



Whitehead Institute

Center for Microarray Technology

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The WICMT is now offering a microRNA expression profiling service utilizing a microarray system that was developed specifically for microRNA detection.

Arrays:

The array content we have printed is distributed by Exiqon. Information about the array can be found here:

<http://exiqon.com/SEEEMS/26.asp>

The arrays contain approximately 1,500 unique probes printed in duplicate on CodeLink slides from GE biosystems. The probes represent all known microRNAs in all organisms as defined by the miRBase 8.1 database at The Wellcome Trust Sanger Institute. A unique feature of the array is that the probes are made of Locked Nucleic Acid (LNA) which gives higher Tms than DNA which is important when trying to capture small targets.

Labeling:

Currently we are offering the labeling kit from Exiqon. The kit works by ligating a labeled primer onto the RNA transcripts and directly hybridizing them to the array. It requires 5ug of total RNA and actually works best with NO size enrichment. The kit is two channel (cy3/cy5) offering the opportunity for ratio-metric analysis of two samples on one array.

Other labeling protocols may also work with these arrays. If anybody finds the Exiqon kit to be inadequate there are other options we can pursue. The Exiqon kit is very easy to use but may have limitations. For instance, the ligation-labeling technique does not seem to work well on size fractionated samples, possibly due to concentration effects.

RNA:

Most companies now have kits available for purification of RNA that will retain small RNAs. For example:

Qiagen's new miRNA easy mini kit:

<http://www1.qiagen.com/Products/RnaStabilizationPurification/microRNA/miRNeasyMiniKit.aspx?ShowInfo=1>

or

Ambion's mirVana kit:

<http://www.ambion.com/catalog/CatNum.php?1560>

Other RNA purification techniques may also work however it is important to make sure the small RNAs are retained in the sample. It is also important that ALL traces of phenol be removed as this is the most common source of labeling failure in expression profiling.

Hybridization:

We intend to use the Agilent SureHybe system for these arrays.

[http://exiqon.com/uploads/Agilent_SureHyb_protocol\(3\).pdf](http://exiqon.com/uploads/Agilent_SureHyb_protocol(3).pdf)

There are two arrays printed on each slide so with the segmented chambers and two channel analysis we can profile 4 distinct samples on each slide.

We are also evaluating the Tecan and MAUI automated hybridization stations, so this may be another option in the future but for now the Agilent system seems to work very well.

Analysis:

Normalization and analysis may be a little tricky on these arrays due to the relatively small number of probes. There are spike-in controls that might be useful, but we need to gain more experience before we know which strategies work best. Users should be prepared to spend some time analyzing their data. Exiqon has expressed willingness to help with analysis and hopefully their expertise with the platform will help us determine the best way to analyze the data.

Sample Submission:

We are ready to begin accepting sample for full service expression profiling. We ask users to provide 6ug of total RNA. We will use 1ug for RNA quality control with the Bioanalyzer and 5ug to label and hybe. The kit claims to work with as little as 1ug of total RNA but we have not tested this yet. If you cannot get 5ug for the hybe, talk to us and we can try it with less. Please use the enclosed form for sample submission. If you have questions about sample submission, please contact Sumeet Gupta (sgupta@wi.mit.edu, phone: 258-8803)

Pricing:

Based on the cost of printing and the array content itself, as well as the expected volume of sales we have set the cost of the arrays at \$200 per slide. There are 2 arrays on each slide so the array cost is \$100 each, or \$50 per sample with two color analysis.

Full-service including all reagents, labor and equipment charges are approximately \$90 per sample (\$180 per array, \$360 per slide). Volume discounts are available beginning at 12 samples when submitted at the same time.

Cost summary:

Full-Service including array.

Per sample: ~\$140

Per Array (2 samples): ~\$275

Per slide (4 samples): ~ \$550

Please feel free to contact me or Sumeet Gupta (sgupta@wi.mit.edu) with any questions.

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