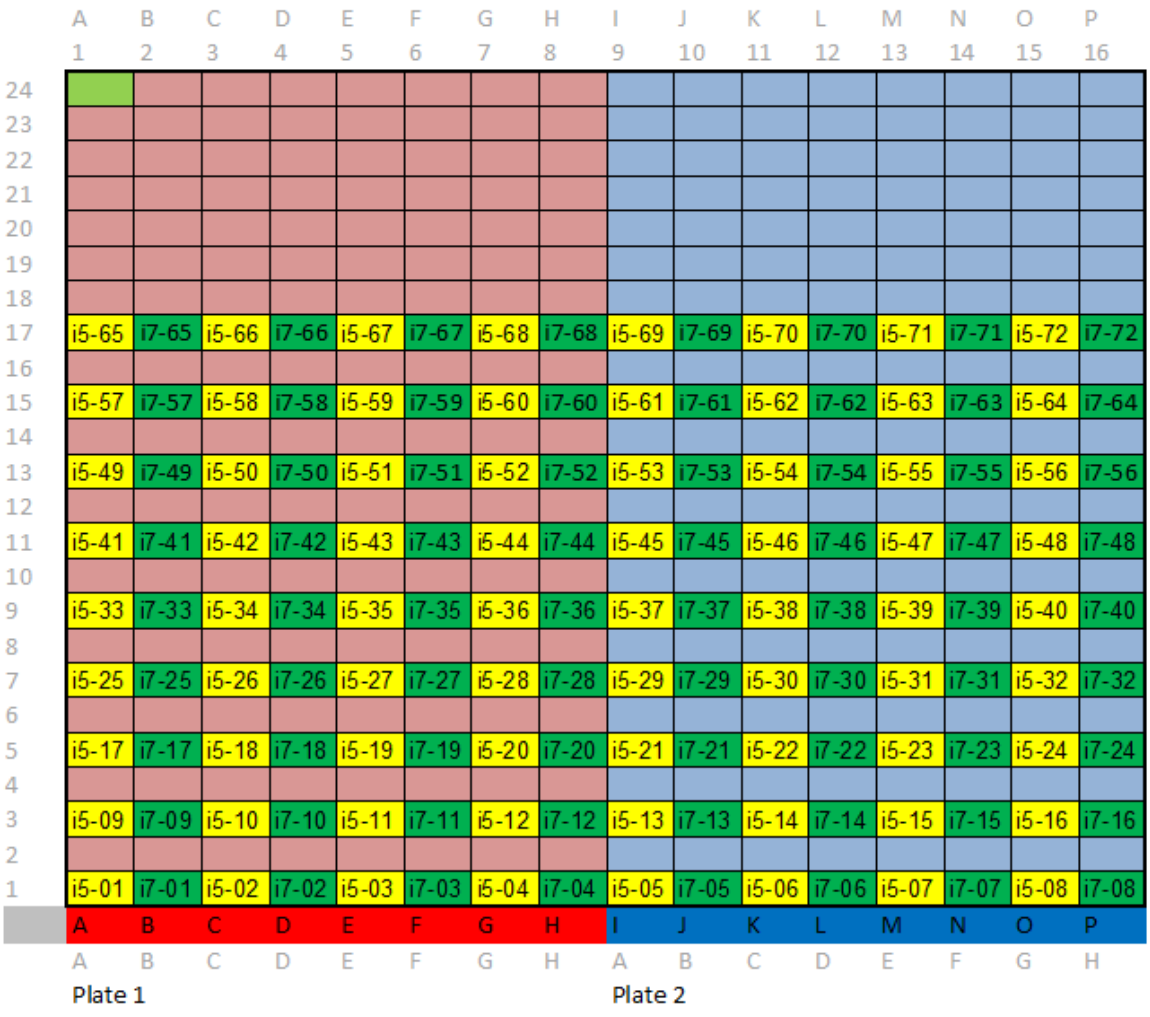
Primer spotting (SCI)

Note: Primer spotting takes ~5hrs for just spotting (not including priming and prep, ~2hrs), break up this protocol into at least two work days.

Thaw and aliquot primers

Primers are aliquoted and diluted from the two 100uM IDT stock plates into two 20uM 96 well PCR plates with 0.1% Tween 20, Plate 1 and Plate 2. These plates should be aliquoted into a Armadillo plate for spotting (Plate 1 A-H into A-H, Plate 2 A-H into I-P).

primer (100uM stock -> 20uM)	40uL
Tween-20 (2% stock -> 0.1%)	10uL
PCR water	150uL
total	**200uL**



1. Get out the two 20uM primer plates from freezer to thaw. Spin down plates.
2. Aliquot 15uL of all 144 primers into an Armadillo plate using a multichannel.
3. Aliquot 15uL of one primer into A24 for spot optimization.
4. Seal all plates, spin down the Armadillo to avoid bubbles. Place in metal plate holder and put on spotter probe location, cut foil to reveal Day 1 (Plate 1) primers.

Robot set up

1. Degas system liquid as outlined in general robot SOP and prime with PDC70 type 1 nozzle.
2. Start chiller.
3. Set dewpoint control to +1.00 (this is different than usual, so the probe plate won't evaporate, but the chips will dry out).
4. Fill fresh water bottle and empty waste water bottle.
5. Fill large ~3.5mL (3.5mL water + 17.5uL SciClean) SciClean basin and make up 5 mL (5mL water + 25uL Sciclean) extra in an eppie for top-ups.

Spot optimization

1. In spot run, check spot with the primer in A24, ensure it is stable and approximately 350-360pL (it will likely drop to 350 over the course of spotting).
2. Once you have a stable drop (est 74/51 for current nozzle) wash out primer as usual (wash flush strong, sciclean wash, wash flush strong).

Run primer spotting

1. Set run to: 2_SmartChip_primers_5uL_FTR_TweenRes. This protocol does 9 washes in the 2% Tween reservoir only (Res2), and the 10th wash is in the SciClean basin.
    - Note: This protocol does 9 washes in the 2% Tween reservoir only (Res2), and the 10th wash is in the SciClean basin. This wash protocol was used from 20170515 onwards, all previous runs were with SciClean at every wash.
2. Set target fld: C:\Users\Scienion AG\sciFLEX_S3 -SCI\Pattern\ SmartChip-R-i5-C-i7-72x72-day-1-plate1.fld or SmartChip-R-i5-C-i7-72x72-day-2-plate2 (files are also attached to this guide as is entire fld of 72x72). #* Note: ***With these flds, primers are spotted into arrays with i5s in rows (i5-01 in row 1 and so on), and i7 in columns (i7-01 in column 1 and so on). Use the attached SmartChipData file for image analysis as it contains these primers. Runs before 20161004 were spotted differently!***
3. Place all chips on block for spotting and ensure that the FTR works on all chips. You can spoof the spotter with a flashlight if it doesn't, but the settings are fairly permissive.
4. Run for all chips. Watch first few primers for monitoring and endtime. Endtime is an underestimate because the second set of primers take longer as they track around the chip more than the second set.
    - Note that for columns, with these flds, the spotter does them in two passes, which looks strange but it is going to all the wells it should.
5. Come back and check on spotter every couple hours at first, it needs monitoring. Check:
    a. Dewpoint (+1.00)
    b. Spot volume/stability(flight path)
    c. 2% tween-20 and SciClean level (top up during washes)
    d. System liquid level compared to sticker on pump tower (can prop up to meet level at ~8 hour mark)
    e. Wash bottle (sufficient water? need ~15L for an entire run)
    f. Waste bottle (empty it if it is getting full)

Data entry

Record the IDs of the batch of primer chips (can take a photo as long as the IDs are readable) and store in F:\SCI\05 Primer Spotting Batches.

Next...

- **Cell preparation and spotting (SCI)**