester,_(R)-_[CAS]
8	10	2.09	A10	sample	55234	72421	223550	227680	0.218	0.167	4.665	3.97	SAM001247078_Acetamide,_2-amino-N-(1-methyl-1,2-diphenylethyl)-,_(+/-)-_[CAS]
8	22	0.34	B10	sample	38876	45128	150410	147150	-0.638	-0.978	1.897	1.308	SAM001247080_Altanserin
8	34	0.66	C10	sample	46857	88850	115680	129610	-0.221	0.856	0.583	0.729	SAM001247084_Betaxolol-hydrochloride
8	46	2.29	D10	sample	83806	70241	175850	247950	1.713	0.075	2.86	4.64	SAM001246563_Indirubin

8	58	1.54	E10	sample	66223	82509	160930	228190	0.793	0.59	2.296	3.987	SAM001247082_Azasetron
8	70	1.21	F10	sample	65106	100938	128020	161510	0.734	1.363	1.05	1.783	SAM001247087_GR_89696
8	82	1.66	G10	sample	50965	66890	188020	231400	-0.006	-0.065	3.321	4.093	SAM001246897_DELTA1-HYDROCORTISONE_21-HEMISUCCINATE_SODIUM_SALT
8	94	2.01	H10	sample	48965	64728	211110	232320	-0.11	-0.156	4.194	4.124	SAM001246872_DIAZOXIDE
8	11	0.12	A11	sample	38246	41110	174340	135390	-0.671	-1.147	2.803	0.92	SAM001246886_2-CHLOROADENOSINE
8	23	1.51	B11	sample	64026	99533	180200	159560	0.678	1.304	3.025	1.719	SAM001246873_ORNIDAZOLE
8	35	-0.59	C11	sample	44353	48100	78480	102840	-0.352	-0.854	-0.824	-0.156	SAM001246914_1,1-DIMETHYL-4-PHENYLPIPERAZINIUM_IODIDE
8	47	0.63	D11	sample	49830	49541	135460	162460	-0.065	-0.793	1.332	1.814	SAM001246893_PIRENPERONE
8	59	0.01	E11	sample	33580	56856	113730	172760	-0.915	-0.486	0.51	2.155	SAM001246866_MESTRANOL
8	71	0.33	F11	sample	53379	50658	125340	124010	0.121	-0.746	0.949	0.543	SAM001246867_2-(2-AMINOETHYL)PYRIDINE
8	83	1.36	G11	sample	53901	44785	193500	185310	0.148	-0.993	3.528	2.57	SAM001246868_BENACTYZINE_HYDROCHLORIDE
8	95	0.22	H11	sample	32535	50983	131270	181460	-0.97	-0.733	1.173	2.442	SAM001246870_DICHLOROACETIC_ACID
8	12	5.63	A12	merbb4-f	169053	351630	234538	248680	6.174	11.879	5.081	4.664	control
8	24	6.19	B12	merbb4-f	210812	269810	152624	229070	8.359	8.447	1.981	4.016	control
8	36	6.22	C12	merbb4-f	211353	233740	179970	274350	8.387	6.934	3.016	5.513	control
8	48	6.75	D12	merbb4-f	251022	246840	259255	231650	10.463	7.483	6.016	4.101	control
8	60	22.71	E12	bace-1	797148	554730	761280	508070	39.04	20.399	25.013	13.239	control
8	72	21.33	F12	bace-1	512997	565400	676880	580120	24.171	20.847	21.819	15.62	control
8	84	18.87	G12	bace-1	726980	465840	656955	515010	35.368	16.671	21.065	13.468	control
8	96	16.75	H12	bace-1	727106	497390	510186	519150	35.375	17.994	15.511	13.605	control
9	1	-0.45	A01	baseline	49310	46560	48910	81040	0.15	-1.101	-0.509	-0.383	control
9	13	-	B0	baseline	68990	67180	59700	73540	1.31	-0.103	-0.014	-0.563	control

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9	25	0.57	C01	baseline	69620	72760	81290	96410	1.348	0.167	0.977	-0.014	control
9	37	-1.2	D01	dapt	37020	38160	33660	47180	-0.575	-1.508	-1.209	-1.196	control
9	49	-0.96	E01	dapt	44670	52770	31400	50510	-0.124	-0.801	-1.313	-1.116	control
9	61	-0.63	F01	dapt	45280	51700	50680	62020	-0.088	-0.852	-0.428	-0.839	control
9	73	-1.08	G01	tapi-1	32580	57790	26710	41800	-0.836	-0.558	-1.528	-1.325	control
9	85	-0.62	H01	tapi-1	46170	58330	44540	54320	-0.035	-0.531	-0.71	-1.024	control
9	2	3.41	A02	sample	180300	150070	123330	133610	7.873	3.909	2.907	0.88	SAM001246806_BESTATIN
9	14	-0.61	B02	sample	28940	50900	62480	82990	-1.051	-0.891	0.114	-0.336	SAM001246774_TOREMIFENE_CITRATE
9	26	-0.24	C02	sample	46460	75090	40900	77970	-0.018	0.28	-0.877	-0.456	SAM001246784_GOSERELIN_ACETATE
9	38	0.79	D02	sample	77150	123490	55560	82280	1.791	2.622	-0.204	-0.353	SAM001246678_SECOISOLARICIRESINOL
9	50	0.36	E02	sample	59400	81460	62940	72910	0.745	0.588	0.135	-0.578	SAM001246796_RALTITREXED
9	62	0.12	F02	sample	44160	76060	73570	93630	-0.154	0.327	0.623	-0.08	SAM001246792_DOXAPRAM_HYDROCHLORIDE
9	74	0.24	G02	sample	41500	71080	80140	113580	-0.31	0.086	0.924	0.399	SAM001247095_RU_24969
9	86	-0.14	H02	sample	34350	66610	119080	90430	-0.732	-0.131	2.712	-0.157	SAM001246865_Brucine
9	3	0.18	A03	sample	53770	63510	67180	98410	0.413	-0.281	0.329	0.034	SAM001246862_TRYPTOLINE
9	15	-1.76	B03	sample	7840	26410	28710	49260	-2.295	-2.076	-1.436	-1.146	SAM001246863_Calmansial
9	27	-1.55	C03	sample	17680	40590	21880	50300	-1.715	-1.39	-1.75	-1.121	SAM001246791_PALONOSETRON_HCl
9	39	0.25	D03	sample	74660	83220	40360	89630	1.645	0.673	-0.902	-0.176	SAM001246874_NAPROXEN_SODIUM
9	51	0.52	E03	sample	87450	100290	47080	77800	2.399	1.5	-0.593	-0.46	SAM001246875_MEPIVACAINE_HYDROCHLORIDE
9	63	1.11	F03	sample	59050	91300	86850	144820	0.724	1.064	1.232	1.149	SAM001246877_3-[3,5-DIBROMO-4-HYDROXYBENZOYL]-2-ETHYLBENZOFURAN
9	75	1.34	G03	sample	49520	101600	84320	169780	0.162	1.563	1.116	1.748	SAM001246890_NIMODIPINE

9	87	0.86	H03	sample	52460	78080	115240	151330	0.336	0.424	2.535	1.305	SAM001246892_ROLITETRACYCLINE
9	4	-0.19	A04	sample	39860	76990	60110	80900	-0.407	0.372	0.005	-0.386	SAM001246891_MEPIRIZOLE
9	16	-0.18	B04	sample	62480	69690	43490	81370	0.927	0.018	-0.758	-0.375	SAM001246876_6-AZAURIDINE
9	28	-0.69	C04	sample	39480	57810	42250	57220	-0.43	-0.557	-0.815	-0.955	SAM001246889_ReichsteinÂ's_substanceÂ S
9	40	-0.21	D04	sample	49130	69970	46890	77790	0.139	0.032	-0.602	-0.461	SAM001246894_3-PYRIDINEMETHANOL
9	52	0.31	E04	sample	55400	67070	62520	125070	0.509	-0.108	0.115	0.675	SAM001246979_Haloperidol
9	64	0.59	F04	sample	66460	78370	49140	128170	1.161	0.439	-0.499	0.749	SAM001247042_Stiripentol
9	76	0.52	G04	sample	56230	103260	70390	90000	0.558	1.643	0.477	-0.167	SAM001247034_Fluperlapine
9	88	0.8	H04	sample	62080	83610	127540	125980	0.903	0.692	3.1	0.696	SAM001246887_OXYPHENONIUM_BROMIDE
9	5	-0.3	A05	sample	39530	55350	83140	89720	-0.427	-0.676	1.062	-0.174	SAM001246869_Homoveratrylamine
9	17	-0.79	B05	sample	34970	44020	49000	60020	-0.695	-1.224	-0.505	-0.887	SAM001246913_TINIDAZOLE
9	29	-0.12	C05	sample	49200	46310	67480	80940	0.144	-1.113	0.343	-0.385	SAM001246912_XANTHINOL_NICOTINATE
9	41	0.11	D05	sample	48390	79180	39860	102090	0.096	0.478	-0.925	0.123	SAM001246896_SYNEPHRINE
9	53	-0.06	E05	sample	27100	65190	61850	176040	-1.159	-0.199	0.085	1.898	SAM001246888_Resveratrol
9	65	-0.03	F05	sample	45120	62890	60830	107170	-0.097	-0.311	0.038	0.245	SAM001246871_118-71-8
9	77	0.37	G05	sample	43820	63880	86070	135300	-0.174	-0.263	1.196	0.92	SAM001246854_6-AMINOINDAZOLE
9	89	1.12	H05	sample	64630	85110	135240	146330	1.053	0.765	3.453	1.185	SAM001246856_ENROFLOXACIN
9	6	-1.04	A06	sample	38200	43710	33220	61280	-0.505	-1.239	-1.229	-0.857	SAM001246857_DEHYDROCHOLIC_ACID
9	18	-0.87	B06	sample	26430	48600	59900	66510	-1.199	-1.002	-0.005	-0.732	SAM001246884_CEFACLOR
9	30	-0.72	C06	sample	31290	54970	43920	73660	-0.912	-0.694	-0.738	-0.56	SAM001246858_1-BENZYLIMIDAZOLE
9	42	-1.26	D06	sample	15920	40180	35830	63180	-1.819	-1.41	-1.11	-0.811	SAM001246523_DULOXETINE_HCl
9	54	-1.28	E06	sample	25250	31240	31740	55360	-1.269	-1.843	-1.297	-0.999	SAM001246573_VARDENAFIL_CITRATE

9	66	0.21	F06	sample	51900	56760	74950	102190	0.303	-0.607	0.686	0.125	SAM001246524_ROPIVACAINE_HCl
9	78	0.29	G06	sample	52830	59970	64720	164960	0.358	-0.452	0.216	1.632	SAM001246525_ANASTROZOLE
9	90	0.45	H06	sample	27830	69940	87830	133240	-1.116	0.03	1.277	0.871	SAM001246984_KETOTIFEN_FUMARATE
9	7	-0.38	A07	sample	27100	55030	58660	97200	-1.159	-0.691	-0.062	0.005	SAM001246906_MEDROXYPROGESTERONE
9	19	-0.5	B07	sample	36180	59470	48710	86420	-0.624	-0.476	-0.518	-0.253	SAM001246907_Pinacidil_monohydrate
9	31	-0.36	C07	sample	47920	53100	85080	22240	0.068	-0.785	1.151	-1.795	SAM001246908_7-NITROINDAZOLE
9	43	0.03	D07	sample	45080	90530	51760	103600	-0.099	1.027	-0.378	0.159	SAM001246909_5-Methoxytryptamine
9	55	0.23	E07	sample	47480	58850	68970	153920	0.042	-0.506	0.411	1.367	SAM001246855_PHENOTHIAZINE
9	67	-1.27	F07	sample	35040	42030	29870	46160	-0.691	-1.32	-1.383	-1.22	SAM001246526_2-CHLORO-2'-DEOXYADENOSINE
9	79	0.06	G07	sample	56310	71970	58160	96520	0.563	0.129	-0.085	-0.011	SAM001246527_GRANISETRON_HCl
9	91	0.19	H07	sample	45540	77030	79030	97360	-0.072	0.374	0.873	0.009	SAM001247094_Rimcazole
9	8	-0.72	A08	sample	34880	52370	48700	66450	-0.701	-0.82	-0.519	-0.733	SAM001247092_Nafadotride
9	20	-0.13	B08	sample	37770	70060	59120	87580	-0.53	0.036	-0.041	-0.226	SAM001246885_DESOXIMETASONE
9	32	0.33	C08	sample	45370	49080	103450	127570	-0.082	-0.979	1.994	0.735	SAM001246557_DEXCHLORPHENIRAMINE_MALEATE
9	44	0.21	D08	sample	52360	88600	57020	100940	0.33	0.934	-0.137	0.095	SAM001247088_Guanidine,_N-cyano-N'-(1,1-dimethylpropyl)-N''-3-pyridinyl-_[CAS]
9	56	0.21	E08	sample	48220	93860	62840	109220	0.086	1.188	0.13	0.294	SAM001247090_L-694,247
9	68	0.64	F08	sample	35330	58950	99010	193410	-0.674	-0.501	1.79	2.316	SAM001247081_AM-251
9	80	0.64	G08	sample	48870	69900	85150	172770	0.124	0.029	1.154	1.82	SAM001247089_HTMT
9	92	1.72	H08	sample	38640	118970	153390	140240	-0.479	2.404	4.286	1.039	SAM001247083_Benzo[a]phenanthridine-10,11-diol,_5,6,6a,7,8,12b-hexahydro-,_trans-_[CAS]
9	9	-0.16	A09	sample	48020	61310	74080	73700	0.074	-0.387	0.646	-0.559	SAM001247091_Methanesulfonamide,_N-[4-[[1-[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl]phenyl]-,_dihydrochloride_[CAS]
9	21	-0.45	B09	sample	35320	48030	89160	87720	-0.675	-1.03	1.338	-0.222	SAM001247076_2H-Indol-2-one,_1,3-dihydro-1-phenyl-3,3-bis(4-pyridinylmethyl)-_[CAS]

9	33	0.09	C09	sample	36380	50180	77430	134670	-0.612	-0.926	0.8	0.905	SAM001246898_Beclomethasone
9	45	1.03	D09	sample	66320	109470	74450	134460	1.153	1.944	0.663	0.9	SAM001246900_OMEPRAZOLE
9	57	0.12	E09	sample	35790	80630	53250	120020	-0.647	0.548	-0.31	0.553	SAM001246842_DOLASETRON_MESYLATE
9	69	3.04	F09	sample	101590	139830	121970	195640	3.232	3.413	2.844	2.369	SAM001246841_Zolmitriptan
9	81	-2.09	G09	sample	5790	24430	16210	102290	-2.416	-2.172	-2.01	0.128	SAM001246852_TREMULACIN
9	93	-2.71	H09	sample	850	920	840	530	-2.707	-3.31	-2.716	-2.316	SAM001246846_DACTINOMYCIN
9	10	-0.42	A10	sample	41040	58770	73130	63460	-0.338	-0.51	0.602	-0.805	SAM001246847_Tramadol
9	22	0.04	B10	sample	77580	76380	54410	66830	1.817	0.342	-0.257	-0.724	SAM001246815_CHLORDIAZEPOXIDE
9	34	0.06	C10	sample	47070	77880	48530	101180	0.018	0.415	-0.527	0.101	SAM001246816_CEFIXIME_TRIHYDRATE
9	46	0.17	D10	sample	51010	71290	48730	125060	0.25	0.096	-0.518	0.674	SAM001246818_CEFDINIR
9	58	-0.26	E10	sample	34980	65320	52690	143960	-0.695	-0.193	-0.336	1.128	SAM001246820_LOFEXIDINE_HCl
9	70	0.83	F10	sample	64570	83160	70360	138350	1.05	0.67	0.475	0.993	SAM001246804_BALSALAZIDE
9	82	0.71	G10	sample	86320	98610	48700	96750	2.332	1.418	-0.519	-0.005	SAM001246802_OLOPATADINE_HCl
9	94	-2.08	H10	sample	7770	15770	19270	63690	-2.299	-2.591	-1.87	-0.799	SAM001246803_ITAVASTATIN_Ca
9	11	0.47	A11	sample	62160	69810	82240	81130	0.908	0.024	1.021	-0.38	SAM001246882_CORTISONE
9	23	-2.27	B11	sample	1540	16420	17010	38930	-2.666	-2.56	-1.973	-1.394	SAM001246883_Cyproheptadine-hydrochloride
9	35	-2.7	C11	sample	910	1110	1220	1880	-2.704	-3.301	-2.698	-2.283	SAM001246822_HOMOHARRINGTONINE
9	47	-0.17	D11	sample	57810	75470	46260	58780	0.651	0.298	-0.631	-0.917	SAM001246879_CORTICOSTERONE
9	59	0.23	E11	sample	61870	67480	49510	120190	0.891	-0.089	-0.482	0.557	SAM001246821_VECURONIUM_BROMIDE
9	71	0.09	F11	sample	65890	68930	49650	105630	1.128	-0.018	-0.475	0.208	SAM001246801_TIBOLONE
9	83	0.39	G11	sample	51230	87330	57060	118840	0.263	0.872	-0.135	0.525	SAM001246860_NICOTINAMIDE
9	95	0.97	H11	sample	63860	88440	95580	105770	1.008	0.926	1.633	0.211	SAM001246861_NIALAMIDE

9	12	2.41	A12	merbb4-f	83480	237070	89330	207530	2.165	8.12	1.346	2.655	control
9	24	2.79	B12	merbb4-f	100540	208370	104300	197670	3.171	6.731	2.033	2.418	control
9	36	2.32	C12	merbb4-f	88400	165660	107590	156320	2.455	4.664	2.184	1.425	control
9	48	2.49	D12	merbb4-f	81190	163740	96990	220190	2.03	4.571	1.698	2.959	control
9	60	8.29	E12	bace-1	213350	381280	189150	378630	9.822	15.1	5.928	6.763	control
9	72	10.17	F12	bace-1	201180	419360	291710	501380	9.104	16.943	10.635	9.71	control
9	84	11.05	G12	bace-1	227650	608040	281300	573400	10.665	26.076	10.157	11.44	control
9	96	11.7	H12	bace-1	271870	543840	259070	518690	13.272	22.969	9.137	10.126	control
10	1	1.68	A01	baseline	71250	78430	111480	112490	2.869	1.635	1.733	1.295	control
10	13	2.71	B01	baseline	121210	97190	140740	139820	6.244	2.683	2.737	2.119	control
10	25	4.45	C01	baseline	129680	169580	124130	113090	6.816	6.729	2.167	1.313	control
10	37	-0.12	D01	dapt	55000	59720	36860	38350	1.771	0.59	-0.828	-0.942	control
10	49	-0.42	E01	dapt	27640	46400	41220	39010	-0.077	-0.155	-0.678	-0.922	control
10	61	-0.73	F01	dapt	27830	34430	42530	27180	-0.064	-0.824	-0.633	-1.279	control
10	73	0.02	G01	tapi-1	39720	64180	40590	46110	0.739	0.839	-0.7	-0.708	control
10	85	1.44	H01	tapi-1	80010	76190	100720	83960	3.461	1.51	1.364	0.434	control
10	2	-1.06	A02	sample	16130	16910	31580	32640	-0.855	-1.803	-1.009	-1.114	SAM001246568_VINDESINE_SULFATE
10	14	-0.86	B02	sample	22860	18680	31910	45290	-0.4	-1.704	-0.997	-0.733	SAM001246570_VINCRISTINE_SULFATE
10	26	-0.44	C02	sample	38080	42250	41490	53370	0.628	-0.387	-0.669	-0.489	SAM001246648_LACIDIPINE
10	38	-0.4	D02	sample	24870	35500	45490	66470	-0.264	-0.764	-0.531	-0.094	SAM001246659_MIRTAZAPINE
10	50	0.12	E02	sample	44250	49890	60860	75870	1.045	0.04	-0.004	0.19	SAM001246707_AMPIROXICAM
10	62	-0.19	F02	sample	34610	49770	47020	55990	0.394	0.034	-0.479	-0.41	SAM001246710_GLIMEPIRIDE

10	74	-0.81	G02	sample	8830	43400	39470	40370	-1.348	-0.322	-0.738	-0.881	SAM001246705_AMLODIPINE_BASE
10	86	0.66	H02	sample	72690	65930	65110	82140	2.966	0.937	0.142	0.379	SAM001246619_RABEPRAZOLE
10	3	-0.15	A03	sample	14300	37520	102690	81160	-0.978	-0.651	1.432	0.349	SAM001246878_CLOFAZIMINE
10	15	-0.95	B03	sample	17710	34010	30290	26190	-0.748	-0.847	-1.053	-1.309	SAM001246598_IRINOTECAN_HCl_)trihydrate)
10	27	0.36	C03	sample	39500	57460	57680	78180	0.724	0.463	-0.113	0.26	SAM001246544_LANSOPRAZOLE
10	39	-1.08	D03	sample	10160	24990	34870	46910	-1.258	-1.351	-0.896	-0.684	SAM001246545_8-Chloro-11-piperidin-4-ylidene-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridine
10	51	0.53	E03	sample	31740	46530	86320	99030	0.2	-0.148	0.87	0.889	SAM001246904_1,3,5(10)-ESTRATRIEN-3-OL-17-ONE_SULPHATE,_SODIUM_SALT
10	63	-0.24	F03	sample	14610	41960	62780	67140	-0.957	-0.403	0.062	-0.073	SAM001246881_MIFEPRISTONE
10	75	-1.91	G03	sample	7580	9120	5070	6520	-1.432	-2.238	-1.918	-1.902	SAM001246880_Etoposide
10	87	1.4	H03	sample	40630	83790	86020	177600	0.8	1.935	0.859	3.259	SAM001247105_Sibutramine
10	4	0.2	A04	sample	58420	54900	57370	72130	2.002	0.32	-0.124	0.077	SAM001247107_Clobenpropit
10	16	-0.02	B04	sample	33290	61960	50820	52640	0.305	0.715	-0.348	-0.511	SAM001246851_HUPERZINE_A
10	28	-0.27	C04	sample	25460	44930	41150	59470	-0.224	-0.237	-0.68	-0.305	SAM001246592_SIBUTRAMINE_HCl
10	40	0.1	D04	sample	21980	48570	67510	99450	-0.459	-0.034	0.224	0.901	SAM001246833_Lorazepam
10	52	0.22	E04	sample	39630	50230	72200	67920	0.733	0.059	0.385	-0.05	SAM001247108_8-Azaspiro[4.5]decane-7,9-dione,_8-[2-[[(2,3-dihydro-1,4-benzodioxin-2-yl)methyl]amino]ethyl]-,_monomethanesulfonate_[CAS]
10	64	1.45	F04	sample	22220	73910	105190	193600	-0.443	1.383	1.517	3.741	SAM001247106_Adenosine,_N-(2-hydroxycyclopentyl)-,_(1S-trans)-_[CAS]
10	76	-0.42	G04	sample	23860	39990	58580	37870	-0.332	-0.513	-0.082	-0.956	SAM001246646_AMIODARONE_HYDROCHLORIDE
10	88	0.15	H04	sample	39450	37990	69970	69110	0.721	-0.625	0.309	-0.014	SAM001246644_mevastatin
10	5	0.49	A05	sample	35740	58850	75590	58350	0.47	0.541	0.502	-0.339	SAM001246622_IMATINIB_MESYLATE
10	17	-0.2	B05	sample	29590	43600	58510	55680	0.055	-0.311	-0.085	-0.419	SAM001247102_Metylperon
10	29	-0.1	C05	sample	27190	40320	67020	66430	-0.107	-0.495	0.207	-0.095	SAM001246773_PARECOXIB_Na

10	41	-0.54	D05	sample	25950	42570	40270	46330	-0.191	-0.369	-0.711	-0.701	SAM001247070_PERGOLIDE MESYLATE
10	53	-0.8	E05	sample	16870	33400	37690	55020	-0.805	-0.881	-0.799	-0.439	SAM001246793_ATRACURIUM_BESYLATE
10	65	0	F05	sample	27350	65740	60650	70040	-0.097	0.926	-0.011	0.014	SAM001246799_ARTEMETHER
10	77	0.97	G05	sample	47890	58730	82000	110230	1.291	0.534	0.722	1.226	SAM001247071_EBSELEN
10	89	0.77	H05	sample	40280	62860	103150	91630	0.777	0.765	1.447	0.665	SAM001247066_CGS_12066B
10	6	-0.69	A06	sample	22210	26580	33540	62610	-0.444	-1.262	-0.941	-0.21	SAM001246805_TELITHROMYCIN
10	18	0.71	B06	sample	27970	44840	121290	118360	-0.055	-0.242	2.07	1.472	SAM001247065_CCPA
10	30	0.48	C06	sample	23850	66260	61090	112350	-0.333	0.955	0.004	1.29	SAM001247067_PD_81723
10	42	0.53	D06	sample	31620	61660	71530	111220	0.192	0.698	0.362	1.256	SAM001246590_Stanozolol
10	54	0.86	E06	sample	29710	56180	103430	113450	0.063	0.392	1.457	1.323	SAM001246641_Zaleplon
10	66	0.78	F06	sample	41810	64010	82160	74000	0.88	0.829	0.727	0.133	SAM001246840_Prostaglandin_E1
10	78	1.41	G06	sample	37740	71920	106010	141540	0.605	1.271	1.545	2.171	SAM001246921_Testosterone
10	90	2.32	H06	sample	49080	96810	124020	151900	1.371	2.662	2.163	2.483	SAM001246922_DEHYDROEPIANDROSTERONE
10	7	0.11	A07	sample	40380	59850	49950	49360	0.784	0.597	-0.378	-0.61	SAM001247099_SDM25N
10	19	0.46	B07	sample	33080	73230	62730	90640	0.29	1.345	0.06	0.635	SAM001247101_Thiophene,_5-bromo-2-(4-fluorophenyl)-3-[4-(methylsulfonyl)phenyl]-_[CAS]
10	31	-1.87	C07	sample	900	1040	6980	25020	-1.883	-2.69	-1.853	-1.344	SAM001247103_5-Nonyloxytryptamine
10	43	-0.89	D07	sample	5850	21530	53940	96420	-1.549	-1.545	-0.241	0.81	SAM001247096_Salmeterol
10	55	0.36	E07	sample	26880	53180	75710	120670	-0.128	0.224	0.506	1.541	SAM001247097_SB_205607
10	67	1.58	F07	sample	61260	64620	97370	132640	2.194	0.863	1.249	1.902	SAM001247098_R(+)-SCH-23390_hydrochloride
10	79	NA	G07	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	91	NA	H07	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	8	NA	A0	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA

			8										
10	20	NA	B08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	32	NA	C08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	44	NA	D08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	56	NA	E08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	68	NA	F08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	80	NA	G08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	92	NA	H08	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	9	NA	A09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	21	NA	B09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	33	NA	C09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	45	NA	D09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	57	NA	E09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	69	NA	F09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	81	NA	G09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	93	NA	H09	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	10	NA	A10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	22	NA	B10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	34	NA	C10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	46	NA	D10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	58	NA	E10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	70	NA	F10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA

10	82	NA	G10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	94	NA	H10	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	11	NA	A11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	23	NA	B11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	35	NA	C11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	47	NA	D11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	59	NA	E11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	71	NA	F11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	83	NA	G11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	95	NA	H11	empty	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	12	5.22	A12	merbb4-f	98850	277940	77020	258730	4.733	12.784	0.551	5.706	control
10	24	7.24	B12	merbb4-f	128870	346170	105900	325750	6.761	16.597	1.542	7.728	control
10	36	8.11	C12	merbb4-f	126230	304460	138100	388750	6.583	14.266	2.647	9.628	control
10	48	5.56	D12	merbb4-f	89730	295030	90650	301620	4.117	13.739	1.018	7	control
10	60	20.71	E12	bace-1	410460	540880	356460	587950	25.783	27.478	10.14	15.637	control
10	72	13.18	F12	bace-1	231110	662950	333930	490510	13.667	34.299	9.367	12.698	control
10	84	15.28	G12	bace-1	250400	637310	270380	586420	14.971	32.866	7.186	15.591	control
10	96	14.25	H12	bace-1	237500	580070	245360	547100	14.099	29.668	6.327	14.405	control

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15 Eidesstaatliche Versicherung

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Ich erkläre hiermit an Eides statt,

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