Materials List for:
Visualizing RNA Localization in *Xenopus* Oocytes

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Materials

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|---|---|
|10X Tx buffer| • 60 mM MgCl_2
• 400 mM Tris-HCl (pH 7.5)
• 20 mM spermidine-HCl |
|20x cap/NTP mix| • 10 mM CTP
• 10 mM ATP
• 9 mM UTP
• 2 mM GTP
• 20 mM G(PPP)G Cap Analog (New England Biolabs) |
|G-50 column| • Hydrate 5 g Sephadex G-50 beads (Sigma Aldrich) in 100 ml deionized H_2O. DEPC-treat for 30 min. and autoclave. Store incomplete stock at room temperature. Before use, add the following RNase-free solutions:
• 0.5 ml 0.2 M EDTA
• 1 ml 1 M Tris pH 8.0
• 0.5 ml 20% SDS
• Store complete G-50 solution at 4 °C.
• Remove and discard the plunger from a 3 ml syringe (BD Biosciences) and place the barrel of the syringe into a 15 ml conical tube (Corning). Plug the syringe with a small amount of glass wool (a plug about half the size of a penny).
• Swirl complete G-50 solution to resuspend beads.
• Add 2 ml G-50 solution to the empty column.
• Spin for 1 minute at 1,000 x g in benchtop centrifuge.
• Add 200 μl DEPC-treated deionized H_2O to each column. Spin.
• Repeat wash twice more for a total of three washes.
• Remove syringe barrel to a fresh 15 ml conical tube. |
|Collagenase solution| • 75 mg collagenase from *Clostridium histolyticum* (Sigma Aldrich)
• 25 ml 0.1 M KPO_4+ (pH 7.4) |
|MBSH buffer| • 88 mM NaCl
• 1 mM KCl
• 2.4 mM NaHCO_3
• 0.82 mM MgSO_4 \times 7H_2O
• 0.33 mM Ca(NO_3)_2 \times 4H_2O
• 0.41 mM CaCl_2 \times 6H_2O
• 10 mM HEPES (pH 7.6) |
|Oocyte Culture Medium| • 50% L15 medium
• 15 mM HEPES (pH 7.6)
• 1 mg/ml insulin |
- 100 mg/ml gentamicin
- 50 U/ml nystatin
- 50 U/ml penicillin
- 50 mg/ml streptomycin

**MEMFA solution**

- 0.1 M MOPS (pH 7.4)
- 2 mM EGTA
- 1 mM MgSO$_4$
- 3.7% formaldehyde

**Computing RNA yield**

- Determine CPM in "input" and "incorporated" samples using a standard scintillation counter.
- incorporation = ("incorporated") / (10 x "input")
- Typical incorporation values range between ~0.03 and 0.10.
- Maximum theoretical yields for different polymerases: T7, T3, SP6 - 2.64 μg
- Reaction yield in μg = (maximum yield of polymerase used) X (incorporation)
- Dilute RNA to 50 nM = (μg RNA) / 320 / (length of RNA in bases) / (5×10$^{-8}$)
- The reaction usually yields ~50-100 μl of RNA at 50 nM.