

UVxFisH of Yeast in Glyoxal [Last update: 6/4/2019]

Grow Culture

1. Prep 25ml cells/sample in YPD to OD 0.2-0.4

Glucose Starvation (30min)

1. Centrifuge culture at 3000rpm for 5min 4°C
2. Resuspend pellet in 1ml of SD-Glucose media, wash twice 3000rpm 5min
3. Resuspend pellet in 5ml of SD-Glucose media, transfer to small glass culture tube and incubate 15min, 30°C on rotor.
4. Take a 10µl sample and image cells for p-bodies
 - If there are fewer than 50% p-bodies, incubate cells for another 5 min, 30°C on rotor. If still not enough p-bodies, start over.

UV-Crosslinking and Fixation (90min)

1. Transfer to 15ml Falcon tube, centrifuge 3000rpm 5min
2. Resuspend in 4ml 3%Glyoxal, transfer to a glass petri dish
3. UV Crosslink 1mJ/cm³ (~15min)
4. Transfer to clean falcon tube and incubate 30 minutes on ice
 1. NOTE: After transferring to tube, use an extra ml fixative to get any spare yeast cells.
5. Incubate 30 minutes at RT, nutating
6. UV Crosslink 1mJ/cm³
7. Take a 10µl sample for imaging
 - It's a good idea to check p-body retention at this step. If fewer than 50% p-bodies, you may want to start over.

Spheroplast formation and Hybridization (150min + O/N)

1. Centrifuge samples 2400rpm 5min
2. Resuspend in 1ml cold Buffer B (on ice), transfer to eppendorf tube
3. Digest cell wall with 2.5µl Zymolyase (2.5mg/ml) at 30°C with rotation one hour
4. Wash with 1ml cold buffer B
5. Resuspend in 1ml 70% EtOH, incubate 1 hour at 4°C
 - Can also incubate O/N at 20°C but this may lead to 15-20% sample loss.
6. Warm hybridization solution to RT before opening (prevents formamide oxidation)
7. Centrifuge samples, resuspend in 1ml 10% Formamide/2xSSC
8. Let stand 2 minutes (for formamide annealing)
9. Centrifuge, resuspend in 200µl hybridization solution
10. Add 0.5µl probe per 100µl, wrap in foil, incubate O/N at 37°C

Washing/Mounting (30min)

1. Add 1ml 10% Formamide/2xSSC to samples, wash 2400rpm 5min
2. Resuspend in 1ml 10%Formamide/2xSSC, incubate 30min at 30°C
3. Resuspend sample in 100µl 2xSSC
4. Image

40ml Glyoxal (store at RT, good for 4 weeks) <ul style="list-style-type: none"> - 28.35 ml ddH₂O - 7.89 ml ethanol (absolute, for consistency w/Richer et al.) - 3.13 ml glyoxal (40% stock solution from Sigma-Aldrich, #128465) - 0.3ml Acetic Acid (glacial) Bring to pH 5 with 1M NaOH	10ml Hybridization Buffer (store aliquots -20°C): <ul style="list-style-type: none"> - 1ml Formamide (100%) - 2.5ml Dextran Sulfate (40%) - 1ml 20xSSC - 20µl BSA (10mg/ml) - 100µl active VRC (vanadyl-complex, 200mM) - 1ml E. Coli tRNA (10mg/ml) - 4.38ml MilliQ H₂O
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