

Riboprobes (making and using)

General Notes

Ref: J. M. Liu Post-doc, experiment 94

Always practice RNase-Free technique when working with RNA

Use RNA-only pipet tips and tubes

Wipe off counter tops and pipetmen with RNaseZap

Filter-sterilize solutions prior to use

Use either DEPC-H₂O or MQ-H₂O

Keep RNA cold (4 °C or lower)

Reagents:

Forward and Reverse primers (Forward should have T7 sequence at 5' end:
GGATCCTAATACGACTCACTATAGGG)

Reagents for PCR (buffer, polymerase, 2.5 mM dNTP mix)

T7 RNA Polymerase (Promega P2075) with 5x Buffer and 100 mM DTT

2.5 mM ATP, CTP, GTP mix

2.5 mM UTP

2.5 mM biotin-16-UTP (Roche 11-388-908-910)

RQ1 DNase (Promega M6101)

DTR cartridges (Edge Biosystems)

Protocol:

Prepare DNA template (for MtlS riboprobe – 042208A and 042208B)

1. Set up the following reaction in a PCR tube:
 - 2 µL gDNA
 - 1 µL 50 µM Forward Primer
 - 1 µL 50 µM Reverse Primer
 - 5 µL 10x Buffer
 - 4 µL 2.5 mM dNTPs
 - 36 µL H₂O
 - 0.5 µL DNA Polymerase
2. Run PCR reaction 30 cycles, 55 °C 30s; 72 °C 30s
3. Run 5 µL reaction on a 2% agarose gel (product runs around 120 nt)
4. Column purify PCR reaction – elute with 50 µL 1 mM TRIS
5. Quantitate (5-10 ng/µL is good)
6. DNA can be stored at -20 °C

Make riboprobe

1. Set up the following reaction in a 1.5 mL RNase-free tube:
 - 2 μ L PCR-generated DNA template
 - 4 μ L 5x Buffer
 - 2 μ L 100 mM DTT
 - 4 μ L 2.5 mM rNTP mix
 - 2.4 μ L 2.5 mM UTP
 - 1.6 μ L 2.5 mM biotin-16-UTP
 - 3 μ L H₂O
 - 1 μ L T7 RNA Polymerase
2. Let reaction incubate at 37 °C for 1-3 hours
3. Add 1 μ L RQ1; incubate at 37 °C for 30 minutes
4. Clean up reaction with DTR cartridge
5. Add all of riboprobe to hybridization buffer

General procedure on Northern blots with riboprobes (MtlS)

Run out RNA on 10% TBE-Urea gel in 1xTBE (200 V, 55 m)

Transfer RNA to nylon+ membrane in 1xTBE (200 mA, 1h)

Wash membrane with 6x SSC; crosslink; wash membrane with 1x SSC

Prehybridize membrane (standard size) with 6 mL UltraHyb (Ambion) at 65 °C, for at least 30 minutes

Hybridize overnight at 65 °C

Detection of probe (see protocol in BrightStar BioDetect kit (Ambion))