

Report



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Gene expression Project Manager / EAT /
Françoise de Longueville

subject:

MAQC Study: Data Generation Plan

Dualchip™ from Eppendorf: Gene Expression Platform

to:

MAQC consortium

only for information:

EAT Christophe Van Huffel
 José Remacle

Test Sites

- 1) Test Site : Eppendorf Array Technologies
Contact : Françoise de Longueville
Address : 20, Rue du Séminaire, 5000 Namur, Belgium
Phone : 0032 (81) 725615
- 2) Test Site : Cold Spring Harbor
Contact : Eli Hatchwell
Address : Genome research Center, 500 Sunnyside blvd, 11797 Woodbury
Phone : 001 (516)4224121
- 3) Test Site : MD Anderson
Contact : Lajos Pusztai
Address : Department of breast medical oncology, PO BOX 301439 /
HOUSTON, Texas 77230-1439
Phone : 001 (713)792-2817

Materials required

Eppendorf Products

- Dualchip™ kit (3 kits will be provided per site)
- cMaster® RTplus Labeling kit, Eppendorf (25 reactions) (2 kits will be provided)
- Biotin-11-dCTP, 5 mM (Perkin Elmer, customized) (2 tubes will be provided)
- Biotin-11-dATP, 5 mM (Perkin Elmer, customized) (2 tubes will be provided)
- Molecular Biology Grade Water, (e.g. Eppendorf) (3 bottle will be provided)
- Cy3-conjugated IgG Anti biotin, Jackson Immuno Research laboratories, Inc (#200-162-096) (2 tubes will be provided)
- Tween 20, e.g. Sigma (# P1379) (Not provided by Eppendorf)
- Thermomixer hybridization equipment (Eppendorf Thermomixer comfort and Thermoblock for slides DC) (3 equipments will be provided)
- Eppendorf CombiSlide Adapter (Not provided by Eppendorf)
- DualChip evaluation software (Eppendorf) (1 CD will be provided)

Non-Eppendorf Products

- Total RNA, Brain (Ambion)(AB) – 100 µg
- Total RNA, Universal Human Reference RNA (Stratagene) (SUHRR) - 100 µg

User Manuals

The manual for the Dualchip™ gene expression platform will be provided for each test site. The manual of Dualchip™ kit (version 2.0) is in annexe 1.

Experimental Design

Four RNA samples (2 references RNAs and 2 mixtures)

RNA	Description
A	Stratagene UHRR (SUHRR)
B	Ambion Brain Reference RNA
C	25% Brain / 75% SUHRR
D	75% Brain / 25% SUHRR

Five replicates hybridization per sample with independent labelling reactions will be conducted for the MAQC main study. This experimental design will be conducted on each test site.

Total RNA Preparation and Analysis

SUHRR will be provided by Stratagene in a solution of 70% ethanol and 0.1 M sodium acetate. This should be prepared as per the procedure recommended in Stratagene's Catalog #740000.

AB will be provided in aqueous solution ready for use.

RNA quantitation and purity assessment: RNA should be quantitated using a NanoDrop ND-100 UV-VIS or equivalent. A260 and A280 should be recorded for reporting to the MAQC study (Appendix 3: "MACQ_RNA_Quality_report_template.xls").

RNA intactness assessment: 200 ng quantities of SUHRR and AB should be run on the Bioanalyzer 2100, three replicates each. Bioanalyzer traces should be saved, and rRNA Ratio[28S/18S] and RIN values recorded, for reporting to the MAQC study (Appendix 3: "MACQ_RNA_Quality_report_template.xls").

Sample labelling

Five replicate labeling reactions should be set up for each sample as follows:

- Procedure described in Eppendorf Dualchip kit manual (point 8.3.2.1.A, page 19) should be followed (Annexe 1).
- 10 µg of total RNA should be input per reaction.
- All 20 target preparations (4 RNAs X 5 replicates) should be processed together starting on the same day by a single person to minimize run-to-run variability.

Sample Hybridization

Five replicate hybridization per sample should be performed.

- Procedure described in Eppendorf Dualchip kit manual (point 8.3.2.1.A, page 21) should be followed (Annexe 1).
- All 20 hybridizations (20 arrays, 10 slides (2 array per slides)) should be processed together starting on the same day by a single person to minimize run-to-run variability (in one batch).
- During all processing steps, the order of the 20 microarrays being used should be randomized, i.e., Hybridization Solutions should be hybridized to randomly selected microarrays, scan order should be randomized.
- Microarrays should be hybridized at 60°C for 16 hours.
- Microarrays should be washed in one batch of 10 slides .
- Microarrays should be washed with Eppendorf Washing Buffer 1 as described in Eppendorf Dualchip kit manual (point 8.3.2.1.C, page 26) (Annexe 1).
- Slides should be scanned as soon as possible after washing.

Data Acquisition

Microarray scanning: All 20 microarrays should be scanned following the procedure described in Eppendorf Dualchip kit manual (point 8.4.1., page 41) (Annexe 1).

Data extraction: Data should be extracted using Dualchip Evaluation software, version 1.0 using the procedure described in Eppendorf Dualchip kit manual (point 8.4.2. A. B., page 41) (Annexe 1).