Library construction (SCI)

Always spin down chip after spotting reagents. Put a drop of mineral oil on the flatbed of the thermalcycler and place the chip on the drop before all incubations. This ensures good thermal contact.

Seals are BioRad microseal A films cut to size and applied with a chip stamper (set to 45 PSI) unless otherwise indicated.

Robot set up

1. Degas system liquid as outlined in general robot SOP and prime with PDC70 type 4 nozzle.
2. Start chiller.
3. Set dewpoint control to -1.00.
4. **If the dewpoint cannot be set to -1.00, use the humidifer at 37% and fixed control temperature at 4°C to protect against evaporation.**
5. Fill fresh water bottle, humidifier if using, and empty waste water bottle. Use fresh water daily to protect against contamination.
6. Fill 2mL SciClean basin (20uL SciClean + 2 mL PCR water).

Library construction

1. Prepare cell lysis buffer (lysis buffer can be spotted over entire chip if cells are being called over lysis, if cells are Poisson spotted, spot entire chip to protect against evaporation on the thermalcycler):

Direct lysis buffer	25	uL
Qiagen Protease	2.5	uL
Glycerol (100%->5%)	1.5	uL
Pluronic (10%->0.1%)	0.3	uL
TOTAL	*29.3*	*uL*
Spot cell lysis buffer	**10**	**nL**

2. Seal chip, **spin 2min at 4000rpm (or higher) to lyse cells *critical step***, incubate in lysis buffer overnight (20hr) in the fridge for library prep the next day.

3. The next day. Spin 2min, incubate at 50°C in the waterbath for 1hr in a chip submarine, then 75°C for 15min in a thermalcycler.

4. Spin chip and check wells for lysis on microscope:

- Lysed wells show no cells, but diffuse fluorescence.
- Visible cells means the lysis was not successful.

5. Spot 2nL (~4 drops) of 6.6pg/nL Salmon Sperm gDNA controls in the appropriate wells.

6. Prepare tagmentation mix*:

	6.5	**3.5**	**2.2**	
TD Buffer	11.335	**14.335**	9	uL
Water	0	0	6.635	uL
Tween 20 (10%)	0.165	**0.165**	0.165	uL
TDE1	6.5	**3.5**	2.2	uL
TOTAL	*18*	*uL*		
Spot tagmentation mix	**18**	**nL**		

*Increased TDE1 results in better copy number profiles but higher GC bias. Use 3.5 tagmentation mix as default.

7. Seal chip, spin 30s, then incubate 55°C for 10 min, cool to 10°C.

8. Spin chip 1 min, put chip on cold chuck, remove seal.

9. Prepare neutralization mix:

Qiagen Protease	8	uL
Tween20 (10%)	0.16	uL
Water	7.84	uL
TOTAL	*16*	*uL*
Spot neutralization mix	**8**	**nL**

10. Seal chip, spin 30s, then incubate 15 min at 50°C, 15 min at 70°C, cool to 10°C.

11. Spin chip 1min, put chip on cold chuck, remove seal.

12. Prepare PCR mix:

NPM (3.3 > 1x)	39	uL
(Index 1 (20mM --> 400mM))	pre-spotted	
(Index 2 (20mM --> 400mM))	pre-spotted	
PPC (5uM>500nM)	13	uL
Tween 20 (10% > 0.1%)	1.3	uL
PCR water	24.70	uL
TOTAL	*78*	*uL*
Spot PCR mix	**39**	**nL**

13. Seal chip, spin 30s, then incubate:

- 72°C for 3 min, 95°C for 30s
- 8 cycles of :
  - 95°C for 10s
  - 55°C for 30s
  - 72°C for 30s
- 72°C for 3 min, cool to 10°C

14. Spin chip 1min, remove seal. If <200 wells, spot 30nL water per well before recovery.

15. If eluting multiply separate library pools from the same chip, replace seal with high stick seal provided by Wafergen, heat to 55°C to seal, flip and cut edges so it will fit in funnel, spin down, cut and peel to expose each library individually.

16. Place chip face down in new funnel with attached eppie, spin down 4000rpm, 2min to recover library.

17. Store library at -20°C for clean-up, and Bioanalyzer QC.

Data entry

Record any library details in Colossus in "Add Libraries" under "Library Construction Information". This might include: locations of spotting that went poorly, experimental conditions, timing of different incubations or spotting.

Next...

- **Library clean-up and QC (SCI)**