Gene expression profiling after radiation induced DNA damage is highly predictive of *BRCA1* mutation carrier status

Materials and Methods.

Poly-L-lysine Coating of the Slides

Slides coated with poly-L-lysine have a surface that is both hydrophobic and positively charged. The hydrophobic character of the surface minimises spreading of the printed spots, and the charge appears to help position the DNA on the surface in a way that makes cross-linking more efficient.

Reagents:

GoldSeal slides	BDH [48300-240]
Poly-L-lysine	Sigma [P8920]
10X PBS	Life Technologies [14200-059]

1. Dissolve 75g NaOH in 300 ml H_2O. Add 450 ml ethanol and stir until the solution clears. If the solution does not clear, add H_2O until it does.
2. Place slides into a slide rack, and place the rack in a glass tank with 500 ml of cleaning solution made in step 1. It is important to wear powder free gloves when handling the slides and to change gloves frequently, as random contact with skin and surfaces transfers grease to the gloves. Incubate for at least 1 hour with vigorous agitation (but not too long, otherwise the solution turns yellow).
3. Pour out the cleaning solution and repeat steps 1 & 2
4. Plunge racks up and down in 95% Ethanol 5 times.
5. Wash the slides in 750 ml H_2O for five minutes. Repeat the wash four times.
6. Mix 75 ml poly-L-lysine with 7.5 ml 10x PBS and 667.5ml H_2O in a very clean plastic container.
7. Quickly transfer the slides and gently agitate for one hour.
8. Plunge the rack 5 times up and down in 300 ml H_2O.
9. Repeat steps 6-8
10. Spin at 500 rpm for 10 mins and place in 67°C oven for 10 mins.
11. Transfer the slides in boxes and store them for a few weeks at room temperature.

Slide boxes used for long term storage should be plastic and free of cork lining. The glue used to affix the cork will leach out over time and give slides stored in these types of boxes a greasy film that has a high degree of autofluorescence. Clean all glassware and racks used for slide cleaning and coating with highly purified H_2O only. Do not use detergent. Dedicate glassware and racks to specific steps.

Post Processing (Blocking)

Reagents:

succinic anhydride	Aldrich [23,969-0]
1-methyl-2-pyrrolidinone	Aldrich [32,863-4]
boric acid	Sigma [B6768]
96% ethanol	BDH [10476]

1. Crosslink the gridded DNA in a Stratalinker at 65 mJ (650 on display).
2. Dissolve completely 6 g of succinic anhydride in 275 ml of n-methyl-pyrrolidinone in a dry 500-ml Duran bottle.
3. Add 75 ml of 0.2M Sodium Borate pH 8.0 (made by dissolving boric acid in water and adjusting the pH with NaOH), and pour into a glass staining trough.
4. Immediately transfer the slide rack containing the arrays in this solution. Plunge the rack 5 times. Soak for 20 mins.
5. Gently immerse the arrays 2 min in 300 ml 95°C water in a plastic container.
6. Plunge the arrays five times in 96% ethanol and leave to dry.
7. The arrays can be used immediately or stored in a dark box at room temperature.

RT Labelling from Total RNA

Nucleotide Mix: 10X low-dC dNTPs (use 100mM dNTPs from Amersham Biosciences [27-2035-02])

	µl	mM final (1/10) concentration
dGTP	25	0.5
dATP	25	0.5
dTTP	25	0.5
dCTP	10	0.2
water	415	
total volume	500	

Reagents:

Primer	Amersham Biosciences oligo $(dT)_{12-18}$ [27-7858-01]
Unlabelled nucleotide mixes	Prepared from Pharmacia 100 mM stocks
RNase inhibitor	Boehringer Mannheim [0799017]
Reverse transcriptase	BRL SuperScript II [18064-014]
0.1X RNA storage buffer	Ambion [7001]; dilute the 1x tenfold
5X buffer	Supplied with the enzyme
0.1M DTT	Supplied with the enzyme
Cy3- and Cy5- dCTP	Amersham Biosciences
CoT-1 DNA	Life Technologies [15279-011]
0.5M EDTA	Sigma [E7889]

Material:

Microcon 30 columns	Millipore [424110]

The quality of the template RNA used for reverse transcription is critical. There should not be any traces of contaminants (e.g., ethanol, lipids, polysaccharides, guanidium, phenol).

1. Mix together:	8 µg total RNA	X µl
	oligo $(dT)_{12-18}$ (500 µg/ml)	1 µl
	Random primer (0.1ul/ug)	< 0.8 µl

Make up to 16 µl with HPLC water. Spin down briefly. Incubate 10 min at 70°C. Cool down to room temperature.

Mix together for each reaction:

Component	µl
5X first strand buffer	8
10X low-dC dNTP mix	4
Fluor dCTP (1 mM)	4
0.1 M DTT	4
SuperScript II (200 units/µl)	4
	22

Add to the annealed RNA, vortex and spin down briefly. Add 4 µl SuperScript II and incubate overnight at 42°C in a PCR block with a heated lid.

2. Add 5 µl of 0.5M EDTA to stop the reaction and mix.
3. Add 10 µl of 0.1M NaOH. Mix and spin down briefly. Incubate 10 min at 70^{o}C to hydrolyse residual RNA.
4. Add 25 µl of 0.1M HCl, pH 7.4 and 400 µl TE. Add 20 µl of CoT-1 DNA (1 mg/ml)(need 3ug/ul RNA) and mix.
5. Transfer each probe to a Microcon 30 column. Spin for 7 mins at 14,000g. Remove eluate, keep for purification. Add 400 µl TE to the column and spin again at 14,000g for 7 mins, then concentrate down to <10 µl.
6. Recover the probes by inverting the concentrators over clean collection tubes and spinning 3 min at 1,000g. The probes can be stored at this stage at –20 °C if not ready for hybridisation.

Hybridisation

Blocking Species:	poly-dA_{40-60}	Pharmacia [27-7988-01]	resuspend at 8 mg/ml
	yeast tRNA	Sigma [R8759]	resuspend at 4 mg/ml

20X SSPE, pH 7.4	Sigma [85637]
0.5M EDTA	Sigma [E6889]
20X SSC	CSSD
SDS	BDH
100X Denhardts	2% Ficoll-400, 2% PVP, 2% BSA type V
0.22 µm Ultrafree-MC filters	Millipore [UFC30GV25]
Hybri-Slip, 22x60 mm	Sigma [Z37,027-4]

1. Mix together:

Probes + CoT1	X µl
Poly-dA_{40-60}	2 µl
yeast tRNA	2 µl
20X SSPE	12 µl
0.5M EDTA	1.1 µl
10% SDS	1 µl
H_2O to	40 µl

2. Incubate 2 min at 98°C, add 2µl of 100X Denhardt's and spin for 15 mins at 13,500 rpm.
3. Carry out a formamide wash on the slides, 2 mins in 70% Formamide/2xSSC, then dip in 70%, 80%, 100% ethanol.
4. Add ~150 µl of 6X SSPE on the bottom of an hybridisation chamber, lay a slide at the bottom of the chamber, gridded side up, and warm up to 45°C.
5. Pipette the probe mix onto the array and carefully place a 22x60 mm Hybri-Slip over it.
6. Close the chamber and incubate overnight at 65°C in a water bath.

Washing

1. Remove the Hybri-Slip from each slide by immersing 2 min in 50 ml 2X SSC/100mM EDTA solution 37°C in a 50-ml centrifuge tube. Wait until the coverslip drops off.
2. Rinse each slide in a 50-ml centrifuge tube with gentle shaking at room temperature:
   2 min in 50 ml 2X SSC/100 mM EDTA
   2 min in 50 ml 1X SSC/50mM EDTA
   2 min in 50 ml 0.1X SSC
3. Immediately dry the slide with nitrogen gas by pushing the residual liquid off the slide.
   Use fresh solutions for each slide.
4. Scan using a GenePix 4000B (Axon).