DropSynth bead barcoding protocol (version 2.0 Aug 2019)

This protocol can be performed using 1x 384-well plate to generate 384 unique barcoded beads, or 4x 384-well plates to generate 1536 unique barcoded beads. Though the process can be done by hand, it is helpful to use a Rainin Liquidator 96 for liquid handling steps.

Reagents Required (384-plex):

1. 240 µL 100 µM anchor oligo (Integrated DNA Technologies)
2. 240 µL 100 µM ligation oligo (Integrated DNA Technologies)
3. 1,056 µL 10X T4 Ligase Buffer (New England Biolabs)
4. 1 µL of each 100 µM barcoded oligo (Integrated DNA Technologies)
5. 24 µL T4 Ligase (New England Biolabs)
6. 240 µL T4 PNK (New England Biolabs)
7. 1500 µL Streptavidin M270 Dynabeads (Invitrogen)
8. >10 mL UltraPure Distilled Water (Invitrogen)
9. >10 mL 2X B&W Buffer

Reagents Required (1536-plex):

1. 960 µL 100 µM anchor oligo (Integrated DNA Technologies)
2. 960 µL 100 µM ligation oligo (Integrated DNA Technologies)
3. 4,224 µL 10X T4 Ligase Buffer (New England Biolabs)
4. 1 µL of each 100 µM barcoded oligo (Integrated DNA Technologies)
5. 96 µL T4 Ligase (New England Biolabs)
6. 960 µL T4 PNK (New England Biolabs)
7. 6,000 µL Streptavidin M270 Dynabeads (Invitrogen)
8. >40 mL UltraPure Distilled Water (Invitrogen)
9. >40 mL 2X B&W Buffer

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

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

1. Hybridize the anchor, ligation and barcoded oligos:
    - Add to the first row of 96-well deep well plate:
        - 20 µL 100 µM anchor oligo
        - 20 µL 100 µM ligation oligo
        - 80 µL 10X T4 Ligase Buffer
        - 640 µL UltraPure Distilled Water
    - Using a Rainin P200 12-channel pipette, add 95 µL of master mix to all rows of master 96-well plate.
    - Using a Rainin Liquidator 96, distribute 19 µL of master mix from master 96-well plate to all wells of a new 384-well plate. The 384-well plate can be adjusted to 4 corners using a Rainin Plate Adapter 384, allowing all wells to be filled from the 96-well master plate.
    - Using a Rainin Liquidator 96, transfer 1 µL from every well of the 100 uM barcoded oligo plate to every well of the 384-well plate.

    - Anneal the mixed oligos on each plate using the following conditions:
        - 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
2. Ligate the barcoded oligo to the ligation oligo:
    - Add to the first row of a 96-well plate:
        - 2 µL T4 Ligase
        - 8 µL 10X T4 Ligase Buffer
        - 70 µL UltraPure Distilled Water
    - Using a Rainin P20 12-channel pipette, add 10 µL of master mix to all rows of a master 96-well plate.
    - Using a Rainin Liquidator 96, distribute 2 µL master mix from the master 96-well plate to all wells of the 384-well plate.
    - Incubate plate at 16ºC for 1 hr or longer, followed by 65ºC for 20 min to heat inactivate the ligase.
3. Phosphorylate the barcoded oligo:
    - Add to first row of 96-well plate:
        - 20 µL T4 PNK
        - 60 µL UltraPure Distilled Water
    - Using a Rainin P20 12-channel pipette, add 10 µL of master mix to all rows of a master 96-well plate.
    - Using a Rainin Liquidator 96, distribute 2 µL master mix from the master 96-well plate to all wells of the 384-well plate.
    - Incubate the plate at 37ºC for 40 min (or longer), followed by 65ºC for 20 min to heat inactivate the PNK.
4. Bind to beads:
    - Prepare 1500 µL stock Dynabeads M270 Streptavidin, washed, and resuspended in 3000 µL 2X B&W buffer.
    - Add 200 µL to first row of 96-well plate.
    - Using a Rainin P200 12-channel pipette, add 25 µL of master mix to all rows of a master 96-well plate.
    - Using a Rainin Liquidator 96, add 5 µL resuspended beads to each well of the 384-well plate.
    - Mix overnight with shaking (>2000 RPM) at room temperature.
5. Pool beads:
    - Using a Rainin Liquidator 96, wash each well with 20 µL 2X B&W buffer 8 times.
    - Using a Rainin Liquidator 96, resuspend each well in 5 µL 2X B&W buffer.
    - Mix 5 µL of each well together, making a 1920 µL mixed barcoded bead pool for each plate. Store these at 4ºC when not in use.