**MATQ-seq Protocol**

Zong Lab

Baylor College of Medicine

**1. Cell lysis**

Lysis solution:

0.2% Triton-X in DEPC H2O 1 μL

0.1 M DTT 0.05 μL

RnaseOUT 0.05 μL

Primer mix\*\* (page purified GAT27dT 1.5 μM, GAT275n3G 5 μM, GAT275n3T 5 μM) 0.4 μL

10 mM each dNTP 0.1 μL

Pipette single cell in lysis solution, briefly centrifuge, then put on PCR block 72ºC 3 min, then quickly put on ice for at least 1 min

**2. Reverse transcription**

Add RT mix containing:

5X FS buffer 0.8 μL

0.1 M DTT 0.2 μL

RnaseOUT 0.1 μL

Superscript III 0.15 μL

Rnase-free H2O 1.15 μL

Mix well by flicking the tubes 4-5 times then brief centrifuge

Run following program:
10 cycles of

8ºC 12s

15ºC 45s

20ºC 45s

30ºC 30s

42ºC 2 min

50ºC 3 min

End cycle

50ºC 15 min

4 ºC Forever

**3. Digestion of primers** (do not go to low temperature during these steps)

50ºC 1 min

Add 0.2 μL T4 DNA polymerase, mix well by flicking the tubes 4-5 times, then brief centrifuge

37ºC 40 min

75ºC 20 min

4 ºC Forever

**4. RNA digest**

Add 0.1 μL RnaseH, 0.1 μL RnaseI, mix well by flicking the tubes 4-5 times, then brief centrifuge

37ºC 15 min

72ºC 15 min

4 ºC Forever

**5. Tailing**

make following TdT mix:

10X TdT buffer 0.4 μL

100 mM dCTP 0.4 μL

TdT ter minal transferase 0.1 μL

H2O 3.1 μL

Heat the samples to 72ºC for 1 min, pause at 37ºC

Add the TdT mix into sample at 37ºC, mix well by flicking the tubes 4-5 times, then brief centrifuge

37ºC 15 min

72ºC 15 min

4 ºC Forever

**6. Second strand synthesis** (important to add polymerase after 48ºC hold)

Make following 2nd stand mix:

10X Thermopol buffer 1.5 μL

dNTP (10 mM each) 1.25 μL

100μM GAT21 6n3G 0.125 μL

H2O 13.1 μL

Add the mix into samples,

95ºC 30s

48ºC hold for at least 20s, add Deepvent exo- DNA polymerase 0.4 μL, mix well by flicking the tubes 4-5 times, then brief centrifuge

10 cycles of

48ºC 20s

72ºC 1 min

End cycle

72ºC 2 min

4 ºC Forever

**8. Amplification**

25 μL 2nd strand product

13 μL 10X Thermopol Buffer,

0.8 μL 100 μM GAT27 primer

3 μL 10 mM each dNTP,

116 μL PCR grade water,

3 μL Deepvent exo- DNA polymerase

Split into 4 PCR tubes

95ºC 1 min

24 cycles of

95 ºC 15s

63 ºC 20s

72 ºC 2 min

End cycle

72 ºC 5 min

4 ºC Forever

Use QIAquick PCR Purification kit or 1.2X Ampure beads to purify

**8. 3NGAT24 Double strand conversion** (For better cluster identification during sequencing)

200ng of amplified cDNA

5 μL 10X Thermopol buffer

1.25 μL of dNTP

0.25 μL of 100 μM 3NGAT24 primer\*\*

1 μL Deepvent exo- DNA polymerase

Add H2O to total 50 μL

95ºC 30s

20 cycles of

62 ºC 20s

72 ºC 1 min

End cycles

72 ºC 3 min

95ºC 30s

20 cycles of

62 ºC 20s

72 ºC 1 min

End cycles

72 ºC 3 min

4 ºC Forever

Use 1.2X Ampure XP beads (60 μL) to purify

\*\* Primer sequence (Integrated DNA Technologies, PAGE purified):

GAT27dT: GTG AGT GAT GGT TGA GGA TGT GTG GAG NNNNN TTTTTTTTTTTTTTTTTTTT

GAT27 5N3G: GTG AGT GAT GGT TGA GGA TGT GTG GAG NNNNN GGG

GAT27 5N3T: GTG AGT GAT GGT TGA GGA TGT GTG GAG NNNNN TTT

GAT21 6N3G: GAT GGT TGA GGA TGT GTG GAG NNNNNN GGG

GAT27 PCR: GTG AGT GAT GGT TGA GGA TGT GTG GAG

3NGAT24: NNN AGT GAT GGT TGA GGA TGT GTG GAG