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# How to Handle Plates with 3RAD v2 Adapter Aliquots

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When you receive the adapters, there is 1.6 nmol of each oligo pair dried in each well, but they are **NOT** annealed. You will need to reconstitute them to the appropriate volume (64 µL -> 25 µM), anneal them, then dilute again (to 2.5 µM) & aliquot them.

Liquid for reconstitution & annealing (10 mM Tris pH 8, 0.1 mM EDTA, 100 mM NaCl):

For 50 mL of salty TLE, add the following to a 50mL conical:
40 mL $dH_2O$
500 µL 1M Tris pH 7.5 to 8
20 µL 0.5M EDTA pH 8
1 mL of 5 M NaCl (or 5 mL of 1M NaCl)
Fill with distilled water to 50 mL mark.

## Protocol:

1) Centrifuge the dry 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 at a time to reconstitute.

3) Add 64 µL of the liquid from above to each well.
- Use the pipet tip to help scrape the bottom of the well to dislodge any of the adapters that is stuck.
- Skloosh (pipette up & down) several times to mix.
- Wait a few minutes.
- Skloosh several more times to mix.
- Let the adapters sit in the liquid at room temperature for at least 5 minutes.

The adapters are now at 25µM.

4) Anneal the adapters together:
- Use thermalcycler to denature (95°C for 1 min.) & cool slowly (e.g., 0.1°C per sec.).

5) Dilute aliquots of the annealed adapters into new labeled strip tubes:
- Add 10 µL of annealed adapters to 90 µL of salty TLE (final conc. = 2.5µM), for SIX separate strips. [Note: This will leave ~4 µL behind in the oligo plates (could be worth

getting as much as possible for the Read1 Adapters – they are limiting; but the Read2's are in excess, so leave that behind). Each strip (with 100 µL) can do FOUR full plates.]

6) Store the strip tubes of adapters at -20°C.

7) Before each use, take adapter aliquots out to thaw and **skloosh well before using!**