Black lab Mouse CRISPR/Cas9 protocol

We basically follow the protocol described in this Jaenisch paper:
http://www.cell.com/abstract/S0092-8674(13)00467-4
One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering

A few notes:

1) In vitro transcription of Cas9 mRNA and sgRNA

Cas9 and sgRNA are transcribed and "cleaned up" in vitro (just like making an in situ probe, except that the Cas9 mRNA need to be capped). We use the following kits.

→for Cas9 mRNA: mMACHINE T7 ULTRA kit (Life Technologies)
→for sgRNA: MEGAshortscript T7 kit (Life Technologies)
→for cleanup: MEGAclear kit (Life Technologies)

Note: We purchased the Cas9 mRNA from SBI and generated a cDNA and then plasmid from it, and we are under an agreement not to provide this material, but it is pretty easy to get Cas9 mRNA or plasmid from any number of sources. If you want to get it from SBI, here is the info: http://www.systembio.com, cat# CAS500A-1

2) RNA mixture preparation

We mix capped Cas9 mRNA (50-100ng/μl) and sgRNA [or RNAs if making a deletion] (10-20ng/μl) in pronuclear injection buffer (5 mM Tris-HCl, pH=7.4, 0.1 mM EDTA).

Note: According to the Jaenisch paper, Cas9 mRNA can be injected at as high a concentration as 200-300ng/μl without affecting viability, but we haven't found this necessary, and in fact, we observe too much deletion (i.e. both alleles) if we go too high.

3) Injection

Identical to regular pronuclear injection except that we inject into the cytoplasm.