

# MIAME Checklist-TB

## 1. Experiment design

### Type of experiment

Comparison of expression profiles of brain and testis tissues

### Experimental variables

Type of tissue

### Hybridizations

GTA-TB1: brain (Cy3) vs. testis (Cy5)

GTA-TB2: testis (Cy3) vs. brain (Cy5)

### Quality control steps taken

Dye-swap for all extracts

Duplicated spots on arrays

## 2. Samples used, extract preparation and labeling

### Biological sample description

Organism: *Mus Musculus*

Strain: 129S4

Developmental stage: 4-month old adult

Tissue sources: whole brain and testis

### Preparation of hybridization extracts

Total RNA were extracted twice with TriZol reagent (Invitrogen) according to protocols provided by the manufacturer. RNA quality was checked with gel electrophoresis and Bioanalyzer (Agilent).

### Labeling protocol

Reverse transcription was performed by mixing 30  $\mu$ g total RNA with oligo(dT)<sub>18</sub>, Superscript II-RT enzyme and buffer (Invitrogen), RNaseOUT RNase Inhibitor (Invitrogen), 25 mM of each dATP, dCTP and dGTP, 12.5 mM dTTP and 12.5 mM amino-allyl-dUTP (Sigma). Incubate at 42 degree C for 2 hours. Add 10  $\mu$ l of NaOH and 10  $\mu$ l of 0.5 M EDTA. Incubate at 65 degree C for 15 minutes. Neutralize by adding 25  $\mu$ l 1M HEPES pH7.4. Filter on Microcon-30 concentrator. Dry on speed-vac, resuspend in water. Cy3 and Cy5 Monoreactive dyes (Amersham) were resuspended in DMSO, activated by Sodium Bicarbonate and incubated with purified cDNA for 1 hour. Coupling reactions were quenched by addition of Hydroxylamine and incubation for 15 minutes. Combine Cy3 and Cy5 labeled cDNA pairs and purify with QIAquick PCR purification columns. Final elute was dried in speed-vac and resuspended in 20  $\mu$ l water. Add 4  $\mu$ l 20X SSC and 2  $\mu$ l of 10 mg/mL poly(dA) (Roche). Filter through Millipore 0.45 micron spin membranes. Mix with SDS to final concentration before hybridization.

## 3. Hybridization procedures and parameters

### Hybridization buffer

3X SSC, 0.23% SDS, 1 mg/ml poly(dA)

Slide blocking

No prehybridization

Probe blocking

1 mg/ml poly(dA) during hybridization

Quantity of labeled target used

All labeled cDNA generated with 30  $\mu$ g total RNA

Hybridization time, volume, temperature

16 h, 26  $\mu$ l, 63 degrees C

Hybridization instrument

Manual, TeleChem hybridization chambers in water bath

Wash procedure

Wash 1: 1X SSC, 0.03% SDS

Wash 2: 1X SSC, dip slides 15 times

Wash 3: 0.2X SSC, shake slides 75 rpm for 20 minutes

Wash 4: 0.05X SSC, shake slides 75 rpm for 10 minutes

Spin-dry slides in centrifuge 50 g for 5 minutes.

**4. Measurement data and specifications**

Scanning hardware

Axon GenePix4000B

Scanning software

Axon GenePix Pro 3.0

Image analysis software

Axon GenePix Pro 3.0

Image analysis output files

Attached (GTA-TB1.gpr and GTA-TB2.gpr)

Data selection and transformation

For each array, spot intensity signals were filtered and removed if the values did not exceed 3 S.D. above the background signal in at least one signal channel or if a spot was flagged as questionable by the GenePix Pro software. Spot-level ratios (Cy5/Cy3) were  $\log_2$  transformed and a loess normalization ( $f = 0.67$ ) strategy was applied using S-Plus (MathSoft, Cambridge, MA) to correct for observed intra-array intensity-dependent ratio biasing. For each sample comparison, data were analyzed by first averaging the spot-level, normalized log ratios of the two reverse-complement arrays, and then taking the average of the intra-array,

gene-level duplicate features. At the spot-level averaging step, only those features possessing both fluor-orientation data points were carried through the analysis.

#### Gene expression data table

Attached (GTA-TB.xls)

### **5. Array design**

#### Array information

Array name: F.H.C.R.C. Mouse 2K-GTA  
Array provider: in-house FHCRC  
Platform type: spotted  
Surface type: glass  
Surface coating: poly-lysine  
Physical dimensions of slides: 40 x 18 mm  
Number of blocks on the array: 16  
Number of spots on the array: 4608  
Spot dimension: ~130 micrometers  
Attachment type: electrostatically  
Spot-reporter matching system: included in each .gpr file

#### Reporter information

Reporter type: cDNA  
Preparation method: 3' RACE RT-PCR  
Single or double stranded: double-stranded  
Approximate length: 0.5-2.0 kb

#### Production protocol

The 2K-GTA set was constructed using nested 3' RACE-PCR products amplified from individual clones of an ES cell gene trap library, using anchoring primers and primers specific to the gene trap tag as described below. Individual PCR products were verified by gel electrophoresis and Southern hybridization against DNA contamination and aberrant splicing. Quality products were selected and purified using Multiscreen-PCR filtration system (Millipore) and mechanically spotted, in duplicate, in 3X SSC (450 mM sodium chloride and 45 mM sodium citrate, pH 7.0) onto poly-lysine coated microscope slides using an OmniGrid high-precision robotic gridded (GeneMachines, San Carlo, CA). Printed slides (arrays) were post-processed prior to use using the following procedure. Printed arrays were re-hydrated over a simmering water bath for 2-5 sec and immediately snap-dried on an inverted heating block at 100°C. Slides were then placed in a UV Stratalinker 2400 (Stratagene, Inc) and exposed to 65 mJ of UV radiation. Following UV cross-linking, arrays were "blocked" by submerging slides in a solution composed of 5.5g of succinic anhydride, 25 ml of 1M sodium borate (pH 8.0), and 325 ml 1-methyl-2pyrrolidinone for 30 minutes with shaking. Following the blocking step, arrays were rinsed and denatured using two independent water baths, each at 90°C, in which the arrays were submerged for 2 minutes each. Arrays were removed from the water bath, transferred to a slide dish containing ethanol (200-proof), and subsequently spun dry at 500 rpm using a centrifuge. Arrays were either used immediately or stored vacuum packed until needed.

Oligos used for 3' RACE:

Anchoring oligo QT: CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGCT(17)  
Anchoring primer QA: CCAGTGAGCAGAGTGACGAGGAC  
Gene trap-specific primer HYG: ACTCGTCCGAGGGCAAAGGAATAGG  
Nested anchoring primer QB: GACGAGGACTCGAGCTCAAGC  
Nested gene trap-specific primer SDEXF: GCTAGCGCGTTCGTCCTCACTCT