

Standardised protocol for visualisation of single transcripts (submitted by Edouard Bertrand)

General procedure

Coating of coverslips

Soak coverslips in a 1N solution of HCl. Boil for 15 min in HCl and then wash ten times in water (e.g. 0.5 – 1l water/100-200 coverslips). Sterilise by autoclaving and store at 4°C.

Wash the coverslips with acetone and let dry.

Put the coverslips in 6-well culture dishes.

Put 300µl of 0.1% Poly-lysine on coverslips.

Incubate 5 to 10 minutes then wash with buffer B (see below) before adding the yeast suspension.

Fixation

Inoculate 40ml YEP + Glucose 2% with 10ml overnight yeast culture and grow to OD₆₀₀ of 0.5. Cells are fixed for 30 minutes at room temperature by directly adding 5ml of 32% (v/v) paraformaldehyde to the medium.

Pellet cells and wash once in ice-cold buffer B pH7 (1.2M Sorbitol, 0.1M Potassium phosphate, pH7).

Spheroplasting

Re-suspend yeast cell pellet in 2ml of buffer B. Take 10µl in an Eppendorf tube and keep as an untreated control.

Add 4µl of 14.35M β mercaptoethanol and 20µl of 10mg/ml stock of Zymolase 20T (Final concentration = 100µg/ml). Incubate for 30-45 minutes at 30°C.

To verify spheroplasting, add 10µl of culture (with and without Zymolase) to 1ml 10% (w/v) SDS and measure the OD₆₀₀: OD₆₀₀ without zymo = 1

OD₆₀₀ with zymo = 0.1

⇒ 90% efficiency of digestion

Then, centrifuge 2 minutes at 3000rpm at 4°C, wash with ice-cold buffer B and resuspend cells in 1.2ml of buffer B (0.6 ml for 6 coverslips).

Wash the poly-lysine coated coverslips with 100µl of buffer B. Add 100µl of the suspension on each coverslip. Let cells attach 30 minutes at 4°C and remove excess liquid.

Permeabilise the cells by slowly adding 3ml of 70% (v/v) ethanol. Leave overnight or longer at 4°C.

Optimised condition for the MS2 probe

Sequence of MS2 probe:

MS2_bis :

5' AT*GTCGACCTGCAGACAT*GGGTGATCCTCAT*GTTTTCTAGGCAATT*A

T* are conjugated with Cy3.

Hybridisation

Wash coverslips in PBS 2 minutes then in 1xSCC, 30% formamide for 30 minutes.

During this time, prepare as follows an aliquot of the hybridisation solution (50µl/coverslip):

Solution A:

- 30% formamide
- 1xSCC
- 1.6µl tRNA 20µg/µl
- 20ng each oligo
- Add water (RNase-free) to give a final volume of 50µl

Heated to 80°C for 1 min and cool at RT before adding:

- 1µl BSA (RNase free 1mg/ml)
- 1µl Ribonucleoside Vanadyl Complex 200mM
- 48µl 20% dextran sulfate.

An aliquot of 50µl of the hybridisation solution is placed on a petri dish and the coverslip turned over it. Place in a humidified chamber (1xSCC, 30% formamide) to hybridise overnight at 37°C.

The next day the coverslips are washed twice with 1xSCC, 30% formamide for 30 min at 37°C and twice 1 hour in the same solution.

Then, wash coverslips in PBS for 2 minutes and mount in glycerol 90%; PBS; p-phenylenediamine 1mg/ml; 0.1µg/ml DAPI.