SIGNAL-seq: Barcoding Plate Generation Protocol v1.0

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DNA Barcoding Plate Generation

Notes:

- The IDT Stock deep well plates can take a long time to thaw if they are full. Thaw at RT and then place on ice immediately after thawing.
- Spin plates at 500 g for 30 seconds to bring all primer solutions to the bottom of the well.

Required reagents:

- 3x 96 well master stock plates from IDT – RT1 barcode primers, L2 barcode primers, and L3 barcode primers in IDT Stock DNA Oligo deep-well plates (100 µM).
- 2x linker oligos - BC_0335 L2 barcode linker primer, BC_0284 L3 barcode linker primer (*Note: these are assumed to be in stock concentrations of 1 mM, be sure to correct for volume if only have 100 µM stocks).*
- 12x 96-well PCR plates (3 stock plates that will last at least 10 experiments, and 3x3 active use plates for each of the first 3x barcoding protocol experiments).
  Note: This will generate 100 µL of DNA barcodes for each well in the stock plates. Each SPLiT-seq experiment requires 4 µL/well of the RT1 primer solution which will last ~20-25 experiments. Each SPLiT-seq experiment requires 10 µL/well of the L2 and L3 barcode/linker primer solution. These plates will last for ~8-10 experiments.

RT1 Primer Plate Generation

Round 1 reverse transcription barcoded primers (final concentrations of 12.5 µM random hexamer and 12.5 µM 15dT primers in each of 48 wells, i.e. 1:1 ratio).

1. Using a multichannel pipette, add 12.5 µL of rows A-D in the IDT Reverse Transcription Barcode Primers to rows A-D of the BC Stock 96 well PCR plate.
2. Using a multichannel pipette, add 12.5 µL of rows E-H in the IDT Reverse Transcription Barcode Primers to rows A-D of the BC stock 96 well PCR plate (mixing Oligo-dT with random hexamer primer here).
3. Add 75 µL of water to rows A-D of the BC stock 96 well PCR plate.

Aliquot out 4 µL of each of the 48-wells (A-D) of RT1 barcodes from the stock plate into 3x new 96 well PCR plates for active use in barcoding experiments. Cover **ALL plates** with a PCR seal when not in use and store at -80C. Spin plates briefly in the centrifuge to bring primer solution to the bottom of the well.

L2 and L3 Primer Plate generation

Ligation Round 2 ligation (L2) (Final concentrations of 12 µM barcodes, 11 µM linker-BC_0335)

1. Using a multichannel pipette, add 12uL of IDT L2 Barcodes to L2 Stock 96 well PCR plate.
2. Add 138.6ul of BC_0335 (1mM) to 10.9494mL water in a basin (BC_0335_dil)
3. Using a multichannel pipette, add 88uL BC_0335_dil to each well of R2 Stock 96 well PCR plate.

Ligation Round 3 (L3) (Final concentrations of 14 µM barcodes, 13 µM linker-BC_0284)

1. Using multichannel pipette, add 14 µL of Round 3 Barcodes to R3 Stock 96 well PCR plate.
2. Add 163.8 µL of BC_0284 (1 mM) to 10.6722 mL water in a basin (BC_0284_dil).
3. Using a multichannel pipette, add 86 µL BC_0284_dil to each well R3 Stock 96 well PCR plate.

For each ligation plate (L2 and L3, not including RT1 barcodes), anneal the barcode and linker oligos with the following thermocycling protocol:

1. Heat to 95C for 2 minutes
2. Ramp down to 20C for at a rate of -0.1C/s to 4C

Aliquot out 10 µL of each barcode/linker stock plate into 3x new 96 well PCR plates. These are the Ligation L2 and L3 plates that should be used for DNA barcoding in the split-pool ligation steps in the protocol. Cover **ALL plates** with a PCR seal and store at -80C. Spin plates briefly in the centrifuge to bring primer solution to the bottom of the well.

Remember to cover the IDT deep well master stock plates with a PCR seal or caps and store at -80. Log the removal volume of oligo from the IDT master stock plate to keep track of the remaining volume using the IDT spec sheet with synthesis yields and the resulting volumes.