

Yeast in situ Hybridization

This is a modification of the protocols on the RH Singer lab web site, www.singerlab.org. The original reference, Long RM, et al. RNA 1995 Dec;1(10):1787-1794 should be cited.

Grow culture to OD600= 0.4-0.8 in YPD or SD Medium (Approx. 120 Klett)

1. Fix cells by adding 20% Paraformaldehyde directly to culture with final concentration of 4% (12ml chemostat culture + 3ml paraformaldehyde). Keep at room temp for 45 minutes.
2. Spin cells at 3000x g for 5 min. Transfer pellet to 2ml tube.
3. Wash cells 3x 1.8ml with Buffer B.
4. Resuspend pellet in 1ml spheroplast buffer plus lyticase (890ul Buffer B + 100ul VRC + 10ul Lyt + 2ul β ME).
5. Incubate at 37C for 30-40min. Monitor digestion: ~90% phase dark (digested) cells.
6. Spin 5 min at 3500rpm (do not spin too hard as cells are fragile due to lyticase).
7. Wash with 1 ml Buffer B.
8. Resuspend pellet in 500 μ l Buffer B and keep on ice.
9. Remove 150 μ l and drop onto poly-L-lysine coverslips so that the whole coverslip is covered. Gently pipette up and down on the coverslip for 1 min. After 1 min take up as much of the cell suspension as possible from the coverslip and return it to the stock on ice.
10. Gently resuspend the stock solution of cells before repeating on the next coverslip. Repeat for all coverslips.
11. Gently wash cell-covered coverslips with 2-3ml Buffer B.
12. Gently add 2ml 70% ethanol.
13. Incubate at -20C for several hours or overnight.

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- Prepare solutions F and H, 2x SSC, 40% Formamide/2x SSC, and probes. Dry probes.
14. Remove ethanol and rehydrate coverslip by adding 2-3ml 2x SSC at room temp for 5 min. Repeat.
 15. Wash once with 40% Formamide/2x SSC at room temp for 5 min.
 16. Add 12ul solution F to probe tube (10ul if probes not dry, e.g only up to 2 μ l probe + tRNA/DNA). Heat at 95C for 3 min.
 17. Add 12ul solution H to same tube (hybridization mix).
 18. Drop 23ul hybridization mix onto parafilm. Place coverslip with cells side down on the drop.
 19. Cover coverslips with parafilm and seal it around coverslip to avoid evaporation. Incubate at 37°C overnight in the dark.

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- Preheat 40% Formamide/2x SSC at 37°C.
20. Put 2 ml 40% Formamide/2x SSC in new 12 well tissue culture dish.
 21. Place cover slips, cells side up, back into tissue culture dish with 40% Formamide/2x SSC. Incubate at 37C 15 min. Steps 21 and 22 with rocking.
 22. Dump out and wash again with 40% Formamide/2x SSC at 37°C 15 min.

23. Wash with 2x SSC 0.1% Triton X-100 at room temp 15 min on shaker.
24. Wash with 1x SSC at room temp 15 min on shaker (wash twice if preferable).
25. Wash with 1xPBS plus DAPI (0.01µg/ml final) at room temp 2 min.
26. Wash with 1xPBS at room temp 2 min.
27. Remove coverslips and dip in 1x PBS to wash off remaining DAPI. Then dip in 95-100% ethanol to help dry for mounting.
28. Place coverslips, cells side up, on kimwipes to dry and place 2-3ul mounting medium in center of slide (do not want any bubbles in mounting medium).
29. After coverslip completely dries place coverslip on mounting medium cell side down.
30. Seal outside of coverslip to slide with nail polish remover and take a look!

Solutions

Buffer B

- 1.2M Sorbitol
- 100mM KHPO4 pH 7.5
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- e.g. 1MKH2PO4 8ml
- 1MK2HPO4 41.5ml
- Sorbitol 109.3g

Spheroplast Buffer

- 1.2M Sorbitol
- 100mM KHPO4 pH 7.5
- 20mM Ribonucleoside-vanadyl complex (VRC; NEB #S1402S)
- 50U lyticase per OD of cells

Lyticase

- Sigma cat# L5263; resuspend in 1x PBS to 25000U per ml. Store at -20C in single use aliquots. Use 50U in 1 ml spheroplast buffer for slow growing cells.

Solution F

- 160ul Formamide
- 2ul 1M NaHPO4 pH 7.5
- 38ul H2O
- 200ul

Solution H

Can vary concentration of Formamide from 40-50% depending on probe.

For final Formamide concentration of 50% in hybridization:

- 60ul H2O
- 40ul 20x SSC
- 40ul BSA (10mg/ml)
- 20ul VRC (100mM)
- 40ul Formamide
- 200ul

Probe

Use 1ng of each probe per *in situ*. Use 20 µg of E. coli tRNA/ssDNA mix (1:1) per *in situ* (tRNA Roche 10 109 541 001, ssDNA Sigma D9156-1ml).

Preparation:

- Mix probe/s and 20ug tRNA/DNA mix
- Open lids and dry down in speed vac. If using only up to 2 μ l total it's not necessary to dry down.

Mounting medium:

- modification of yeast immunofluorescence mounting medium; Methods in Yeast Genetics, Cold Spring Harbor Labs, 2000 edition
- dissolve 5mg p-phenylenediamine in 0.5ml PBS, pH not adjusted.
- add 4.5ml glycerol and store in small aliquots at -80deg. C. Can be reused if not brown

Preparation of Coverslips

1. Put one box of 18mm round coverslips into 500ml 0.1N HCL
2. Boil for 10 min
3. Rinse extensively with H₂O
4. Place in H₂O and autoclave
5. Store in 70% ethanol

Before Use:

6. Drop 150ul of 0.01% poly-L-lysine on coverslip (Sigma #P8920, dilute 1/10).
Leave at room temp for 5 min
7. Remove poly-L-lysine and let air dry
8. Wash 3x with H₂O
9. Dump out H₂O and let air dry