I. Probe Labeling and Hybridization

1.取 RNA 40 ug for Cy5, 20 ug for Cy3 + 2 ul poly dT (0.5 ug/ul in DEPC ddH2O)
補 DEPC ddH2O 至 19 ul

2. Mix well and heat to 65℃ 10-15 min to denature RNA (prepare supertanscript reation mixture), cool down on ice

3. Supertanscript reation mixture

8 ul 5 x first strand burffer
2 ul 20 X low T-dNTP (10 mMA, C and GTP, 4 mM dTTP in DEPC water)
4 ul 0.1 M DTT
1 ul RNasin
2 ul superscript II
4 ul 1 mM Cy3- or Cy5- dUTP 最後才加入 (之後的操作避光進行)

total reaction volume 40 ul in 100ul 微離心管中 (RNase free) --- > 42℃, 60 min

Stop transcription reaction and hydrolyze RNA : + 5 ul 500 mM EDTA --- > + 10 ul 1M NaOH --- > 65℃ ~45-60 min --- >
Neutralize the pH : + 25 ul 1M Tris (pH 7.6)

4. Pre-hybridization

Prepare 100 ul pre-hybridizaton buffer : 5 X SSC , 0.1% SDS and 1 % BSA
Pre-hybrid the microarray chip in 37 ul pre-hybridizaton buffer, 42℃ in chamber , 1 hr. (water bath 需保持水平).
Wash off the pre-hybridizaton buffer by rapid dunking in ddH2O 2 min --- > in Isopropanol 2 min --- > spin 600 r.p.m. 5 min to dry

5. Purify labeled probe

Vortex Bio-Spin 6 columns to mix well gel --- > 3000 r.p.m., 2 min to remove buffer (gel 呈現白色) --- > 去掉下層,並且更換 new 1.5 ml eppendorf to collect --- > 各別 loading labeled probes (~80 ul) into each column --- > 3000 r.p.m. , 4 min

Wash and concentrate purified probe: + 200 ul TE (pH7.6) to microcon YM 30 ---> + 80 ul elution (from Bio-6 column) 此時將 Cy3 an Cy5 labeled probe mix to the one microcon, pipette to mix --- > 14000 r.p.m. 15 min --- > + 400 ul TE to

wash --- > 14000 r.p.m., 15 min --- > 重複 washing --- > 14000 r.p.m., 6 min to concentrate to ~17 ul (用 TE buffer 補足) --- > recover the concentrator over 1 clean collection tube --- > 4000 r. p. m., 3 min

6. ~ 17 ul The purufied probe
   1 ul COT-1 DNA (10ug/ul)
   1 ul yeast tRNA (4 ug/ul)
   1 ul poly A (1 ug/ul)
   total 20 ul , denature at 90-94℃, 2min (only treat before hybridization)

7. Hybridization
   Make fresh 2 X hybridization buffer
   50 ul 100% Formamide
   50 ul 20 X SSC
   2 ul 10% SDS

   20 ul hybridization buffer + 20 ul sample (denature) mix, loading ~37 ul on coverslip, cover with slide, 放入 chamber , 42℃ , 16~ O/N

II. Washing and Scanning
(將 slide 從 chamber 拿出後, washing 至上機過程全程避光,最好關掉 lab 日光燈)
Warm up the scanner (~20 min) and prepare washing buffer

2 X SSC, 0.1% SDS	(400 ml in 染色壺)	10 min
1 X SSC, 0.1% SDS	(400 ml in 染色壺)	10 min
0.2 X SSC	(400 ml in 染色壺)	10 min
0.05 X SSC	(400 ml in 染色壺)	2 min

--- > spin 600 r.p.m. 5 min