1.4 Reagents

A.

CB 5X

RRL	Final	WG	Final
400 mM HEPES pH 7.5	80 mM	400 mM HEPES pH 7.5	80 mM
75 mM MgCl2	15 mM	70 mM MgAc2	14 mM
10 mM Spermidine	2 mM	10 mM Spermidine	2 mM

Chloride ions are inhibitory to the WG translation system, so we should try making CB with acetate reagents. I expect transcription will be fine with acetate, and our linked translations in WG may be improved).

NOTE: These values are probably alright for most of the plasmids in the lab. However, if you are going to translate a new plasmid, it is best to do a titration for these reagents (esp. the MgCl2) to determine which amounts give optimal levels of transcription. For example, plasmid #572 has an optimal MgCl2 concentration of 12 mM.

B.

(WG) **4 NTP (5X) capped** 15 mM of each ATP, CTP, UTP; 1.5 mM GTP Final concentration: 3.0 mM; 0.3 mM

Be sure and use the ribonucleotide triphosphates, NOT the deoxy ones!! Prepare 1 ml at a time and keep the solution frozen at -70 in small aliquots, say 100 ul each, using up one before going to the next. When thawed, always keep on ice and refreeze soon.) Remember, only baked spatulas into powder reagents! Make up each nucleotide stock solution at 0.1 M stock in H2O, without adjusting pH. Make the 4 triphosphate cocktail adjusted to pH approx 7 as follows:

0.1 M ATP	150 uL
0.1 M CTP	150 uL
0.1 M UTP	150 ul
0.1 M GTP	15 uL
H2O	535 uL

Check pH by spotting 0.5 ul onto pH 6-8 pH paper. If necessary, add 1 ul aliquots of 2 M Tris Base to adjust pH to approx 7.

(RRL) **4 NTP (5X) uncapped** 15 mM of each ATP, CTP, UTP, and GTP.

Make and pH cocktail as above.

C.

Cap (10X) 5 mM GpppG (diguanosine triphosphate)

Order from PL-Pharmacia in vials with 25 A250 units dried down. 21 A250 units = 1 mg, so 25 A250 = 1.2 mg. Mol wt is approx. 750 so 1.2 mg/300 ul = 5 mM. Add 300 ul 20 mM Tris pH 8, cap tightly, shake it well, spin down for a few seconds by low speed centrifugation, usually recovering about 250 ul. Check and if necessary adjust pH as above. This is another labile reagent which should be aliquoted into 50 ul aliquots and stored at -80 except for brief stays on ice. G cap is used because this is the first base of SP6 transcripts - its probably being partially capped in the translation extract - but is several fold stimulated by capped transcripts. Xenopus oocyte microinjection requires capped transcript for expression - it's probably rapidly degraded without the protection of the cap.

D.

DTT 10X 0.1 M dithiothreitol

A reducing reagent which I store in aliquots at -80. Half an ml will last your life for this purpose.

E.

tRNA 50X 10 mg/ml Boehringer calf liver transferase RNA

This provides any stray RNase something to chew on besides your transcript! There is usually a communal stock of aliquots in -70, so you should not make it up fresh yourself.

F.

RNasin 25X 27 U/ul RNase inhibitor RNA guard

Use straight out of the misc enzyme rack, following usual freezer enzyme handling rules. Extremely oxidation sensitive, so take what you need and cap tightly immediately.

G.

SP6 pol 10 U/ul SP6 polymerase

Use straight out of misc enzyme rack as above. Always check units. Sometimes its sold very concentrated, e.g. if it is 20 units/ul you should use only half the volume as you would at 10 U/ul.

H.

DNA - Double stranded plasmid DNA either supercoiled or linearized downstream from the end of your coding region.

DNA can either be used straight out of the plasmid stocks or if linearized, must be repurified by a round of phenol extraction and ethanol precipitation. Use in transcription reaction at final concentration of 0.1 mg/ml (or 1 ug per 10 ul reaction). Cell free translation will go well with either linear or supercoiled DNA, while Xenopus oocyte microinjection requires linear DNA. Sometime we should optimize the DNA concentration better than we have. I suspect that more than this is inhibitory, less might be better.