RNP Nucleofection for cell lines using Lonza 4D Nucleofector

Protocol:

1. For each 20ul nucleofection aliquot 200k cells into 15 mL conical tubes.

2. Prepare RNP mix. Cas9 buffer is kept in the TC hood and must be kept sterile.
    a. Bring 100 pmol of Cas9 (15ug) to a final volume of 5 µL using Cas9 buffer (20 mM HEPES-KOH pH 7.5, 150 mM KCl, 10% glycerol, 1 mM TCEP). Add TCEP fresh each time.
    b. Bring 120 pmol sgRNA (~4ug) to a final volume of 5 µL using Cas9 buffer.
    c. Add Cas9 to sgRNA very, very slowly while swirling pipette tip, should take 30 s to 1 minute.
    d. Allow RNP complex to form at room temp for 10-20 minutes. During this step move on to preparing cells below.

3. Prepare Cells
    a. Spin cells at 100 x g (or below) for 10 minutes to pellet cells softly. While the cells are spinning, prepare plate and cuvette.
    b. Prepare a 12-well-plate with 1mL media per well, and pre-warm in the incubator.

4. Nucleofection
    a. Prepare and label wells on 20uL nucleofection strips. Configure Lonza 4d using recommended cell-type program.
    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 µL of nucleofector solution using a P200.
    d. Add the entire 10 µL RNP mix to the 20 µL resuspension and mix.
    e. Optional: Add 1uL of 100uM donor DNA (100 pmoles) and mix well.
    f. Add nucleofection mixes to the multiwell cuvette, and cap.
        a. Pay attention to the orientation of the cap and cuvette in the nucleofector, which is noted in the manufacturer's instructions.
        b. Add carefully to one short side of the well, at an angle.
        c. Do not produce any bubbles. The solution does not need to be completely filling the well as long as there are no bubbles.
    g. Insert cuvette into nucleofector and run nucleofection program.
    h. Allow cells to sit in nucleofection strips for 10 minutes post-nucleofection. This is supposed to increase efficiency.
    i. Add 80uL 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 moving on to downstream analysis.