Materials List for:

Visualizing RNA Localization in *Xenopus* Oocytes

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Materials

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_3+ (pH 7.4)

MBSH buffer

- 88 mM NaCl
- 1 mM KCl
- 2.4 mM $NaHCO_3$
- 0.82 mM $MgSO_4$ X $7H_2O$
- 0.33 mM $Ca(NO_3)_2$ X $4H_2O$
- 0.41 mM $CaCl_2$ X $6H_2O$
- 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) / ($5X10^{-8}$)
- The reaction usually yields ~50-100 µl of RNA at 50 nM.