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How to Handle Aliquoted Plates of iTru i5 & i7 Primers

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Primer aliquot plates have 1.25 nmoles of dried primer in each well. You will need to reconstitute them to the appropriate volume (125 µL -> 10 µM). The iTru5 sets of 8 are in columns (A-H) and iTru7 sets of 12 are in rows (1-12); refer to the relevant plate map.

Acceptable liquids to use for reconstitution are:

1) 10 mM Tris pH 8 (or pH 7.5), or
2) TLE (home made or IDTE from IDT = 10 mM Tris pH 8 & 0.1 mM EDTA)

Protocol:

1) **Centrifuge the plates to get all the primer to the bottom of the wells.**

2) To limit contamination, peel back the foil cover from the plate one row or column (depending on loading scheme) at a time to reconstitute.

3) Add 125µL of your chosen liquid (e.g., TLE) to each well. Then, using a multichannel pipetter:
- Skloosh (i.e., pipette up & down) several times & use the pipet tip to help scrape the bottom of the well to dislodge any of the primer that is stuck.
- Skloosh several more times.
- Wait a few minutes and then skloosh several more times.
- Let the primers sit in the liquid at room temperature for at least 5 minutes.

4) Once the primers have been fully dissolved, transfer the liquid to new strip tubes.
- It is wise to use tubes of different color for each set. Be certain to maintain left/right orientation of the strips.
- It is wise to aliquot each row/column into multiple strips (i.e., so you have <125 µL per primer in each tube). You want these primers to undergo freezing & thawing as few times as possible, so small aliquots are best (in practice 25 - 62.5 µL aliquots are reasonable).

5) Each primer is now at 10 µM, and needs to be diluted down to 5 µM (25µL TLE and 25µL 10µM primer for a 50µL aliquot). Now they are ready to use.
- It is wise to Nanodrop or otherwise quantify your primers. These primer aliquots are quite small, so they are easy to miss. If you miss the tiny dried amount in a well, your primers aren't at 5 µM & your library PCRs won't work!
- Use 2.5µL for each 50 µL reaction; you started with enough for 100 reactions.

6) Store the primers at -20°C

7) Take primers out to thaw shortly before use and **<u>skloosh well before using</u>!**