Summary of the MAQC Project Meeting

The MAQC Project: Calibrated RNA Samples, Reference Datasets, and QC Metrics/Thresholds for Microarray Quality Control

Meeting Date: May 2-3, 2005
Meeting Place: FDA Parklawn Building, 5600 Fishers Lane, Rockville, MD 20857
Summary Date: May 10, 2005
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Day 1: 8:00 AM – 5:00 PM, Monday, May 2, 2005

What Makes the MAQC Project Different from Other Efforts?

Felix Frueh (FDA/CDER) gave a brief summary of the aggressive progress of the MAQC project. Leming Shi (FDA/NCTR) reiterated the goals of the MAQC project and why the MAQC project is complementary to other ongoing efforts (*e.g.*, ERCC and NIST Gene Expression Metrology Program) aimed at microarray quality control and standardization. Leming Shi emphasized again the ultimate importance of the **availability** and **accessibility** of the two RNA samples used in the MAQC project to **the general microarray community beyond the MAQC project**. It was also stressed that for RNA samples from human donors it is essential to make an RNA batch in a quantity that is **available for the microarray community to use for several years**. The availability of the same RNA samples used in the MAQC project, the large reference datasets by different microarray platforms and other independent technologies, and the derived QC metrics/thresholds will help individual microarray laboratories to better assess performance and to avoid procedural failures. Studies (including those published in *Nature Methods*, May 2005) aimed at microarray standardization and quality control would have been much more valuable if the same RNA materials were available to the microarray community.

Leming Shi outlined the goals of the meeting:

1. To select two RNA samples for the main study from four candidates (A. Ambion Brain RNA; B. Ambion Liver RNA; C. Clontech UHRR; and D. Stratagene UHRR);
2. To design the main study; and
3. To select 1,000 genes for QRT-PCR verification

by reviewing the datasets generated in the MAQC pilot study.

Leming Shi also reiterated the six criteria originally proposed during the February 11, 2005 MAQC project meeting on RNA sample selection:

1. Available in large quantity;
2. Reproducibility in production;
3. High quality;
4. Accessibility (commercial source);
5. Wide gene presence; and
6. Large fold changes for a number of genes.

The MAQC pilot study was designed to provide quantitative information to evaluate the candidate RNA samples based on (5) gene presence, (6) fold change (differential gene expression), and (3) RNA quality.

Presentations on the Analysis of the MAQC Pilot Datasets

Jacques Retief (Affymetrix) and Weida Tong (FDA/NCTR) co-chaired the morning's session on the analysis of the pilot study datasets. Yaron Turpaz (Affymetrix), Paul Wolber (Agilent), Mike Wilson (Ambion), Lu Zhang (Applied Biosystems), Rich Shippy (GE Healthcare), Leming Shi (NCTR), Walter Liggett (NIST), Rob Clum (Stratagene), and Rick Jensen (UMass Boston) gave a brief presentation on each organization's analysis results. Clontech did not provide the analysis results and Laurence Lamarcq (Clontech) could not attend the meeting. Shawn Baker (Illumina) could not attend the meeting but provided a short presentation with analysis results that was shown to the attendees. Each presentation was followed by ~5 mins discussion. After the presentations, there was a general discussion for about 30 mins before lunch. It was emphasized that the ranking of the four RNA samples by quality and gene presence was straightforward and different data analysis sites reached very consistent ranking. However, the tricky issue was how to rank the six sample pairs by *differential* gene expression. The diversity of the analysis methods applied for identifying genes with differential expression for the six sample pairs resulted in inconsistent ranking of sample pairs, as expected. It was decided at the end of the discussion that instead of directly ranking the four candidate RNA samples, the selection should be focusing on the ranking of the six sample pairs.

Paul Wolber co-chaired the afternoon's sessions on RNA sample selection (with Jim Fuscoe of NCTR), sample-mixing strategies (with Uwe Scherf of CDRH), and independent verification (with Federico Goodsaid of CDER).

It was proposed (by *e.g.* Lu Zhang and Rich Shippy) and accepted by the MAQC group that the balance of the number and magnitude of up- and down-regulated genes should also be considered as a criterion for selecting the best RNA sample pair. All attendees were invited to vote for their favorite sample pairs based on the following three criteria:

1. Gene coverage;
2. Differential gene expression (fold change);
3. Balance of the number and magnitude of up- and down-regulated genes.

The results are shown in Table 1.

Table 1. Voting results on RNA sample pairs

Criteria	A-D Pairing	A-B Pairing	C-D Pairing
Gene coverage	18	5	
Differential gene expression (fold change)	16	9	
Balance of up- and down-regulated genes	19	2	2

A second round of voting between A-D and A-B pairs confirmed the preference of the MAQC group on the choice of A (Ambion brain RNA) and D (Stratagene UHRR) for the MAQC main study. The MAQC group reiterated that all the four RNA samples were of good quality compared to samples one would normally work with in the real biological experiment. The MAQC group emphasized that the selection of the two RNA samples for the MAQC study did not imply that these two samples were superior to other products. Instead, it only reflected the factor that they best met the criteria set specifically for the MAQC project.

Stratagene has a stock of over 2 grams of UHRR, which is a mixture of RNAs from 10 human cell lines. Stratagene has demonstrated that the cell line based UHRR could be produced at reasonable batch-to-batch reproducibility. Ambion is fully committed to producing the brain RNA sample with the highest quality and reasonable quantity (*e.g.*, each batch will be enough for the microarray community to use for about three years). The regeneration of relatively consistent brain RNA sample was discussed during the MAQC meeting, and Ambion is going to use a fairly substantial number of donors to minimize the batch-to-batch variation.

During the session on Titration Strategies, Rich Shippy (GEHC) presented a strategy of mixing two RNA samples for assessing the accuracy of microarrays and the bioinformatic approach for visualizing the expected and observed ratios. Scott Pine (FDA/CDER) described the project led by Karol Thompson (FDA/CDER) that created two rat mixture RNA samples from four tissue RNAs with defined ratios. Doug Lane (ViaLogy) described an ongoing titration study aimed at assessing the performance of individual probes on the Affymetrix platform.

During the session on Verification with Independent Platforms, Lu Zhang (Applied Biosystems) described a series of criteria for selecting a subset of 1,000 genes for TaqMan® verification to cover genes with appropriate distributions in terms of fold change and intensity. During the discussion session, Federico Goodsaid (FDA/CDER) stressed the importance of selecting a certain number of genes that do not show cross-platform ratio concordance from the pilot datasets. Scott suggested using RefSeq as the basis for identifying the set of genes that are commonly probed by each commercial platform used in the MAQC project. For each selected gene, Applied Biosystems will conduct TaqMan® verification in four replicates. Yuling Luo (Genospectra) described the QuantiGene™ assay platform for differential gene expression analysis. Genospectra will provide QuantiGene™ assay data for 200-400 genes to be selected by the MAQC group.

Day 2: 8:00 AM – 12:00 PM, Tuesday, May 3, 2005

What to Expect from the MAQC Project

The second day of the MAQC meeting was co-chaired by Wendell Jones (Expression Analysis) and Uwe Scherf (FDA/CDRH) and was focused on the design of the MAQC main study. Leming Shi (FDA/NCTR) reiterated the goals of the MAQC project:

1. To assess the performance that is achievable by the same microarray platform within the same laboratory;
2. To assess cross-laboratory comparability of the same microarray platform;
3. To assess cross-platform comparability of the microarray technology;
4. To develop a set of metrics/thresholds for the objective assessment of microarray performance;
5. To assess the advantages and disadvantages of various data analysis methods;
6. To provide practical tools (calibrated reference RNA samples, reference datasets, QC metrics/thresholds, orthogonal measurements, *etc*.) to microarray end-users (including those not involved in the MAQC project) to avoid procedural failures and to ensure laboratory proficiency;
7. To provide a foundation for platform providers to further improve the microarray technology.

To better design the MAQC main study, the attendees were given a chance to express their main expectations from participating in the MAQC meeting:

1. Jacques Retief (Affymetrix): To develop reference materials available in a tube for the quality control of microarray hybridizations;
2. Yaron Turpaz (Affymetrix): To identify sources of variability so that confounding factors can be blocked in experimental design;
3. Scott Pine (FDA/CDER): Regulatory needs for data analysis, proficiency tests;
4. Paul Wolber (Agilent): How different platforms compare; If not comparable, identify the main sources of discrepancies; To establish reference materials for QC;
5. Jim Fuscoe (FDA/NCTR): Reference materials for proficiency tests; datasets for developing guidelines for microarray data analysis;
6. Bud Bromley (ViaLogy): To establish microarray performance baseline; To ensure that all labs are operating within the norms;
7. Mike Wilson (Ambion): How to build microarray "standards";
8. Susan Lundquist (EPA): To help policy decision and risk assessment;
9. Federico Goodsaid (FDA/CDER): To adequately address technical concerns so that we can move to biology;
10. Don Jin (Gene Logic): To demonstrate that consistent data can be generated;
11. Walter Liggett (NIST): To get the sources of variation out;
12. Ron Peterson (Novartis): To build confidence in microarray data for regulatory submissions;
13. Uwe Scherf (FDA/CDRH): To help regulatory review of IVD's.

Design of the MAQC Main Study (Microarrays)

1. A survey was conducted and 22 people favored the use of the identical protocol recommended by the manufacturer for the same platform, whereas five people voted for using any protocol at the test site's choice. A decision was made for all test sites of the same platform to use exactly the same protocol recommended by the manufacturers.
2. For the same platform, each test site should use the same chip lot and the same reagent/kit lot. Data should be generated by one operator per test site.
3. It was decided not to include "day effect" in the design.
4. Each platform will be tested at 3 sites to be chosen by the array manufacturer.
5. Each of the two samples (Ambion Brain RNA and Stratagene UHRR) will be tested in five replicates at each test site.
6. Two RNA mixtures will be tested. A subcommittee will be formed to decide the details on RNA-mixing. The vendors will test the mixing first before the test sites perform testing on mixtures.
7. Each test site is expected to conduct 20 hybridizations (5 replicates x 4 samples (Brain, UHRR, and two mixtures), and each platform provider is expected to contribute 60 arrays for the MAQC main study.
8. Each hybridization should start from independent cRNA synthesis/labeling reactions.
9. cRNA yield, labeling efficiency, and size distribution (Bioanalyzer profile) should be recorded by the test sites and cRNA samples should also be sent to Ambion for independent testing. Such information is expected to help establish QC metrics/thresholds for RNA quality control.

Design of the MAQC Main Study (Validation)

1. Validation by independent platforms such as TaqMan® and QuantiGene™ will be conducted.
2. RNA titration will be used to assess the behavior of a subset of genes.
3. Based on the mapping of probes to RefSeq, about 8K RefSeq sequences are in common to the four platforms used in the MAQC pilot study (thanks to Scott for this information).
4. *In silico* analysis will be conducted to finalize the selection of genes for independent validation. *In silico* simulation will also be conducted to examine the titration behavior of the brain and UHRR samples.

Other Issues

Teleconferences will be scheduled once every two weeks to finalize the design of the MAQC main study and to keep track of the progress of the project. The timeline of the MAQC project will be determined by the availability of the larger batch of brain RNA sample to be manufactured by Ambion. Ambion is figuring out the logistic details and will keep the MAQC group updated.

MAQC for rodents (mouse and rat) was listed in the meeting agenda but was not discussed because of the limitation of time. Because of the importance of rodents to the EPA and FDA, these issues will be actively explored.

Organizations should address issues such as MTAs individually when required.

A list of attendees is shown in Table 2.

Table 2. A list of participants of the MAQC project meeting on May 2-3, 2005

No.	Organization	Name	No.	Organization	Name	No.	Organization	Name
1	Affymetrix	Liu, Chunmei	16	FDA/CDER	Thompson, Karol	31	NIST	Liggett, Walter
2	Affymetrix	Retief, Jacques	17	FDA/CDRH	Elespuru, Rosalie	32	NIST	Satterfield, Mary
3	Affymetrix	Turpaz, Yaron	18	FDA/CDRH	Scherf, Uwe	33	Novartis	Peterson, Ron
4	Agilent	Wolber, Paul K.	19	FDA/CDRH	Tezak, Zivana	34	Stratagene	Clum, Rob
5	Ambion	Setterquist, Robert	20	FDA/CVM	Harbottle, Heather	35	Stratagene	Fischer, Gavin M.
6	Ambion	Wilson, Mike	21	FDA/NCTR	Fuscoe, James	36	Stratagene	Smith, Cheryl
7	Applied Biosystems	Lee, Kathy Y.	22	FDA/NCTR	Shi, Leming	37	Umass Boston	Jensen, Roderick
8	Applied Biosystems	Zhang, Lu	23	FDA/NCTR	Tong, Weida	38	Umass Boston	Lombardi, Michael
9	EPA	Gallagher, Kathryn	24	GE Healthcare	Shippy, Richard	39	ViaLogy	Bromley, Bud
10	EPA	Lundquist, Susan	25	Gene Logic	Jin, Donald F.	40	ViaLogy	Lane, Doug
11	Expression Analysis	Jones, Wendell	26	Genospectra	Luo, Yuling			
12	FDA/CBER	Han, Jing	27	Genospectra	Ma, Yunqing			
13	FDA/CDER	Frueh, Felix W.	28	Luminex	Calvin, Ted			
14	FDA/CDER	Goodsaid, Federico	29	NCBI	Herman, Damir			
15	FDA/CDER	Pine, Scott	30	NIH/NCI	Kawasaki, Ernest			

*Those attending the meeting via teleconference are not listed.