MIAME Checklist-PDGF

1. Experiment design

Type of experiment

Treatment on wild-type and mutant cells in a time course

Experimental variables

Time and genetic variations

Hybridizations

Hybridization ID	Genotype of Cells	Target Label (Cy3)	Target Label (Cy5)	Time (hr)
GTA-P1	Wild-type	Ref	Untreated	0
GTA-P2	Wild-type	Untreated	Ref	0
GTA-P3	Wild-type	Ref	Treated	0.5
GTA-P4	Wild-type	Treated	Ref	0.5
GTA-P5	Wild-type	Ref	Treated	1
GTA-P6	Wild-type	Treated	Ref	1
GTA-P7	Wild-type	Ref	Treated	2
GTA-P8	Wild-type	Treated	Ref	2
GTA-P9	Wild-type	Ref	Treated	4
GTA-P10	Wild-type	Treated	Ref	4
GTA-P11	PDGFR□-/-	Untreated	Treated	1
GTA-P12	PDGFR□-/-	Treated	Untreated	1
GTA-P13	PDGFR□-/-	Untreated	Treated	4
GTA-P14	PDGFR□-/-	Treated	Untreated	4
GTA-P15	PDGFR□-/-	Untreated	Treated	1
GTA-P16	PDGFR□-/-	Treated	Untreated	1
GTA-P17	PDGFR□-/-	Untreated	Treated	4
GTA-P18	PDGFR□-/-	Treated	Untreated	4

- Treated: treated with PDGF-BB, collected at indicated times
- Untreated: not treated, collected at time 0
- Ref: wild-type cells, not treated, collected at time 0

Quality control steps taken

Dye-swap for all extracts
Duplicated spots on arrays
Cells are individually isolated from 6-8 embryos and pooled
Reference RNA is pooled from multiple individual extracts
Each differential verified by Northern with independently isolated cells

2. Samples used, extract preparation and labeling

Biological sample description

Organism: *Mus Musculus*
Strain: 129S4 X C57BL/6J
Genotypes: Wild-type, PDGFR□-/-, PDGFR□-/-
Cell source: Mouse embryonic fibroblasts (MEFs) isolated from individual E12.5 embryos

Biological sample manipulation

MEFs of each genotype were grown in DMEM with 10% FCS (fetal calf serum), 50 U/ml penicillin, 50 □g/ml streptomycin at 37 degree C in 5% CO2 on 15 cm tissue culture plates during passage, and were pooled at passage 3. Subconfluent cultures were brought to quiescence by growing in low serum medium (0.5% FCS) for 48 h, treated with PDGF-BB (30 ng/ml), and collected at indicated times for RNA extraction.

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 □g total RNA with oligo(dT)18, 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 □l of NaOH and 10 □l of 0.5 M EDTA. Incubate at 65 degree C for 15 minutes. Neutralize by adding 25 □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 □l water. Add 4 □l 20X SSC and 2 □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 □g total RNA

Hybridization time, volume, temperature

16 h, 26 □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-P1.gpr to GTA-P18.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-PDGF.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 cloride and 45 mM sodium citrate, pH 7.0) onto poly-lysine coated microscope slides using an OmniGrid high-precision robotic gridder (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 HYGF: ACTCGTCCGAGGGCAAAGGAATAGG
Nested anchoring primer QB: GACGAGGACTCGAGCTCAAGC
Nested gene trap-specific primer SDEXF: GCTAGCGCGTTCGTCCTCACTCT