PANArray™ miRNA expression profiling kit

Ver. 1: Expression profiling of **cancer**-related miRNAs [PM-1001]

Ver. 2: Expression profiling of **stem cell**-related miRNAs [PM-2001]

Instruction manual

Storage: Room temperature for slides and buffers and -20°C for labeling kit
Warning: For research use only

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I. PRODUCT SUMMARY

Contents and storage

Microarray slides [product # PM-1001A or PM-2001A]

PANArray™ miRNA expression profiling kit consists of positive control probes and 135 or 124 probes for cancer- or stem cell-related miRNAs, respectively, in human as annotated in miRBase Release 10.1.

This kit contains five microarray glass slides which are ready for use. You can test four samples each slide, 20 test per kit.

Storage: Room temperature

Hybridization buffer [product # PA1102]: 1 ml X 2 tubes

Storage: Room temperature

Washing buffer (20X) [product # PA1103]: 250 ml X 1 bottle

Storage: Room temperature

Labeling kit [product # PM-1001B or PM-2001B]

- pCp-Cy3: 63 µl X 1 tube
- T4 RNA ligase: 23 µl X 1 tube
- 10X T4 RNA ligase buffer: 250 µl X 1 tube
- 0.1% BSA: 250 µl X 1 tube

Storage: - 20°C

II. INTRODUCTION

MicroRNAs (miRNAs) are a class of small noncoding RNA, typically 18-25 nt in length that controls gene expression at the post-transcriptional level such as development, cell proliferation, differentiation and metabolism (1-3). Recently, the data base (http://microrna.sanger.ac.uk/ release 10.1: 2007) contains over 5,000 validated miRNAs sequences from 58 species (2). Growing evidence suggests that miRNAs are important regulators of cell division and differentiation as well as human cancer.

Detection of miRNAs the most commonly found in cancer

miRNAs modulate a variety of cellular pathways including development, differentiation, cell proliferation and apoptosis, and regulation of miRNA expression underlies specific oncogenic events in human cancer. It has been shown that miRNA signatures can distinguish normal from tumor tissue, cancer type and tissue of origin, and may also correlate with disease outcome. There is a general down and up regulation of a number of miRNAs in tumors compared with normal tissues in multiple human cancers. Hierarchical clustering analysis of miRNA expression profiles is able to distinguish tumor from normal pancreas, pancreatitis and cell lines. miRNA signatures have also been reported in chronic lymphocytic leukemia (CLL), lung cancer, pituitary adenomas, uterine leiomyomas and adult acute myeloid leukemia (AML). Of considerable interest has been the demonstration that miRNA expression profiles successfully classify poorly differentiated tumors. In addition, miRNA expression profiles have been shown to correlate with disease outcome (4-7). PANArray™ miRNA expression profiling kit is a PNA-based microarray for expression profiling of stem cell-related miRNAs including novel miRNA labeling method. Peptide nucleic acid (PNA) is an artificial nucleotide, in which the entire negatively charged sugar-phosphate backbone is replaced with a neutral N-(2-amino ethyl)-glycine units repeatedly linked by peptide bonds. Because PNA is uncharged unlike DNA, the electrostatic repulsion is greatly reduced between PNA/RNA duplex compared to its DNA/RNA equivalent, which results in a stronger binding affinity

between PNA/RNA strands. The neutral character of PNA provides a constant binding strength independent of the changes of ionic strength and pH. Also PNA is highly stable chemically and biologically.

We have developed our own proprietary labeling technique, ‘labeling on chip’ which directly labels mature miRNAs after hybridization on microarray slide. Total RNA is hybridized to the slide and then labeling process is performed by using the bound miRNAs as enzymatic ligation with pCp-Cy3.

The advantage of this labeling technique is improved that enhances specificity, the specificity of a standard hybridization assay with the high discrimination power (7).

III. ADVANTAGES

The PANArray™ miRNA expression profiling kit has the following advantages.

1. High specificity

'Labeling on chip' method is more specific for signal intensity than conventional 'labeling in suspension' method.

2. High sensitivity

PANArray™ miRNA expression profiling kit detects miRNAs at <0.5 amole.

3. High reproducibility

PANArray™ miRNA expression profiling kit is highly reproducible and can be repeatedly used after a washing-off that eliminates 'chip-to-chip' variation.

4. Fast hybridization (4 hours)

PANArray™ miRNA expression profiling kit needs only 4 hours for hybridization, whereas other miRNA array systems need from 16 to 20 hours.

5. Small amount of sample

PANArray™ miRNA expression profiling kit uses 400 ng total RNA.

IV. PROCEDURES

Denaturation

Step 1. Denaturation reaction

Hybridization

Step 2. Hybridization reaction

Step 3. Wash the microarray slides

Labeling

Step 4. Ligation reaction

Step 5. Wash the microarray slides

Scanning

Step 6. Scan the microarray slides

PANArray™ miRNA expression profiling kit uses cyanine 3-labeled targets to measure miRNAs in experimental and control samples. Figure 1 shows a standard workflow for sample preparation, hybridization and labeling processes.

Workflow

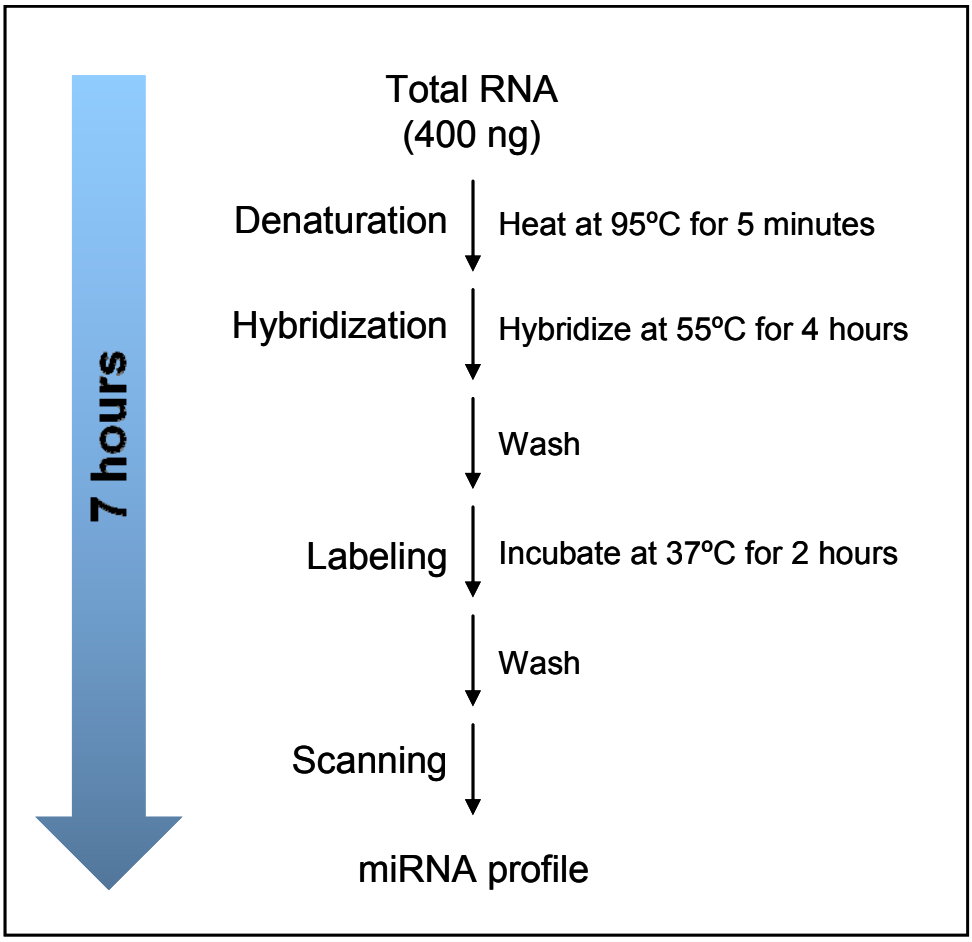

Figure1. Workflow of PANArray™ miRNA expression profiling kit

PANArray™ miRNA expression profiling kit generates fluorescent miRNAs with a sample input of 400 ng of total RNA. This method involves ligation of one pCp-Cy3 molecule to the 3' end of a RNA molecule with greater than 95% efficiency. The pCp-Cy3 is provided in the labeling kit (product #: PM-1001B or PM-2001B).

Denaturation

Step 1. Denaturation reaction

1. Prepare the total RNA denaturation mixture by gently mixing the components listed in Table 1 and maintain on ice.

Table 1. Denaturation mixture

Components	Amount
Total RNA sample	400 ng
RNase-free water	Up to 15 µl

2. Incubate at 95°C in a circulating water bath or heat block for 5 minutes.

CAUTION!

Incubate the sample for no less than 5 minutes and no more than 10 minutes, or the labeling efficiency of the sample may be affected.

3. Immediately transfer to ice.

4. Continue to the next step immediately.

Hybridization

Step 2. Hybridization reaction

1. Add 85 µl of hybridization mixture (listed in Table 2) to each sample for a total volume of 100 µl.

Table 2. Hybridization mixture

Components	Volume (µl)
Denatured RNA sample	15
Hybridization buffer	85
Total volume	100

2. Mix well and gently vortex for 2-3 seconds.
3. Preheat an incubator, such as a hybridization oven, to 55°C.
4. Set up a slide chamber on microarray slide.
5. Add all the mixtures into each well.
6. Hybridize at 55°C for 4 hours. Prevent desiccation by maintaining humidity.

Step 3. Wash the microarray slides

1. Dilute the 20X washing buffer to 1X washing buffer of 250 ml.
2. Add 1X washing buffer with a magnetic stir bar in the glass jar.
3. Carefully remove the slide chamber from the microarray slides.
4. Load the slides into a slide holder and immerse them in 1X washing buffer in a glass jar.
5. Wash the slides at room temperature for 5 minutes in 1X washing buffer.
6. Decant the buffer and repeat this washing step with fresh washing buffer.
7. Spin dry at 1,000 rpm for 5 minutes.

Labeling

Step 4. Ligation reaction

1. Warm the 10X T4 RNA ligase buffer at 37°C and vortex until all precipitates are dissolved.

CAUTION!
Ensure that the 10X T4 RNA ligase buffer is cooled to room temperature before you proceed. Failure to do so may affect the T4 RNA ligase activity, and thus the labeling efficiency.

2. Prior to use, prepare the ligation mixture by gently mixing the components listed in Table 3 and maintain on ice.

Table 3. Ligation mixture

Components	Volume (µl) per reaction	Volume (µl) per 4 reactions
10X T4 RNA ligase buffer	10	40
0.1% BSA	2	8
pCp-Cy3	3	12
T4 RNA ligase (10 U/µl)	1	4
RNase-free water	84	336
Total volume	100	400

CAUTION!
Be sure to use the ligation mixture within 15 minutes after mixing all the components in Table 3. Failure to do so may affect the labeling efficiency.

3. Set up a slide chamber on the hybridized microarray slide.
4. Immediately add 100 µl of the ligation mixture into each well.
5. Incubate at 37°C for 2 hours. Keep humidity.

Step 5. Wash the microarray slides

1. Immediately proceed to the washing step (step 3 on page 10).
2. Scan slides immediately. If necessary, store slides in the dark until use.

Scanning

Step 6. Scan the microarray slides

[Scanner: GenePix4000B Axon Instruments]

1. Check the default preferences for the laser power (100% each), PMT Gain 700 and 532 nm.
2. Scan.
3. Run the appropriate protocol.
4. Open the scanned tif with the gal file.
5. Convert ‘GPR file’ to ‘Excel file’.

V. REFERENCES

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6. Mack, G.S. (2007) MicroRNA gets down to business. *Nat Biotechnol*, **25**, 631-638.
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VI. SCHEMATIC DIAGRAM-1 (cancer-related miRNAs)

PM		miR-1	miR-1	miR-124a	miR-124a	miR-133b	miR-133b	miR-142-5p	miR-142-5p
U6	U6	miR-100	miR-100	miR-125a	miR-125a	miR-134	miR-134	miR-143	miR-143
let-7a	let-7a	miR-101	miR-101	miR-125b	miR-125b	miR-135a	miR-135a	miR-145	miR-145
let-7b	let-7b	miR-103	miR-103	miR-126	miR-126	miR-135b	miR-135b	miR-146a	miR-146a
let-7c	let-7c	miR-106a	miR-106a	miR-127	miR-127	miR-136	miR-136	miR-146b	miR-146b
let-7d	let-7d	miR-106b	miR-106b	U6	U6	miR-137	miR-137	miR-148a	miR-148a
let-7e	let-7e	miR-107	miR-107	miR-127-5p	miR-127-5p	miR-140-3p	miR-140-3p	miR-149	miR-149
let-7f	let-7f	miR-10a	miR-10a	miR-128	miR-128	miR-140-5p	miR-140-5p	miR-150	miR-150
let-7g	let-7g	miR-10b	miR-10b	miR-132	miR-132	miR-141	miR-141		
let-7i	let-7i	miR-122	miR-122	miR-133a	miR-133a	miR-142-3p	miR-142-3p		PM

PM		miR-17-3p	miR-17-3p	miR-188-5p	miR-188-5p	miR-196b	miR-196b	miR-200c	miR-200c
U6	U6	miR-181a	miR-181a	miR-18a	miR-18a	miR-197	miR-197	miR-202	miR-202
miR-151	miR-151	miR-181b	miR-181b	miR-18b	miR-18b	miR-198	miR-198	miR-203	miR-203
miR-153	miR-153	miR-181c	miR-181c	miR-190b	miR-190b	miR-199a	miR-199a	miR-204	miR-204
miR-154	miR-154	miR-181d	miR-181d	miR-191	miR-191	miR-199a-3p	miR-199a-3p	miR-205	miR-205
miR-155	miR-155	miR-182	miR-182	U6	U6	miR-199b	miR-199b	miR-206	miR-206
miR-15a	miR-15a	miR-183	miR-183	miR-192	miR-192	miR-19a	miR-19a	miR-20a	miR-20a
miR-15	miR-15	miR-185	miR-185	miR-194	miR-194	miR-19b-3	miR-19b-3	miR-21	miR-21
miR-16	miR-16	miR-186	miR-186	miR-195	miR-195	miR-200a	miR-200a		
miR-17	miR-17	miR-188-3p	miR-188-3p	miR-196a	miR-196a	miR-200b	miR-200b		PM

PM		miR-221	miR-221	miR-27b	miR-27b	miR-30c	miR-30c	miR-7	miR-7
U6	U6	miR-222	miR-222	miR-28-5p	miR-28-5p	miR-31	miR-31	miR-9	miR-9
miR-210	miR-210	miR-223	miR-223	miR-296-3p	miR-296-3p	miR-342-3p	miR-342-3p	miR-9*	miR-9*
miR-214	miR-214	miR-224	miR-224	miR-296-5p	miR-296-5p	miR-342-5p	miR-342-5p	miR-92a	miR-92a
miR-215	miR-215	miR-23a	miR-23a	miR-29a	miR-29a	miR-34a	miR-34a	miR-92b	miR-92b
miR-216a	miR-216a	miR-24	miR-24	U6	U6	miR-368	miR-368	miR-93	miR-93
miR-216b	miR-216b	miR-25	miR-25	miR-29b	miR-29b	miR-372	miR-372	miR-95	miR-95
miR-218	miR-218	miR-26a	miR-26a	miR-29c	miR-29c	miR-373	miR-373	miR-99a	miR-99a
miR-219-5p	miR-219-5p	miR-26b	miR-26b	miR-30a	miR-30a	miR-375	miR-375		
miR-22	miR-22	miR-27a	miR-27a	miR-30b	miR-30b	miR-488	miR-488		PM

VI. SCHEMATIC DIAGRAM-2 (stem cell-related miRNAs)

PM		miR-1	miR-1	miR-122	miR-122	miR-130a	miR-130a	miR-137	miR-137
let-7a	let-7a	miR-100	miR-100	miR-124a	miR-124a	miR-130b	miR-130b	miR-138	miR-138
let-7b	let-7b	miR-101	miR-101	U6	U6	miR-132	miR-132	miR-141	miR-141
let-7c	let-7c	miR-103	miR-103	miR-125a	miR-125a	miR-133a	miR-133a	miR-142-3p	miR-142-3p
let-7d	let-7d	miR-106a	miR-106a	miR-125b	miR-125b	miR-133b	miR-133b	miR-142-5p	miR-142-5p
let-7e	let-7e	miR-106b	miR-106b	miR-126	miR-126	miR-134	miR-134	miR-146a	miR-146a
let-7f	let-7f	miR-107	miR-107	miR-127-3p	miR-127-3p	miR-135a	miR-135a	miR-146b	miR-146b
let-7g	let-7g	miR-10a	miR-10a	miR-127-5p	miR-127-5p	miR-135b	miR-135b		
let-7i	let-7i	miR-10b	miR-10b	miR-128	miR-128	miR-136	miR-136		PM

PM		miR-17	miR-17	miR-18b	miR-18b	miR-199a	miR-199a	miR-20a	miR-20a
miR-149	miR-149	miR-17-3p	miR-17-3p	miR-191	miR-191	miR-19a	miR-19a	miR-20b	miR-20b
miR-150	miR-150	miR-181a	miR-181a	miR-192	miR-192	miR-19b	miR-19b	miR-21	miR-21
miR-153	miR-153	miR-181b	miR-181b	miR-193a-5p	miR-193a-5p	miR-200a	miR-200a	miR-22	miR-22
miR-154	miR-154	miR-181c	miR-181c	miR-193b	miR-193b	miR-200b	miR-200b	miR-210	miR-210
miR-155	miR-155	miR-181d	miR-181d	miR-195	miR-195	miR-200c	miR-200c	miR-214	miR-214
miR-15a	miR-15a	miR-182	miR-182	miR-196a	miR-196a	miR-205	miR-205	U6	U6
miR-15	miR-15	miR-183	miR-183	miR-196b	miR-196b	miR-206	miR-206		
miR-16	miR-16	miR-18a	miR-18a	miR-197	miR-197	miR-208b	miR-208b		PM

PM		miR-24	miR-24	miR-30d	miR-30d	miR-369-5p	miR-369-5p	miR-488	miR-488
miR-215	miR-215	miR-25	miR-25	miR-32	miR-32	miR-370	miR-370	miR-7	miR-7
miR-218	miR-218	miR-26a	miR-26a	U6	U6	miR-371-3p	miR-371-3p	miR-9	miR-9
miR-219-5p	miR-219-5p	miR-26b	miR-26b	miR-339-5p	miR-339-5p	miR-371-5p	miR-371-5p	miR-92	miR-92
miR-221	miR-221	miR-301a	miR-301a	miR-33a	miR-33a	miR-372	miR-372	miR-93	miR-93
miR-222	miR-222	miR-302a	miR-302a	miR-34a	miR-34a	miR-373	miR-373	miR-96	miR-96
miR-223	miR-223	miR-30a	miR-30a	miR-367	miR-367	miR-375	miR-375	miR-99a	miR-99a
miR-23a	miR-23a	miR-30b	miR-30b	miR-368	miR-368	miR-424	miR-424		
miR-23b	miR-23b	miR-30c	miR-30c	miR-369-3p	miR-369-3p	miR-452	miR-452		PM