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Dicer によるマイクロ RNA 前駆体 (pre-miRNAs) 切断反応に  
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## **A Study of The Effects of Small Molecules on Dicer- mediated Cleavage of Precursor miRNAs (pre-miRNAs)**

Ph.D. Thesis

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## ABSTRACT

MicroRNA (miRNA) is a small (18-22 nucleotides) and evolutionary conserved non-coding RNA that is expressed as a post-transcriptional regulator of living cells and is known to be implicated in various cellular processes and diseases. On the normal condition, miRNA was tightly regulated in the cells, however, the aberrant expression of this type of non-coding RNA would result in cancer, and this type of miRNA is commonly referred to as oncomirs. Inhibition is preferred to overcome this unfavorable overexpression.

A combination screening strategy was used to find a small molecule with the potential to be an inhibitor of miRNA production. This type of screening strategy was a hybrid of target-based screening and cell-based screening. The goal of this combination was to overcome the limitations of target-based screening, which did not take into account the physiological aspects of the living organism, and also cell-based screening, that considered as mechanism-agnostic screening. The combination of both types of screening would increase the relevance of screening results to physiological conditions and make it easier to track the mechanism of action behind the inhibition.

Uterine corpus endometrial carcinoma (UCEC) was chosen to apply those screening concepts due to the lack of alternative drugs available to cure this kind of cancer. Several UCEC-associated miRNAs reported by Favier A., et al. then were used to demonstrate the inhibition of small molecules to the production of UCEC-associated miRNAs (miR-182, miR-31, miR-30d). Nakatani group, on the other hand, developed small molecules with nucleobase recognition sites, such as guanine recognition by N-Acyl-2-amino-7-methyl-1,8-naphthyridine, adenine recognition by 7-methyl-2-oxo-1,8-naphthyridine (azaquinolone), and cytosine recognition by protonated 2-amino-7-methyl-1,8-naphthyridine. These small molecules are designed to specifically bind the complementary nucleobase on the nucleic acids. If those small molecules can bind to miRNA precursors, they may inhibit oncogenic miRNA production.

The first chapter of this dissertation described the framework and background of the research. In chapter 2, the use of Real-time PCR (qPCR) to determine the kinetical properties of an *in-vitro* Dicer reaction was investigated. However, due to a number of challenges, the results of this experiment did not meet the expected results.

In chapter 3, We demonstrated a multistep qPCR-based screening of an in-house chemical library that targets UCEC-associated miRNA production using the screening strategy that

combined the target-based screening and cell-based screening assay. *In-vitro* Dicer-mediated processing of pre-miR-182, pre-miR-31, and pre-miR-30d was used for the initial screening. The first screening yielded 48 different compounds with significant inhibition effects on miRNA production. The inhibitory effect of the identified compounds on the biogenesis of the miRNA targets on the cells was then investigated. We discovered eight hit compounds with potential inhibitory effects on pre-miR-182 and pre-miR-31 processing in vitro and in cells. Furthermore, the interaction of eight compounds with pre-miRNAs was studied using the Surface Plasmon Resonance (SPR) assay and gel analysis. Among eight hit compounds, only 2 compounds showed favorable binding to pre-miRNAs. This suggested two possible ways of inhibition may occur, first, the compounds bind to the pre-miRNA and interfere with the Dicer-mediated cleavage processing, or the compounds directly interact with Dicer and affected the cleavage processing.

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## Abbreviation

AGO I	Argonaute RISC Component 1
ATP	Adenosine Triphosphate
BSA	Bovine Serum Albumin
CCK-8	Cell-counting kit 8
cDNA	complementary DNA
CT value	Cycle Threshold Value
DANP	N,N'-bis(3-aminopropyl)-2,7-diamino-1,8-naphthyridine
dNTP	deoxynucleoside triphosphate
EDTA	Ethylenediaminetetraacetic acid
FDA	Food and Drug Administration (agency within the United States Department of Health and Human Services)
IC <sub>50</sub>	Inhibition Concentration- 50
K <sub>m</sub>	Michaelis Constant
MBLs	Mismatch Binding Ligands
miR-X	microRNA-X
miRNA	microRNA
MOA	Mechanism of Action
NA	Naphtiridine Azaquinolone
ND	Naphtiridine Dimer
PCR	Polymerase Chain Reaction
pre-miRNA	precursor microRNA
qPCR	quantitative / Real-time Polymerase Chain Reaction
RISC	RNA-Induced Silencing Complex
RNA	Ribonucleic Acids
RNA Pol II	RNA Polymerase II
RT	Reverse Transcription
RT-qPCR	Reverse Transcription - quantitative / Real-time Polymerase Chain Reaction
SA	Streptavidin
SPR	Surface Plasmon Resonance
TCGA	The Cancer Genome Atlas
TRBP	Transactivation Response element RNA-binding Protein
UCEC	Uterine Corpus Endometrial Carcinoma
V <sub>max</sub>	Maximum rate of reaction
WST	Water-soluble Tetrazolium

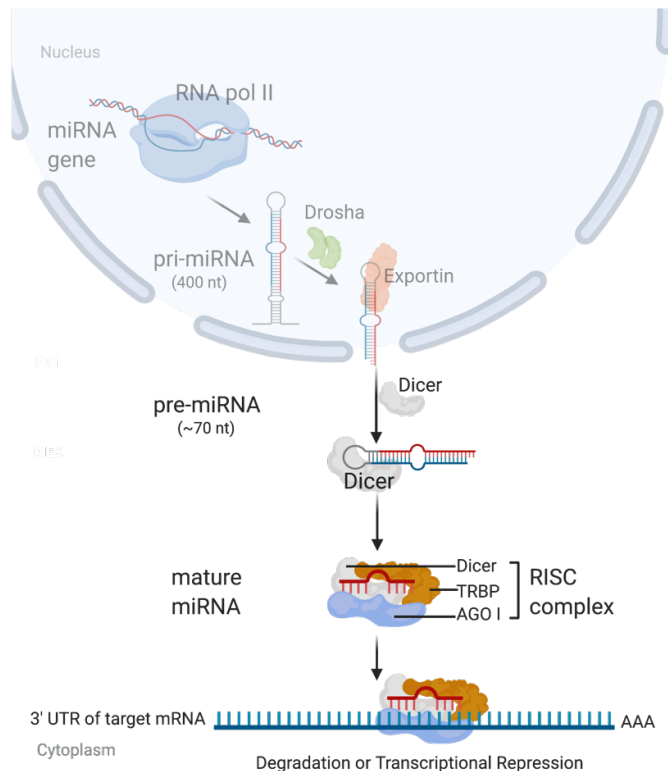


# CHAPTER I

## General Introduction

### 1.1 MicroRNA (miRNA) as a Post-transcriptional Gene Regulator and Cancer Therapeutics

miRNAs are a class of evolutionary conserved small non-coding RNA with 18-22 nucleotides in length, which regulate gene expression through translational repression and mRNA degradation. (1,2) The biogenesis of miRNAs as illustrated in Figure 1.1, begins with the transcription of the miRNA gene in the nucleus by RNA Pol II, and the primary miRNAs (pri-miRNAs) are capped, spliced, and polyadenylated. RNase III-type enzyme Drosha cleaves long pri-miRNAs into precursor miRNAs (pre-miRNAs), and the resulting pre-miRNAs are exported from the nucleus to the cytoplasm by Exportin. Another RNase III-type enzyme Dicer then processes the pre-miRNA to produce mature miRNA. The RNA-induced silencing complex (RISC), which includes TRBP and AGO 1, complements target mRNAs for post-transcriptional gene silencing. (3)



**Figure 1.1** microRNA Biogenesis

The expression of miRNA is tightly regulated in normal cells, but dysregulation of miRNA expression has been observed in many types of cancer (oncogenic miRNA / oncomirs). The dysregulation of miRNA expression can cause some types of cancer including breast cancer, pancreatic cancer, prostate cancer, and lung cancer. (3-8) Several strategies have been developed to treat these cancers, by targeting the oncogenic miRNAs.

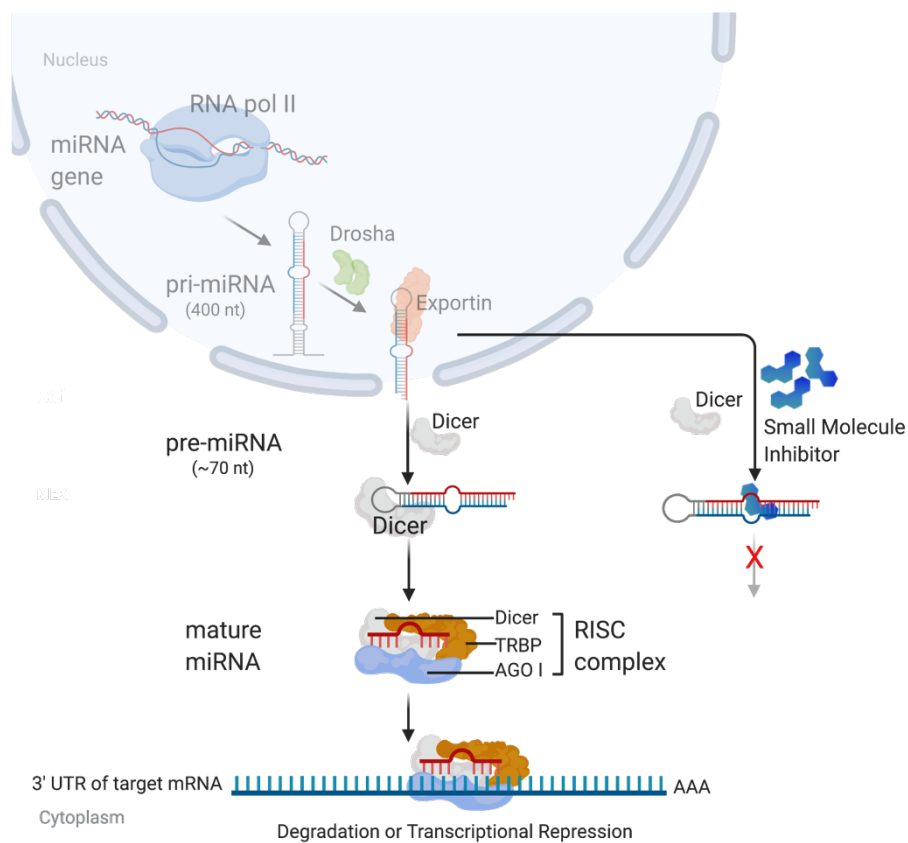
Krutzfeldt et al. developed antisense oligonucleotides (antagomirs), which are miRNA inhibitors that work by annealing to the mature miRNA's guide strand and inducing degradation or stoichiometric duplex formation, to block the overexpressed oncomirs. Their research focuses on improving the stability and specificity of antagomirs by adopting 2'-OMe-modified nucleotides with a phosphorothioate linkage that complements miR-122. (9,10) The antagomirs against miR-122 (antagomir-122) were administered to mice, and could decrease endogenous miR-122 level to undetectable level as long as 23 days post-injection. (11,12) Another strategy for inhibiting oncomir overexpression is genome editing using CRISPR/Cas9. Zhao et al used this strategy to downregulate miR-17-92 cluster and miR-21 by in vitro experiment. (13) Other researchers applied Cas9 mRNA and guide RNA into zebrafish embryos, as the result they reported chromosomal deletions and inversions. (14)

These strategies reported previously are shown to have adverse effects. Galbraith W.M., et al. reported that after administering antagomirs to monkeys, peripheral white blood cell counts decreased. Another issue raised by the use of antisense oligonucleotides is that these miRNA inhibitors may be unable to distinguish between members of the same family of miRNA. In contrast to the antisense oligonucleotide strategy, CRISPR/Cas9 gene-editing results in permanent gene modifications. However, the off-target effects have not been precisely profiled, this genome editing method must be improved further before being used as a substitute for cancer/disease therapy. (15,16,17)

Due to the challenges that come with those miRNA inhibitors, it may be useful to develop another strategy for inhibiting miRNA functions. Numerous strategies could be used to discover a novel method of inhibiting oncomirs overexpression. It is not necessary to develop inhibitors from scratch, rather, it is possible to repurpose currently available small molecules/drugs for miRNA inhibition by screening for the most suitable inhibitor candidates while also obtaining a drug candidate.

Target-based screening or biochemical assay is one of the strategies that are usually used if the target was identified. Coussens N.P. et al. describe this type of assay as having a variety of schemes, for example, to detect the binding between a small molecule and the target can be observed directly using fluorogenic products, or it can be coupled to a reporter that facilitates detection, such as luciferases. This biochemical assay is frequently used in combination with another analytical instrument, such as mass spectrometry, to quantify reaction products. (18-20)

Another screening approach is phenotypic assay. The advantage of target-based screening or biochemical assays is that they are more direct methods for quantifying or analyzing the effect of a small molecules on a target of interest, however, these strategies do not always reflect the biochemical processes in complex cellular environments. It is common for drug candidates to appear potent when tested in biochemical assays but fail to work in the cellular environment. Phenotypic assays cover a wide range of assessment techniques, from the simplest such as cell death or growth arrest to the use of animal models. The limitation of this type of assay is that it is mechanism-agnostic, which means that it cannot be used to determine the mechanism of action. (21-24)

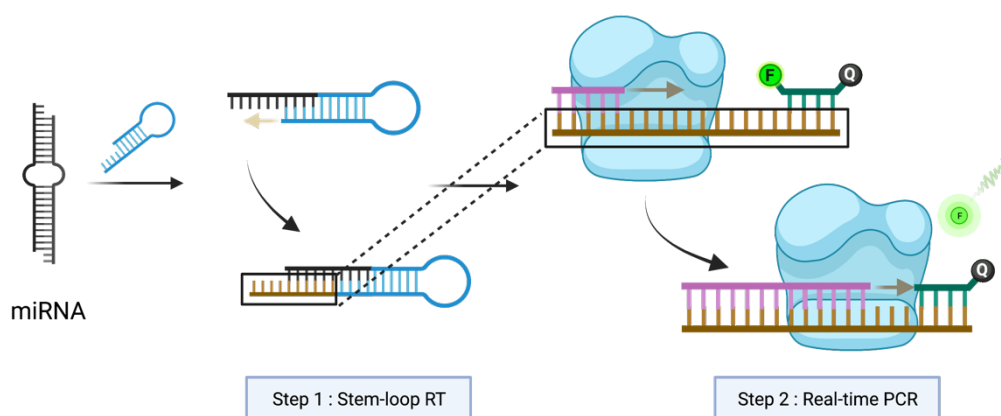


**Figure 1.2** Dicer-mediated pre-miRNA maturation inhibition pathway

With the desire to find a new inhibitor of disease-associated miRNAs, the middle point of those two screening strategies would be preferred. We have attempted to target the biogenesis process of miRNAs by a small molecule (Figure 1.2), to inhibit the disease-associated miRNA. From the miRNA biogenesis process, we chose Dicer-mediated cleavage of pre-miRNA for a target-based screening. We also adopted a cell-based assay to see the effect of small-molecule on endogenous miRNA production. The adoption of a mechanism-specific assay, such as an in-vitro Dicer cleavage reaction, would resolve the phenotypic assay's mechanism-agnostic problem. On the other hand, adopting a cell-based assay would more reflect the biochemical processes in complex cellular environments. (25)

Applying those concepts to tackling the disease, we choose Uterine Corpus Endometrial Carcinoma (UCEC) as a cancer case model. UCEC is a malignant tumor of the female reproductive system that develops from the uterus's inner lining cells and is a major threat to women's health worldwide. (26,27) Additionally, UCEC accounts for approximately 20%-30% of female reproductive system cancers, ranking second only to cervical cancer. (28) Only a few drugs have been approved by the FDA for the treatment of this type of cancer. These drugs includes Pembrolizumab (Keytruda), Dostarlimab-gxly (Jemperly), Lenvatinib (Lenvima), and Megastrol (Megace). Due to the limited alternatives of available drugs, the discovery of novel drug candidates holds great promise for risk management and personalized therapeutic approaches for UCEC. (29)

A study by Favier A. et al. demonstrated a significant increase in the level of several miRNA expressions associated with this type of carcinoma, including miR-182, miR-31, and miR-30d. Similarly, the Cancer Genome Atlas (TCGA) revealed similar findings. In this study, we selected those three miRNAs as a model for implementing the combination of target-based and cell-based assay as described previously. (30)

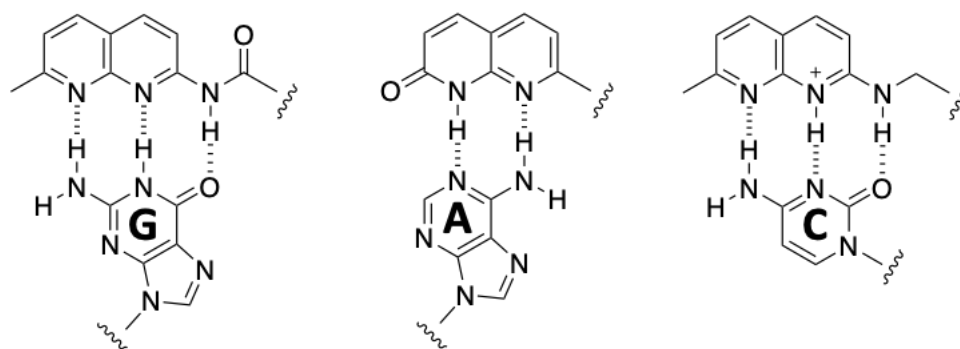


**Figure 1.3** Stem-loop Reverse Transcription Real-Time PCR (RT-qPCR) Scheme

To evaluate miRNA production during the Dicer-mediated cleavage reaction, as well as miRNA expression during the cell-based assay, we used real-time PCR as the main instrument due to its fast and high-throughput detection and quantification of target nucleic acids sequences. (31) Due to specificity and sensitivity to detect and amplify the mature miRNA during reverse transcription (RT) reaction, stem-loop RT primer was used as illustrates in Figure 1.3. (32)

## 1.2 Sequence-Specific Small Molecules

Nakatani group has developed a number of sequence-specific DNA mismatch binding ligands (MBLs) that are capable of recognizing and binding to bulged sites and mismatched base pairs in duplex DNA (Figure 1.4). The developed molecules work by occupying the spaces and forming hydrogen bonds to the mismatched nucleotide bases. These ligands are then stabilized through stacking interactions with adjacent base pairs. (33)



**Figure 1.4** Nucleobase recognition parts. (a) Guanine recognition by *N*-Acyl-2-amino-7-methyl-1,8-naphthyridine, (b) Adenine recognition by 7-methyl-2-oxo-1,8-naphthyridine (azaquinolone), (c) Cytosine recognition by protonated 2-amino-7-methyl-1,8-naphthyridine

Naphthyridine Dimer (ND) is a dimeric form of *N*-acyl-2-amino-1,8-naphthyridine, which was the first compound designed to recognize G-G mismatches. From a structural perspective, this molecule consists of two naphthyridine heterocycles connected by a linker. Two naphthyridine form hydrogen bonding with the guanine, and the linker that contained a secondary amino group offers the appropriate conformational restriction to the dynamic motion of two heterocycles besides providing attractive electrostatic interaction to negatively charged DNA and gained water solubility. (34-36)

8-azaquinolone, which is a complement to adenine in terms of its hydrogen bonding surface was utilized to develop another molecule that could recognize G-A mismatches, naphthyridine-azaquinolone (NA). In another case, a hydrogen bonding rearrangement of the donor-donor-acceptor groups of cytosine was required to develop C-C mismatch recognition, which was achieved through the development of a protonated form of 2-amino-1,8-naphthyridine. This molecule meets the requirement that needed, and it is also reported that 2-amino-1,8-naphthyridine is strongly and selectively bound to C-C mismatch. (37-40)

Due to the specific nucleobase recognition properties of the small molecules synthesized and developed by Nakatani group, it is highly likely that those small molecules will bind to pre-miRNAs that has the unpaired nucleotides in their structure, which may affect the miRNA maturation process. If small molecules could inhibit UCEC-associated oncogenic miRNA production *in vitro* and also in cells, then those small molecules have the potential to be drug candidates.

### **I.3 Objectives**

The objective of this research is to analyze the effect of the small molecule on the Dicer-mediated cleavage of pre-miRNA *in vitro* and in cells, seeking for potential small-molecule candidates for treating UCEC that are associated with miRNA dysregulation. To achieve that objective, we demonstrated a combination of assays between target-based assay and cell-based assay to screen the small molecules that shows the potential inhibitory activity to mature miRNA production.

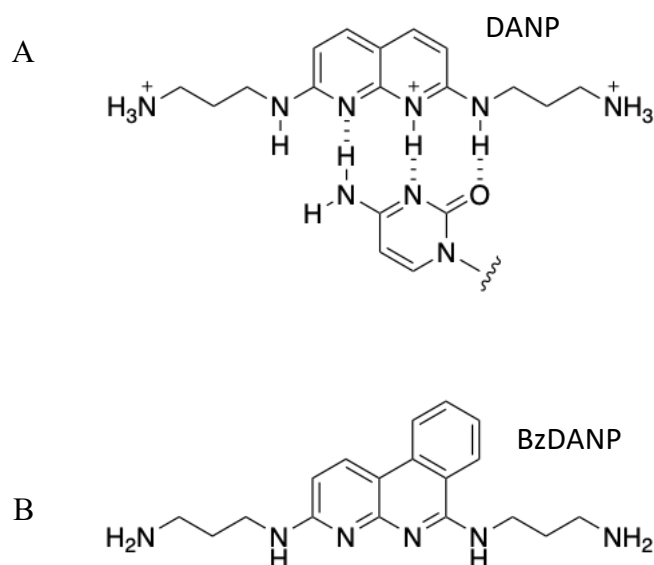
Aside from that, the results and the screening methods from this research could be used for consideration in the development of a high throughput screening system of the small molecules that targeting UCEC-associated miRNA.

## CHAPTER II

### Utilization of Real-Time PCR to determine Kinetical Properties of In-vitro Dicer reaction

#### 2.1 Introduction

A small molecule that binds to a specific pre-miRNA and inhibits or accelerates its processing can be a tool for modulating miRNA-mediated gene regulation. It was previously reported that by protonating the nitrogen in the 2,7-diamino-1,8-naphthyridine chromophore, N,N'-bis(3-aminopropyl)-2,7-diamino-1,8-naphthyridine (DANP) could form hydrogen bonds with cytosine as shown in Figure 2.1 A. (39) This compound, however, has a limitation in that it does not bind to RNA. To address this issue, this compound was modified by the addition of an expanded ring system (BzDANP) (Figure 2.1 B), which may improve stacking interaction with neighboring base pairs in RNA. (40)



**Figure 2.1** DANP could form hydrogen bonding with cytosine (A) and BzDANP (B)

Further studies of BzDANP by Otabe, et.al shows that BzDANP reduced the initial velocities of the pre-miR-136 Dicer cleavage reaction, resulting in a significant decrease in  $V_{max}$ .(41) BzDANP has the potential to be used in other pre-miRNAs, but one of the major



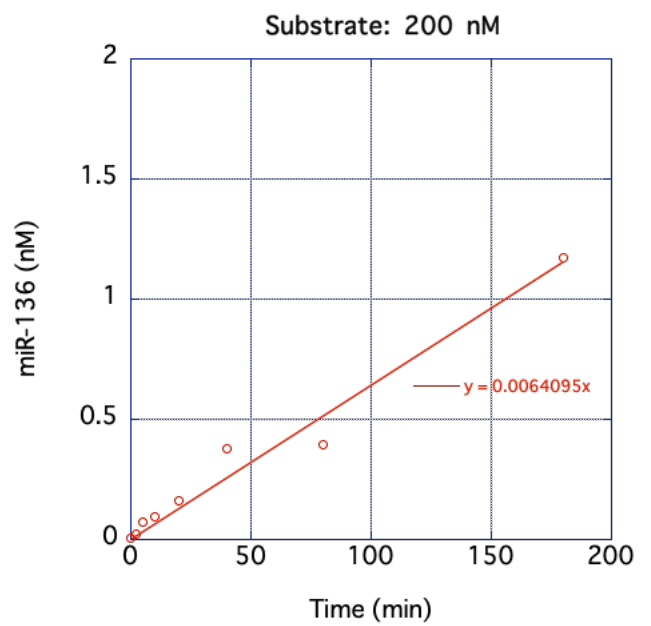
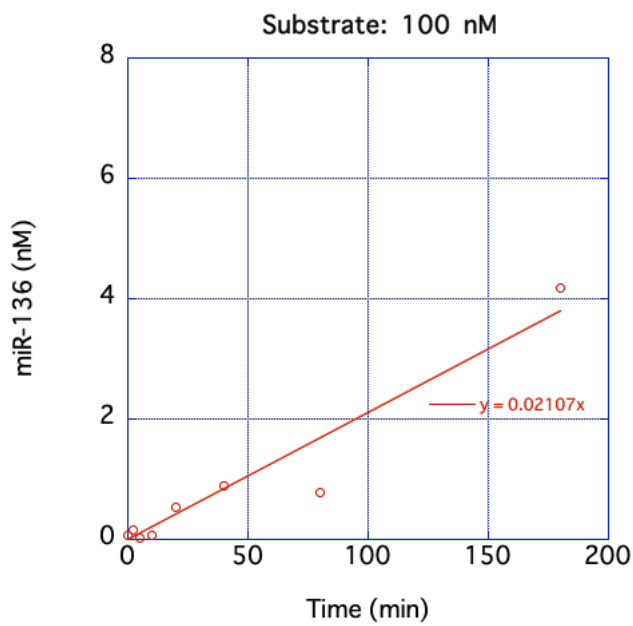
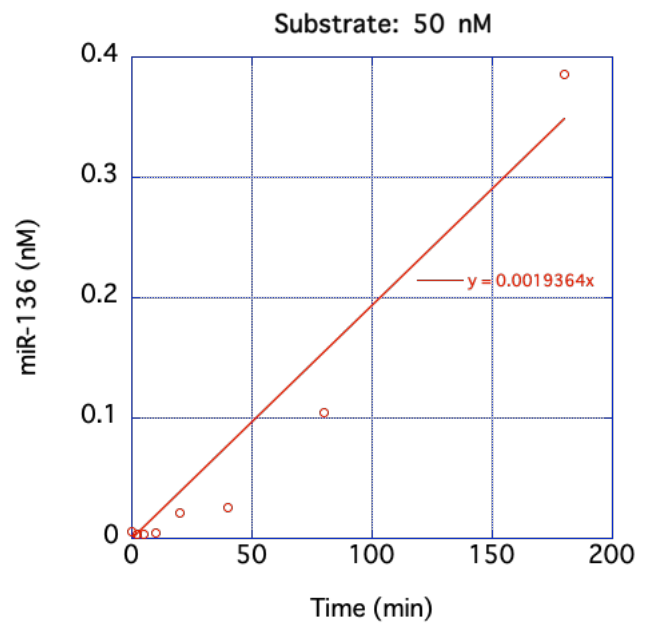
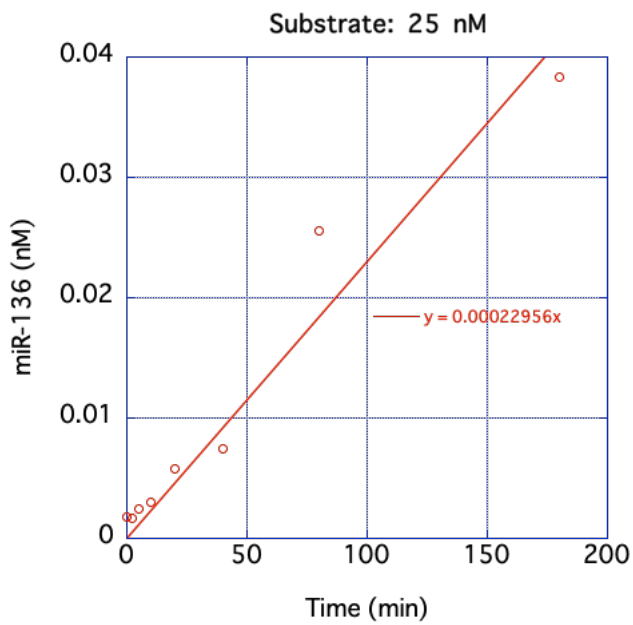
challenges is determining the kinetical properties of this compound in Dicer-mediated cleavage reactions in a more direct and convenient way than isotope labeling. One option is to directly detect the product, which is miRNA, which can be done with real-time PCR. Here we conducted an experiment to determine the kinetical properties by using real-time PCR.

## 2.2 Results and Discussions

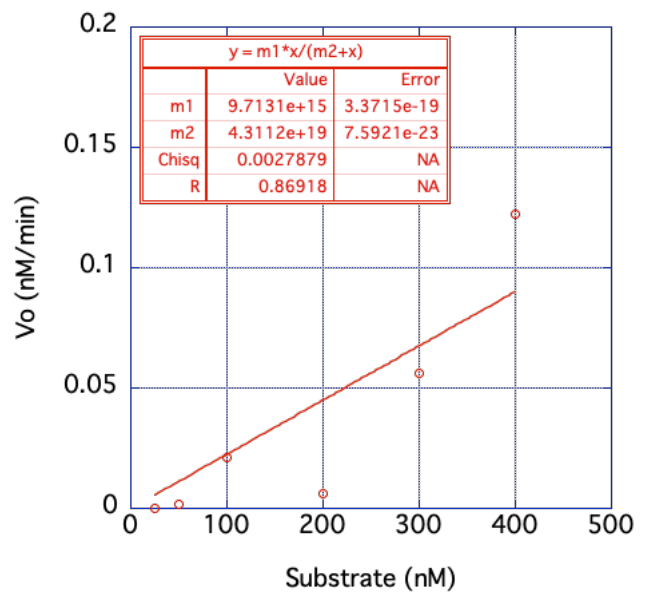
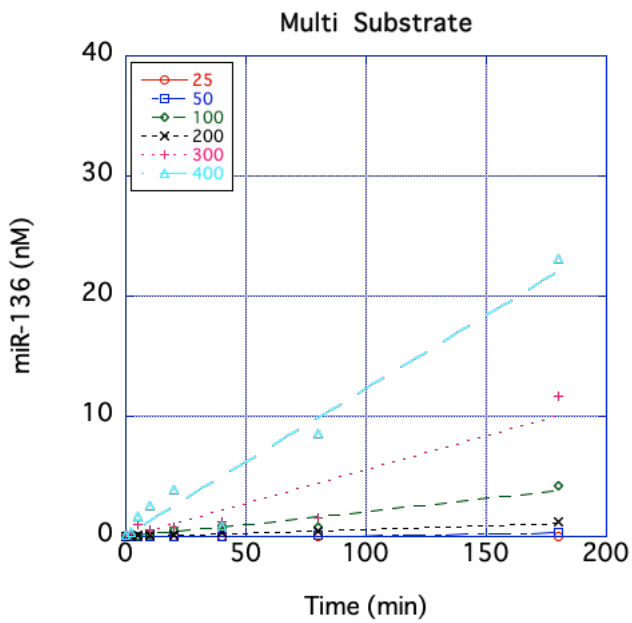
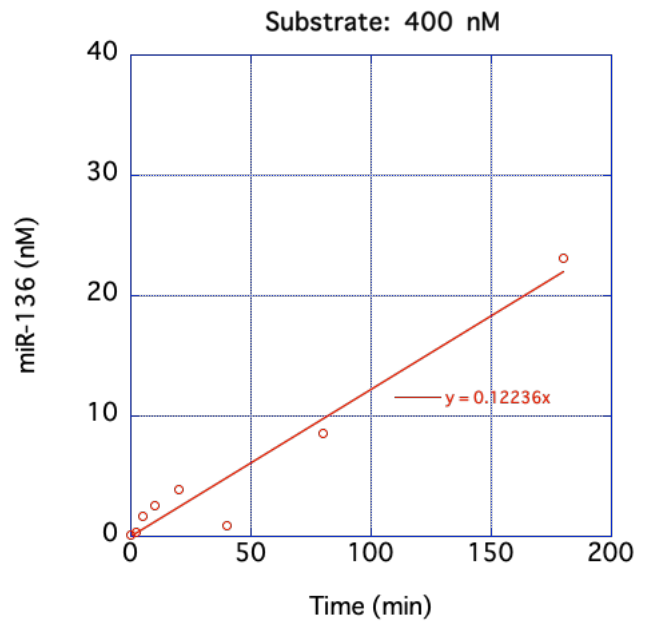
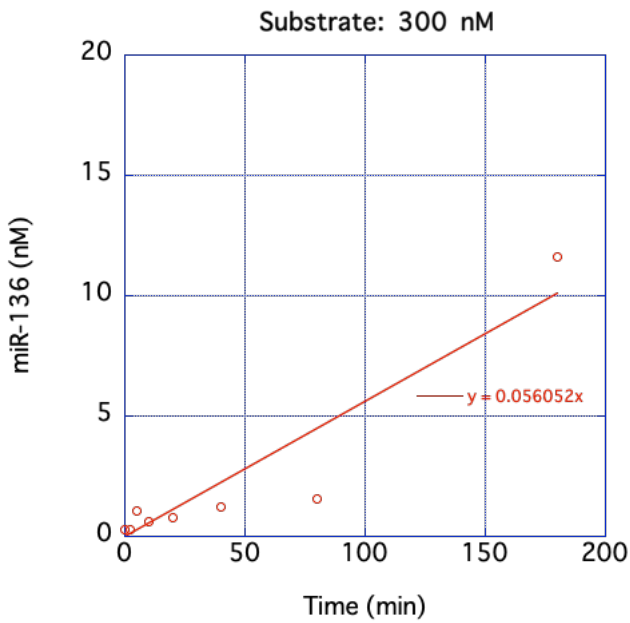
To determine the kinetical properties of *in vitro* dicer reaction, several different concentrations of substrates (pre-miR-136) were used, ranging from 25 nM to 400 nM, as well as different incubation times at the *in vitro* dicer reaction. The aliquots of the reaction solution separated after 2.5, 5, 10, 20, 40, 80, and 180 minutes incubation. Figure 2.2 and 2.3 shows the correlation between concentration miR-136 product and time for every variation concentration of substrate.

Table 2.1 shows every initial velocity from various substrate concentrations. The initial velocity then was plotted against substrate concentrations and the obtained plots were analyzed by least-squares fitting to the Michaelis-Menten equation. The Michaelis-Menten analysis for the *in vitro* dicer reaction of pre-miR-136 yielded a  $K_m$  of  $9.71 \times 10^{15}$  nM, and  $V_{max}$   $4.31 \times 10^{19}$  nM  $\text{min}^{-1}$ . The results obtained here are far different compared to the previous study by Otabe, et.al, in which they obtained  $K_m$  of  $52.6 \pm 8.6$  nM and  $V_{max}$  of  $1.52 \pm 0.08$  nM  $\text{min}^{-1}$ .

The differences between these two result raised from the different methods that used. It is important to note that using qPCR as an instrument to measure the kinetical aspect especially in this *in-vitro* dicer reaction is still needs to be improved. Several critical points often occur during the experiments, the technical handling problem quite often becomes a problem due to multi-step procedures, from the sampling, preparation for reverse transcription, and the preparation for real-time PCR, especially for the standard curve technique. This makes the results are often difficult to reproduce in the same quality. Unfortunately, because of the limitation of time to overcome this problem, it is difficult to continue this experiment.



**Figure 2.2** Velocity plot of *in vitro* Dicer reaction by several different substrate concentration



**Figure 2.3** Velocity plot of *in vitro* Dicer reaction by several different substrate concentrations

**Table 2.1** Initial Velocity of several different substrate concentrations

pre-miR-136 (nM)	25	50	100	200	300	400
Vo (nM/min)	0.0002	0.0019	0.0211	0.0064	0.0561	0.1224

## 2.3 Conclusion

We have demonstrated the utilization of real-time PCR to investigate the kinetical properties of the *in vitro* dicer reaction. Compared to the previous study conducted by Otabe, et.al, we obtain significantly different results. As mentioned in the results and discussion section, the methods for utilizing real-time PCR to investigate the kinetic properties of enzymatic reactions particularly those used in the *in vitro* dicer reaction still require improvement. It would be preferable to reduce the systematical error as possible, for example by using one-step RT-qPCR reagents, or digital droplet PCR which eliminates the need to create a standard curve.

## 2.4 Methods

### 2.4.1 Time-dependent Dicer Cleavage Reaction

In the presence of a buffer reaction, phosphorylated precursor microRNA (25 nM), 50 mM MgCl<sub>2</sub>, 10 mM ATP, 0.05 percent BSA, and Dicer (50 nM) were mixed. 6 different variation concentration (25, 50, 100, 200, 300, 400 nM) of BzDANP was then added, with RNase-free water serving as a negative control. After that, the mixture was incubated at 37°C for several variation time (0, 2.5, 5, 10, 20, 40, 80, 180 minutes). The reaction was then halted by adding 10 mM EDTA.

### 2.4.2 Reverse Transcription Real-time PCR (RT-qPCR)

The 100 mM dNTP Mix, the Multiscribe Reverse Transcriptase, and the 5X Reverse Transcription primer were combined and incubated according to the manufacturer's protocol. After that, the mixture was mixed with the Taqman Master Mix. This mixture was then loaded to the real-time PCR instrument, and the resulting data were analyzed using the real-time PCR instrument's software.



## **CHAPTER III**

# **Study of Mechanism of Action (MOA)-based Screening Assay of Small Molecules Targeting Uterine Corpus Endometrial Carcinoma (UCEC)-associated microRNA**

### **3.1 Introduction**

UCEC is a malignant tumor of the female reproductive system that develops from the cells of the uterus's inner lining. It is a major threat to women's health globally. (26,27) Additionally, UCEC accounts for approximately 20%-30% of female reproductive system cancers, trailing only cervical cancer in this category. (28)

Because the available drugs to treat this type of cancer are still limited, it would be preferable to discover a new drug to treat this type of cancer. From a mechanism of action standpoint, the majority of drugs used to treat UCEC were either PD-1 binders or kinase inhibitors. For this reason, an alternative to searching for a new drug is to identify a potential pathway associated with this cancer that could be used as a starting point for drug discovery.

In this chapter, the screening of small molecules to find the UCEC drug candidates was demonstrated. All of the small molecules that used in this screening shows in Figure 3.1. MicroRNA biogenesis was adopted into the screening system to mimic the Dicer-mediated pre-miRNAs maturation process. This process is conducted in-vitro as previously mentioned as target-based screening. (18,19) The compounds obtained from this screening then continued to the in-cell screening, to know the effect of the small molecules in the living cells environment.

Further analysis was then performed on the small molecules that may possess the potential oncogenic miRNA inhibitors, such as SPR analysis to analyze the small molecule's binding affinity to the pre-miRNA and its viability, which measures the effect such as IC<sub>50</sub>.

2A (2B_N131002)	3A (8D_N131052)	4A (5B_N132028)	5A (2B_N140022)AK041	6A (7E_N140465- Azacylidine)	7A (3C_N160011)DDAP	8A (7H_N160048) AK248	9A (7A_N14241)K106	10A (8H_N14256)K305
2B (2H_N131008)	3B (9H_N131064)	4B (5C_N132027)	5B (3H_N14016)AK112	6B (9A_N14049)P0003	7B (4B_N160018)Am-B2N	8B (8D_N160052) h 039	9B (7B_N14242)K249	10B (9A_N14257)K280
2C (4B_N131018)	3C (10B_N131066)	4C (6F_N132038)	5C (4B_N14018)SA029	6C (8E_N14054)B2DANP	7C (4C_N160019)Am- B2ND	8C (9A_N160057) bAla- (Nap)	9C (7C_N14243)B2NA	10C (9B_N14258)K188
2D (4G_N131023)	3D (11H_N131080)	4D (8H_N132056)	5D (4H_N14024)SM-221	6D (10E_N14069)NC16	7D (4G_N160023)CMBL4	8D	9D (7D_N14244)K253	10D (9F_N14262)K289
2E (6A_N131033)	3E (2A_N132001)	4E (9F_N132082)	5E (5C_N14027)YM011	6E (2B_N160002) 5H-4Q	7E (5A_N160025) CMBL5a	8E	9E (7E_N14245)K107	10E (9H_N14264)K372
2F (6B_N131034)	3F (2C_N132003)	4F (11E_N132077)	5F (5E_N14029)NN146	6F (2D_N160004)PGA- urea	7F (5H_N160032)NCD-C4- SH	8F	9F (7G_N14247)K224	10F (10A_N14265)K380
2G (6G_N131039)	3G (4E_N132021)	4G (1F_N132078)	5G (5G_N14031)NN246	6G (2G_N160007)PGA- C3-amide	7G (6C_N160035)NCD-C3- NH2	8G	9G (8A_N14249)K238	10G (10B_N14268)K344
2H (7C_N131043)	3H (4H_N132024)	4H (11H_N132080)	5H (6G_N14039)Thioflavin	6H (3B_N160010)PGA- urea	7H (7E_N160046)AK216	8H	9H (8B_N14250)K223	10H (10D_N14269)K358

Figure 3.1 67 Library compounds from Nakatani Laboratory group

## 3.2 Results and Discussions

### 3.2.1 *In Vitro* Dicer Reaction Screening

To determine the effects of small molecules in the dicer processing reaction, we performed *in vitro* dicer reaction by using pre-miR-182, pre-miR-31, and pre-miR-30d as the miRNA targets and 67 selected small molecules from the Nakatani laboratory library as tested compounds. The products of this reaction were either promoted or inhibited mature miRNA. However, to measure the product we need to perform a reverse transcription reaction to transcribe miRNA products to cDNA. This cDNA was then quantified using qPCR.

Figure 3.2 , 3.3, and 3.4 shows the qPCR results of three different miRNAs, each of which was replicated twice. The  $2^{-\Delta CT}$  expressions were used to evaluate the amount of cDNA that is directly proportional to the amount of miRNA products affected by each tested compound. For every compound that shows the  $2^{-\Delta CT}$  value greater than 1, this indicates that the compound promoted miRNA production, on the other hand, any compound with  $2^{-\Delta CT}$  value less than 1 inhibits miRNA production during the dicer processing reaction. To screen the compounds that would be used in the next step of screening, we introduced a  $2^{-\Delta CT}$  value of 0.5 as a threshold which is analogous to  $IC_{50}$  which indicates that certain compounds inhibited 50% of miRNA production. In this case, a lower  $2^{-\Delta CT}$  value indicates greater inhibition which is preferable. Aside from  $2^{-\Delta CT}$  expressions, the replication was also considered when selecting compounds for the next step of the screening, only compounds that passed the threshold twice were chosen for the next screening.

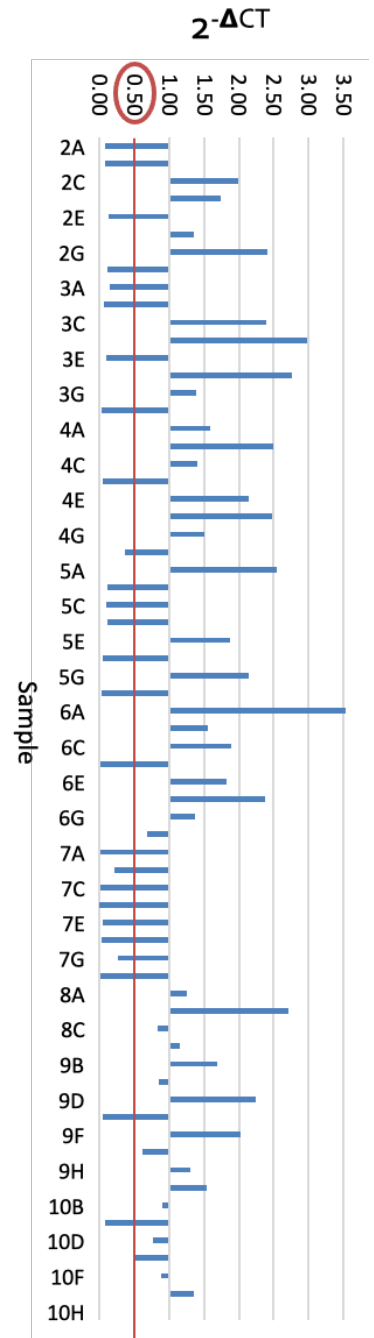
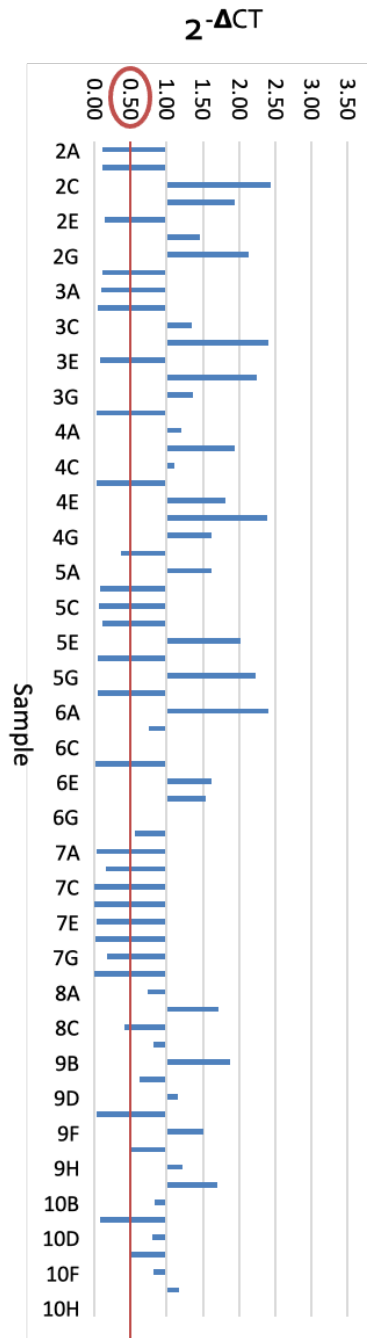
According to the criteria described above, Figure 3.2 demonstrates that 26 compounds pass the 0.5 threshold and also meet the replication criteria, indicating that those 26 compounds may exhibit inhibitory activity against the dicer-mediated pre-miR-182 processing reaction. The inhibition of pre-miR-31 and pre-miR-30d processing reaction shows in Figure 3.3 and 3.4, there are 31 and 22 different compounds that have shown potential inhibitory activity to the pre-miR-31 and pre-miR-30d.

The *in vitro* dicer reaction result was summarized in Figure 3.5, which shows 48 different small molecules inhibiting three different dicer-mediated pre-miRNA processing. All of it could be divided into three categories: compounds that appear to preferentially inhibit



one type of miRNA, compounds that appear to preferentially inhibit two types of miRNAs, and compounds that appear to preferentially inhibit all three types of miRNAs.

Following that, we performed in-cell screening to investigate the biological significance of those 48 compounds in living cells.



**Figure 3.2** *In vitro* Dicer screening of hsa-miR-182

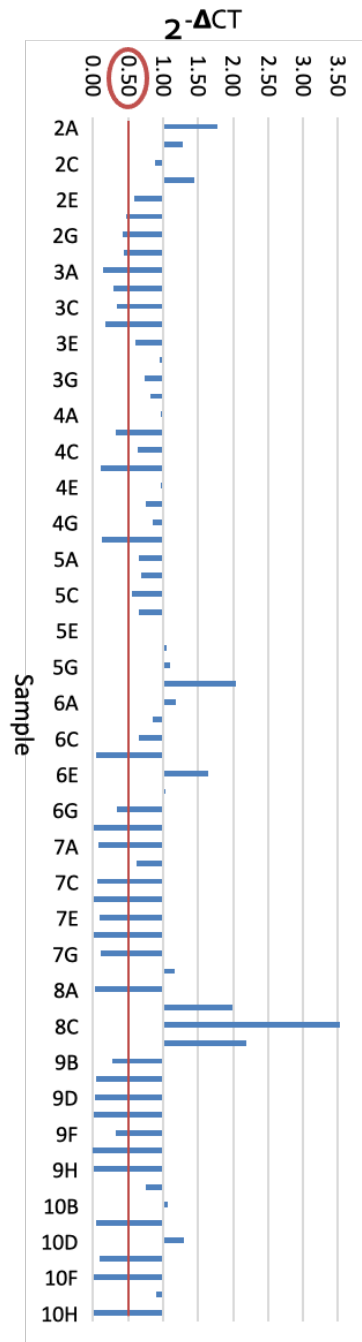
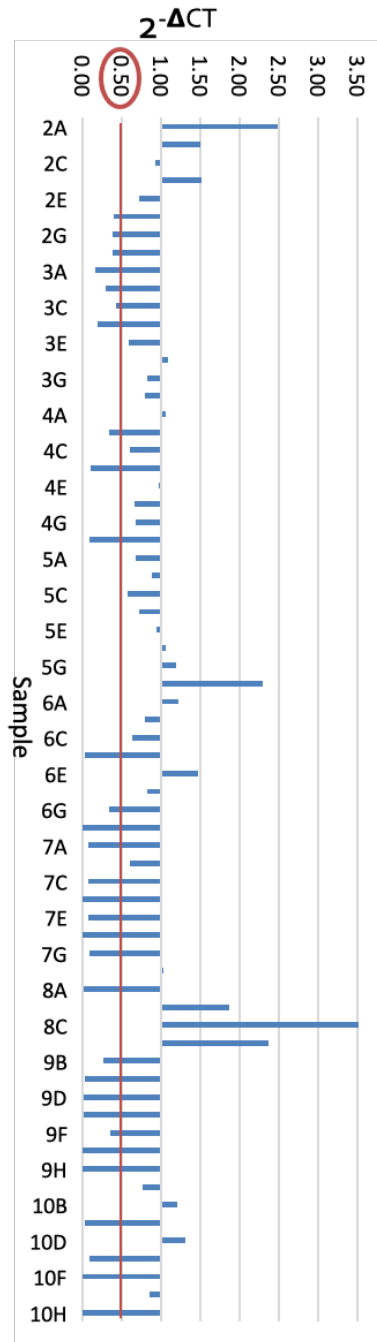


Figure 3.3 *In vitro* Dicer screening of hsa-miR-31

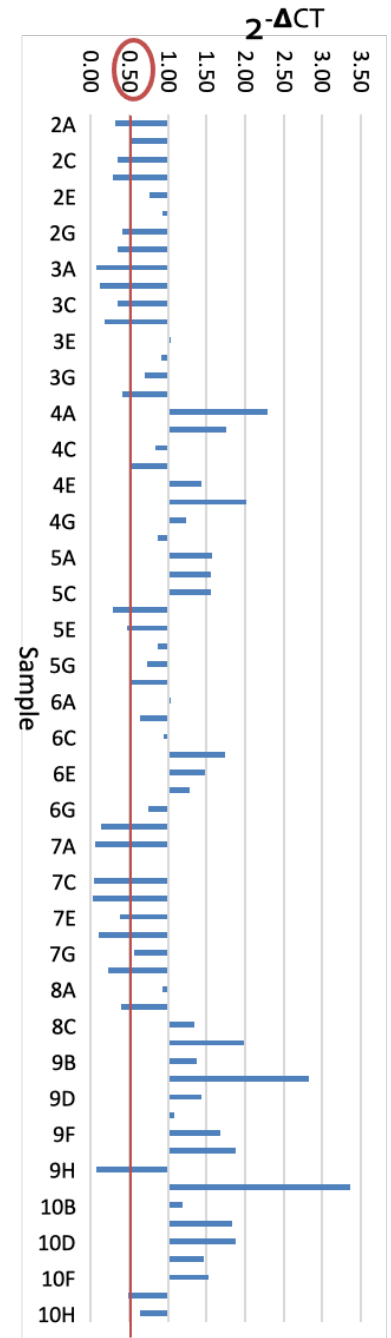
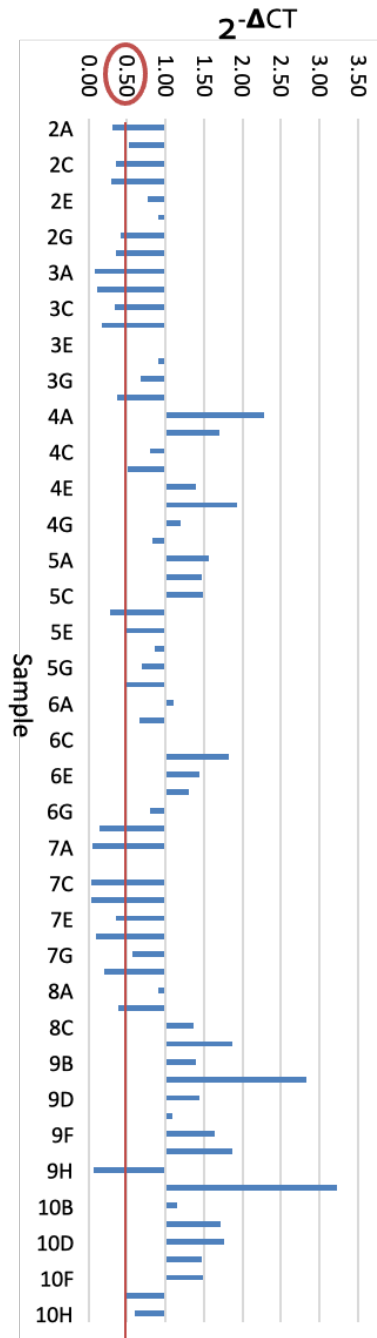


Figure 3.4 *In vitro* Dicer screening of hsa-miR-30d

miR-182	miR-31	miR-30d	miR-182-30d	miR-182-31	miR-30d-31	All
2B 2B (ZL, N131089)	2F 2F (BL, N131034)	2C 2C (BL, N131039)	2A 2A (BL, N131002)	4D 4D (BL, N132050)	2G 2G (BL, N131099)	2H 2H (TC, N131043)
2E (6A, N131033)	4B 4B (CC, N132027)	2D 2D (GL, N131023)	3H 3H (GL, N13204)	4H 4H (HL, N132080)	3C 3C (OL, N131066)	3A 3A (BL, N131052)
3E 3E (TX, N132001)	4G 4G (CC, N160007) POA-C3- amide	5E 5E (CC, N14027) MO-1	5D 5D (GL, N1402) SM-221	6D 6D (OE, N1406) NC-160	3D 3D (TL, N131080)	3B 3B (HL, N131064)
5B 5B (GL, N1401) AK-12	5A 5A (TL, N160048) AK-240	5B 5B (BL, N160052) B-930	7E 7E (TL, N160046) AK-210	7G 7G (CC, N16005) NC-167	6E 6E (BL, N16007) POA-D- NMD	7A 7A (CC, N16001) DM-07
5C 5C (BL, N1401) SMO-039	9B 9B (BL, N1422) R-249	10C 10C (OL, N1426) R-330	9E 9E (TL, N1424) R-107	9F 9F (TL, N1424) R-188	9H 9H (BL, N1425) R-223	7C 7C (CC, N16001) AmB-02
5F 5F (EL, N1402) NN-446	9C 9C (TC, N1424) B-0A0	10B 10B (OL, N1424) R-345	10E 10E (CC, N1424) R-150	10C 10C (BL, N1424) R-184	9I 9I (BL, N1425) R-223	7D 7D (GL, N160023) CM-164
5H 5H (GL, N1403) Th-0A0	9D 9D (TD, N1424) R-233	10F 10F (OL, N1424) R-340	10G 10G (TL, N160052) B-930	10D 10D (CC, N1424) R-188	9J 9J (BL, N1425) R-223	7E 7E (SA, N160023) CM-150
7B 7B (BL, N160013) AmB-01	9F 9F (CC, N1424) R-224	10G 10G (OL, N1426) R-345	10H 10H (TL, N160052) B-930	10E 10E (CC, N1424) R-188	9K 9K (BL, N1425) R-223	7F 7F (SH, N160023) NC-164- S10

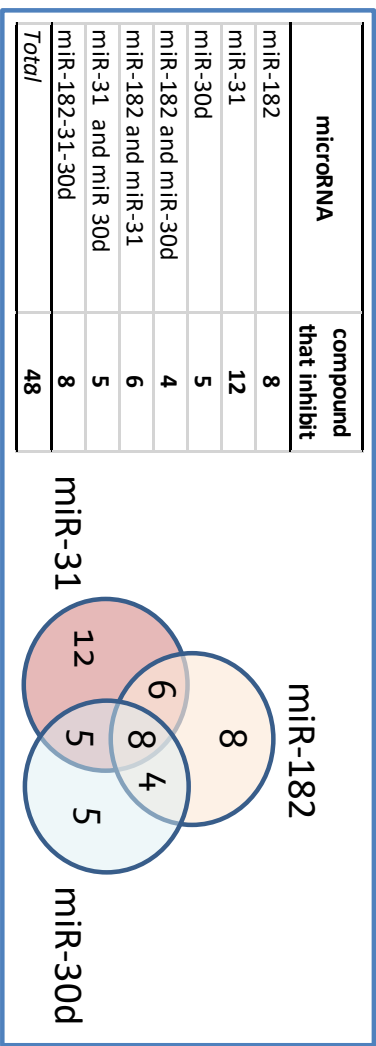


Figure 3.5 In vitro Dicer screening results and its summary

### 3.2.2 Feasibility Assay of HeLa cell-line

In order to confirm the feasibility of HeLa cells as a model organism in this study, we examined the expression of three types of miRNA (hsa-miR-182, hsa-miR-31, and hsa-miR30d) in these cells. The HeLa cells were cultured and lysed using standard technique, and the miRNAs of interest were quantified by applying RT-qPCR. We found that the average CT value of all three types of miRNAs was less than 30 (out of 40) as shown in Table 3.1, implying that the difference in value before and after the addition of the compounds could be easily counted, and we concluded that HeLa cells are viable to use in this study.

CT Value	Replication	microRNA		
		182	31	30d
	I	27.276	20.357	24.583
	II	25.926	20.295	24.928
	III	26.625	20.178	25.307
	<b>Average</b>	<b>26.609</b>	<b>20.277</b>	<b>24.939</b>

**Table 3.1** CT value summary of miR-182, miR-31, and miR-30d in HeLa cells

### 3.2.3 In-Cell Screening

To investigate the effects of previously-screened small molecules on the expression of hsa-miR-182, hsa-miR-31, and hsa-miR-30d in living cells, we added those compounds to the cells and incubated them overnight to reveal their biological effects. After incubation, the cells were lysed, and the expression of miRNAs was quantified using RT-qPCR techniques similar to the *in vitro* dicer reaction.

Figure 3.6, 3.7, and 3.8 shows the expression of hsa-miR-182, hsa-miR-31, and hsa-miR-30d on the triplication of each miRNA, respectively. In contrast to the *in-vitro* dicer reaction, to evaluate the amount of cDNA products, we used  $2^{-\Delta\Delta CT}$  expressions rather than  $2^{-\Delta CT}$  because of the differences in normalization calculation between the two. The CT value of compound-affected miRNA was normalized to the CT value of the untreated product in terms of *in-vitro* dicer reaction. In the cell-based assay, we used two-step normalization, first normalizing the CT value of treated cells to the CT value of untreated cells, then normalizing it again to the internal control gene, in this case, U6 snRNA. Even though the expression used is different, the practical definition of the two is the same. If the  $2^{-\Delta\Delta CT}$  expressions value is less than 1, there is an inhibition effect on miRNA expression and vice versa.

To determine which compounds exhibit potential inhibitory activity, we set the  $2^{-\Delta\Delta CT}$  expressions value of 0.5 as a threshold, and compounds with  $2^{-\Delta\Delta CT}$  values less than the threshold have significant inhibition effects. Similar to the screening criteria in the previous *in vitro* dicer reaction screening, we considered replication in addition to the  $2^{-\Delta\Delta CT}$  expressions value. If a compound showed the  $2^{-\Delta\Delta CT}$  expressions value less than the threshold more than or equal to two times replication, we considered this compound as a potential inhibitor.

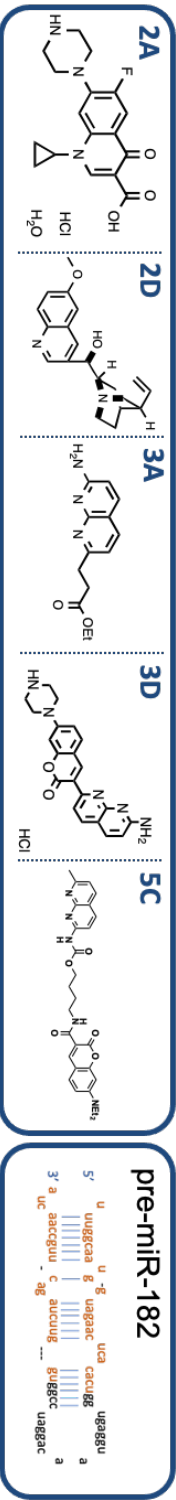
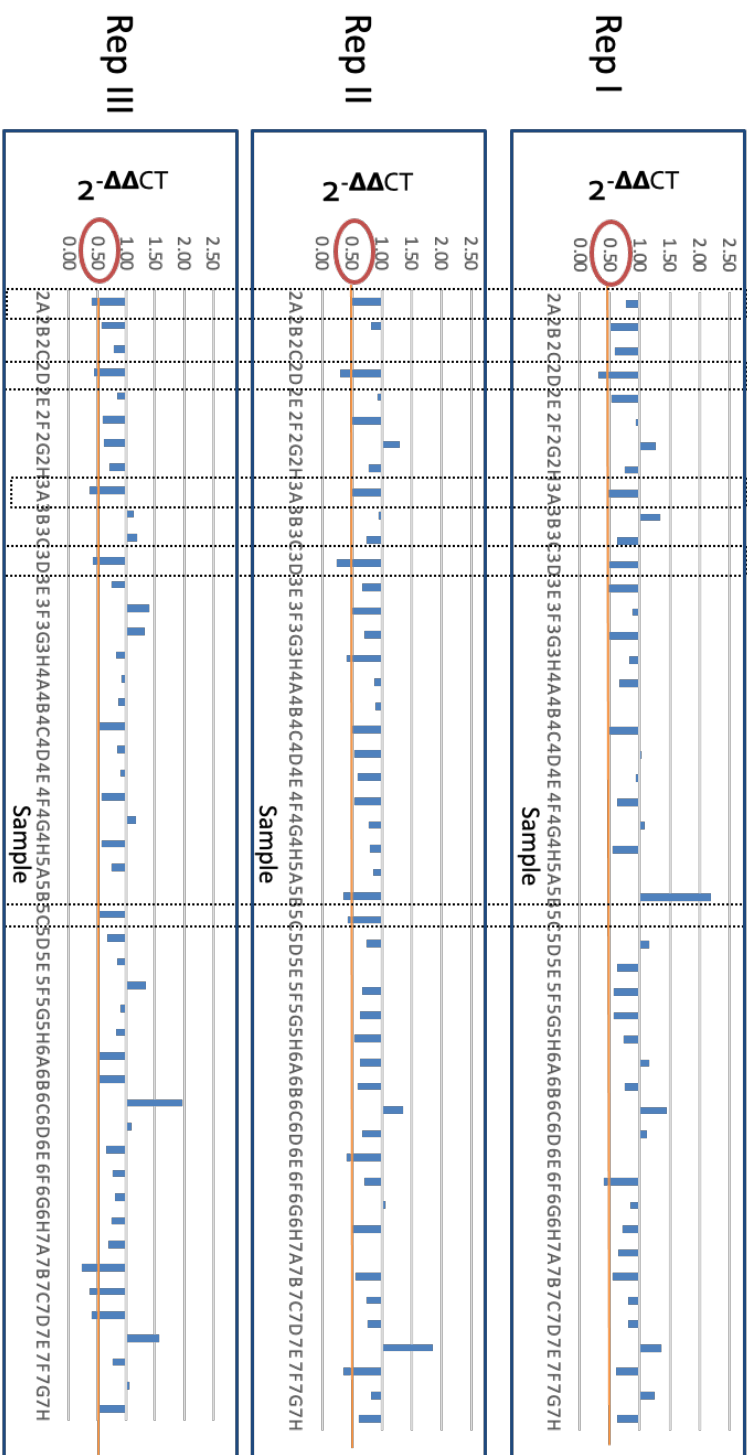


Figure 3.6 *In-Cell* screening results of hsa-miR-182



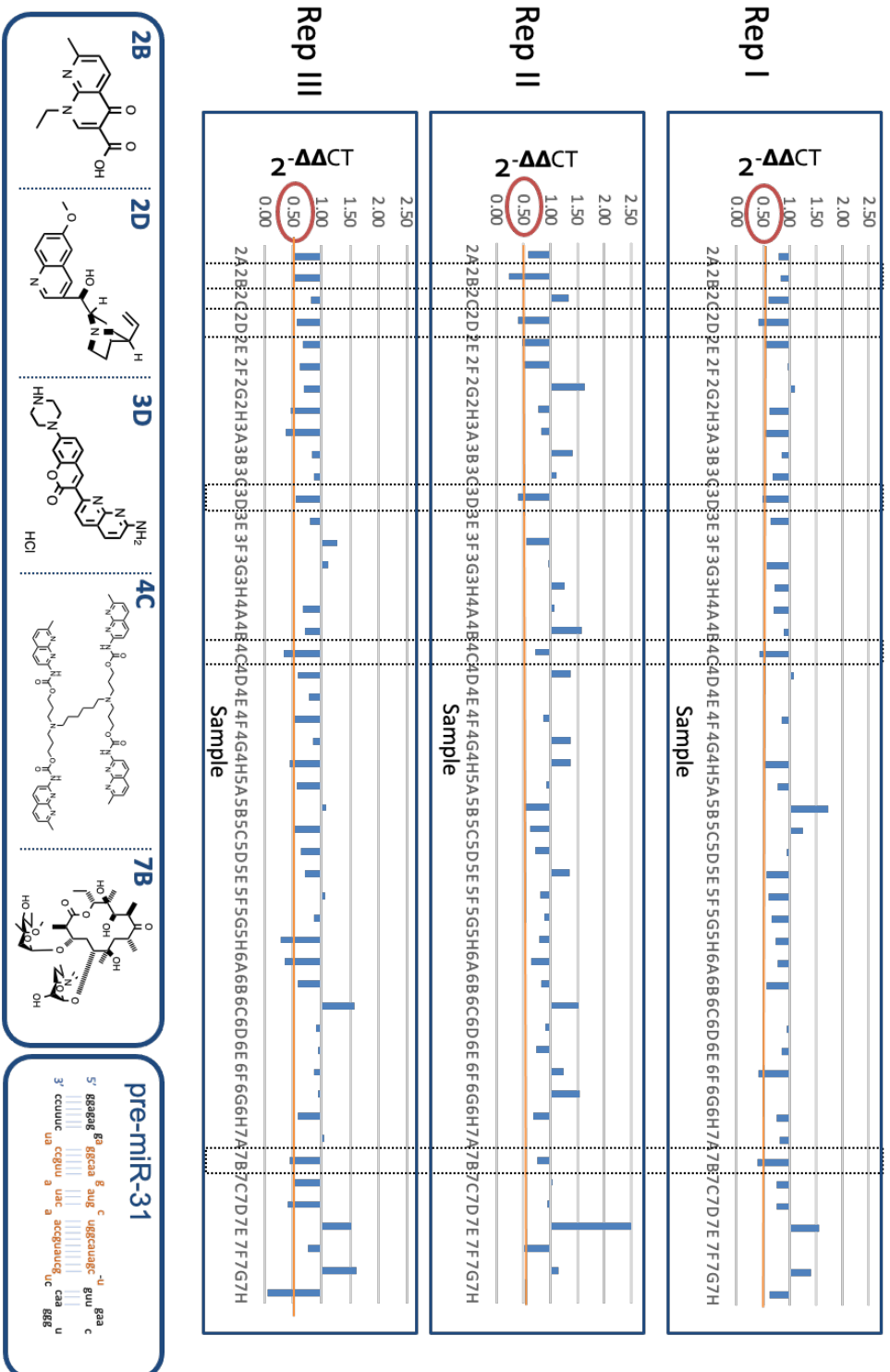


Figure 3.7 In-Cell screening results of hsa-miR-31

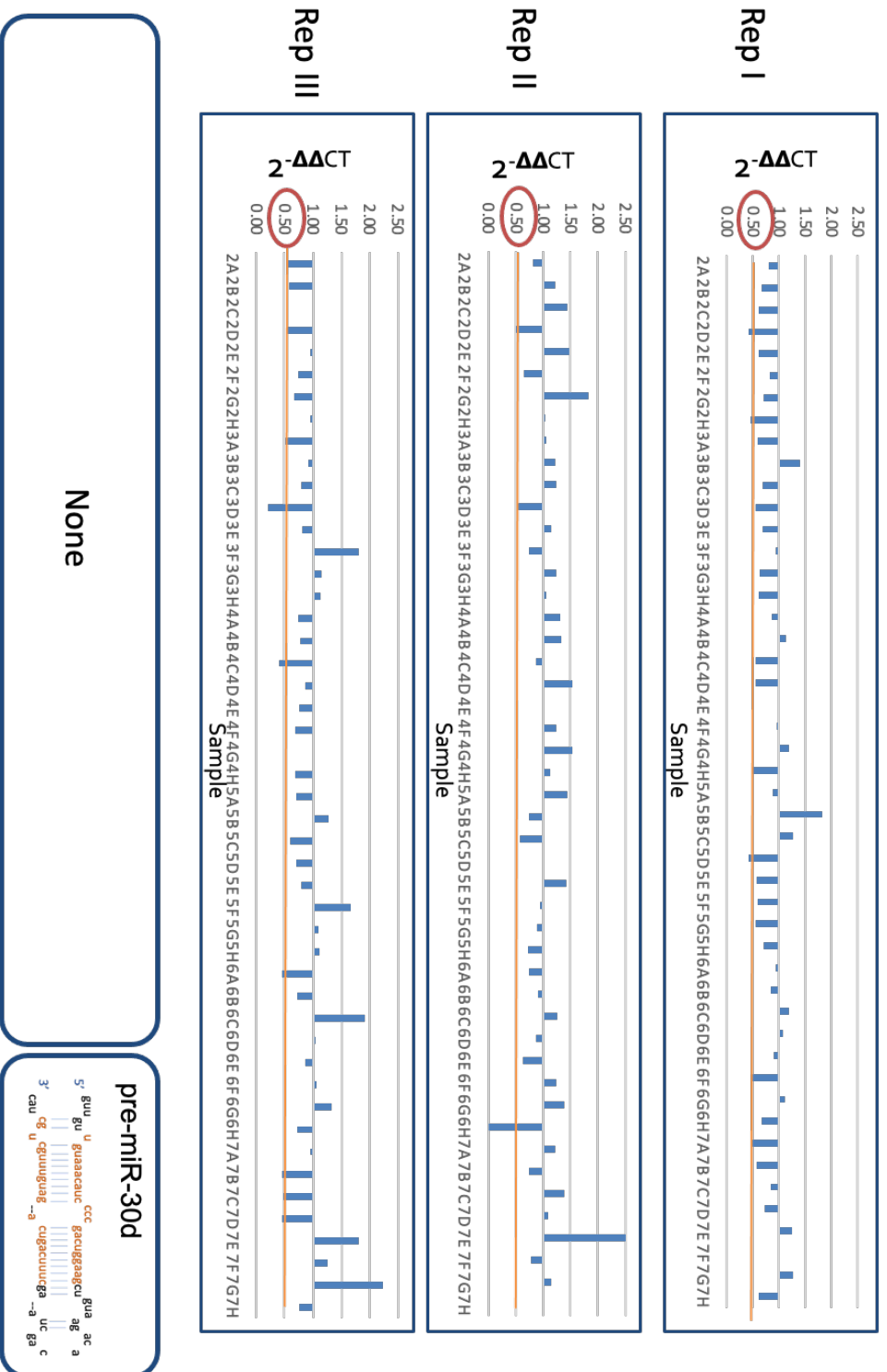
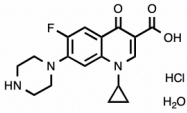
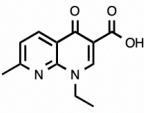
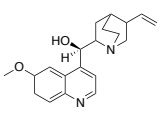
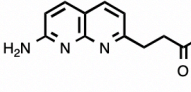
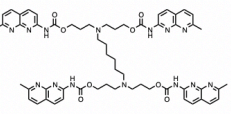
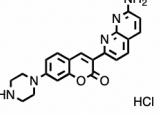
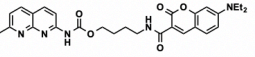
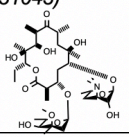


Figure 3.8 In-Cell screening results of hsa-miR-30d

According to the criteria provided above, Figure 3.6 demonstrates that 5 compounds reach the 0.5 threshold and also meet the replication criteria, indicating that these 5 compounds may possess inhibitory activity against the pre-miR-182 processing reaction. The inhibition of pre-miR-31 and pre-miR-30d processing reaction shows in Figure 3.7 and 3.8. While 5 different compounds have demonstrated potential inhibitory activity against hsa-miR-31 production, there are no compounds that meet all of the criteria listed above for hsa-miR-30d production.

The summary of compounds that inhibit hsa-miR-182 and hsa-miR-31 production is shown in Figure 3.9. Compounds 2A, 3A, and 5C inhibit the production of has-miR-182, whereas compounds 2B, 4C, and 7B inhibit the production of hsa-miR-31. In contrast to the other six compounds, which appear to preferentially inhibit one type of miRNA, compounds 2D and 3D inhibit both types of miRNA production.

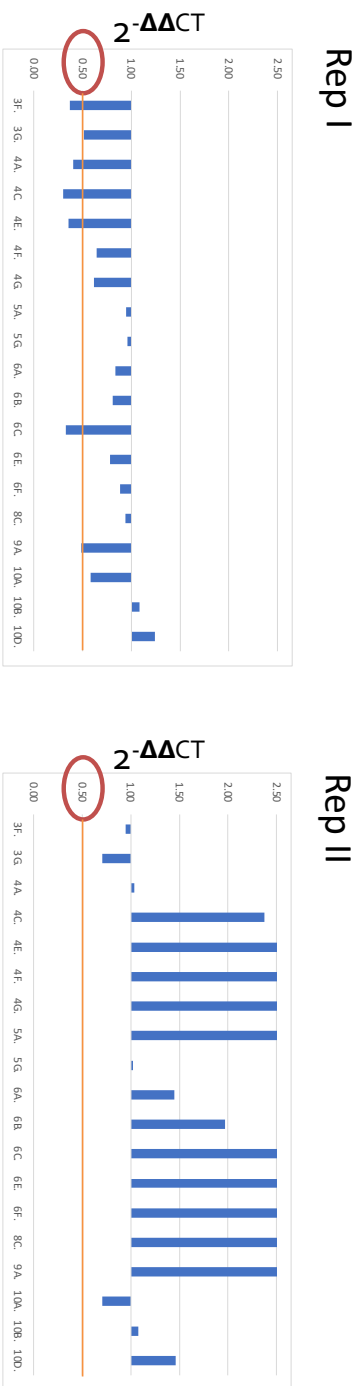
miR-182		miR-31		miR-182 and miR-31	
<b>2A</b>	2A (2B_N131002)  HCl H <sub>2</sub> O	<b>2B</b>	2B (2H_N131008) 	<b>2D</b>	2D (6B_N131034) 
<b>3A</b>	3A (2A_N132001) 	<b>4C</b>	4C (10E_(N14069)NCT6) 	<b>3D</b>	3D (8H_N132056)  HCl
<b>5C</b>	5C (7F_(N160046) AIK216) 	<b>7B</b>	7B (7C_N131043) 		

**Figure 3.9** *In-Cell* screening results summary

### 3.2.4 Confirmation Experiment *In Cell* Reaction

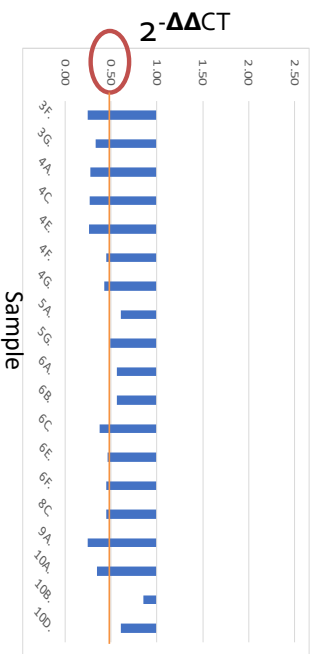
To verify the validity of the criteria used in the *in vitro* dicer reaction screening, we performed a similar experiment on the 19 compounds that were excluded from the in-cell screening. These compounds were used to treat HeLa cells in a similar manner, and the results of this experiment were evaluated similarly to those from the in-cell screening.

As presented in Figure 3.10, 3.11, and 3.12, none of the compounds significantly inhibited has-miR-182, has-miR-31, or has-miR-30d production by failing to pass the  $2^{-\Delta\Delta CT}$  expressions threshold, and even when several compounds did pass the threshold, those compounds did not meet the replication criteria, defined as passing the threshold more than twice. The validity of the screening criteria is concluded as a result of these findings.

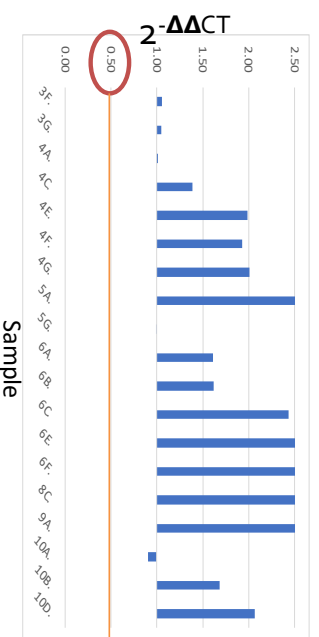


**Figure 3.10** In cell assay of 19 compounds that excluded after first screening to confirm the expression of hsa-miR-182. This results indicates that none of the compounds that pass the threshold and meet the replication criteria

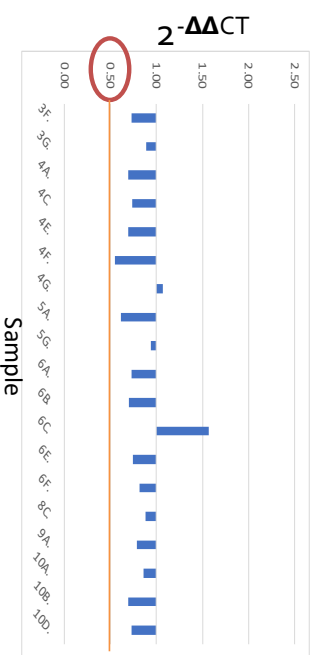
Rep I



Rep II



Rep III

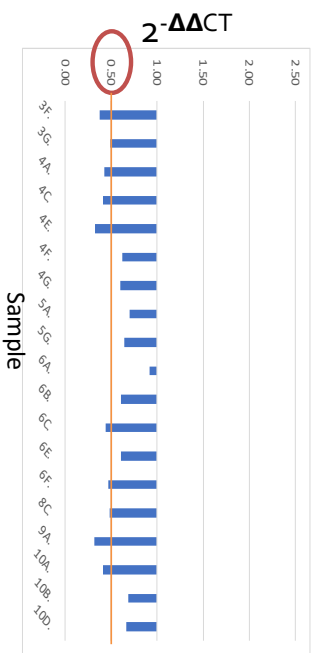


None

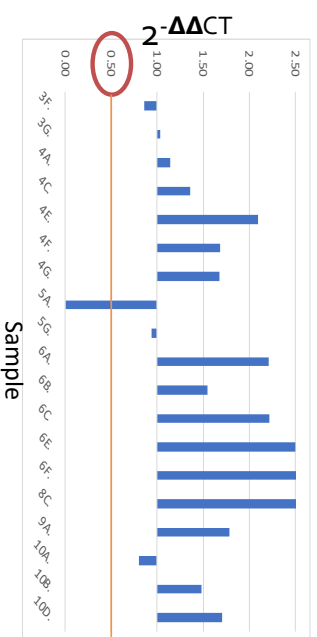


**Figure 3.11** In cell assay of 19 compounds that excluded after first screening to confirm the expression of hsa-miR-31. This results indicates that none of the compounds that pass the threshold and meet the replication criteria

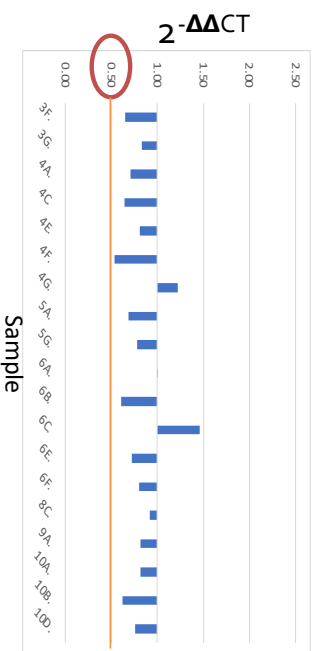
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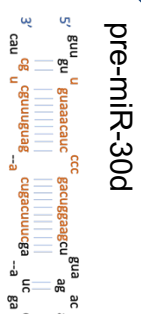
Rep II



Rep III



None



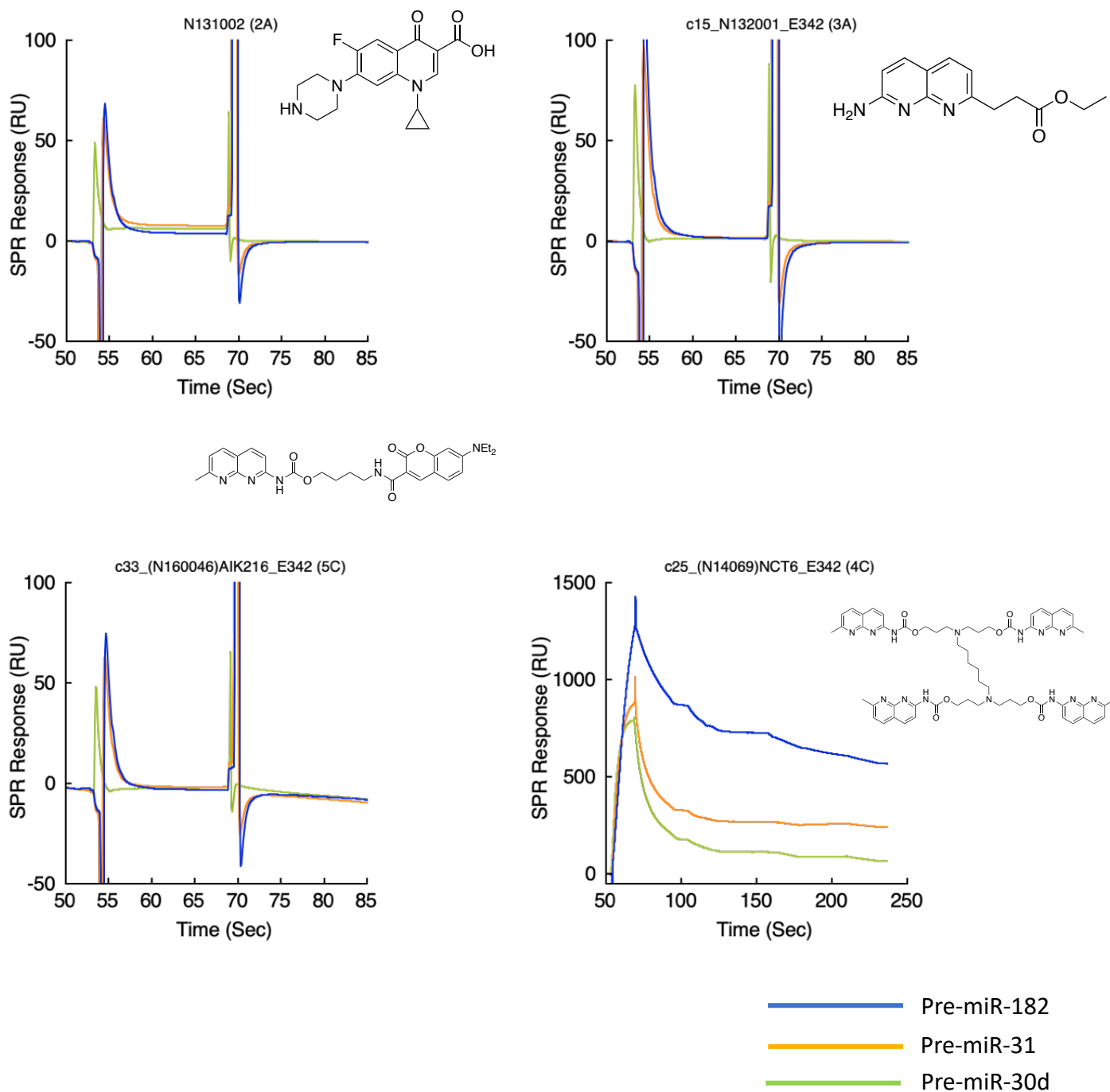
**Figure 3.12** In cell assay of 19 compounds that excluded after first screening to confirm the expression of hsa-miR-30d. This results indicates that none of the compounds that pass the threshold and meet the replication criteria

### 3.2.5 Surface Plasmon Resonance (SPR) Analysis

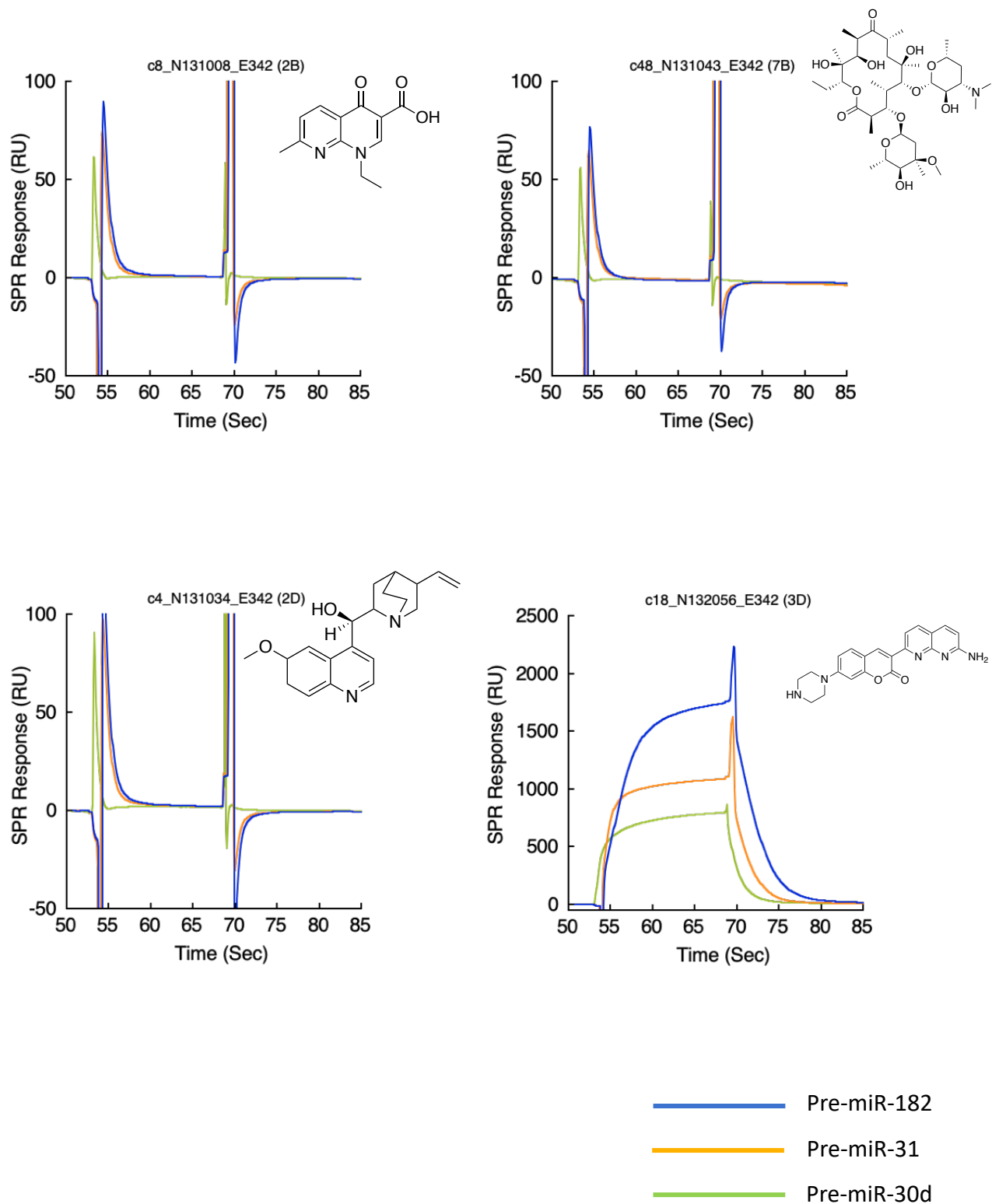
It was previously reported that the small molecule could bind to precursor miRNAs and then interfere with the cleavage processing of the dicer. (41) To verify this possibility, SPR analysis was used to determine the mechanism of action (MOA) of the small molecules that inhibited hsa-miR-182, hsa-miR-31, and hsa-miR-30d production. This study will assess the binding ability of 8 small molecules identified through in-cell screening (2A, 2B, 2D, 3A, 3D, 4C, 5C, and 7B) to the precursors of hsa-miR-182, hsa-miR-31, and hsa-miR-30d.

Binding experiments were generally carried out on the SA chip with amounts of immobilized pre-miR-182, pre-miR-31, and pre-miR-30d corresponding to 1606.8, 2150.0, and 1950.7 RU, respectively. Figure 3.13 and 3.14 depicts typical binding curves monitored during contact time with an 8-compound solution. It demonstrates that compounds 3D and 4C have better binding profiles than the other six compounds. A comparison of compound 3D and 4C binding to pre-miRNAs reveals that pre-miR-182 has the highest response, followed by pre-miR-31 and pre-miR-30d, respectively.





**Figure 3.13** SPR binding assay of compound 2A, 3A, 5C, and 4C to the pre-miR-182/31/30d immobilized surface. Ligand was added at concentration of 50  $\mu$ M. The amount of pre-miR-182/31/30d immobilized on SA chip was 1606.8, 2150, 1950.7 RU respectively

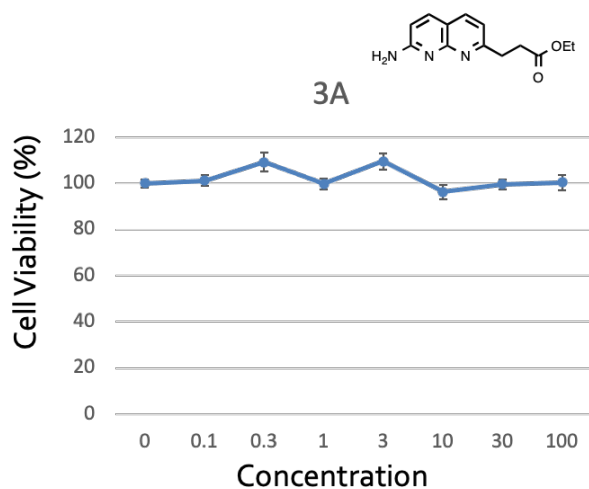
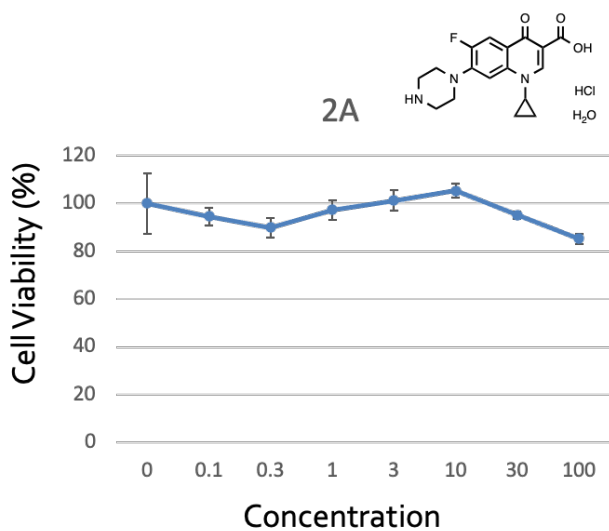
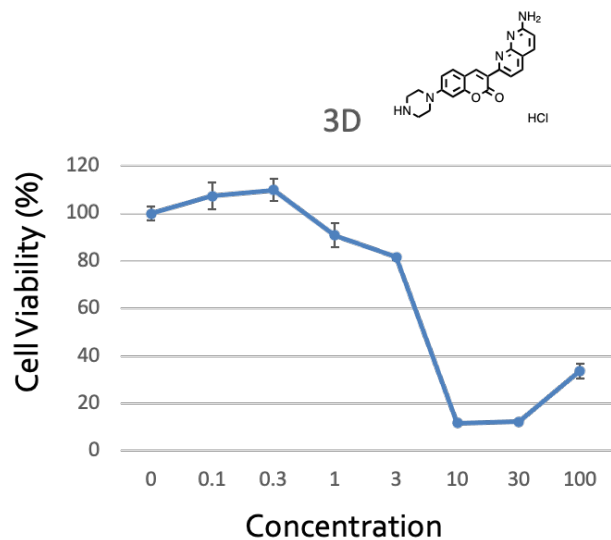
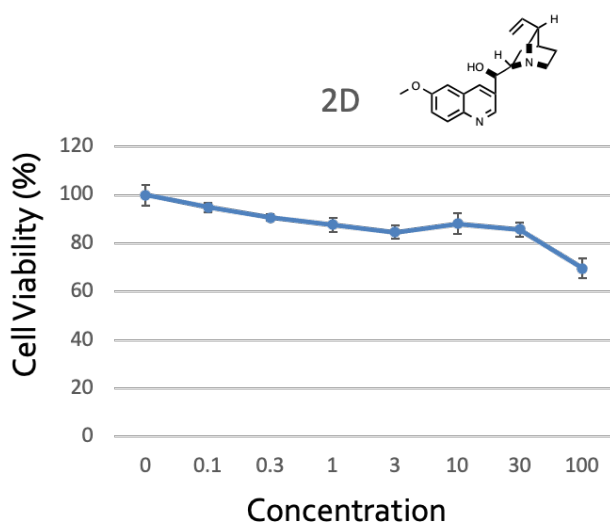


**Figure 3.14** SPR binding assay of compound 2B, 7B, 2D, and 3D to the pre-miR-182/31/30d immobilized surface. Ligand was added at concentration of 50  $\mu$ M. The amount of pre-miR-182/31/30d immobilized on SA chip was 1606.8, 2150, 1950.7 RU respectively

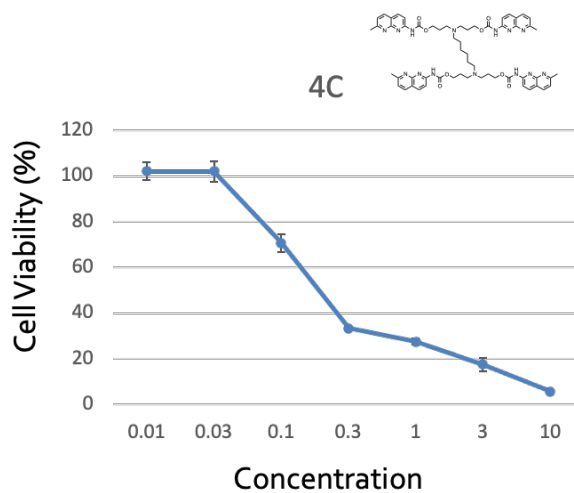
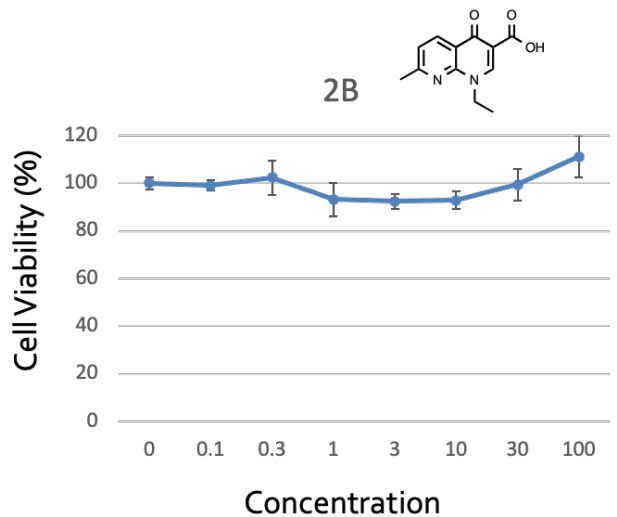
### 3.2.6 WST-8 Viability Assay

To assess the biological significance of eight compounds from the previous screening, we used a WST-8 viability assay to measure cell viability and proliferation. The cells were cultured and pre-incubated before adding varying concentrations of each of the eight compounds ranging from 0 to 100  $\mu\text{M}$ . After another incubation, the cells' absorbance at 450 nm was measured using a microplate reader.

The results of this assay are depicted in Figure 3.15 and 3.16, which shows the effects of compound concentration on cell viability. The common pattern that appears in all eight figures is that the higher the concentration of the compounds, the lower the cell viability. However, among those eight compounds, compounds 3D and 4C show significant inhibition, and based on this data, we can predict that the  $\text{IC}_{50}$  for 3D is around 5  $\mu\text{M}$  and 0.2  $\mu\text{M}$  for 4C.

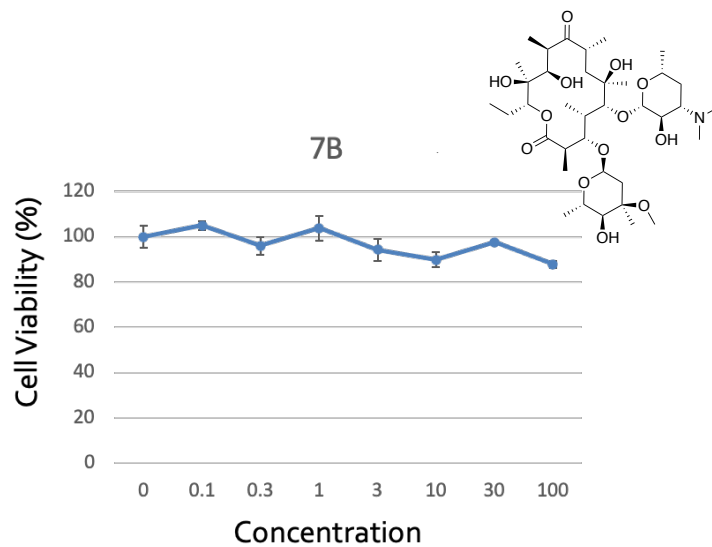
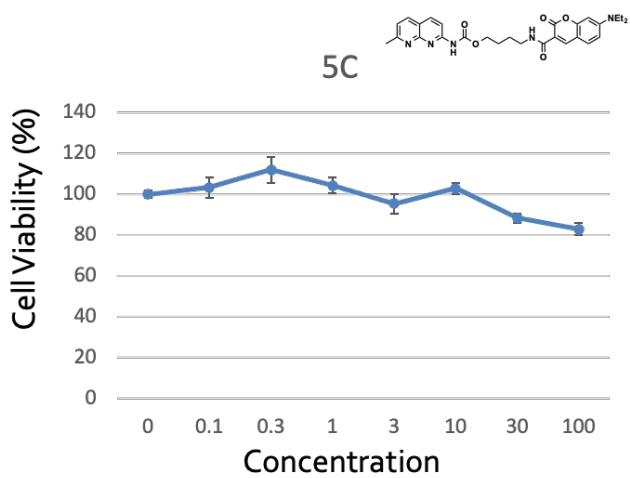


**Figure 3.15** Viability assay of compound 2D, 3D, 2A and 3A



Conc (uM)	0	0.1	0.3	1	3	10	30	100
Via %	100.00	99.10	102.39	93.14	92.49	92.89	99.38	111.17
Std Dev	2.60	2.17	7.29	7.16	3.12	3.55	6.72	8.78

Conc (uM)	0.01	0.03	0.1	0.3	1	3	10
Via %	102.14	102.04	70.69	33.39	27.55	17.60	5.79
Std Dev	3.87	4.59	3.75	0.34	1.34	2.92	0.40



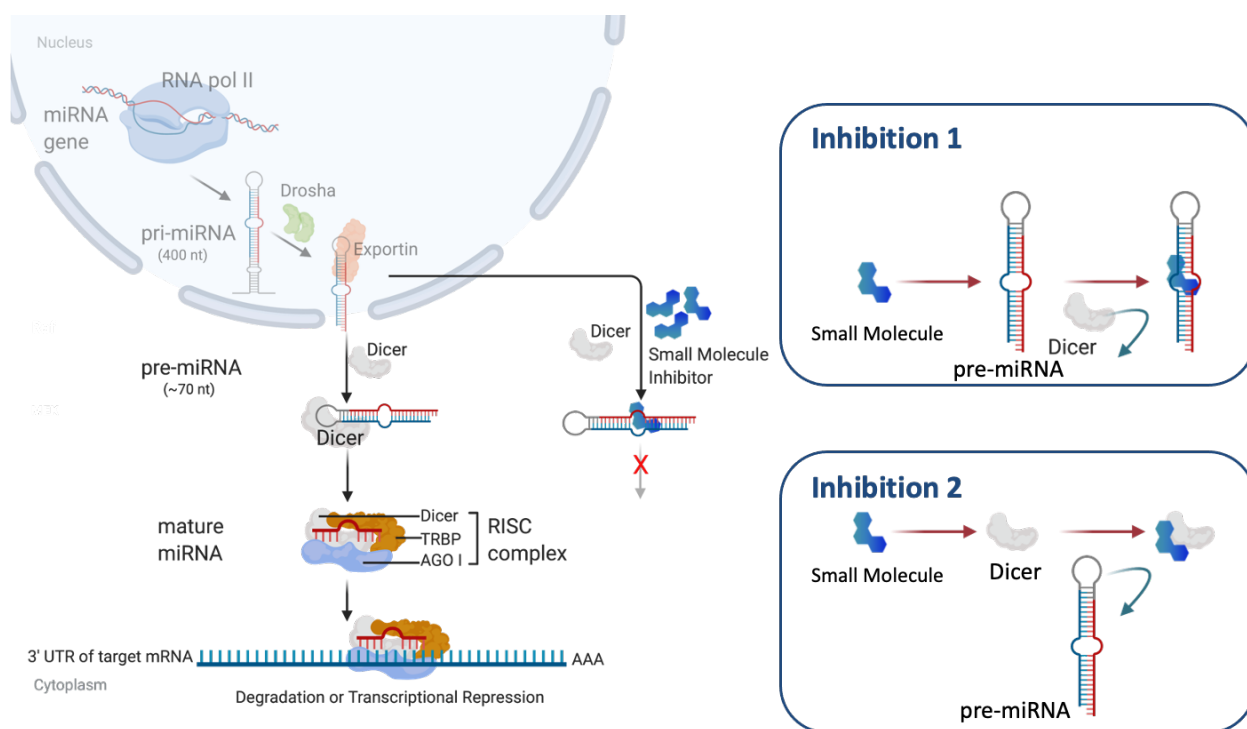
Conc (uM)	0	0.1	0.3	1	3	10	30	100
Via %	100.00	103.32	111.92	104.41	95.42	102.84	88.34	82.93
Std Dev	1.92	5.08	6.22	4.00	4.70	2.58	2.25	2.88

Conc (uM)	0	0.1	0.3	1	3	10	30	100
Via %	100.00	105.05	96.06	103.78	94.24	89.79	97.59	87.81
Std Dev	4.76	1.91	3.96	5.56	4.71	3.29	0.54	1.49

**Figure 3.16** Viability assay of compound 2B, 4C, 5C and 7B

### 3.2.7 The Mechanism of Action

Based on the screening results, it was known that the the miRNA production was inhibited by all of the hit compounds, however, among 8 hit compounds (2A, 2B, 2D, 3A, 3D, 4C, 5C, and 7B), only 2 compounds that shows binding affinity to miRNA precursors (3D and 4C). To understand this phenomenon, we could refer to the first screening assay. From that assay (the *In vitro* dicer reaction), the reaction condition has been limited to the miRNA precursors, Dicer, and buffer. It means there are two possibilities regarding the miRNA production inhibition mechanism as shown in Figure 3.16, either by binding to the miRNA precursors and inhibit the Dicer-mediated cleavage reaction, or these compounds directly affecting the Dicer thereby inhibit the miRNA production.



**Figure 3.17** Inhibition mechanism; (Inhibition 1) small molecule bind to pre-miRNA and inhibit Dicer-mediated cleavage reaction, (Inhibition 2) small molecule affecting the Dicer, and it makes the inhibition occurred.

### 3.3 Conclusions

We performed a screening assay based on our understanding of miRNA biogenesis. We used the *in vitro* dicer cleavage reaction in our primary screening and we were able to find 48 compounds from the initial 67 compounds as potential inhibitors. These 48 compounds then subjected to in-cell screening as the secondary screening, and we were able to obtain 8 compounds (2A, 2B, 2D, 3A, 3D, 4C, 5C, and 7B) with promising results.

Aside from that, we conducted a similar in-cell screening for 19 excluded compounds from the primary screening to evaluate our screening criteria, and the result shows that all 19 compounds did not pass the second screening, indicating that the criteria that were used in the first screening are valid.

Additional assays were also performed to learn more about the biological significance of those eight compounds. According to the SPR analysis and viability assay, compound 3D was the most promising result as an inhibitor to miR-182 production. Compound 4C also shows similar results, however, its low IC<sub>50</sub> value during viability assay makes the second screening of this compound compromised and needs to be analyzed further before using this compound on the further assay.

## 3.4 Methods

### 3.4.1 The Library Compounds and pre-microRNA targets

All chemical compounds and microRNA precursors (pre-miR-182/31/30d) belong to the Nakatani laboratory library. 67 different compounds that were used in this experiment were selected by considering the structural difference of every compound group. To make it easier to identify, the nomenclature in this experiment was used by giving letters and numbers according to the location of the compounds in the library well-plate. The rearrangement of the 48-compounds also applied after the first screening.

### 3.4.2 In Vitro Dicer Cleavage Reaction

In the presence of a buffer reaction, phosphorylated precursor microRNA (0.5  $\mu$ M), 50 mM  $MgCl_2$ , 10 mM ATP, 0.05 percent BSA, and Dicer (50 nM) were mixed. The sample from the library compounds was then added, with RNase-free water serving as a negative control. After that, the mixture was incubated at 37°C for 6 hours. The reaction was then halted by adding 10 mM EDTA. To remove non-nucleic acids, ethanol precipitation was used, followed by RNase-free water to solute the nucleic acids.

### 3.4.3 In-cell Reaction

HeLa cells were cultured and expanded using standard techniques. The cells were then plated in multi-well plates and incubated in the incubator overnight (37°C, 5%  $CO_2$ ). At each well, the cell density is 5000 cells per 100  $\mu$ L. Following incubation, HeLa cells were treated with a final concentration of the target compounds of 1  $\mu$ M. Another overnight incubation was performed to ensure that the desired biological effect was achieved. Following treatment, the lysis and cDNA procedures were carried out in accordance with the kit's manufacturer's instructions.



#### 3.4.4 WST-8 Cell Viability Assay

After dispensing 100uL of cell suspension (5000 cells/well) into a 96-well plate, a 24-hour pre-incubation period in a humidified incubator was performed. Following that, 10 uL of the substances to be tested at various concentrations was added. Another 24-hour incubation period was required to determine the effect of the substances. Then CCK-8 was added and incubated for an additional 1-4 hours. After that, using a microplate reader, determine the absorbance at 450 nM.

## CHAPTER IV

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## SUPPLEMENTARY DATA

- S1 Well plate rearrangement , this arrangement used for 48 compound  
In-Cell screening, SPR analysis, and viability assay
- S2 *In vitro* Dicer reaction detail calculation for hsa-miR-182
- S3 *In vitro* Dicer reaction detail calculation for hsa-miR-31
- S4 *In vitro* Dicer reaction detail calculation for hsa-miR-30d
- S5 *In cell* screening detail calculation for hsa-miR-182
- S6 *In cell* screening detail calculation for hsa-miR-31
- S7 *In cell* screening detail calculation for hsa-miR-30d
- S8 Additional *In cell* Assay detail calculation for hsa-miR-182
- S9 Additional *In cell* Assay detail calculation for hsa-miR-31
- S10 Additional *In cell* Assay detail calculation for hsa-miR-30d
- S11 Detail calculation of 25 nM and 50 nM substrate for velocity plot
- S12 Detail calculation of 100 nM and 200 nM substrate for velocity plot
- S13 Detail calculation of 300 nM and 400 nM substrate for velocity plot

## Well Plate Rearrangement

2A (2B_N131002)	3A (8D_N131052)	4H (11H_N132080)	6G (2G_(N160007) PQA-C3-arnide)	7G (6C_(N160035) NCD-C3-NH2)	9F (7G_(N14247)jix224)
2B (2H_N131008)	3B (9H_N131064)	5B (3H_(N14016)AIK112)	6H (3B_(N160010) PQAD-urea)	7H (7F_(N160046) AIK216)	9G (8A_(N14249)jix238)
2C (4B_N131018)	3C (10B_N131066)	5C (4B_(N14018)SA029)	7A (3C_(N160011) DDAP)	8A (7H_(N160048) AIK248)	9H (8B_(N14250)jix223)
2D (4G_N131023)	3D (11H_N131080)	5D (4H_(N14024)SM-221)	7B (4B_(N160018) Am-BzN)	8B (8D_(N160052) ih039)	10C (9B_(N14258)jix188)
2E (6A_N131033)	3E (2A_N132001)	5E (5C_(N14027)YM011)	7C (4C_(N160019) Am-BzND)	9B (7B_(N14242)jix249)	10E (9H_(N14264)jix372)
2F (6B_N131034)	3H (4H_N132024)	5F (5E_(N14029)NN146)	7D (4G_(N160023) CMBL4)	9C (7C_(N14243)BzNA)	10F (10A_(N14265)jix380)
2G (6G_N131039)	4B (5C_N132027)	5H (6G_(N14039)Thioflavin)	7E (5A_(N160025) CMBL5a)	9D (7D_(N14244)jix253)	10G (10B_(N14266)jix344)
2H (7C_N131043)	4D (8H_N132056)	6D (10E_(N14069)NCT6)	7F (5H_(N160032) NCD-C4-SH)	9E (7E_(N14245)jix107)	10H (10D_(N14268)jix358)

**S1** Well plate rearrangement , this arrangement used for 48 compound In-Cell screening, SPR analysis, and viability assay

Sample	has-miR-182 (CT value)		dCT		2 <sup>-</sup> dCT		Threshold <0.5		R
	I	II	I	II	I	II	I	II	
2A	20.967	20.554	3.558	3.144	0.085	0.113	1	1	OK
2B	20.900	20.571	3.491	3.162	0.089	0.112	1	1	OK
2C	16.413	16.126	-0.996	-1.283	1.995	2.434	0	0	-
2D	16.614	16.456	-0.795	-0.954	1.735	1.937	0	0	-
2E	20.359	20.246	2.950	2.837	0.129	0.140	1	1	OK
2F	16.965	16.861	-0.444	-0.548	1.360	1.462	0	0	-
2G	16.140	16.317	-1.269	-1.092	2.410	2.131	0	0	-
2H	20.438	20.484	3.029	3.075	0.123	0.119	1	1	OK
3A	20.133	20.808	2.724	3.399	0.151	0.095	1	1	OK
3B	21.252	21.725	3.843	4.316	0.070	0.050	1	1	OK
3C	16.153	16.974	-1.257	-0.435	2.389	1.352	0	0	-
3D	15.838	16.146	-1.572	-1.263	2.972	2.400	0	0	-
3E	20.776	20.952	3.367	3.543	0.097	0.086	1	1	OK
3F	15.944	16.240	-1.465	-1.169	2.761	2.248	0	0	-
3G	16.943	16.962	-0.466	-0.447	1.381	1.363	0	0	-
3H	22.494	22.441	5.085	5.032	0.029	0.031	1	1	OK
4A	16.740	17.148	-0.669	-0.261	1.590	1.198	0	0	-
4B	16.091	16.457	-1.318	-0.952	2.493	1.935	0	0	-
4C	16.922	17.267	-0.487	-0.142	1.402	1.104	0	0	-
4D	22.016	22.071	4.607	4.662	0.041	0.040	1	1	OK
4E	16.308	16.554	-1.101	-0.855	2.145	1.808	0	0	-
4F	16.102	16.149	-1.307	-1.260	2.475	2.395	0	0	-
4G	16.819	16.714	-0.591	-0.695	1.506	1.619	0	0	-
4H	18.854	18.835	1.444	1.426	0.367	0.372	1	1	OK
5A	16.063	16.719	-1.346	-0.691	2.543	1.614	0	0	-
5B	20.596	21.048	3.187	3.638	0.110	0.080	1	1	OK
5C	20.689	21.183	3.279	3.773	0.103	0.073	1	1	OK
5D	20.495	20.473	3.086	3.064	0.118	0.120	1	1	OK
5E	16.500	16.400	-0.909	-1.009	1.878	2.013	0	0	-
5F	22.066	21.932	4.657	4.523	0.040	0.044	1	1	OK
5G	16.308	16.252	-1.101	-1.157	2.145	2.230	0	0	-
5H	22.123	22.000	4.714	4.591	0.038	0.041	1	1	OK
6A	15.436	16.143	-1.973	-1.266	3.926	2.405	0	0	-
6B	16.774	17.817	-0.635	0.408	1.553	0.754	0	0	-
6C	16.488	17.383	-0.921	-0.026	1.893	1.018	0	0	-
6D	23.421	24.057	6.012	6.648	0.015	0.010	1	1	OK
6E	16.539	16.712	-0.870	-0.697	1.828	1.621	0	0	-
6F	16.158	16.786	-1.251	-0.623	2.380	1.540	0	0	-
6G	16.958	17.383	-0.451	-0.027	1.367	1.019	0	0	-
6H	17.948	18.259	0.538	0.850	0.689	0.555	0	0	-
7A	22.986	22.429	5.576	5.020	0.021	0.031	1	1	OK
7B	19.617	20.033	2.207	2.624	0.217	0.162	1	1	OK
7C	24.398	24.750	6.989	7.341	0.008	0.006	1	1	OK
7D	26.438	26.395	9.029	8.985	0.002	0.002	1	1	OK
7E	21.963	22.541	4.554	5.132	0.043	0.029	1	1	OK
7F	22.584	22.961	5.175	5.552	0.028	0.021	1	1	OK
7G	19.315	19.964	1.906	2.555	0.267	0.170	1	1	OK
7H	24.096	24.376	6.686	6.967	0.010	0.008	1	1	OK
8A	17.083	17.861	-0.326	0.452	1.254	0.731	0	0	-
8B	15.972	16.623	-1.437	-0.786	2.707	1.724	0	0	-
8C	17.682	18.644	0.273	1.235	0.827	0.425	0	1	-
9A	17.210	17.690	-0.200	0.280	1.148	0.823	0	0	-
9B	16.660	16.498	-0.749	-0.911	1.681	1.880	0	0	-
9C	17.635	18.068	0.226	0.659	0.855	0.633	0	0	-
9D	16.245	17.194	-1.164	-0.215	2.241	1.161	0	0	-
9E	21.765	22.215	4.355	4.806	0.049	0.036	1	1	OK
9F	16.396	16.811	-1.013	-0.598	2.019	1.514	0	0	-
9G	18.118	18.347	0.708	0.938	0.612	0.522	0	0	-
9H	17.034	17.119	-0.375	-0.290	1.297	1.223	0	0	-
10A	16.782	16.640	-0.627	-0.769	1.545	1.704	0	0	-
10B	17.566	17.681	0.157	0.271	0.897	0.829	0	0	-
10C	21.061	20.977	3.652	3.568	0.080	0.084	1	1	OK
10D	17.785	17.729	0.376	0.320	0.771	0.801	0	0	-
10E	18.471	18.423	1.061	1.014	0.479	0.495	1	1	OK
10F	17.587	17.703	0.178	0.293	0.884	0.816	0	0	-
10G	16.964	17.187	-0.445	-0.222	1.361	1.167	0	0	-
10H	17.413	17.448	0.004	0.039	0.997	0.974	0	0	-
Reference	17.409								

S2 *In vitro* Dicer reaction detail calculation for hsa-miR-182



Sample	has-miR-31 (CT value)		dCT		2 <sup>-</sup> dCT		Threshold <0.5		R
	I	II	I	II	I	II	I	II	
2A	15.295	14.664	-0.758	-1.390	1.691	2.620	0	0	-
2B	15.775	15.386	-0.278	-0.667	1.212	1.588	0	0	-
2C	16.301	16.069	0.248	0.015	0.842	0.989	0	0	-
2D	15.596	15.373	-0.457	-0.680	1.373	1.602	0	0	-
2E	16.887	16.431	0.833	0.378	0.561	0.770	0	0	-
2F	17.223	17.270	1.170	1.216	0.444	0.430	1	1	OK
2G	17.367	17.361	1.313	1.308	0.402	0.404	1	1	OK
2H	17.345	17.368	1.292	1.314	0.409	0.402	1	1	OK
3A	18.994	18.526	2.941	2.472	0.130	0.180	1	1	OK
3B	17.907	17.707	1.853	1.654	0.277	0.318	1	1	OK
3C	17.695	17.198	1.642	1.144	0.320	0.452	1	1	OK
3D	18.603	18.340	2.550	2.287	0.171	0.205	1	1	OK
3E	16.841	16.731	0.788	0.678	0.579	0.625	0	0	-
3F	16.216	15.853	0.163	-0.200	0.893	1.149	0	0	-
3G	16.560	16.239	0.507	0.186	0.704	0.879	0	0	-
3H	16.409	16.294	0.356	0.241	0.781	0.846	0	0	-
4A	16.184	15.890	0.131	-0.163	0.913	1.120	0	0	-
4B	17.782	17.509	1.729	1.456	0.302	0.365	1	1	OK
4C	16.778	16.678	0.724	0.624	0.605	0.649	0	0	-
4D	19.425	19.258	3.371	3.204	0.097	0.108	1	1	OK
4E	16.188	16.009	0.135	-0.045	0.911	1.031	0	0	-
4F	16.550	16.550	0.497	0.497	0.709	0.709	0	0	-
4G	16.357	16.523	0.304	0.470	0.810	0.722	0	0	-
4H	19.145	19.328	3.091	3.275	0.117	0.103	1	1	OK
5A	16.758	16.519	0.704	0.466	0.614	0.724	0	0	-
5B	16.663	16.149	0.609	0.096	0.655	0.936	0	0	-
5C	16.964	16.772	0.911	0.719	0.532	0.607	0	0	-
5D	16.755	16.444	0.702	0.391	0.615	0.763	0	0	-
5E	16.103	16.049	0.050	-0.005	0.966	1.003	0	0	-
5F	16.065	15.879	0.012	-0.175	0.992	1.129	0	0	-
5G	15.996	15.716	-0.057	-0.337	1.040	1.263	0	0	-
5H	15.096	14.777	-0.957	-1.276	1.942	2.422	0	0	-
6A	15.897	15.689	-0.157	-0.364	1.115	1.287	0	0	-
6B	16.357	16.302	0.304	0.249	0.810	0.842	0	0	-
6C	16.745	16.631	0.692	0.577	0.619	0.670	0	0	-
6D	20.875	20.556	4.822	4.503	0.035	0.044	1	1	OK
6E	15.414	15.410	-0.639	-0.643	1.557	1.562	0	0	-
6F	16.080	16.247	0.026	0.193	0.982	0.875	0	0	-
6G	17.685	17.540	1.632	1.487	0.323	0.357	1	1	OK
6H	22.738	22.976	6.685	6.923	0.010	0.008	1	1	OK
7A	19.979	19.712	3.926	3.658	0.066	0.079	1	1	OK
7B	16.809	16.687	0.755	0.634	0.592	0.644	0	0	-
7C	20.206	19.769	4.153	3.716	0.056	0.076	1	1	OK
7D	24.883	24.534	8.830	8.481	0.002	0.003	1	1	OK
7E	19.597	19.508	3.544	3.454	0.086	0.091	1	1	OK
7F	22.339	22.534	6.286	6.481	0.013	0.011	1	1	OK
7G	19.407	19.319	3.353	3.265	0.098	0.104	1	1	OK
7H	15.908	15.934	-0.145	-0.119	1.106	1.086	0	0	-
8A	21.585	21.387	5.532	5.334	0.022	0.025	1	1	OK
8B	15.136	15.071	-0.917	-0.982	1.888	1.975	0	0	-
8C	13.753	13.855	-2.300	-2.198	4.924	4.588	0	0	-
9A	14.995	14.727	-1.058	-1.326	2.082	2.508	0	0	-
9B	17.979	17.846	1.926	1.793	0.263	0.289	1	1	OK
9C	20.919	20.884	4.866	4.831	0.034	0.035	1	1	OK
9D	21.625	21.392	5.572	5.339	0.021	0.025	1	1	OK
9E	22.151	22.021	6.098	5.967	0.015	0.016	1	1	OK
9F	17.755	17.440	1.702	1.387	0.307	0.382	1	1	OK
9G	35.006	35.217	18.953	19.164	0.000	0.000	1	1	OK
9H	23.997	23.881	7.943	7.828	0.004	0.004	1	1	OK
10A	16.557	16.341	0.504	0.288	0.705	0.819	0	0	-
10B	16.034	15.694	-0.020	-0.359	1.014	1.283	0	0	-
10C	20.795	20.586	4.742	4.532	0.037	0.043	1	1	OK
10D	15.751	15.574	-0.302	-0.479	1.233	1.394	0	0	-
10E	19.609	19.448	3.555	3.394	0.085	0.095	1	1	OK
10F	24.209	24.154	8.156	8.101	0.004	0.004	1	1	OK
10G	16.289	16.197	0.236	0.144	0.849	0.905	0	0	-
10H	24.786	24.592	8.733	8.539	0.002	0.003	1	1	OK
Reference	16.053								

S3 *In vitro* Dicer reaction detail calculation for hsa-miR-31

Sample	hsa-miR-30d (CT value)		dCT		2 <sup>-</sup> dCT		Threshold <0.5		R
	I	II	I	II	I	II	I	II	
2A	16.119	16.099	1.683	1.663	0.311	0.316	1	1	OK
2B	15.342	15.393	0.906	0.957	0.533	0.515	0	0	-
2C	15.956	15.953	1.520	1.517	0.349	0.349	1	1	OK
2D	16.281	16.199	1.845	1.763	0.278	0.295	1	1	OK
2E	14.843	14.807	0.407	0.371	0.754	0.773	0	0	-
2F	14.607	14.538	0.171	0.102	0.888	0.932	0	0	-
2G	15.714	15.709	1.278	1.273	0.412	0.414	1	1	OK
2H	15.927	15.978	1.491	1.542	0.356	0.343	1	1	OK
3A	18.211	18.168	3.776	3.732	0.073	0.075	1	1	OK
3B	17.709	17.613	3.273	3.177	0.103	0.111	1	1	OK
3C	16.028	15.960	1.593	1.524	0.332	0.348	1	1	OK
3D	17.012	16.931	2.576	2.496	0.168	0.177	1	1	OK
3E	14.540	14.374	0.104	-0.062	0.930	1.044	0	0	-
3F	14.597	14.560	0.161	0.124	0.895	0.918	0	0	-
3G	15.076	14.933	0.641	0.497	0.641	0.709	0	0	-
3H	15.966	15.736	1.531	1.301	0.346	0.406	1	1	OK
4A	13.268	13.226	-1.168	-1.210	2.247	2.313	0	0	-
4B	13.739	13.604	-0.697	-0.831	1.621	1.780	0	0	-
4C	14.857	14.675	0.421	0.239	0.747	0.847	0	0	-
4D	15.444	15.368	1.009	0.932	0.497	0.524	1	0	-
4E	13.995	13.906	-0.440	-0.530	1.357	1.444	0	0	-
4F	13.558	13.410	-0.878	-1.026	1.837	2.036	0	0	-
4G	14.236	14.118	-0.200	-0.318	1.148	1.247	0	0	-
4H	14.778	14.629	0.342	0.193	0.789	0.875	0	0	-
5A	13.822	13.768	-0.614	-0.668	1.530	1.588	0	0	-
5B	13.964	13.787	-0.471	-0.649	1.386	1.568	0	0	-
5C	13.951	13.786	-0.485	-0.650	1.400	1.569	0	0	-
5D	16.310	16.231	1.874	1.795	0.273	0.288	1	1	OK
5E	15.535	15.498	1.100	1.062	0.467	0.479	1	1	OK
5F	14.681	14.615	0.245	0.179	0.844	0.884	0	0	-
5G	15.053	14.882	0.618	0.446	0.652	0.734	0	0	-
5H	15.582	15.378	1.146	0.942	0.452	0.521	1	0	-
6A	14.212	14.371	-0.224	-0.065	1.168	1.046	0	0	-
6B	14.971	15.073	0.535	0.638	0.690	0.643	0	0	-
6C	14.440	14.501	0.004	0.065	0.997	0.956	0	0	-
6D	13.529	13.621	-0.907	-0.815	1.876	1.759	0	0	-
6E	13.957	13.850	-0.478	-0.586	1.393	1.501	0	0	-
6F	14.037	14.064	-0.399	-0.372	1.318	1.294	0	0	-
6G	14.676	14.832	0.241	0.397	0.846	0.760	0	0	-
6H	17.277	17.367	2.841	2.932	0.140	0.131	1	1	OK
7A	18.869	18.787	4.433	4.351	0.046	0.049	1	1	OK
7B	14.476	14.409	0.040	-0.027	0.973	1.019	0	0	-
7C	19.365	19.372	4.929	4.936	0.033	0.033	1	1	OK
7D	19.639	19.637	5.203	5.201	0.027	0.027	1	1	OK
7E	15.990	15.823	1.554	1.387	0.340	0.382	1	1	OK
7F	17.933	17.837	3.498	3.401	0.089	0.095	1	1	OK
7G	15.260	15.242	0.824	0.806	0.565	0.572	0	0	-
7H	16.791	16.621	2.355	2.185	0.195	0.220	1	1	OK
8A	14.628	14.518	0.193	0.082	0.875	0.945	0	0	-
8B	15.901	15.776	1.465	1.340	0.362	0.395	1	1	OK
8C	13.991	13.990	-0.444	-0.446	1.361	1.362	0	0	-
9A	13.627	13.432	-0.809	-1.004	1.752	2.005	0	0	-
9B	13.970	13.957	-0.466	-0.479	1.381	1.394	0	0	-
9C	12.953	12.914	-1.482	-1.522	2.794	2.871	0	0	-
9D	13.908	13.898	-0.528	-0.538	1.442	1.451	0	0	-
9E	14.314	14.315	-0.121	-0.121	1.088	1.088	0	0	-
9F	13.760	13.675	-0.676	-0.761	1.597	1.695	0	0	-
9G	13.556	13.504	-0.880	-0.932	1.840	1.908	0	0	-
9H	18.375	18.328	3.939	3.892	0.065	0.067	1	1	OK
10A	12.831	12.665	-1.605	-1.771	3.042	3.413	0	0	-
10B	14.277	14.173	-0.159	-0.263	1.117	1.200	0	0	-
10C	13.769	13.541	-0.667	-0.894	1.587	1.859	0	0	-
10D	13.731	13.506	-0.705	-0.930	1.630	1.905	0	0	-
10E	13.895	13.871	-0.541	-0.565	1.455	1.480	0	0	-
10F	13.913	13.802	-0.523	-0.634	1.436	1.551	0	0	-
10G	15.696	15.445	1.260	1.009	0.417	0.497	1	1	OK
10H	15.260	15.076	0.824	0.641	0.565	0.641	0	0	-
Reference	14.436								

**S4** *In vitro* Dicer reaction detail calculation for hsa-miR-30d

Sample	U6			hsa-miR-182			Average			dCT 182-U6			ddCT (dCT sample-dCT 0)			2-d-ddCT			Score X<0.5			R	
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III		
2A	13.656	12.798	13.272	25.245	25.108	25.262	13.242	25.238	13.664	25.328	11.689	12.309	11.990	0.869	1.110	1.356	0.774	0.463	0.391	0	0	1	1.0K
2B	13.685	13.517	13.066	25.639	24.989	25.199	13.664	25.326	13.664	25.326	12.278	11.472	11.464	0.959	0.272	0.831	0.514	0.828	0.562	0	0	0	0
2C	13.212	13.819	14.066	25.314	25.048	25.045	13.669	25.326	13.669	25.326	12.022	11.229	10.979	0.783	0.029	0.346	0.581	0.920	0.787	0	0	0	0
2D	12.777	12.297	13.451	25.296	25.246	25.260	12.842	25.434	13.018	13.018	12.318	12.949	11.809	1.699	1.769	1.176	0.308	0.297	0.443	1	1	1	1.0K
2E	13.040	13.680	13.571	25.272	24.976	24.461	12.901	24.904	12.231	11.296	11.296	11.296	10.890	0.256	0.096	0.566	0.532	0.335	0.837	0	0	0	0
2F	13.849	13.478	13.550	25.260	24.741	24.967	13.292	24.889	11.411	12.262	11.411	12.262	11.418	0.092	1.063	0.784	0.398	0.479	0.581	0	1	0	0
2G	14.007	13.920	13.483	24.973	24.720	24.862	13.075	24.862	10.809	12.066	10.809	11.354	-0.354	-0.390	0.720	1.278	1.311	0.607	0.581	0	0	0	0
2H	13.175	13.311	13.875	24.915	24.862	25.204	13.875	24.862	11.400	11.400	11.400	11.400	11.400	0.421	0.351	0.415	0.747	0.784	0.700	0	0	0	0
3A	13.124	12.947	12.899	25.480	25.138	24.968	12.960	25.195	12.555	12.191	12.555	12.191	12.069	1.036	0.991	1.455	0.488	0.503	0.370	1	0	0	1.0K
3B	14.250	14.032	14.224	25.335	25.300	25.300	14.169	25.037	10.985	12.952	10.985	11.268	0.435	0.068	0.068	-0.181	1.352	0.954	1.134	0	0	0	0
3C	13.469	13.456	13.932	25.477	25.084	24.677	13.619	24.962	12.099	11.627	11.627	10.931	0.689	0.428	0.428	-0.242	0.620	0.743	1.183	0	0	0	0
3D	13.160	12.202	13.578	25.489	25.388	25.484	12.980	25.454	12.320	13.186	13.186	11.905	1.010	1.010	1.272	0.486	0.486	0.232	0.416	1	1	1	1.0K
3E	13.160	13.227	13.727	25.584	24.992	24.792	13.275	25.123	12.424	11.766	11.766	11.056	1.108	0.566	0.422	0.465	0.676	0.676	0.726	1	0	0	0
3F	13.981	12.711	14.627	25.381	24.871	24.822	13.523	24.932	12.321	11.161	11.161	10.156	0.181	0.961	-0.478	0.882	0.514	1.392	1.392	0	0	0	0
3G	13.061	13.385	14.186	25.392	24.603	24.970	13.448	25.099	11.296	12.465	11.296	10.242	0.485	0.485	-0.398	0.886	0.715	1.398	1.398	1	0	0	0
3H	13.312	13.157	13.875	24.908	25.603	24.788	13.448	25.099	11.296	12.465	11.296	10.910	0.277	1.246	0.376	0.825	0.422	0.836	0.836	0	1	0	0
3I	13.246	13.958	13.624	25.448	24.979	24.390	13.589	24.939	11.303	11.381	11.381	10.767	0.384	0.181	0.133	0.697	0.882	0.932	0.932	0	0	0	0
4B	14.024	13.883	13.923	25.248	25.229	24.729	13.944	25.107	11.303	11.347	11.347	10.840	-0.017	0.147	0.107	1.012	0.203	0.827	0.827	0	0	0	0
4C	12.975	12.898	12.776	25.305	25.049	24.729	12.985	24.855	12.530	12.156	12.156	11.594	0.956	0.867	0.207	1.037	0.516	0.529	0.529	0	0	0	0
4D	14.252	13.858	13.813	25.519	25.925	24.704	13.647	25.024	11.250	12.067	11.250	10.891	-0.053	0.867	0.257	1.037	0.548	0.837	0.837	1	0	0	0
4E	14.070	13.183	13.687	25.493	25.108	24.470	13.647	25.024	11.423	11.924	11.924	10.783	0.104	0.725	0.149	0.930	0.605	0.902	0.902	0	0	0	0
4F	13.528	13.163	13.455	25.533	25.230	24.897	13.382	25.220	12.005	12.067	11.441	11.441	10.686	0.867	0.807	0.807	0.622	0.548	0.571	0	0	0	0
4G	14.286	13.824	14.022	25.495	25.381	24.442	14.044	25.106	11.205	11.557	11.557	10.420	-0.110	0.358	-0.214	1.079	0.780	1.160	1.160	0	0	0	0
4H	12.958	13.353	12.901	25.135	24.860	24.351	13.071	24.988	12.718	11.507	11.507	11.450	0.858	0.307	0.816	0.552	0.808	0.568	0.568	0	0	0	0
5A	13.866	13.692	13.583	25.206	25.104	24.654	13.720	24.988	11.320	11.412	11.071	11.071	10.001	0.001	0.212	0.438	1.000	0.863	0.738	0	0	0	0
5B	14.894	12.948	14.698	25.080	25.605	25.354	14.180	25.346	10.820	12.657	10.820	10.656	-1.133	1.457	1.022	1.193	0.364	0.985	0.985	0	0	1	0
5C	14.165	12.737	13.186	25.482	25.113	24.859	13.363	25.131	11.317	11.317	11.317	11.672	-0.002	1.176	1.039	1.002	0.442	0.487	0.487	0	1	1	1.0K
5D	13.954	13.067	13.742	25.558	24.668	24.950	13.588	24.950	11.104	11.601	11.601	11.208	-0.215	0.401	0.574	1.161	0.757	0.672	0.672	0	0	0	0
5E	13.188	14.035	13.728	25.188	25.217	24.629	13.650	25.011	12.000	11.183	11.183	10.901	0.680	-0.017	0.267	0.624	1.012	0.831	0.831	0	0	0	0
5F	13.132	13.007	14.664	25.238	24.795	24.872	13.601	24.968	12.007	11.788	11.788	10.208	0.787	0.588	-0.426	0.579	0.655	1.344	1.344	0	0	0	0
5G	13.079	13.042	13.967	25.192	24.890	24.750	13.362	24.922	12.114	11.848	11.848	10.783	0.795	0.640	0.150	0.577	0.638	0.902	0.902	0	0	0	0
5H	13.318	12.884	13.776	25.089	24.974	24.705	13.326	24.922	11.771	12.090	11.771	12.090	0.452	0.890	0.295	0.731	0.540	0.815	0.815	0	0	0	0
6A	13.693	12.970	12.847	24.826	24.826	24.436	13.170	24.683	11.096	11.855	11.589	11.589	-0.224	0.656	0.955	1.168	0.635	0.516	0.516	0	0	0	0
6B	13.570	13.251	13.503	25.300	25.198	25.148	13.441	25.215	11.730	11.947	11.645	11.645	0.411	0.747	1.011	1.111	0.752	0.596	0.496	0	0	0	0
6C	14.169	13.984	15.023	25.218	24.734	24.675	14.392	24.787	10.783	10.749	10.749	9.651	-0.537	-0.451	-0.982	1.451	1.367	1.976	1.976	0	0	0	0
6D	14.063	13.282	14.230	25.418	25.065	24.733	13.858	25.005	11.154	11.783	11.783	10.503	-0.165	0.583	-0.131	1.121	0.668	1.095	1.095	0	0	0	0
6E	13.996	12.978	13.881	25.329	25.471	25.159	13.618	25.320	11.333	12.493	11.278	11.278	0.014	1.294	0.991	0.408	0.640	0.640	0.640	0	1	0	0
6F	12.914	13.589	13.963	25.330	25.484	24.901	13.489	25.259	12.616	11.682	11.011	11.011	1.297	-0.091	0.378	0.407	0.716	0.770	0.770	0	0	0	0
6G	13.917	13.792	14.110	25.295	25.295	25.123	13.940	25.146	11.808	11.109	11.109	10.942	0.247	0.489	0.308	0.843	1.065	0.808	0.808	0	0	0	0
6H	13.487	12.943	13.317	25.295	25.441	24.389	13.249	24.936	11.808	12.180	11.072	11.072	0.489	0.980	0.980	0.713	0.507	0.738	0.738	0	0	0	0
7A	13.707	13.053	14.262	25.641	24.790	25.445	13.844	25.292	11.395	11.227	11.183	11.183	0.615	0.027	0.550	0.653	0.981	0.683	0.683	0	0	0	0
7B	13.312	13.054	12.959	25.485	25.104	25.797	13.844	25.292	12.173	12.050	12.050	11.838	0.854	0.851	2.204	0.553	0.553	0.217	0.217	0	0	0	0
7C	13.551	13.618	13.234	25.189	25.247	24.835	13.468	25.263	11.638	11.629	11.629	12.118	0.319	0.429	1.484	0.802	0.743	0.357	0.357	0	0	0	0
7D	13.552	13.435	12.889	25.160	25.035	24.835	13.292	25.010	11.638	11.600	11.600	11.945	0.289	0.400	1.312	0.818	0.758	0.403	0.403	0	0	0	0
7E	14.333	14.985	14.894	25.398	25.289	24.875	14.737	25.121	10.866	10.304	9.981	9.981	-0.454	-0.896	-0.653	1.369	1.861	1.572	1.572	0	0	0	0
7F	13.816	12.613	14.140	25.834	25.292	25.173	13.523	25.433	12.018	12.679	12.018	12.018											

Sample	U6			hsa-miR-31			Average 31			DCT 31-U6			ddCT (DCT sample - dCT 0)			2Δ-ddCT			Score X<0.5			R	
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III		
2A	13.656	12.798	13.272	19.008	19.092	18.987	13.242	13.644	13.029	5.351	6.294	5.715	0.328	0.745	0.942	0.797	0.597	0.521	0	0	0	1	OK
2B	13.686	13.517	13.729	18.972	21.173	19.516	13.644	13.887	13.887	5.287	7.656	5.787	0.544	2.107	1.013	0.833	0.232	0.495	0	0	0	1	OK
2C	13.212	13.819	14.066	18.592	18.592	19.131	13.699	13.014	19.014	5.747	5.133	5.065	0.723	-0.316	0.287	0.006	1.334	0.817	0	0	0	0	OK
2D	12.777	12.297	13.451	19.052	19.171	19.131	12.862	19.088	19.088	6.775	6.875	5.620	1.251	1.416	0.847	0.420	0.399	0.556	1	1	1	0	OK
2E	13.040	13.680	13.571	18.966	20.288	18.917	13.431	13.394	19.394	5.925	6.618	5.446	0.301	1.069	0.572	0.365	0.477	0.673	0	0	1	0	OK
2G	13.849	12.478	13.550	18.906	18.906	19.034	13.803	13.002	19.002	6.899	4.836	5.302	-0.173	-0.713	0.528	1.091	1.639	0.693	0	0	0	0	OK
2H	14.007	13.920	13.483	18.906	18.757	18.784	13.803	13.816	18.816	5.697	5.918	5.945	0.673	0.598	1.141	0.627	0.774	0.453	0	0	0	0	OK
3A	13.175	13.311	13.875	18.872	19.250	19.790	12.959	19.790	12.959	5.918	5.829	6.192	0.894	0.280	0.280	0.538	0.823	0.374	0	0	0	0	OK
3B	14.250	14.022	14.224	19.408	19.076	19.275	14.169	19.283	19.283	5.247	5.004	5.051	0.244	-0.505	0.278	0.856	1.419	0.825	0	0	0	0	OK
3C	13.469	13.456	13.932	19.042	18.851	18.920	12.980	18.937	18.937	5.773	5.394	5.968	0.349	-0.155	0.214	0.883	1.113	0.882	0	0	0	0	OK
3D	13.160	12.202	13.578	19.194	19.051	19.245	12.980	19.164	19.164	6.093	6.849	5.667	1.011	1.300	0.894	0.466	0.466	0.338	1	1	1	0	OK
3E	13.160	12.277	13.737	19.194	18.755	18.848	13.375	18.808	18.808	5.660	5.528	5.111	0.368	-0.021	0.337	0.644	1.015	0.792	0	0	0	0	OK
3F	13.881	12.711	14.667	18.916	19.137	19.094	13.753	19.049	19.049	5.035	6.427	4.427	0.011	0.878	-0.347	0.999	0.544	1.272	0	0	0	0	OK
3G	13.061	13.285	14.136	18.899	18.880	18.823	13.514	18.867	18.867	5.838	5.595	4.627	0.814	0.046	-0.147	0.569	0.569	1.107	0	0	0	0	OK
3H	13.712	13.157	13.875	18.818	18.818	18.700	13.448	18.631	18.631	5.218	5.218	4.825	0.483	-0.331	0.052	0.716	1.258	0.965	0	0	0	0	OK
4A	13.546	13.598	13.624	19.093	19.035	18.968	13.589	19.032	19.032	5.347	5.437	5.344	0.323	0.571	0.571	0.686	1.081	0.673	0	0	0	0	OK
4B	14.024	13.883	13.925	19.229	18.768	19.185	13.584	19.061	19.061	5.205	4.885	5.240	0.181	-0.664	0.486	0.882	1.385	0.714	0	0	0	0	OK
4C	12.975	12.694	12.776	19.204	18.900	19.119	12.882	19.074	19.074	6.229	6.007	6.343	1.205	0.458	1.569	0.434	0.728	0.337	1	1	1	0	OK
4D	14.252	13.858	13.813	19.128	18.953	19.365	13.647	19.165	19.165	5.094	5.094	5.552	-0.098	-0.455	0.778	1.070	1.371	0.583	0	0	0	0	OK
4E	14.070	13.438	13.687	19.123	18.707	18.808	13.647	18.879	18.879	5.053	5.524	5.121	0.029	-0.025	0.348	0.980	1.018	0.786	0	0	0	0	OK
4F	13.328	13.433	13.452	18.799	18.812	19.295	13.382	18.990	18.990	5.271	5.724	5.804	0.247	0.200	1.030	0.843	0.870	0.490	0	0	0	0	OK
4G	14.286	13.824	14.022	19.269	18.915	19.041	14.044	19.075	19.075	4.982	5.092	5.019	-0.041	-0.457	0.245	1.029	1.373	0.844	0	0	0	0	OK
4H	12.958	13.353	12.901	18.977	18.497	18.848	13.071	18.754	18.754	6.019	5.083	5.947	0.396	-0.466	1.173	0.502	1.381	0.443	0	0	0	0	OK
4I	13.886	13.692	13.583	19.278	19.534	19.165	13.720	19.266	19.266	4.233	5.662	5.582	0.368	0.113	0.808	0.775	0.925	0.571	0	0	0	0	OK
4J	14.894	12.948	14.698	18.874	18.938	19.368	14.180	19.295	19.295	4.708	6.245	5.720	-0.790	0.699	0.892	1.244	0.617	0.515	0	0	0	0	OK
4K	14.165	12.977	13.186	19.127	19.298	19.188	13.363	18.924	18.924	5.109	6.040	5.446	0.085	0.491	0.672	0.949	0.711	0.627	0	0	0	0	OK
4L	13.354	13.067	13.742	19.063	19.108	19.188	13.588	19.120	19.120	5.109	6.040	5.446	0.085	0.491	0.672	0.949	0.711	0.627	0	0	0	0	OK
4M	13.188	14.035	13.728	19.030	19.150	18.996	13.601	19.059	19.059	5.842	5.115	5.268	0.818	-0.434	0.494	0.567	1.351	0.710	0	0	0	0	OK
4N	13.132	13.007	14.664	18.887	18.858	19.347	13.601	19.024	19.024	5.755	5.831	4.682	0.731	0.282	-0.091	0.602	0.823	1.065	0	0	0	0	OK
4O	13.078	13.967	13.967	18.718	18.758	18.949	13.362	18.808	18.808	5.714	5.874	4.982	0.616	0.185	0.209	0.653	0.892	0.885	0	0	0	0	OK
4P	13.019	13.042	13.776	18.770	18.758	19.154	13.362	19.256	19.256	5.452	5.874	4.982	0.428	0.325	0.209	0.743	0.798	0.285	0	0	0	0	OK
4Q	13.693	12.970	12.847	19.068	19.060	19.049	13.441	19.181	19.181	5.864	5.809	5.346	0.460	0.280	0.773	0.784	0.540	0.346	0	0	0	0	OK
4R	13.370	13.251	13.503	19.434	19.454	19.049	13.441	19.181	19.181	5.809	5.809	5.346	0.460	0.280	0.773	0.784	0.540	0.346	0	0	0	0	OK
4S	14.169	13.884	13.826	19.230	18.926	19.132	14.392	19.088	19.088	5.098	4.942	4.109	0.012	-0.607	-0.665	0.992	1.323	1.386	0	0	0	0	OK
4T	14.063	13.822	14.230	19.139	18.973	19.150	14.392	19.088	19.088	5.098	5.691	4.920	0.142	0.142	0.142	0.854	0.731	0.942	0	0	0	0	OK
4U	13.996	12.978	13.881	19.247	18.914	18.741	13.618	18.989	18.989	5.251	6.001	4.860	0.227	0.452	0.086	0.854	0.731	0.942	0	0	0	0	OK
4V	12.914	13.589	13.983	19.220	18.814	18.996	13.469	18.996	18.996	6.306	5.224	4.895	1.282	-0.325	0.220	0.411	1.252	0.839	0	0	0	0	OK
4W	13.917	12.943	13.317	18.934	18.707	18.897	13.469	18.996	18.996	5.016	4.915	4.876	-0.008	-0.694	0.103	1.005	1.252	0.931	0	0	0	0	OK
4X	13.487	13.943	13.317	18.934	19.049	18.883	13.249	18.876	18.876	5.440	6.096	5.546	0.416	0.546	0.773	0.750	0.884	0.585	0	0	0	0	OK
4Y	13.407	13.563	14.262	19.147	19.149	18.977	13.844	19.049	19.049	5.310	5.384	5.981	0.288	0.035	0.715	0.820	0.976	1.041	0	0	0	0	OK
4Z	13.112	13.054	12.959	19.713	19.005	18.947	13.108	19.222	19.222	6.041	5.951	5.988	1.377	0.402	1.215	0.385	0.797	0.431	1	1	1	0	OK
7A	13.551	13.024	13.234	18.982	19.111	18.941	13.468	19.022	19.022	5.431	5.493	5.746	0.407	-0.056	0.973	0.763	1.040	0.510	0	0	0	0	OK
7B	13.552	13.443	12.889	18.966	19.072	18.973	13.292	19.004	19.004	5.414	5.636	6.004	0.391	0.089	1.310	0.763	0.941	0.403	0	0	0	0	OK
7C	14.333	14.985	14.894	18.717	18.311	19.057	14.737	18.895	18.895	4.984	3.926	4.183	-0.640	-1.623	-0.611	1.558	3.081	1.327	0	0	0	0	OK
7D	14.316	12.613	14.140	18.819	19.113	19.321	13.523	19.064	19.064	5.003	6.493	4.183	-0.021	-0.950	-0.408	1.014	3.517	0.754	0	0	0	0	OK
7E	14.139	13.692	14.952	18.664	18.985	19.038	13.214	18.896	18.896	4.523	5.334	4.086	-0.099	-0.196	-0.688	1.413	1.145	1.611	0	0	0	0	OK
7F	13.113	13.059	13.226	18.904	18.520	19.153	13.178	20.080	20.080	5.950	5.344	5.944	0.866	-0.005	4.697	0.830	1.009	0.039	0	0	0		

Sample	U6			hsa-miR-30d			Average 30d			DCT 30d-U6			ddCT (DCT sample - dCT 0)			Zn-ddCT			Score X<0.5			R
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	
2A	13.656	12.798	13.272	22.071	21.973	22.537	13.242	22.194	22.345	8.415	9.175	9.265	0.316	0.290	0.881	0.804	0.818	0.543	0	0	0	0
2B	13.685	13.517	13.729	22.352	22.320	22.920	13.644	22.464	22.466	8.666	8.603	8.766	0.568	-0.281	0.806	0.674	1.215	0.572	0	0	0	0
2C	13.212	13.819	14.066	22.008	22.169	22.448	13.699	22.205	22.055	8.795	8.350	8.372	0.666	-0.335	-0.012	0.617	1.449	1.008	0	0	0	0
2D	12.770	12.997	13.451	22.147	22.163	22.695	12.842	22.335	22.335	9.569	9.866	9.244	1.270	0.982	0.860	0.415	0.566	0.551	1	0	0	0
2E	13.040	13.680	13.571	22.167	22.066	22.029	13.431	21.967	22.156	8.326	8.457	8.457	0.727	-0.559	0.073	0.604	1.473	0.951	0	0	0	0
2G	13.809	13.920	13.483	22.232	21.975	22.351	13.293	22.186	22.186	8.383	9.407	8.801	0.794	0.612	0.801	0.821	0.654	0.746	0	0	0	0
2G	13.175	13.311	13.875	22.599	21.940	22.437	13.803	22.325	22.325	8.592	8.020	8.954	0.492	-0.865	0.570	0.711	1.821	0.674	0	0	0	0
2H	13.175	13.311	13.875	22.407	22.137	22.330	13.454	22.291	22.291	8.425	8.455	8.455	1.132	-0.059	0.071	0.456	1.042	0.952	1	0	0	0
2H	13.175	13.311	13.875	22.071	21.753	22.233	12.990	22.233	22.233	8.847	8.806	8.334	0.748	-0.079	0.950	0.595	1.056	0.518	0	0	0	0
2H	14.250	14.032	14.224	21.971	22.087	22.738	14.169	22.409	22.409	7.617	8.590	8.515	-0.482	-0.295	0.131	1.397	1.227	0.913	0	0	0	0
2H	13.469	13.456	13.932	22.068	22.037	22.639	13.575	22.258	22.258	8.628	8.581	8.707	0.529	-0.304	0.322	0.689	1.324	0.805	0	0	0	0
2D	13.160	12.202	13.578	22.139	22.019	22.433	12.980	22.792	22.792	8.960	9.799	10.656	0.800	0.915	0.152	0.543	0.531	0.207	0	0	0	0
2D	13.160	13.277	13.737	22.823	21.909	22.433	13.375	22.055	22.055	8.663	8.682	8.682	0.633	-0.202	0.312	0.622	1.151	0.805	0	0	0	0
2E	13.881	12.711	14.667	22.069	22.069	22.200	13.753	22.096	22.096	8.188	9.308	7.533	0.888	0.423	-0.851	0.941	0.746	1.803	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.136	21.864	22.377	13.514	22.029	22.029	8.785	8.579	8.181	0.685	-0.305	0.203	0.622	1.236	1.151	0	0	0	0
2E	13.312	13.157	13.875	22.136	21.973	22.433	13.448	22.132	22.132	8.320	8.815	8.196	0.725	-0.069	0.427	0.605	1.307	1.139	0	0	0	0
2E	13.061	13.285	14.196	22.1																		

Sample	U6			hsa-miR-182			Average			DCT 182-U6			ddCT (DCT sample-DCT 0)			2 <sup>-ΔΔCT</sup>			Score X<0.5			R
	I	II	III	I	II	III	U6	182		I	II	III	I	II	III	I	II	III				
3F.	12.893	13.033	13.389	24.219	24.560	23.935	13.038	24.238	11.526	11.527	10.546	1.435	0.083	0.597	0.370	0.944	0.661	1	0	0	-	
3G.	13.053	13.221	13.758	24.102	25.166	24.499	13.344	24.589	11.049	11.945	10.741	0.958	0.500	0.792	0.515	0.707	0.577	1	0	0	-	
4A.	12.722	13.177	13.606	24.130	24.567	24.169	13.169	24.289	11.408	11.390	10.563	1.317	-0.055	0.614	0.401	1.039	0.653	1	0	0	-	
4C.	12.793	13.574	13.579	24.621	23.769	24.227	13.315	24.206	11.828	10.195	10.648	1.738	-1.249	0.699	0.300	2.377	0.616	1	0	0	-	
4E.	12.767	14.211	13.885	24.339	23.905	24.425	13.621	24.223	11.572	9.694	10.539	1.482	-1.750	0.590	0.358	3.365	0.664	1	0	0	-	
4F.	13.669	14.144	13.480	24.379	23.670	24.009	13.761	24.019	10.720	9.526	10.528	0.529	-1.918	0.579	0.546	3.760	0.669	0	0	0	-	
4G.	13.752	14.099	14.411	24.546	23.841	24.248	14.087	24.212	10.795	9.742	9.837	0.704	-1.703	-0.111	0.614	3.255	1.080	0	0	0	-	
5A.	14.825	16.062	13.626	24.797	24.144	24.167	14.771	24.370	10.153	8.082	10.541	0.082	-3.362	0.592	0.945	10.284	0.663	0	0	0	-	
5G.	13.837	13.240	13.855	23.990	24.652	23.894	13.644	24.179	10.453	10.911	10.039	0.065	-0.032	0.090	0.957	1.023	0.940	0	0	0	-	
6A.	14.315	13.934	13.800	24.659	24.845	24.601	14.016	24.702	10.344	10.911	10.802	0.253	-0.533	0.853	0.839	1.447	0.554	0	0	0	-	
6B.	13.911	13.833	13.480	24.304	24.289	23.891	13.741	24.165	10.394	10.466	10.411	0.503	-0.978	0.462	0.811	1.970	0.726	0	0	0	-	
6C.	13.281	14.380	14.723	24.908	23.897	23.982	14.121	24.282	11.707	9.517	9.259	1.817	-1.927	-0.890	0.326	3.803	1.813	1	0	0	-	
6E.	13.817	14.653	14.066	24.270	24.771	24.172	14.179	24.404	10.453	10.118	10.104	0.363	-1.327	0.156	0.778	2.508	0.898	0	0	0	-	
6F.	13.038	15.354	13.932	24.210	24.012	23.815	14.441	24.045	10.269	8.658	9.884	0.178	-2.786	-0.065	0.884	6.897	1.046	0	0	0	-	
8C.	13.941	15.029	14.231	24.123	24.484	24.412	14.400	24.340	11.182	9.455	10.181	0.091	-1.989	0.232	0.339	3.971	1.851	0	0	0	-	
9A.	13.472	14.950	14.227	24.601	24.438	24.504	14.216	24.514	11.129	9.488	10.277	1.039	-1.957	0.328	0.487	3.881	0.797	1	0	0	-	
10A.	13.211	12.768	13.786	24.079	24.716	24.126	13.285	24.307	10.868	11.948	10.341	0.777	-0.504	0.392	0.583	0.705	0.762	0	0	0	-	
10B.	14.454	13.806	13.763	24.427	25.146	23.923	14.008	24.499	9.974	11.340	10.160	-0.117	-0.105	0.211	1.084	1.075	0.864	0	0	0	-	
10D.	14.432	13.954	13.872	24.214	24.850	23.879	14.086	24.314	9.781	10.896	10.006	-0.309	-0.548	0.058	1.239	1.462	0.961	0	0	0	-	
Sample 0	15.058	13.158	14.322	25.148	24.602	24.271	14.179	24.674	10.090	11.444	9.949	0.000	0.000	0.000	1.000	1.000	1.000	0	0	0	-	

S8 Additional *In cell* Assay detail calculation for hsa-miR-182

Sample	U6			hsa-miR-31			Average			dCT 31-U6			ddCT (dCT sample-dCT 0)			2~ddCT			Score X<0.5			R
	I	II	III	I	II	III	U6	31	31	I	II	III	I	II	III	I	II	III	I	II	III	
3F-	12.693	13.033	13.989	18.193	17.998	17.679	13.038	18.057	18.057	5.500	4.965	4.580	2.041	-0.073	0.450	0.2430	1.0520	0.7322	1	0	0	-
3G-	13.093	13.221	13.758	18.098	18.192	18.068	13.344	18.119	18.119	5.045	4.971	4.310	1.586	-0.067	0.169	0.3531	1.0474	0.8892	1	0	0	-
4A-	12.722	13.177	13.606	18.049	18.200	18.280	13.169	18.176	18.176	5.327	5.022	4.674	1.869	-0.016	0.533	0.2738	1.0111	0.6910	1	0	0	-
4C-	12.793	13.574	13.579	18.140	18.135	18.161	13.315	18.145	18.145	5.348	4.562	4.581	1.889	-0.476	0.441	0.2700	1.3910	0.7369	1	0	0	-
4E-	12.767	14.211	13.885	18.175	18.258	18.548	13.621	18.327	18.327	5.408	4.047	4.663	1.949	-0.991	0.522	0.2589	1.9877	0.6965	1	0	0	-
4F-	13.659	14.144	13.480	18.268	18.235	18.492	13.761	18.342	18.342	4.610	4.091	5.011	1.151	-0.947	0.871	0.4503	1.9277	0.5469	1	0	0	-
4G-	13.752	14.099	14.411	18.440	18.132	18.456	14.087	18.342	18.342	4.688	4.033	4.045	1.229	-1.005	-0.096	0.4265	2.0075	1.0690	1	0	0	-
5A-	14.625	16.062	13.626	18.809	18.300	18.465	14.771	18.525	18.525	4.184	2.238	4.839	0.726	-2.800	0.698	0.6048	6.9646	0.6163	0	0	0	-
5G-	13.837	13.240	13.855	18.323	18.285	18.085	13.644	18.231	18.231	4.486	5.045	4.229	1.028	0.007	0.089	0.4905	0.9952	0.9404	1	0	0	-
6A-	14.315	13.934	13.800	18.602	18.284	18.393	14.016	18.427	18.427	4.287	4.350	4.593	0.829	-0.688	0.453	0.5631	1.6110	0.7307	0	0	0	-
6B-	13.911	13.833	13.480	18.198	18.173	18.132	13.741	18.157	18.157	4.288	4.340	4.652	0.829	-0.687	0.511	0.5629	1.6217	0.7017	0	0	0	-
6C-	13.261	14.830	14.723	18.123	18.134	18.215	14.121	18.355	18.355	4.862	3.754	3.482	1.404	-1.284	-0.649	0.3779	2.4349	1.5680	1	0	0	-
6E-	13.817	14.653	14.068	18.392	18.037	18.636	14.179	18.355	18.355	4.575	3.383	4.589	1.117	-1.655	0.428	0.4612	2.4349	0.7434	1	0	0	-
6F-	14.038	15.354	13.932	18.663	18.190	18.970	14.441	18.408	18.408	4.825	2.937	4.438	1.167	-2.201	0.298	0.4454	4.9884	0.8136	1	0	0	-
8C-	13.941	15.029	14.231	18.559	18.232	18.549	14.400	18.446	18.446	4.918	3.203	4.318	1.159	-1.835	0.177	0.4478	3.5881	0.8847	1	0	0	-
9A-	13.472	14.950	14.221	18.993	18.465	18.717	14.216	18.712	18.712	5.482	3.515	4.480	2.023	-1.523	0.349	0.2461	2.8731	0.7849	1	0	0	-
10A-	13.211	12.768	13.766	18.190	17.949	18.147	13.255	18.095	18.095	4.979	5.181	4.361	1.520	-0.143	0.220	0.3487	1.9054	0.8583	1	0	0	-
10B-	14.454	13.806	13.763	18.141	18.094	18.424	14.008	18.240	18.240	3.688	4.288	4.660	0.725	-0.750	0.519	0.8532	1.6817	0.6976	0	0	0	-
10D-	14.432	13.954	13.872	18.616	17.943	18.461	14.086	18.340	18.340	4.184	3.989	4.588	0.725	-1.049	0.447	0.6048	2.0690	0.7333	0	0	0	-
Sample 0	15.058	13.158	14.322	18.516	18.196	18.463	14.179	18.391	18.391	3.459	5.038	4.141	0.000	0.000	0.000	1.0000	1.0000	1.0000	0	0	0	-

S9 Additional *In cell* Assay detail calculation for hsa-miR-31

Sample	U6			hsa-miR-30d			Average		DCT 30d-U6			ddCT (dCT sample -dCT 0)			2 <sup>-ΔΔCT</sup>			Score X<0.5			R
	I	II	III	I	II	III	U6	30d	I	II	III	I	II	III	I	II	III	I	II	III	
3F.	12.693	13.033	13.389	23.001	23.487	23.481	13.038	23.323	10.308	10.454	10.093	1.397	0.217	0.625	0.3797	0.8603	0.6484	1	0	0	0
3G.	13.053	13.221	13.756	22.979	23.408	23.491	13.344	23.293	9.926	10.187	9.733	1.014	-0.050	0.265	0.4951	1.0354	0.8319	1	0	0	0
4A.	12.722	13.177	13.606	22.853	23.223	23.565	13.169	23.214	10.131	10.046	9.989	1.220	-0.191	0.491	0.4294	1.1419	0.7113	1	1	0	0
4C.	12.793	13.574	13.579	22.988	23.369	23.699	13.315	23.348	10.195	9.796	10.110	1.284	-0.441	0.642	0.4107	1.3578	0.6407	1	0	0	0
4E.	12.787	14.211	13.865	23.300	23.380	23.684	13.621	23.448	10.532	9.189	9.778	1.621	-1.008	0.311	0.3292	2.0967	0.8062	1	0	0	0
4F.	13.659	14.144	13.480	23.262	23.588	23.594	13.761	23.581	9.603	9.484	10.373	0.692	-0.753	0.906	0.6190	1.6857	0.5336	0	0	0	0
4G.	13.752	14.099	14.411	24.051	23.956	23.641	14.087	23.529	9.426	9.489	9.183	0.741	-0.748	-0.285	0.5985	1.6796	1.2183	0	0	0	0
5A.	14.625	16.082	13.626	24.051	32.956	23.641	14.771	26.863	9.540	16.894	10.015	0.515	6.657	0.548	0.7000	0.0099	0.6842	0	1	0	0
5G.	13.837	13.240	13.855	23.376	23.567	23.682	13.644	23.542	9.034	9.092	9.462	0.628	0.090	0.359	0.6470	0.0396	0.7796	0	0	0	0
6A.	14.315	13.934	13.800	23.349	23.026	23.262	14.016	23.212	9.622	9.086	9.827	0.122	-1.145	-0.006	0.9187	2.2119	1.0041	0	0	0	0
6B.	13.911	13.833	13.480	23.533	23.438	23.664	13.741	23.545	9.622	9.092	10.184	0.711	-0.631	0.716	0.6109	1.5489	0.6086	0	0	0	0
6C.	13.261	14.380	14.723	23.342	23.468	23.644	14.121	23.485	10.081	9.088	8.921	1.170	-1.149	-0.546	0.4445	2.2171	1.4604	1	1	0	0
6E.	13.817	14.653	14.068	23.444	23.570	24.002	14.179	23.672	9.627	8.916	9.935	0.715	-1.321	0.467	0.6090	2.4979	0.7235	0	0	0	0
6F.	14.038	15.029	13.932	24.040	23.723	23.710	14.441	23.833	10.002	8.396	9.779	1.091	-1.841	0.311	0.4696	3.5829	0.8060	1	1	0	0
8C.	13.941	15.029	14.231	23.897	23.723	23.824	14.400	23.815	9.956	8.694	9.593	1.045	-1.543	0.125	0.4847	2.9148	0.9166	1	1	0	0
9A.	13.472	14.960	14.227	24.045	24.350	23.991	14.216	24.129	10.573	9.400	9.764	1.662	-0.837	0.296	0.3161	1.7865	0.8143	1	1	0	0
10A.	13.211	12.768	13.786	23.407	23.319	23.545	13.265	23.424	10.196	10.551	9.760	1.284	0.314	0.292	0.4105	0.8044	0.8166	1	0	0	0
10B.	14.454	13.806	13.763	23.912	23.480	23.923	14.008	23.772	9.459	9.673	10.160	0.547	-0.564	0.692	0.6845	1.4762	0.6190	0	0	0	0
10D.	14.432	13.954	13.872	23.925	23.419	23.733	14.086	23.692	9.493	9.465	9.860	0.581	-0.772	0.393	0.6684	1.7080	0.7617	0	0	0	0
NMI RT	15.058	13.158	14.322	23.969	23.395	23.789	14.179	23.718	8.912	10.237	9.468	0.000	0.000	0.000	1.0000	1.0000	1.0000	0	0	0	0

**S10 Additional *In cell* Assay detail calculation for hsa-miR-30d**



Substrate 25 nM

Time Sampling (min)	CT mean Value		Conc (uM) mean		Δ CT to 0 min
	Ct Value	SD	Conc (uM)	SD	
0	33.488	0.7355	0.00710	7.59.E-06	0.0000
2.5	32.258	0.1585	0.00684	3.53.E-06	1.2301
5	32.156	0.4772	0.00959	9.18.E-06	1.3323
10	30.820	0.0661	0.01216	4.20.E-06	2.6681
20	30.128	0.0519	0.02293	2.63.E-05	3.3602
40	30.147	0.0193	0.02960	1.52.E-05	3.3411
80	29.109	0.0460	0.10214	1.28.E-04	4.3797
180	27.467	0.0142	0.15325	1.61.E-04	6.0209

Substrate 50 nM

Time Sampling (min)	CT mean Value		Conc (uM) mean		Δ CT to 0 min
	Ct Value	SD	Conc (uM)	SD	
0	32.855	0.0989	0.00546	3.76.E-05	0
2.5	31.698	0.1315	0.00323	3.15.E-06	1.156601
5	32.045	0.1335	0.00294	1.08.E-05	0.809743
10	31.241	0.1030	0.00473	2.30.E-05	1.613736
20	28.993	0.1065	0.02149	3.23.E-05	3.861771
40	28.943	0.0782	0.02607	2.38.E-05	3.912268
80	27.624	0.0773	0.10497	5.74.E-04	5.230887
180	26.126	0.0885	0.38511	2.24.E-03	6.728572

**S11** Detail calculation of 25 nM and 50 nM substrate for velocity plot

Substrate 100 nM

Time Sampling (min)	CT mean Value		Conc (uM) mean		Δ CT to 0 min
	Ct Value	SD	Conc (uM)	SD	
0	29.508	0.0486	0.06489	1.43.E-04	0
2.5	30.811	0.0916	0.15440	9.02.E-05	0.32676
5	32.382	0.1918	0.02403	3.15.E-05	-1.19671
10	30.717	0.0719	0.07559	8.01.E-05	-0.30246
20	30.246	0.1046	0.52890	1.47.E-04	1.909919
40	30.136	0.0210	0.89781	2.55.E-04	2.727061
80	29.867	0.1016	0.76997	2.91.E-03	3.364975
180	27.966	0.0899	4.18384	4.63.E-03	5.561035

Substrate 200 nM

Time Sampling (min)	CT mean Value		Conc (uM) mean		Δ CT to 0 min
	Ct Value	SD	Conc (uM)	SD	
0	28.963	0.1185	0.00630	1.98.E-02	0
2.5	27.124	0.1121	0.02376	2.06.E-01	1.83901
5	24.958	0.0143	0.07054	1.26.E-01	4.004938
10	24.538	0.0639	0.09258	2.83.E-01	4.425247
20	23.639	0.0941	0.16015	4.96.E-01	5.324462
40	22.217	0.0538	0.37632	9.31.E-01	6.74645
80	22.257	0.0316	0.39424	2.23.E+00	6.705735
180	20.325	0.1173	1.17342	1.07.E+00	8.638033

**S12** Detail calculation of 100 nM and 200 nM substrate for velocity plot

Substrate 300 nM

Time Sampling (min)	CT mean Value		Conc (uM) mean		$\Delta$ CT to 0 min
	Ct Value	SD	Conc (uM)	SD	
0	26.884	0.0249	0.25513	4.85.E-04	0
2.5	26.780	0.1028	0.26920	4.11.E-04	0.103791
5	24.641	0.0516	1.03634	1.20.E-03	2.24324
10	26.052	0.0258	0.60742	3.12.E-03	0.831655
20	25.140	0.0174	0.78602	1.79.E-03	1.744209
40	24.442	0.0210	1.21573	2.58.E-03	2.442131
80	24.021	0.0653	1.56371	2.72.E-03	2.862636
180	20.999	0.0176	11.62720	3.76.E-02	5.884878

Substrate 400 nM

Time Sampling (min)	CT mean Value		Conc (uM) mean		$\Delta$ CT to 0 min
	Ct Value	SD	Conc (uM)	SD	
0	29.001	0.0494	0.07517	2.35.E-04	0
2.5	27.045	0.0270	0.37456	2.12.E-03	1.955842
5	23.948	0.0034	1.64587	4.58.E-03	5.052423
10	23.198	0.0114	2.53681	5.79.E-03	5.802208
20	22.416	0.0187	3.92261	3.66.E-03	6.585011
40	24.739	0.0251	0.93875	8.95.E-04	4.262106
80	21.759	0.0095	8.59525	4.47.E-02	7.241521
180	19.526	0.0501	23.16750	1.72.E-02	9.474163

**S13** Detail calculation of 300 nM and 400 nM substrate for velocity plot

## List of presentations

1. **Muhammad Nurrohman Sidiq**, Asako Murata, and Kazuhiko Nakatani, "Screening of small molecules that interfere dicer-mediated processing of pre-miR-182/31/30d", Japan Society of Chemical Biology, virtual meeting, June 2021 (Poster)
2. **Muhammad Nurrohman Sidiq**, Asako Murata, and Kazuhiko Nakatani, "qPCR-based screening methods for small molecules that modulate dicer-mediated pre-miR-182/31/30d", The 48<sup>th</sup> International Symposium of Nucleic Acids Chemistry, virtual meeting, November 2021 (Poster)