## 1.5 Troubleshooting

This is a highly reproducible and reliable approach to making large quantities of RNA. As long as your DNA is clean (phenol extracted and ethanol precipitated miniprep DNA and the enzymes haven't been abused, e.g. left out on the bench, etc. all should go well. When using miniprep DNA remember you must omit the RNAse step or any RNA you synthesize will get chewed up. Keep all of your key reagents (e.g. 4NTP -cap, cap, 0.1 M DTT, etc.) in small aliquots frozen at -80øC and work off of the same aliquot each time, putting a dot with magic marker on the side of the tube each time it's thawed and refrozen. That way you have some idea how many times its been used. When a problem develops, you can pull out a fresh, unused aliquot of everything and be confident that they should work. If not, suspect any newly prepared reagent, or get/give transcript from/to someone else. If the problem is corrected it suggests a problem with either your reagents or your assays for expression.

Transcription reaction mRNA products may be quantitated by fluorimetry (see appropriate section of lab manual). Radiolabelled RNA can also be produced by incorporating a nucleotide labelled with 32P or 35S at the à position. For a reasonable signal, 0.5 uL of (à-35S) UTP (10 mCi/mL) in a 10 uL reaction is sufficient. The 5X NTP mix will not have to be adjusted. 1 uL of this plus 9 uL of deionized formamide can be run on an 10% acrylamide/8 M urea TBE gel and visualized overnight by autoradiography.

The SP6 polymerase 10 u/uL stock will sometimes freeze if the freezer is much below -20øC. Adjust freezer temperature to -20øC, or keep a more concentrated stock.