RACE Protocol

1. Cell clone lysis and poly-A isolation

Lysis buffer: 100mM Tris/HCl pH 8.0
500mM LiCl
10mM EDTA
1% LIDS
5mM DTT

1.1. mix the cell lysates with lysis buffer, pipet up and down a few times
1.2. transfer 25 µl of lysate into 96-well plate
1.3. add 5µl of oligo d(T)-Biotin solution
Biotin solution: 5µl od(T)Biotin in 1000µl 10mMTris-0,05%DEPC-water
(stock: 2nmol/20µl, i.e. 100pmol/µl)
1.4. incubate 15-30 mins at 37°C
1.5. transfer to streptavidin-tubes or plates (Roche/ Advanced Biotechnology)
1.6. incubate for 15-30 mins at 37°C
1.7. wash 3 x with ice-cold 100 µl of 1x wash-buffer (diluted to 1x with 0.05% DEPC-water)
1x wash buffer: 10mM, Tris 8.3
200mM LiCl
1mM EDTA
1.8. add 15 µl random primer mix
random primer mix: 0.5 µl random primer (400pmol/µl)
14.5 µl 10mM Tris (DEPC-water)
1.9. incubate at 70°C for 10 mins (primer annealing)
1.10. transfer samples onto ice

2. First strand cDNA synthesis

2.1. prepare master mix as follows (final reaction volume 25 µl)

for 1 sample:

5x Reverse Transcriptase buffer	5.0 µl
Nuc's 10mM stock	1.25 µl
RNase-inhibitor (40units/µl; 25,2U/sample)	0.63 µl
ReverseTranscriptase (200units/µl; 126U/s)	0.63 µl
Bidest-water	2.5 µl

2.2. add 10 µl cDNA-mix to each sample

2.3. incubate at:

30°C for 10 mins
42°C for 40 mins
51°C for 10 mins
70°C for 1 min

3. cDNA purification Cleaning using Millipore-Multiscreen-PCR-Filter-plates

3.1. add 25 µl Bidest-water to Multiscreen-PCR-plate to wet the membrane
3.2. dilute the 25 µl cDNA with 100 µl Bidest and transfer it into the Multiscreen-PCR-plate
3.3. place the Multiscreen PCR-plate on top of the MS-manifold, place a plate for waste
3.4. apply vakuum at 15 inches for 10 mins

4. Poly d(C) tailing

CTP-mix for 1 sample

Bidest-water	12.5 ul
5x tailbuffer	5.0 µl
2mM CTP	2.5 ul

4.1. remove plate from MS-manifold
4.2. add 22 µl CTP mix to the dry cDNA on Multiscreen-PCR-plate
4.3. dissolve sample by shaking roughly 5 mins and pipetting up and down
4.4. retrieve 20 µl of cDNA and transfer to a new plate
4.5. preheat PCR-machine and incubate samples at 70°C for 5mins
4.6. chill rapidly to 4°C on ice-bath (≥1min)
4.7. prepare tailing-enzym

for 1 sample

5x tailbuffer	0.2ul
TdT (15units/µl)	0.3ul
Bidest-water	4.5ul

4.7. add 5 µl of this reaction mix to each well
4.8. incubate at 37°C for 10 mins, and 72°C for 10 mins
4.9. continue to 1st round PCR

5. First round PCR

component	amount (µl)
tailed cDNA	1.00
sterilized, destilled water	3.50
10x reaction buffer	0.75
25mM MgCl2	0.60
2mM dNTP mix	0.75
1st specific primer (10pmol/µl)	0.50
1st anchor primer (10pmol/µl)	0.25
Red Hot Taq Polymerase (ABgene)	0.15
final volume	7.50

23x(96°C/15s; 60°C/10-15s; 65°C/180s) + 72°C/10mins

6. Second round PCR

component	amount (µl)
1/10 diluted first round PCR product	1.00
sterilized, destilled water	3.50
10x reaction buffer	0.75
2 mM dNTP mix	0.75
2nd specific primer (10pmol/µl)	0.50
2nd anchor primer (10pmol/µl)	0.25
Taq Polymerase (Eppendorf)	0.07
final volume	7.50

30x(96°C/15s; 63°C/15s; 72°C/180s) + 72°C/10mins

7. Primers

Number	Sequence (5´ -> 3´)	Primer type	Tm value
69	CTA CTA CTA CTA GGC CAC GCG TCG ACT AGT ACG GGI IGG GII GGG IIG	1st (5') anchor primer (including G tail)	66.9
36	CTA CTA CTA CTA GGC CAC GCG TCG ACT AGT AC	2nd anchor primer	66.2
M13(-21)	CGA CGT TGT AAA ACG ACG GCC AGT	Sequencing primer	61.4
pT1betageo			
73	GCC AGG GTT TTC CCA GTC ACG A	1st specific primer	61.5
57	TGT AAA ACG ACG GCC AGT GTG AAG GCT GTG CGA GGC CG	2nd specific primer; seq. with M13(-21)	71.7
204	GCC AGT GTG AAG GCT GTG CGA G	Altern. sequencing primer	63.4
ROSAbetageo & U3geo			
135	GCC ATT CAG GCT GCG CAA	1st specific primer	57.3
133	CAAGGCGATTAAGTTGGGTAACG	2nd specific primer	57.9
203	GGG TTT TCC CAG TCA CGA CG	Sequencing primer	59.6
FlipROSAbetageo			
353	CAG GGT TTT CCC AGT CAC GAC	1st specific primer	59.6
354	TGT AAA ACG ACG GGA TCC GCC	2nd specific primer, seq. with 361	59.6
361	GTC ACA GAT CAT CAA GCT TAT CG	Sequencing primer for 354	56.1
360	TGT AAA ACG ACG GCC AGT TTG TAA AAC GAC GGG ATC CG	2nd specific primer, seq. with M13(-21)	67.3
Ceo-(CD2)-vectors			
349	CAA GTT GAT GTC CTG ACC CAA G	1st specific primer	57.8
371	CCA AGG CAC CCC AGG TTT C	2nd specific primer, seq. with 372	59.6
372	CCC CAG GTT TCC AAG GCA TTC	Sequencing primer for 371	59.6
352	TGT AAA ACG ACG GCC AGT ACC CCA GGT TTC CAA GGC ATT	2nd specific primer, seq. with M13(-21)	68.2