**SYNTHESIS OF DS ADAPTERS**

(Adapted from doi:10.1038/nprot.2014.170)

**MATERIALS:**

- DS adapters (PAGE purified, hand mixed [IDT]):

|  |  |
| --- | --- |
| **new MWS51** | 5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3′ |
| **new MWS55** | 5′-TCTTCTACAGTCA NNNNNNNN AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3′ |

- Klenow fragment (3′→5′exo − ; New England Biolabs (NEB) cat. no. M0212L, includes NEB buffer #2).

- HpyCH4III (NEB, cat. no. R0618; includes CutSmart buffer).

- IDTE (1X TE Solution) buffer from IDT

**PROCEDURE:**

The adapter synthesis protocol generates a sufficient amount of adapters for several hundred samples.

**1.** Anneal the two oligonucleotides (MWS51 and MWS55 in Table 3) by combining 100 μl of each 100 μM oligonucleotide in a 0.2-ml PCR tube; heat the tube to 95 °C for 5 min in a thermocycler with a heated lid. Turn off the machine and leave it for 1 h. Remove and save 1 μl for quality control; label it as ‘annealed adapters’.

**CRITICAL STEP** It is extremely important that the oligonucleotides be allowed to cool slowly. Make sure that the thermocycler does not automatically cool after the heating cycle.

**2.** Extend the annealed adapters by mixing the components in the table below by gently pipetting up and down, and then splitting into two 0.2-ml PCR tubes and incubating for 1 h at 37 °C.

**Reagent Volume (ul) Final concentration**

DNA from step 1 199 Variable

10× NEB buffer #2 28 1×

10 mM dNTP mix 28 1 mM

ddH2O 11.6 N/A

Klenow exo − (5 U/μl) 11.6 0.5 U/μl

**3.** Ethanol-precipitate\* the DNA and resuspend it with 200 μl of ddH2O. We recommend saving 1 μl of the resuspended adapters for quality control purposes; label as ‘extended adapters’.

**Reagent Volume (ul) Final concentration**

3M Sodium Acetate ph5.2 31 0.3

100% ethanol 930 (3x total volume)

* Mix the tube by inversion.
* Incubate at -20 for 30 minutes.
* Centrifuge the mixture for 30 min at 4°C at >10,000g.
* Put at -20 for 30 mins, spin max in cold centrifuge, remove ethanol
* Remove the supernatant, add 1 ml of 70% (vol/vol) ethanol to each tube and mix by inversion.
* Centrifuge the tube for 30 min at 4 °C at >10,000g.
* Remove the supernatant. Spin and remove the rest with a small tip. Dry the tube for 10 min inverted on a paper towel, and then for 5 min upright. **!** DON’T OVERDRY
* Resuspend in 200ul of water
* Save 1ul: extended adapters

**4.** Cleave the extended adapters by mixing the components in the table below by carefully pipetting up and down and then dividing into four 0.2-ml PCR tubes. Incubate the tubes for **16 h at 37 °C** in a thermocycler with a heated lid. After 16 h, remove and save 1 μl for quality control purposes; label as ‘cut adapters’.

**Reagent Volume (ml) Final concentration**

DNA from step 3 200 Variable

10× NEB CutSmart buffer 50 1×

ddH 2 O 235 N/A

HpyCH4III (5 U/μl) 15 0.5 U/μl

**5.** Ethanol-precipitate twice the DNA and resuspend it with 200 μl of ddH2O.

* Add 55ul 3M Sodium Acetate (pH=5.2) (550 μl final volume)
* Split into two tubes (2x275 μls each)
* Add 825 μls of ethanol, mix the tubes by inversion.
* Incubate at -20 for 30mins
* Centrifuge the mixture for 30 min at 4°C at >10,000g.
* Remove the supernatant, add 1 ml of 75% (vol/vol) ethanol to each tube and mix by inversion.
* Centrifuge the tube for 30 min at 4 °C at >10,000g.
* Remove the supernatant. Spin and remove the rest with a small tip. Dry the tube for 10 min inverted on a paper towel, and then for 5 min upright.
* Resuspend the pellet in each tube with 112.5 μl TE low. Pool together (total volume = 225ul)
* Add 25ul 3M Sodium Acetate, mix and add 750 of ethanol.
* Incubate at -20 for 30mins
* Centrifuge the mixture for 30 min at 4°C at >10,000g.
* Remove the supernatant, add 1 ml of 70% (vol/vol) ethanol to each tube and mix by inversion.
* Centrifuge the tube for 30 min at 4 °C at >10,000g.
* Remove the supernatant. Spin and remove the rest with a small tip. Dry the tube for 10 min inverted on a paper towel, and then for 5 min upright.
* Resuspend in 200ul of water.
* Remove and save 1 μl for quality control; label as ‘final adapters’.

**6.** Divide the adapters into 50 μl aliquots and place them at −80 °C for long-term storage. The final concentration will be ~50 μM