

Swift 2S Turbo DNA library

1/4 version

Materials needed

- Ice
- PCR machines

Sample preparation

>5 ng/μl DNA in Low EDTA TE buffer.

K part

1. Start Swift One on PCR.
2. Make K Master Mix.

	x1	x100	x200
Buffer K1	0.75	75	150
Reagent K2	0.375	37.5	75
Enzyme K3	1.5	150	300

3. Add 2.63 μl **K master mix** to 4.9 μl DNA sample.
4. Put samples to Swift 1 PCR program (8 μl - 40 min timer).
5. Take out W1, W5.

Adapter ligation

1. Make Swift 2S Turbo v2 master mix.

	x1	x100	x200
Buffer W1	3	300	600
Enzyme W3	1	100	200
Reagent W5	1.25	125	250
Low EDTA TE	2.25	225	450

2. Add 7.5 μl **S2 master mix** to samples.
3. Put samples to Swift 2 PCR program, for 20 min (15 μl).
4. Take beads to RT.

Adapter cleanup

1. Add 12 μl **vortexed beads** to each sample. Seal, mix, spin and incubate for 5 min at RT.
2. Take UDI primers and PCR mix to room temp.
3. Prepare 80% ethanol.

	x1	plate
EtOH	72	3.6 ml (0.9 x 8)
H ₂ O	18	0.9 ml (0.9 x 2)

4. Place on magnet for 2 min until clear supernatant.
5. Remove supernatant.
6. Add 45 μl **80% ethanol**. Incubate for 30 sec and remove.
7. Repeat ethanol wash.
8. Spin plate and remove any remaining ethanol.
9. Add 8 μl **Low EDTA TE** and resuspend pellet.
10. Place on magnetic rack for 2 min until solution clears.
11. Transfer 5 μl supernatant into new plate (below step 3, in new pcr plate with primers).

Stubby Y PCR indexing

1. Add 6.5 **PCR mix** to 5 μl sample.

2. Add 1.25 μl **UDI** to a new plate.
3. (Add 5 μl supernatant from cleanup).
4. Run in Swift PCR program (13 μl).

PCR cleanup

1. Add 8.125 μl **vortexed beads** to each sample. Seal, mix, spin and incubate for 5 min at RT.
2. Prepare 80% ethanol

	x1	plate
EtOH	72	3.6 ml (0.9 x 8)
H ₂ O	18	0.9 ml (0.9 x 2)

3. Place on magnet for 2 min until clear supernatant.
4. Remove supernatant.
5. Add 45 μl **80% ethanol**. Incubate for 30 sec and remove.
6. Repeat ethanol wash.
7. Spin plate and remove any remaining ethanol.
8. Add 10 μl **Low EDTA TE** and resuspend pellet.
9. Place on magnetic rack for 2 min until solution clears.
10. Transfer 9 μl into new plate.

PCR programmes

Swift One: 4 min 4°C, 11⁽¹⁾ min 32°C, 30 min at 65°C, forever at 4°C. Heated lid at 70°C.

Swift Two: 20 min at 20°C, no heated lid.

Swift PCR: 45s 98°C, 7 cycles of 15s 98°C, 30s at 60°C, 30s at 72°C, 5 min 72°C, 4°C forever. Heated lid 105°C.

⁽¹⁾ for 350 bp insert size.