Summary of 1st MAQC Project Meeting

Microarray QC Metrics and Thresholds (MAQC):
Calibrated RNA Samples, Microarray Datasets, and qRT-PCR Datasets

Meeting Date: February 11, 2005, 8:30 am – 3:00 pm CST
Location: FDA/NCTR, Jefferson, Arkansas
Participants: 60 (on-site: 30, video: 10, and tel: ~20). Contact information is attached.
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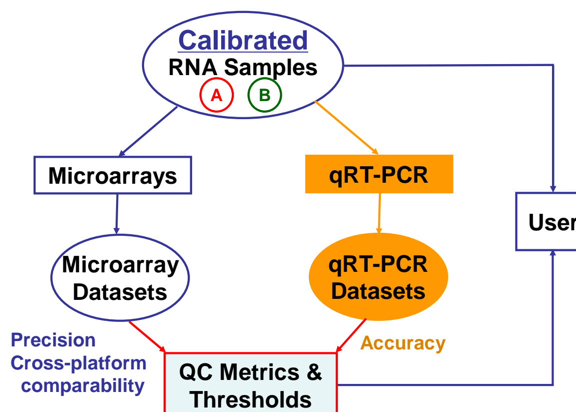
Date: February 16, 2005

BACKGROUND

The U.S. Food and Drug Administration's (U.S. FDA) Critical Path white paper (www.fda.gov/oc/initiatives/criticalpath/) identifies pharmacogenomics and toxicogenomics as promising techniques in advancing medical product development and personalized medicine, and a draft guidance for the industry on pharmacogenomic data submissions has been released (www.fda.gov/cder/guidance/5900dft.pdf). However, standardization is much needed before DNA microarrays – a core technology in pharmacogenomics and toxicogenomics – can be reliably applied in clinical practice and regulatory decision-making. Many commercial and in-house microarray platforms are in use, and a natural question is whether the results from different platforms are comparable and reliable (E. Marshall, *Science* 306:630-631, 2004). A paramount challenge to the microarray community is to ensure that experiments at individual laboratories are conducted to the performance that should be achieved and that the resulting data are properly analyzed.

The MicroArray Quality Control Metrics and Thresholds (MAQC) project aims to establish QC metrics and thresholds for objectively assessing the performance achievable by various microarray platforms and evaluating the merits of various data analysis methods. Two RNA samples will be selected for each species (human, rat, and mouse) and differential gene expression levels between the two samples will be calibrated with both microarrays and qRT-PCR. The resulting microarray datasets will be used for assessing the precision and cross-platform comparability of microarray platforms. The qRT-PCR datasets will enable evaluating the nature and magnitude of any systematic biases that may exist between the two measurement technologies. Importantly, the calibrated RNA samples will be made readily accessible to the microarray community for proficiency testing so that procedural failure can be more easily identified and corrected. It is also anticipated that the calibrated RNA samples will be profiled using other technologies (*e.g.*, SAGE). Overall, the MAQC project is expected to improve microarray technology and foster its proper use.



SUMMARY

1. Dan Casciano (Director, FDA/NCTR) chaired the opening session and welcomed participants to the 1st MAQC project meeting. Representatives from FDA headquarters (CDER, CDRH, and CBER) gave brief overviews of each Center's current genomics activities. Federico Goodsaid (CDER) reviewed PG data submissions, IPRG (Interdisciplinary PG Review Group), and ArrayTrack (being evaluated as a review tool for PG data submissions). Uwe Scherf outlined CDRH's views in reviewing genomics-based medical devices; the industry is encouraged to discuss with CDRH/OIVD before submitting applications. Jing Han described CBER's capabilities in conducting genomics research; CBER uses both customized arrays and the Affymetrix platform.
2. Leming Shi (FDA/NCTR) outlined the scope of the *Microarray QC Metrics and Thresholds (MAQC)* project, covering project overview, RNA sample selection, microarray data generation, qRT-PCR reference measurements, data sharing, QC metrics/thresholds, and timetable. LS emphasized that the MAQC project is aimed at objectively assessing the measurement accuracy (precision and bias) that is achievable on each platform by establishing two calibrated RNA samples per species (human, rat, and mouse) and generating microarray and qRT-PCR datasets. The datasets from these two technologies will provide individual microarray laboratories the standards needed to assess proficiency as well as guide correction of deficiencies. Data analysts can cross-validate the merits of various computational methods. The reliability and accessibility of the calibrated RNA samples to the microarray community is crucial to the success of the MAQC project.
3. Karol Thompson (FDA/CDER) presented results from an FDA project on the "*Application of a Cross-platform RNA Standard for Assessing Microarray Data*" where tissue-selective genes were identified from four rat tissues, which were mixed with defined composition (hence defined input fold changes for tissue-selective genes). Jacques Retief (Affymetrix) described an ANOVA-based approach for evaluating the resulting cross-platform microarray datasets.
4. Janet Warrington (Affymetrix) and David Dorris (Ambion) chaired the 1st (10:00 am – 12:00 pm CST) and 2nd (1:00 pm – 3:00 pm) discussion sessions, respectively, which were extensive and focused on the goals of the MAQC study, RNA sample selection, and the reliability of using qRT-PCR as a reference method. **It was agreed that an independent technology (*e.g.*, qRT-PCR) is essential to assess the accuracy (precision and bias) of microarray results**, and the assessment of the reliability of qRT-PCR itself constitutes a large study. There was considerable debate surrounding the need to work with rat and mouse arrays. However, because of the popularity of human arrays used by the general microarray community, **the decision was made to begin the project with human RNA arrays while actively planning similar studies with rat and mouse arrays**. There were many discussions regarding which two human RNA samples should be selected to be developed as the "calibrated" RNA samples. LS suggested selection criteria and options for both RNA samples (slide 5 of his presentation) as follows: (1) available in large quantity (*i.e.*, enough for the microarray community to use for several years) and/or reproducible in production (*i.e.*, with acceptable batch-to-batch variability); (2) high quality; (3) readily accessibility to the general public (*e.g.*, from commercial sources); (4) wide gene coverage; and (5) large fold change for a number of genes. Several options for RNA sample selection were also listed: (1) two universal reference RNAs (URRs); (2)

two tissue RNAs; (3) two cell line RNAs; and (4) any combination of a URR, a tissue RNA, or a cell line RNA. LS emphasized his preference of selecting two URR samples because of their wide gene coverage, broad fold changes, and existing acceptance by the microarray community (in particular for two-color platforms). Advantages and options of mixing different tissue RNAs were also extensively discussed. At the end of the meeting, **consensus was reached to conduct a pilot study to provide enough expression data for selecting two RNA samples for the ultimate MAQC study**.

ACTIONS NEEDED

1. Conduct a pilot study to determine which two human RNA samples will be used in the MAQC study. See "Pilot Study" for more information.
2. Distribute the list of contact information for attendees. (LS)
3. Provide shipping list for RNA and array distribution. (LS)
4. Provide meeting summary and action item list. (LS)
5. David Dorris (Ambion, ddorris@ambion.com) will coordinate the distribution of RNA samples and arrays. RNA samples from Clontech and Stratagene will be sent to Ambion and then distributed to test sites. Samples will be QC'd at each site with Agilent Bioanalyzer. (DD)
6. Platform providers (Affymetrix, Agilent, Applied Biosystems, GE Healthcare, and Illumina) will provide FDA/NCTR with array type information (*e.g.*, probe IDs and sequences) to map genes across multiple platforms.
7. All data images, non-normalized data, and .CEL files will be sent to FDA/NCTR for analysis. In addition, each site performing array hybridization will be able to access all data generated in this pilot study and conduct independent analysis.
8. Set up an FTP site to permit exchange of data by all parties involved in data generation and analysis. Make ArrayTrack available, visible to participants (www.fda.gov/nctr/science/centers/toxicoinformatics/ArrayTrack/).
9. Schedule conference calls every 2 weeks to keep the MAQC project on track.
10. Schedule a face-to-face meeting in about 2 months to discuss data from the pilot study and make final decision on RNA sample selection for the actual MAQC study. Will be coordinated with ERCC's next meeting (most likely in DC, April 2005).
11. Consider inviting other sites and pharmas to participate in the project.

PILOT STUDY

The pilot study is being planned to generate microarray data to determine gene coverage and ratio dynamic range for the sample pair to be used in the actual MAQC study. The six (6) candidate human RNA samples are (Set I: A, B, and C; Set II: D, E, and F):

A: Universal reference (Stratagene)
B: Universal reference (Clontech)
C: Mix universal reference (Stratagene 20%, Clontech 80%)
D: Brain (Ambion)
E: Liver (Ambion)
F: Mix tissue (Brain 20%, Liver 80%)

30 arrays (5 replicates for each sample) will be processed for each platform (Affymetrix, Agilent, GE Healthcare, and Illumina) at the platform provider's laboratory. Optionally, the same number of arrays will be processed at additional sites:

Platform	Site 1	Site 2	Site 3
Affymetrix	Affymetrix	Ambion	
Agilent	Agilent	FDA/NCTR	
GE Healthcare	GE Healthcare	Ambion	U Mass Boston
Illumina	Illumina		

Microarray data from the pilot study are expected to be centralized within ArrayTrack and analyzed at the FDA/NCTR by the end of March, 2005. The results of this analysis will be distributed to participating laboratories within 1 week after FDA/NCTR receives data. Based on the results of the pilot study, a list of 1000 genes will be selected for conducting qRT-PCR assays.

NOTES ADDED AFTER THE MEETING

1. Feedback to the meeting and MAQC project has been extraordinarily positive. Participating organizations are enthusiastic to move the MAQC project forward.
2. Applied Biosystems has confirmed its commitment of providing qRT-PCR (TaqMan®) assay data for at least 1000 genes on the two RNA samples to be used in the ultimate MAQC study.
3. Applied Biosystems decided not to generate microarray data for the pilot study, but is committed to participate in the ultimate study using its genome survey arrays.
4. Clontech has been contacted and committed to participate in the pilot study by providing free RNA samples.
5. RNA sample providers are being requested to prepare more detailed information regarding the quantity, reproducibility, and quality of their RNA samples that may be selected as the "calibrated" RNA samples after the pilot study. The exchange of such information will be a critical component of next teleconference at the end of February, 2005.
6. Suggestions have been received on the design of the ultimate MAQC study. The design will be discussed during follow-up teleconferences while the pilot study is in progress.
7. Suggestions have also been made to follow ISO accepted metrological terminology in exchanging information related to the MAQC study (thanks to David Duewer of NIST).
8. There were complaints about audio quality during the discussion sessions when on-site attendees did not always use the microphones when they spoke. It was also pointed out that teleconference attendees were not given enough opportunities to contribute to the discussions during the 2nd session. Improvements will be made.
9. There is a strong preference of the EPA and FDA to use rat and mouse arrays. Sample selection for rat and mouse should be concurrently considered.
10. LS will distribute the Meeting Summary to the **Gene-Arrays** mailing list to solicit suggestions from the microarray community.

Attachment: Contact list.