**3.9.2014 ATAC-seq 16 CLL 1**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cup | Name | cells | ID | Add info | Nr cells | Lysis/wash | Ct 1/4th max intensity | Enrich | Adapter | Cq |
| 1 | ATAC\_16-1 | CLL | 1-5-36904 | 30’ tagment | 50 K | ATAC | 8.88 | 8 | 3 | 9.20 |
| 2 | ATAC\_16-2 | CLL | 1-5-36904 | 30’ tagment | 50 K | ATAC+wash with 1x MgCl buffer (vortex in wash step) | 9.8 | 9 | 9 | 9.82 |
| 3 | ATAC\_16-3 | CLL | 1-5-36904 | 60’ tagment | 50 K | ATAC | 7.3 | 8 | 1 | 7.56 |
| 4 | ATAC\_16-4 | CLL | 1-5-36904 | 60’ tagment | 50 K | ATAC+wash with 1x MgCl buffer (vortex in wash step) | 7.3 | 8 | 10 | 7.37 |
| 5 | ATAC\_16-5 | CLL | 1-5-42480 | 30’ tagment | 50 K | ATAC | 9.26 | 9 | 6 | 9.39 |
| 6 | ATAC\_16-6 | CLL | 1-5-42480 | 30’ tagment | 50 K | ATAC+wash with 1x MgCl buffer (vortex in wash step) | 10.15 | 10 | 11 | 10.3 |
| 7 | ATAC\_16-7 | CLL | 1-5-42480 | 60’ tagment | 50 K | ATAC | 8.16 | 8 | 2 | 8.32 |
| 8 | ATAC\_16-8 | CLL | 1-5-42480 | 60’ tagment | 50 K | ATAC+wash with 1x MgCl buffer (vortex in wash step) | 8 | 8 | 12 | 8.01 |

Prepare nuclei and tag DNA (For pipetting, use 200 ul tips and cut off the tip a bit to not to lyse nuclei)

1. Thaw Nextera kit components on ice
2. Prepare nuclei: Spin 50,000 cells at 300g for 5 min 4°C
3. Wash cells once in 50 μL cold 1x PBS and spin 300 x g for 5 min at 4°C.
4. Gently lyse cells by resuspending them in 50 μL cold lysis buffer
5. Immediately after lysis spin nuclei at 300 x g for 10 min using a refrigerated centrifuge. To avoid losing cells during the nuclei prep, use a fixed-angle centrifuge and carefully pipetted away from the pellet after centrifugations.
6. Wash samples 2,4,6,8 with 50 ul homemade 1x MgCl transposase buffer, vortex 2x5s medium speed
7. Immediately following the nuclei prep, resuspend the pellet in the transposase reaction mix (10.5 μL nuclease-free water, 12.5 μL 2× TD, 2 μL TDE1 (Illumina)).
8. Incubate 30/60 min at 37 °C, then put on ice
9. Purify the sample with a Qiagen MinElute kit and elute in 11 μL EB 🡺 safe stop -20

1. Perform qPCR with the following program (“ ATAC qPCR “):
   1. 72°C 5 minutes
   2. 98°C 30 seconds
   3. 25 cycles of:
      1. 98°C 10 seconds
      2. 63°C 30 seconds
      3. 72°C 1 minutes
   4. hold at 4 °C

|  |  |
| --- | --- |
| Component qPCR | volume |
| H2O | 2.9 μL |
| Index primer 1 noMX | 0.5 μL |
| Index primer 2 barcode | 0.5 μL |
| 100x SYBR green (fresh) | 0.1 μL |
| NEBnext HF 2x ready MM | 5 μL |
| Tagmented sample | 1 μL |

1. Perform PCR with the following program (“ ATAC seq“):
2. 72°C 5 minutes
3. 98°C 30 seconds
4. x cycles of:
   * 1. 98°C 10 seconds
     2. 63°C 30 seconds
     3. 72°C 1 min
5. 72°C 1 min final ext
6. hold at 4 °C

|  |  |
| --- | --- |
| Component qPCR | volume |
| H2O | 15 μL |
| Index primer 1 noMX | 2.5 μL |
| Index primer 2 barcode | 2.5 μL |
| NEBnext HF 2x ready | 25 μL |
| Tagmented sample | 5 μL |

1. Purify with sample:SPRI 1:1.8, then size select 0.45/1.8 double SPRI