DropSynth bead barcoding protocol

Prepare 2X Binding and Wash buffer (2M NaCl, 1mM EDTA, 10mM Tris)
2X B&W 40mL:

- 4.675g NaCl salt
- 400 uL UltraPure 1M Tris-HCl, pH 7.5 (Invitrogen)
- 80uL UltraPure 0.5M EDTA, pH 8.0 (Invitrogen)
- UltraPure Distilled Water (Invitrogen) to 40 mL

This protocol can be done on a single 384 well plate or 4x 96 well plates, the latter protocol is provided.
Reagents required:

- 384 uL 100 uM anchor oligo (Integrated DNA Technologies)
- 384 uL 100 uM ligation oligo (Integrated DNA Technologies)
- 1 uL 100 uM of each barcode oligo (Integrated DNA Technologies)
- 1,576 uL 10X T4 ligase buffer (New England Biolabs)
- 384 uL T4 PNK (10,000 U/mL) (New England Biolabs)
- 40 uL T4 ligase (concentrated 2,000,000 U/mL) (New England Biolabs)
- 1,920 uL stock Dynabeads M270 Streptavidin (Invitrogen)

For each of the four 96-well plates:

1. Mix 96 uL 100 uM anchor oligo and 96 uL 100 uM ligation oligo.
2. Prepare the 96 well plate. In each well add:
    - 2 uL of mixed anchor and ligation oligo
    - 1 uL 100 uM barcoded oligo
    - 4 uL 10X T4 Ligase buffer
    - 33 uL UltraPure Distilled Water
    - TOTAL: 40 uL
3. Anneal the mixed oligos on each plate using using the following conditions (30 min total):
    - 3 min at 70ºC
    - Ramp down to 60ºC for 1 min, 0.1ºC/sec
    - Ramp down to 50ºC for 1 min, 0.1ºC/sec
    - Ramp down to 40ºC for 1 min, 0.1ºC/sec
    - Ramp down to 30ºC for 1 min, 0.1ºC/sec
    - Put plate on ice
4. Ligate the barcoded oligo to the ligation oligo:
    - Make a 1:10 T4 Ligase dilution:
        10 uL T4 Ligase (concentrated 2,000,000 U/mL)
        10 uL 10X T4 ligase buffer
        80 uL H_2O
        TOTAL: 100 uL
    - Add 1 uL T4 Ligase (1:10 dilution) to each well
    - Incubate plate at 16ºC for 1 hr or longer, followed by 65ºC for 20 min to heat inactivate the ligase
5. Phosphorylate the barcoded oligo:
    - Add 1 uL T4 PNK into each well
    - Incubate the plate at 37ºC for 40 min (or longer), followed by 65ºC for 20 min to heat inactivate the PNK
6. Bind to beads:
    - Prepare 480 uL stock Dynabeads M270 Streptavidin, washed, and resuspended in 960 uL B&W buffer
    - Add 10 uL resuspended beads to each well. (~3.25E6 beads/well and ~18.5E6 molecules/bead)

    - Mix overnight with shaking (2000 RPM) at room temperature.
7. Pool beads:
    - Wash each well with 150 uL B&W buffer 5 times.
    - Resuspend in 10 uL B&W buffer
    - 1 uL of each well is mixed together, making a 96 uL mixed barcoded bead pool for each plate.
    - Mix 96 uL from each plate to make a full 384 uL mixed barcoded bead pool. Store these at 4ºC when not in use.