

Yeast protocol

All the references and suppliers mentioned in this protocol are the one we use and recommend. There is no obligation to use these particular products and suppliers.

RNA extraction

- 1- Centrifuge cells at room temperature, wash with sterile water and centrifuge again. Usually, 50ml of culture at OD 0.6-0.8 are enough.
- 2- If necessary, store cell pellets at -80°C.
- 3- Resuspend cell pellets in TES buffer (10mM Tris pH 7.5, 10mM EDTA, 0.5%SDS, autoclaved) (1.2ml buffer for 50 OD of cells).
- 4- Transfer 400ul aliquots of the resuspended cells in three 1.5ml eppendorf tubes.
- 5- Add 400ul of Phenol:Chloroform 5:1 (from Sigma ref: P-1944).
- 6- Incubate at 65°C for 20min and vortex 30sec every 5min (be very careful with hot phenol!!).
- 7- Cool at -80°C for 30min.
- 8- Centrifuge at room temperature for 15min at 15000rpm.
- 9- Keep the aqueous phase and add 400ul of Phenol:Chloroform 5:1.
- 10- Vortex tubes three times for 30sec then centrifuge.
Repeat steps 8 to 10 one more time.
- 11- Add 300µl of Chloroform (ready red from Sigma) to the aqueous phase.
- 12- Vortex tubes three times 20sec.
- 13- Centrifuge 2' at 15000rpm.
- 14- Transfer aqueous phase in tubes containing 30µl of 3M Na Ac pH 5.3 (autoclaved).
- 15- Add 600µl Ethanol absolute.
- 16- Incubate one hour at -20°C.
- 17- Centrifuge 15min at 15000rpm at 4°C.
- 18- Dry pellets and resuspend the three pellets in the same 100µl of bidistilled autoclaved water (we use commercial water from Eurobio, Molecular Biology quality).
- 19- Carefully quantify RNA and use 100µg for RNA clean up.

RNA clean up

- 1- The RNA clean up is an essential step for the quality of the Reverse Transcription. Use a Rneasy minikit (Quiagen, ref : 74106) for RNA clean up. Just follow the recommendations of Quiagen.
- 2- Elute the column with 50µl of water a first time, then re-elute a second time with the same 50µl.
- 3- Quantify your purified RNA. You will need 10µg for cDNA synthesis.

cDNA synthesis and labelling

For the RT reaction, we use a PCR machine (mastercyclerEppendorf) for all the incubation steps.

For each condition (Cy3 and Cy5):

1-Primary mix :

In a 0.2ml PCR tube put :

10µg of your purified total RNA

5µg of random hexamers (Roche, ref :1034731)

2µg of oligo dT (Invitrogen, ref : yo1212)

add bidistilled autoclaved water to a final volume of 23µl

2- Incubate 10min at 70°C.

3-Put immediately on ice.

Add 1µl of Cy3 OR Cy5 (1mM) from Amersham-Pharmacia.

Add 16µl of the following mix to each tube: for one double-labelling reaction (Cy3 tube and Cy5 tube),

16µl of 5X Superscript II buffer (Gibco BRL, ref : 18064-014), 8µl of DTT (0.1M) (supplied with the Gibco BRL Superscript II kit), 4µl of dNTP mix (do it yourself : 2.5mM dATP, dCTP, dGTP, 1.25mM dTTP), 4µl of Superscript II from Gibco BRL (200U/µl).

4- Incubate 10min at 23°C

5- Incubate 2 hours at 42°C

6- RNA Hydrolysis: add 15ul of 0.1M NaOH (made just before use).

7- Incubate 10min at 70°C.

8- pH neutralisation: add 15ul of 0.1M HCl

cDNA Purification

1- Pool the two reactions (Cy3 and Cy5).

2- Add 1/10th volume of Sodium Acetate 3M pH 5.2 and 2.5 volumes of 100% Ethanol.

3- Precipitate at -80°C for 30 minutes.

4- Centrifuge 30 minutes at 13500 rcf at 4°C

5- Pour off all ethanol carefully. You do not need to dry the tube.

Proceed to the following extra-purification step using a Quiaquick column (Quiagen, ref : 28106) to reduce the background. This step is essential to avoid high background on your slide :

6- Resuspend pellet in 40µl of water, add 4µl of Sodium Acetate and 200µl of Quiagen buffer PB.

7- Apply the mixture to a Quiaquick column and centrifuge at 13,500 rcf for 1 minute.

8- Discard flow-through, add 600µl of PE buffer. Spin at 13,500 rcf for 1 minute.

9- Discard flow-through and spin for 2 more minutes at 13,500 rcf to dry filter.

10- Put the column in a new, clean, 1,5ml centrifuge tube.

- 11- Elute by adding 30µl of water preheated to 37°C, let stand 1 minute, spin for 1 minute at 13500 ref. You can repeat this elution step with the same 30µl to ensure better yield of elution.

Pre-hybridisation of the microarray slide

Remember that the spots of DNA are on the face with no barcode tag !!!

This pre-hybridisation step has to be done not more than two hours before you are ready for hybridisation. You should not keep pre-hybridised slides for more than 3 hours.

- 1- Prepare the following blocking solution : 5X SSC, 0.1% SDS, 1% BSA (we recommend the 30% BSA stock solution from Sigma ref : A-9576).
- 2- Preheat blocking solution at 42°C.
- 3- Incubate the microarray in this solution at 42°C for 45 minutes. All the incubation and rinsing steps can be made in a 50ml Falcon tube.
- 4- Rinse the slide five times in bidistilled water (make 10 up and down of the falcon tube for each rinsing step).
- 5- Rinse the slide in isopropanol (make 10 up and down of the falcon tube for each rinsing step) and leave it to dry vertically on a clean bench paper.
- 6- Put the slide in a dust-free box before hybridisation.

Hybridisation of microarray

Remember that the spots of DNA are on the face with no barcode tag !!!

- 1- After Quiaquick purification : mix the 30µl of purified cDNA with 30µl of 2X hybridisation buffer (50% formamide, 10X SSC, 0.2% SDS, preheat at 70°C before use).
- 2- Put the microarray slide in a hybridisation chamber (we use Telechem's, www.arrayIt.com) with 15µl of water in each hole.
- 3- Pre-heat the purified cDNA at 70°C for 2-5 minutes (you can centrifuge to avoid bubbles).
- 4- Apply immediately the cDNA solution on the microarray (it is better make several small deposit all along the array than one big drop at the center) and immediately put on a 22X60mm coverslip angled from bottom (left) to top (right). Avoid bubbles as much as possible when you drop the cDNA mixture but do not try to discard bubbles if some have formed under the coverslip.
- 5- Seal chamber and put it into a 42°C water bath overnight.

Washing slides

Rinse the slides with :

- 1X SSC, 0.2% SDS preheated at 42°C
- 0.1X SSC, 0.2% SDS at room temperature
- 0.1X SSC at room temperature

Proceed the same as for the pre-hybridisation washing step: make 10 up and down of a 50ml falcon tube for each rinsing step. Mind the coverslip during the first washing step : once the coverslip has fallen down in the liquid, put the slide so that the coverslip will be on the side with no DNA spots in the falcon tube, otherwise it will damage the microarray !

After the last washing step, dry the slides by centrifugation (500g for 3 minutes). Put a paper towel at the bottom of the slide holder to absorb liquid during centrifugation. Proceed quickly to avoid drying of the buffer on the slide.

Scanning of the slide

Use your favorite scanner, or the Axon scanners of the SGDB (you have to book it !). Do not forget that the spots are on the side with no barcode tag and that you have to scan the slides so that the bar code tag on the top of the picture, otherwise you will not be able to apply the analysing grid correctly.