

General CRISPR-RNP Nucleofection using a Lonza 4D Nucleofector

Protocol:

1. Prepare RNP mix. Cas9-NLS is stored in -80, sgRNAs are prepped by runoff transcription. Cas9 buffer is kept in the TC hood and must be kept sterile.
 - a. Bring 100 pmol of Cas9 to a final volume of 5 μ L using Cas9 buffer (20 mM HEPES-KOH pH 7.5, 150 mM KCl, 10% glycerol, 1 mM TCEP). For 40 μ M stock: 2.5 μ L.
 - b. Bring 120 pmol sgRNA to a final volume of 5 μ L using Cas9 buffer. This means you will need a minimum sgRNA concentration of 24 μ M.
 - c. Add Cas9 to sgRNA slowly while swirling pipette tip, should take 30 s to 1 minute. This tip comes from the Doudna lab.
 - d. Allow RNP to form for 10-20 minutes.
2. Prepare Cells
 - a. Count cells. (Trypsinize as needed.) For each nucleofection, pipette 200k cells into a 15 mL conical.
 - b. Spin 100 x g for 10 minutes to pellet cells softly. While the cells are spinning, prepare plate and cuvette.
 - c. Prepare a 12-well-plate with 1mL media per well, and pre-warm in the incubator.
3. Nucleofection
 - a. Prepare and label wells on 20 μ L nucleofection strips. Configure Lonza 4d using recommended cell-type program.
 - a. Recommended nucleofection conditions and detailed protocols for most cell types can be found in the LONZA database:
<https://knowledge.lonza.com/>
 - b. Pipette off media from cells, gently but completely, using a P200. The pellet is very soft so be careful.
 - c. Resuspend cells in 20 μ L of nucleofector solution (usually SF media) using a P200.
 - d. Add the entire 10 μ L RNP mix to the 20 μ L resuspension and mix.
 - e. If performing HDR, add 1 μ L of 100 μ M donor DNA (100 pmoles) and mix well.
 - f. Add nucleofection mixes to the multiwell cuvette, and cap. Pay attention to the orientation of the cap and cuvette in the nucleofector, which is noted in the manufacturer's instructions. NOTE: Add carefully to one short side of the well, at an angle. Do not produce any bubbles. Solution need not be completely filling the well as long as there are no bubbles.
 - g. Insert cuvette into nucleofector and zap.
 - h. Allow cells to sit in nucleofection strips for 10 minutes post-nucleofection. This is supposed to increase efficiency.
 - i. Add 80 μ L of pre-warmed media to each well.
 - j. Pipette mixture out with a P200 into your pre-warmed 12-well plate. This should get the vast majority of cells, but if you wish, you may wash out the rest with media from the same well, chemistry-style.
 - k. Allow cells 24 hours to settle and recover before attempted downstream analysis. Consider including un-zapped controls to test viability.