recombinetics

mRNA Transcription from RCIscript-Vectors

Materials:
*Sac*I Restriction Enzyme (NEB High Fidelity recommended)
RNAsecure (Ambion AM7005)
Qiagen miniElute PCR cleanup kit
RNAase free injection buffer (5 mM Tris Cl, pH 7.5; 0.1 mM EDTA)
mMessage Machine T3 kit (Ambion AM1348)
Ambion MEGAClear kit (Ambion AM1908) or Qiagen RNeasy

General remarks:

A. *I have found mRNA generated from Qiagen miniPrep DNA to outperform that from other miniPrep kits. I suspect there are more contaminants (ie. Endotoxin) in non-Qiagen preps.*
B. *Both TALEN monomers can be synthesized in the same reaction with only minor effect on efficiency (>5% difference that synthesizing each individually). I typically add equimolar amounts of both left and right TALEN monomers to the restriction digest (step 1 of "TEMPLATE PREP") and proceed with one reaction per TALEN pair.*

TEMPLATE PREP:

1. Cut 5-10 ug (miniprep DNA >150 ng/ul is sufficient) of RCIscript-GoldyTALEN with 5x Units of *Sac*I-high Fidelity for 2+ hours in a 100 ul reaction.
2. Treat the reaction with 4 ul RNAsecure (Ambion) and incubate at 60° C for ten minutes.
3. Purify the RNAsecure treated DNA using the miniElute PCR cleanup kit from Qiagen. I typically wash once with PB and twice with PE prior to elution in 10-15 ul of RNAase free injection buffer (5 mM Tris Cl, pH 7.5; 0.1 mM EDTA). *!! Important !! Make sure to use clean buffers from the miniElute kit.*

mRNA SYNTHESIS:

1. Follow the standard protocol for mRNA production using the mMessage Machine T3 kit (Ambion). Use 1ug of linearized template as prepared above, and incubate at 37°C 1-2 hours.
2. After the 37°C incubation, add 1 ul of DNAase Turbo and incubate for an additional 15 minutes at 37°C.
3. Purify the mRNA using the Ambion MEGAClear kit and elute 2x with 50ul of heated H2O. I have also successfully purified mRNA using either the RNeasy Mini kit with a single 50 ul elution or for even higher concentrations of mRNA, the RNeasy MinElute Cleanup Kit with 10-20 ul elution.

4. Typical mRNA yield has been >35 micrograms. I typically run ~1 ug on a FA gel to ensure that I get one solid band (you will see some smearing below the band) of the expected size. *I prefer the 10% FA gel recipe in the back of the Qiagen RNeasy handbook. The gel loading buffer provided in the mMessage Machine kit works well in place of the loading buffer recipe in the Qiagen handbook.*