

Spread Sheet for CyScribe cDNA Post Labelling Kit

8 samples ↔ 4 arrays

batch nr.:

Date:

Reference: STR aRNA, 2 rds ampl. (other:)

Samples:, 2 rds ampl.

PREHYBRIDISATION-PROTOCOL WITH FORMAMIDE

Array ID/Barcode	Sample (Cy5)					Reference (Cy3)				
	SMD Exp.-Name	RNA		prim	H ₂ O	ref	RNA		prim	H ₂ O
		ug	μl	μl	μl		ug	μl	μl	μl
		1		1+1		STR	1	1	1+1	8
		1		1+1		STR	1	1	1+1	8
		1		1+1		STR	1	1	1+1	8
		1		1+1		STR	1	1	1+1	8

Samples undiluted or diluted *(1:10)

Primers: for aRNA use 1μl random nonamers (black) + 1μl oligo(dT) primer (white)

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

Pipette: sample + H₂O (ad 9) + 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 ddH₂O, then stable for 1 month

+ 8 μ l MM

+ 1 μ l CyScript RT (red) to each tube

Incubate at 42° C for 1.5 hours

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

Incubate at 37° C for 15 min

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

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

Add sample

Add 75 μ l 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 μ l 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 μ l of RNase-free ddH₂O (RT)

Resuspend one aliquot of CyDye (Cy3 or Cy5, short spin!!!) in 15 μ l 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 post-process the arrays

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

QIAquick PCR Purification Kit (Qiagen)

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

Add 225 μ l of buffer PB to each sample, RT

Add the labelling solution to the column

Spin for 60 sec at 10.000 g, RT

Discard flow-through

Add 750 μ l 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 37° C, waterbath at 65° C (put Washing Solutions 1 and 2 in the waterbath)

V at 10ul with H₂O (nuclease free)

+ 1 µl poly-A-RNA (20 µg)

+ 20 µl human cot-1-DNA (20 µg)

+ 1 µl yeast t-RNA (20 µg)

+ 6.8 µl 20x SSC

+ 1.2 µl 10% SDS

V total = 40 µl

95° C for 1 min (thermoblock)

37° C for 10 min (thermoblock), spin

Place the probe on a microarray

Place some drops (30-40 µl) of 3X SSC in the chamber (humidity)

Cautiously place the cover slip (24x60 mm) on the probe; while lowering the cover slip, avoid air bubbles if possible and try not to scratch the slide

Close the hybridisation-chamber tightly and place it in a waterbath at 65° C overnight (14 hours at minimum)