Oligo-array Protocol for unamplified RNA

Julia Enrich, last modified March 2007

Immobilization

UltraGAPS Oligo-Slides:

1. Place arrays in humidity chamber filled with 80 mL ddH_2O for 5 min.
2. Snap dry the arrays (array up) on 100ºC hot plate for about 3 sec.
3. UV-cross-link (UV Stratalinker) the oligos to the arrays at 600 mJoules (= 6000 x 100 µJoules).

You can keep the arrays in the slide holder until ready for prehybridization (after labeling the cDNAs).

Prehybridization

Prehybridization should be done *immediatel*y before hybridization.

1. Incubate slides in prewarmed *Prehybridization Solution*[1] at 42ºC for one hour.
2. Transfer arrays to *0.1X SSC* and gently agitate at room temperature for 5 min.
3. Repeat step 3 in a fresh bath of *0.1X SSC*.
4. Transfer arrays to ddH20 for 30 sec.
5. Dry arrays by spinning for 10 min.

[1] See composition and preparation of all solutions at the end of the protocol.

CyScribe cDNA Post Labelling Kit Amersham

8 samples ↔ 4 arrays

batch nr.:

Date:

Reference: STR

Samples: ….....…

	Sample (Cy5)					Reference (Cy3)				
Array ID/Barcode	SMD Exp.-Name	RNA		prim	H_2O	ref	RNA		prim	H_2O
		ug	µl	µl	µl		ug	µl	µl	µl
		20		**3**		STR	20		**3**	

Samples undiluted or diluted *(1:10)

Primers: for totalRNA use 3 oligo(dT) primer (white)

0.2 ml tubes, work on ice, always vortex, spin.

Pipette: sample + H_2O (ad 8) + primer → **total volume=11µl**

Incubate at 70° C for 5 min (11µl), take out immediately

Cool to RT for 10 min, **spin** and put on ice

MM	1x	**9x**
5x CyScript buffer (yellow)	4µl	**36**
0.1M DTT (orange)	2µl	**18**
nucleotide mix (green)	1µl	**9**
AA-dUTP (pink)**	1µl	**9**
total	8µl	

** lyophil.; + 30 µl RNase-free ddH_2O, then stable for 1 month

+ 8 µl MM

+ 1µl CyScript RT (red) to each tube

Incubate at 42° C for 1.5 hours

On Ice: Add 2 µl of 2.5M NaOH to each reaction, vortex, spin

Incubate at 37° C for 15 min

Add 10 µl of 2M HEPES free acid to each reaction, vortex, spin, on ice

3 µl of 3M NaOAc in 1.5 ml Epi, put them on ice

Add sample

Add 75 µl of 100% cold ethanol

Incubate 60 min at –80° C *precool centrifuge!!!!!!*

Centrifuge at full speed at 4° C for 40 min

Discard the supernatant (carefully with pipette) and

immediately add 1000 µl of cold 70% ethanol to each sample

Centrifuge at full speed at 4°C for 10 min

Discard the supernatant (pipette)

Air dry the pellet (5 min) *centrifuge at RT again!!!!*

Resuspend the pellet in 15 µl of RNase-free ddH2O (RT)

Resuspend one aliquot of CyDye (Cy3 or Cy5, short spin!!!) in 15 µl fresh 0.1M

Sodium Bicarbonate pH=9.0, immediately prior to use

Immediately add one aliquot of CyDye to the corresponding reaction

Mix and incubate in the dark for 1 hour at room temperature

In the meantime prepare solutions

Switch on SpeedVac, preheat at 45° C (1/2 hour before use)

<u>QIAquick PCR Purification Kit (Qiagen)</u>

Clean Cy3- and Cy5- reactions **seperately**,

Add 225 µl of buffer PB to each sample, RT

Add the labelling solution to the column

Spin for 60 sec at 10.000 g, RT START WITH PREHYBE NOW!

Discard flow-through

Add 750 µl of wash buffer PE (containing ethanol)

Spin for 60 sec at 10.000 g, RT

Discard flow-through

Add 500 µl of wash buffer PE

Spin for 60 sec at 10.000 g, RT

Transfer the column into a new 1.5ml tube (not included in the KIT)

Pipette 50 µl of RNase-free ddH2O to the column

Incubate for 3 minutes at room temperature

Elute by spinning for 60 sec at 10.000 g, RT

labelled cDNA is ready! Sample and Ref. in 50 µl each!!!

Now unify sample and corresponding ref.: V total = 100 µl

Dry down all samples in SpeedVac around 5 µl (45° C, takes more than an hour)

Preheat thermoblock at 95° C, another thermoblock at 40° C, waterbath at 42° C (put Washing Solution 1 in the waterbath)

Hybridization

1. Resuspend dried cDNAs in 25µl of Rnase freeH2O preheated to 40ºC.
2. Prepare *2X Hybridisation Buffer*, keep at 40ºC all the time, to avoid SDS precipitation.
3. Add 25 µl of 2X Hybridisation Buffer to each sample, resuspend thoroughly by pipetting.
4. Boil the samples for 3 min.
5. Meanwhile prepare the hybridization-chambers.
6. Spin down the samples and pipett the probe onto the middle of the array. Carefully place the coverslip onto the array.
7. Place 30-50 µl *3X SSC* in the chamber (humidity). Seal the chamber and incubate in 42ºC waterbath for 24 hours.

Post-Hybridization Washes

1. Take out the hybridization chambers, disassemble them carefully (each seperately)
2. Take out the slides, put them in the first preheated 2X SSC, 0.1% SDS jar, until coverslips freely move away from the slides.
3. Transfer slides to a new jar containing preheated 2X SSC, 0.2% SDS and gently agitate for 5 min.
4. Transfer slides to 0.1X SSC, 0.1% SDS at room temperature and wash for 5 min.
5. Transfer arrays to 0.1X SSC and wash for 5 min.
6. Rinse arrays in 0.01X SSC for 10 sec and immediately spin-dry for 5 min.
7. Scan the arrays.

Solutions Required for Microarray Exepriments :

- Filtered and autoclaved ddH2O

ALL solutions to be filtered (Nalgene filter) and autoclaved.

0.01X SSC :

20X SSC : 400 µl

ddH2O : 799.6 ml

2 Bottles of 0.1X SSC :

20X SSC : 4 ml (x2)

ddH2O : 796 ml (x2)

0.1X SSC, 0.1% SDS :

20X SSC : 4 ml

10% SDS : 8 ml

ddH2O : 788 ml

2X SSC, 0.1% SSC :

20X SSC : 80 ml

10% SDS : 8 ml

ddH2O : 712 ml

Prehybridisation Solution (5X SSC, 25% formamide, 0.1% SDS, 0.1 mg/ml BSA)

20X SSC : 200 ml

10% SDS : 8 ml

BSA : 80 mg

ddH2O : 392 ml

Filter and autoclave. Only after that, add 200 mL formamide.

2X Hybridisation solution (6X SSC, 0.2% SDS, 0.4µg/µg poly(A), 0.4 µg/µl yeast tRNA)

For 200 µl :

20X SSC	10% SDS	4µg/µl Poly(A)	tRNA (2 µg/µl)	Formamide
60 µl	4 µl	20 µl	40 µl	76 µl