**20724 WGBS protocol**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Library No | Cell plate ID | Cell line name | vol of gDNA | conc. of gDNA | unit | 2ug vol. | lambda DNA (10ng/uL) | H2O |
| PY1 | 1\_1 | GM18499 | 50 | 75.2 | ng/uL | 26.60 | 1 | 22.40 |
| PY2 | 1\_2 | GM18499 | 50 | 98.4 | ng/uL | 20.33 | 1 | 28.67 |
| PY3 | 2\_1 | GM18510 | 50 | 86 | ng/uL | 23.26 | 1 | 25.74 |
| PY4 | 2\_2 | GM18510 | 50 | 72 | ng/uL | 27.78 | 1 | 21.22 |
| PY5 | 3\_1 | GM18519 | 50 | 117 | ng/uL | 17.09 | 1 | 31.91 |
| PY6 | 3\_2 | GM18519 | 50 | 74.8 | ng/uL | 26.74 | 1 | 22.26 |
| PY7 | 4\_1 | GM18852 | 50 | 74.4 | ng/uL | 26.88 | 1 | 22.12 |
| PY8 | 4\_2 | GM18852 | 50 | 96.8 | ng/uL | 20.66 | 1 | 28.34 |
| PY9 | 5\_1 | GM18861 | 50 | 81.2 | ng/uL | 24.63 | 1 | 24.37 |
| PY10 | 5\_2 | GM18861 | 50 | 68.6 | ng/uL | 29.15 | 1 | 19.85 |
| PY11 | 6\_1 | GM18870 | 50 | 97.6 | ng/uL | 20.49 | 1 | 28.51 |
| PY12 | 6\_2 | GM18870 | 50 | 101 | ng/uL | 19.80 | 1 | 29.20 |
| PY13 | 7\_1 | GM18909 | 50 | 108 | ng/uL | 18.52 | 1 | 30.48 |
| PY14 | 7\_2 | GM18909 | 50 | 95.4 | ng/uL | 20.96 | 1 | 28.04 |
| PY15 | 8\_1 | GM18916 | 60 | 86.4 | ng/uL | 23.15 | 1 | 25.85 |
| PY16 | 8\_2 | GM18916 | 60 | 81.2 | ng/uL | 24.63 | 1 | 24.37 |
| PY17 | 9\_1 | GM19098 | 60 | 69.2 | ng/uL | 28.90 | 1 | 20.10 |
| PY18 | 9\_2 | GM19098 | 50 | 53 | ng/uL | 37.74 | 1 | 11.26 |
| PY19 | 10\_1 | GM19099 | 50 | 95.8 | ng/uL | 20.88 | 1 | 28.12 |
| PY20 | 10\_2 | GM19099 | 50 | 108 | ng/uL | 18.52 | 1 | 30.48 |
| PY21 | 11\_1 | GM18912 | 75 | 114 | ng/uL | 17.54 | 1 | 31.46 |
| PY22 | 11\_2 | GM18912 | 75 | 120 | ng/uL | 16.67 | 1 | 32.33 |
| PY23 | 12\_1 | GM18913 | 75 | 138 | ng/uL | 14.49 | 1 | 34.51 |
| PY24 | 12\_2 | GM18913 | 75 | 149 | ng/uL | 13.42 | 1 | 35.58 |

**1 Covarsi sonication**

PIP - 30 W  
DF - 15%  
CPB - 1000  
Repeat/Iterations - 18  
Repeat Process Treatment Duration - 10 seconds   
Total Treatment Time per sample - 180 seconds

**Library Construction Protocol**

**1. End Repair and A-Tailing**

**1.1** Assemble each End Repair & A-Tailing reaction as follows in a tube or well of a PCR plate:

|  |  |
| --- | --- |
| Component | Volume |
| Fragmented, double-stranded DNA | 50 μl |
| End Repair & A-Tailing Buffer† | 7 μl |
| End Repair & A-Tailing Enzyme Mix† | 3 μl |
| Total volume | 60 μl |

† The buffer and enzyme mix may be pre-mixed and added in a single pipetting step. Premixes are stable for ≤24 hours at room temperature, for ≤1 week at 4°C, and for ≤3 months at -20°C

**1.2** Mix thoroughly and centrifuge briefly.

**1.3** Incubate in a thermocycler with the following thermal profile:

|  |  |  |
| --- | --- | --- |
| Step | Temp | Time |
| End Repair & A-Tailing | 20°C | 30 min |
| 65°C | 30 min |
| HOLD | 4°C | ∞ |

**1.4** Proceed immediately to the next step.

**2. Adapter Ligation**

**2.1** Dilute adapter as1:10.

**2.2** Assemble each Adapter Ligation reaction as follows:

|  |  |
| --- | --- |
| Component | Volume |
| End Repair & A-Tailing reaction product | 60 μl |
| PCR-grade water† | 5 μl |
| Ligation Buffer† | 30 μl |
| DNA Ligase† | 10 μl |
| **Adapter stock methylated original conc.** | 5 μl |
| Total volume | 110 μl |

† The water, buffer and ligase enzyme may be premixed and added in a single pipetting step. Premixes are stable for ≤24 hours at room temperature, for ≤1 week at 4°C and for ≤3 months at -20°C.

**2.3** Mix thoroughly and centrifuge briefly. Incubate at 20°C for 30 min. 4°C pause. Add 3 ul USER enzyme and 37°C for 30min.

**3. Post-ligation Cleanup:**

1X XP beads in 22ul H2O

**3.1 Zymo gold bisulfite (Cat**#**D5006 )**

**20 uL sample + 130 uL CT conversion**

|  |  |  |
| --- | --- | --- |
| Step | Temp | Time |
| Denature | 98°C | 10 min |
| Bisulfite | 64°C | 2.5 hr |
| HOLD | 4°C | ∞ |

**Purify and elute in 20 uL**

**3.2 2nd Zymo gold bisulfite (Cat**#**D5006 )**

**20 uL sample + 130 uL CT conversion**

|  |  |  |
| --- | --- | --- |
| Step | Temp | Time |
| Denature | 98°C | 10 min |
| Bisulfite | 64°C | 2.5 hr |
| HOLD | 4°C | ∞ |

**Purify and elute in 22 uL**

**4. Library Amplification**

**4.1** Assemble each library amplification reaction as follows:

|  |  |
| --- | --- |
| Component | Volume |
| **2X KAPA HiFi U+ HotStart ReadyMix** | 25 μl |
| E7140S\_UD index | 5 μl |
| Adapter-ligated library | 20 μl |
| Total volume | 50 μl |

**4.2** Mix thoroughly and centrifuge briefly.

**4.3** Amplify using the following cycling protocol:

|  |  |  |  |
| --- | --- | --- | --- |
| Step | Temp | Duration | Cycles |
| Initial denaturation | 95°C | 3 min | 1 |
| Denaturation | 98°C | 20 sec | 4 cycles |
| Annealing† | 62°C | 30 sec |
| Extension | 72°C | 1 min |
| Final extension | 72°C | 1 min | 1 |
| HOLD | 4°C | ∞ | 1 |

**4.4** Store the tube/plate at 4 °C or -20 °C for up to 72 hours, or proceed directly to Step 5: Post-amplification Cleanup.

**5. Post-amplification Cleanup:** Purify twice with 40ul XP beads and elute in 30ul H2O.