PROTOCOL FOR CATALOGUED ANALYSIS OF GENE EXPRESSION

Tao Feng 8/14/01, Edited by JYP 8/13/02

I. Isolation of total RNA

II. Making BNT18-beads

1. 500ul M-280 streptavidin beads (regular beads, mix well) on Magnet Particle Concentrator (MPC).
2. Remove supernatant.
3. Wash with 500ul 1X binding buffer (BB); place in MPC.
4. Remove supernatant.
5. Repeat steps 3 and 4.
6. Resuspend beads in 100ul 1X binding buffer.
7. Add 15ug of BNT18.
8. Mix well; incubate at RT for 10 minutes.
9. Remove the supernatant, save the supernatant (#1) to check the binding efficiency.
10. Wash beads with 200ul 1X BB $\times$ 2 times.
11. Remove supernatant on MPC.
12. Repeat steps 6-11, save the supernatant (#2) to check the binding efficiency.
13. Resuspend beads in 500ul 2X binding buffer, keep @ 4°C.

III. mRNA selection

1. Pick up 200ul above BNT18-beads; remove binding buffer.
2. Resupend BNT18-beads in 100ul 2X binding buffer.
3. Add 100ug of total RNA (final volume is 100ul) above the beads.
4. Mix well; incubate at RT for 10 minutes.
5. Place on MPC; remove the supernatant.
6. Wash beads with 300ul washing buffer.
7. Resuspend beads in 200ul of washing buffer; ready for 1st strand cDNA synthesis.

IV. Synthesis of 1st strand cDNA

1. Wash beads with 1X 1st strand buffer.

Items	1X 50ul

5x first strand buffer	10.0	2.
10mM dNTPs mix	2.5	
Rnasin	1.0	
DTT(100mM)	5.0	
MMLV RT	2.5	
Rnase-free ddwater	29.0	

3. And add the reaction solution above to the beads tube (remove the washing buffer first).
4. Mix well, and keep @ 37°C for 60 minutes.
5. Mount the slide with permount, cover the section with cover slip.

V. Synthesis of 2nd strand cDNA

1. DEPC water 291ul
   dNTPs (10mM) 7.5ul
   10 X 2nd strand buffer 40.0ul
   E.coli DNA pol. I 10ul
   E.coli DNA ligase 1.25ul
2. Add 350ul mixture to the 50ul 1st cDNA tube and mix well.
3. Keep at 16°C for 2 hours.
4. Add 5ul Dnase-free Rnase and keep at 37°C for 30 minutes.
5. Wash 3X with washing buffer.
6. Resupend in 100ul of washing buffer and keep store at -80°C.

VI. Digestion of double strand cDNA with NlaIII

1. Wash beads with 200ul 1X buffer"4" (NEB).
2. 10X buffer 4 (NEB) 10ul
   100 BSA 1ul
   NlaIII (10u/ul) 5ul
   DEPC-H_2O 84ul
3. Total 100ul, transfer the reaction solution into the beads tube, keep at 37°C for 1-2 hours.
4. Place on MPC, remove supernatant.
5. Wash with 300ul 1X ligation buffer.
6. Resuspend beads in 100ul of 1X ligation buffer.
7. Take 25ul for continuous step below (2 tubes).
8. The remaining 50ul beads (cDNA cut by NlaIII) are stored at -80°C.

VII. Ligation with NlaIII linker

1. Ligation reaction

5X ligation buffer	4.0ul
NlaIII linker	1.0ul (1:5 diluted)
ddH_2O	13.0ul
T4 ligase	2.0ul

2. Total 20ul; add into beads tube (after remove the 25ul supernatant)
3. 16°C for 4 hours.
4. Add 100ul ddH_2O, and keep @ -80°C.

VIII. NotI digestion

1. Pick up 50ul solution from the steps above; remove supernatant on MPC.
2. Wash beads with 100ul NotI buffer.
3. Cut beads at 37°C with NotI in 100ul volume for 2 hours.
4. Transfer the supernatant (about 100ul) to another new tube on MPC; keep at -20°C.

IX. PCR with primer "BNT18" and anchor primer

1. Anchor primer
   NAPA,NAPC,NAPG,NAPT,LNAPAA,LNAPAC,LNAPAG,LNAPAT,LNAPCA,LNAPCC,LNAPCG,LNAPCT,LNAPGA,LNAPGC,LNAPGG,LNAPGT,LNAPTA,LNAPTC,LNAPTG,LNAPTT,M13R(control)
2. Primer 2
   BNT18,BNT18A,BNT18C,BNT18G,BNT18T
3. Set up the PCR reaction solution and condition

10X buffer	1.0ul
DMSO	0.6ul
dNTPs (10mM)	0.4ul
Primer 1 (350ng/ul)	0.5ul
Primer 2 (350ng/ul)	0.5ul
ddH2O	6.3ul
Taq E	0.2ul
DNA Template	1.0ul

92°C	2'	1 cycle
92°C	30"	
65°C	30"	30 cycles
70°C	40"	