**AltR Guide Duplex Formation and Cas9 Ribonucleoprotein (RNP) Complexing:**

Guide Duplex Reaction:

1. Re-suspend IDT CRISPR crRNA (unique sequence) and IDT CRISPR tracrRNA (same for each guide) at 100 μM in RNAse free Duplex Buffer
2. Combine equal volumes of re-suspended crRNA and tracrRNA (e.g. 5 μl each) in a PCR tube
3. Incubate at 95C for 5 minutes, then allow to cool to RT on benchtop
4. Nanodrop to QC if desired, however, the concentration can be assumed to be ~2000 ng/μl

Cas9/Guide RNP Complexing:

1. For electroporation preps with multiple guides, assemble guide RNA duplexes with Cas9 individually
   1. Generally, we use **6 μg** of each guide, and a total of **5 μg Cas9** protein in a final volume of **10 μl**, making up any volume with MIJ TE buffer
2. Incubate guide RNA/Cas9 mixtures at 37 C for 15 minutes
3. Combine RNP mixtures into one 10 μl prep