SWIFT SSDNA LIBRARY PREPARATION

Notes:

- Use the filter tips, H_2Odd and PCR tubes exclusively for ssDNA library.
- Ensure that reagent vials are at room temperature and enzyme vials at 4ºC. Before mixing, briefly vortex reagents vials and spin down.
- Add reagents in the order listed when preparing the master mix.
- Add enzymes just before use.
- Master mix is prepared on ice and use the Master mix excel to prepare your master mixes.
- Take the proper amount of AMPure beads in an eppendorf tube and leave it at RT.
- Put Samples at room temperature before mix with AMPure beads.

DENATURATION

1. Prepare the master mix except enzymes for the next step (Adaptase).
2. Pre-heat the thermocycler to 95ºC and transfer 7.5 µl of each sample to a PCR tube. Add Low EDTA TE to reach 7.5 µl if necessary.
3. Incubate the samples in a thermocycler for 2’ at 95ºC.
4. Immediately place the tubes on ice for 2’ and proceed directly to the next step.

ADAPTASE

5. Load the Adaptase thermocycler program and hold it at 37ºC.
6. Prepare the Adaptase master mix:

Adaptase Master Mix	µl
Low EDTA TE	5.75
Buffer G1	2
Reagent G2	2
Reagent G3	1.25
Enzyme G4	0.5
Enzyme G5	0.5
Enzyme G6	0.5
Total	**12.5**

7. Add 12.5ul of the Adaptase master mix to each tube containing 7.5 µl of sample. Mix by pipetting (10 times) and spin down briefly.

8. Run the pre-loaded Adaptase thermocycler program:

T	**time**
37ºC	15'
95ºC	2'
4ºC	-

EXTENSION

9. Load the Extension thermocycler program and hold it at 98ºC.

10. Prepare the Extension master mix:

Extension Master mix	µl
Low EDTA TE	9.25
Reagent Y1	1
Reagent W2	3.5
Buffer W3	8.75
Enzyme W4	1
Total	**23.5**

11. Add 23.5 µl of the Extension master mix to each tube. Mix by pipetting (10 times) and spin down briefly.

12. Run the pre-loaded Extension thermocycler program:

T	**time**
98ºC	30''
63ºC	15''
68ºC	5'
4ºC	-

13. Purify the DNA with AMPure Agencourt XP beads (1:1 ratio, 43.5 µl beads) to 10 µl of Low EDTA TE.

LIGATION

14. Prepare the Ligation master mix (Enzyme B3 must be added right before use):

Ligation Master Mix	µl
Low EDTA TE	2
Buffer B1	2
Reagent B2	5
Enzyme B3	1
Total	**10**

15. Add 10 µl of the Ligation master mix to µl of eluate. Mix by pipetting (10 times) and spin down briefly.

16. Incubate the tubes at 25ºC for 15'.

17. Purify the DNA with AMPure Agencourt XP beads (1:1 ratio, 20 µl beads) to 10 µl of Low EDTA TE.

INDEXING PCR

18. Add 2,5 µl of the appropriate index primer (Index Kit, red tubes) to each sample.

19. Prepare the Indexing PCR master mix (Enzyme W4 must be added right before use):

Indexing PCR master mix	µl
Low EDTA TE	5
Reagent W2	2
Buffer W3	5
Enzyme W4	0.5
Total	**12.5**

20. Add 12.5 µl of the Indexing PCR master mix to each sample. Mix by pipetting (10 times) and spin down briefly.

21. Run the Indexing PCR program for the appropriate amount of cycles:

T	**time**
98ºC	30''
98ºC	10''
60ºC	30''
68ºC	1'
4ºC	-

General PCR program in eSPAN experiments:

-**INPUT** and **BrdUIP** samples: **11 cycles**
-**ChIP** samples: **13 cycles**
-**eSPAN** samples: **15 cycles**

22. Purify the DNA with Agencourt XP beads (1:0.8 ratio, 20 µl beads) to 20 µl of Low EDTA TE. Purify again with 16 µl beads (1:0.8 ratio) and recover 18 µl for Sequencing and 2 µl for Bioanalyzer run or 20 µl for Sequencing, if Bionalyzer is not needed.

AGENCOURT XP BEADS PURIFICATION

1. Mix the beads with the sample by pipetting thoroughly (10 times) and incubate at RT for 7'.
2. Place on magnet and wait 2' until clear.
3. Remove the supernatant with a micropipette, leaving aprox. 5 uL in order to not aspire beads.
4. Add 200ul of 80% ethanol, and wait 30''.
5. Remove the ethanol, with vacuum (15 mbar) and repeat the ethanol wash.
6. Dry beads on the magnet, remove all drops.
7. Resuspend beads with Low EDTA TE and wait 1'.
8. Place tubes on the magnet and wait 2' until clear.
9. Transfer to new tubes.

Notes:

-Put Samples at room temperature before mix with AMPure beads

-If you aspire beads and you can see in the tip, replace the volume in the tube (on the magnet), and take the supernatant again.

-When beads are dried, close PCR tube, to avoid overdrying, and as soon as posible, add Low EDTA TE.

-Extension purification: samples resuspension is difficult, not worry!