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UTAG-seq: accurate detection of somatic mutations by single-cell duplex-sequencing

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We use this protocol and it's working

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Disclaimer

Patent application covering UTAG-seq chemistry has been filed by Baylor College of Medicine.

Abstract

Here, we developed UTAG-seq, a single-cell duplex-sequencing method that enables comprehensive genome coverage. Applying UTAG-seq to individual human umbilical cord blood cells, we validated the somatic mutation calling accuracy to be < 10^{-9}. Together, these results demonstrate that UTAG-seq supports highly accurate single-cell somatic mutation detection and will facilitate the characterization of somatic mosaicism across diverse biological contexts.

Protocol materials

- Protease (20 mg/mL) **Qiagen**
- DEPC-treated water **Ambion** **Catalog #**AM9915G
- 0.5M EDTA **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**E7889-100ML
- 10% Triton X-100 solution **Merck MilliporeSigma (Sigma-Aldrich)**
- 1 M KCl **Thermo Scientific**
- 10% NP40 **Amresco**
- UltraPure™ 1M Tris-HCl, pH 8.0 **Invitrogen - Thermo Fisher** **Catalog #**15568025
- 5M NaCl **Invitrogen - Thermo Fisher** **Catalog #**AM9760G
- Tn5 transposase, unloaded **Diagenode** **Catalog #**C01070010-20
- Glycerol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**G5516-100ML
- 5X TB1 **Illumina, Inc.**
- Q5U® Hot Start High-Fidelity DNA Polymerase **New England Biolabs** **Catalog #**M0515
- dNTP solution mix (10 mM each) **New England Biolabs** **Catalog #**N0447
- 10X ThermoPol Buffer **New England Biolabs**
- Thermolabile Proteinase K **New England Biolabs** **Catalog #**P8111S
- Thermolabile USER II Enzyme - 50 units **New England Biolabs** **Catalog #**M5508S
- 10X T4 Ligase buffer **NEB**
- T4 DNA Ligase **New England Biolabs** **Catalog #**M0202L
- Deep Vent DNA Polymerase - 200 units **New England Biolabs** **Catalog #**M0258S
- Evagreen dye, 20x in water **Biotium** **Catalog ##**31000
- Ampure XP beads **Beckman** **Catalog #**A63881
- NEBNext Ultra II Q5 Master Mix **New England Biolabs** **Catalog #**M0544X

Cell lysis

3h 20m

1 Prepare cell lysis buffer (2.5 uL per cell)

0.075 µL
UltraPure™ 1M Tris-HCl, pH 8.0 **Invitrogen - Thermo Fisher Catalog #**15568025
0.01 µL
0.5M EDTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7889-100ML
0.05 µL
1 M KCl **Thermo Scientific**
0.025 µL
10% Triton X-100 solution **Merck MilliporeSigma (Sigma-Aldrich)**
0.09 µL
10% NP40 **Amresco**
0.12 µL
Protease (20 mg/mL) **Qiagen**
2.13 µL
DEPC-treated water **Ambion Catalog #**AM9915G

2 Sort single cell/nuclei into individual PCR tube or well. Incubate at 50 °C 03:00:00 75 °C 00:20:00 to perform cell lysis. Lysed cells can be stored at -80 °C . 3h 20m

Custom Tn5 transposase assembly

40m

3 Prepare 500 µL 2X Annealing buffer.

40 µL
UltraPure™ 1M Tris-HCl, pH 8.0 **Invitrogen - Thermo Fisher Catalog #**15568025
10 µL
5M NaCl **Invitrogen - Thermo Fisher Catalog #**AM9760G
450 µL
DEPC-treated water **Ambion Catalog #**AM9915G

4 Transposon annealing. 10m

20 µL 2X Annealing buffer
10 µL [M] 200 micromolar (µM) ME_REV
10 µL [M] 200 micromolar (µM) T1ME_U

Oligonucleotides sequences
ME_REV: /5Phos/CTGTCTCTTATACACATCT

T1ME_U: TCGUCGGCAGCGUC AGATGTGTAUAAGAGACAG

90 °C 00:05:00
65 °C 00:05:00 Ramp rate 0.1ºC/s
4 °C Hold, Ramp rate 0.1ºC/s

5 Custom Tn5 assembly. 30m
20 µL Tn5 transposase, unloaded **Diagenode Catalog #**C01070010-20
20 µL Annealed transposon mix
Incubate at 23 °C 00:30:00 . Add 20 µL
Glycerol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G5516-100ML . Mix well and brief centrifuge. Store at -20 °C .

Single-cell UTAG procedure 3h 4m 30s

6 Prepare tagmentation mix (2.5 uL per cell). 55m
1 µL 5X TB1 **Illumina, Inc.**
0.5 µL [M] 10 millimolar (mM) MgCl2
0.2 µL U-Tn5 (1:200 diluted)
0.8 µL DEPC-treated water **Ambion Catalog #**AM9915G

Gently mix and briefly spin down. Incubate at 25 °C 00:50:00 37 °C 00:05:00 .

Note: the activity of purchased Tn5 transposase may vary between different batches. The goal of this step is to tagment gDNA to an average of ~200 bp fragments. Please adjust the amount of enzyme at this step if needed.

7 Tn5 inactivation. 30m
Add 1 µL [M] 0.2 Molarity (M) EDTA. Incubate at 50 °C 00:30:00 .

8 Gap filling.

8.1 Add 1 µL [M] 0.2 Molarity (M) MgCl2.

8.2 Add 3 µL Sealing mix: 5m 30s

1 µL 10X ThermoPol Buffer **New England Biolabs**

1 µL dNTP solution mix (10 mM each) **New England Biolabs Catalog #**N0447

0.1 µL

Q5U® Hot Start High-Fidelity DNA Polymerase **New England Biolabs Catalog #**M0515

0.9 µL DEPC-treated water **Ambion Catalog #**AM9915G

Incubate at 65 °C 00:00:30 . Quench reaction on ice. Add 1 µL [M] 0.1 Molarity (M) EDTA. Mix well and briefly spin down. Incubate at Room temperature 00:05:00 .

8.3 Add 1 µL Thermolabile Proteinase K **New England Biolabs Catalog #**P8111S .
Incubate at 25 °C 02:00:00 20 °C Overnight 55 °C 00:12:00 .

9 USER digestion. 30m
Add 2 µL USER mix:

0.2 µL 10X ThermoPol Buffer **New England Biolabs**

0.5 µL

Thermolabile USER II Enzyme - 50 units **New England Biolabs Catalog #**M5508S

1.3 µL [M] 10 micromolar (µM) T2ME_ssDNA

Incubate at 37 °C 00:30:00 .

Oligonucleotides sequences

T2ME_ssDNA: GTCTCGTGGGCTCGG AGATGTGTAT

10 Ligation adapter annealing. 10m
Add 1 µL MgCl2 mix:

0.3 µL 10X ThermoPol Buffer **New England Biolabs**

0.5 µL [M] 0.2 Molarity (M) MgCl2

0.2 µL DEPC-treated water **Ambion Catalog #**AM9915G

Incubate at 55 °C 00:05:00 25 °C 00:05:00 Ramp rate 0.1ºC/s.

11 Ligation. 40m

Add 6 µL Ligation mix:

2.1 µL 10X T4 Ligase buffer **NEB**

1 µL T4 DNA Ligase **New England Biolabs Catalog #**M0202L

2.9 µL DEPC-treated water **Ambion Catalog #**AM9915G

Incubate at 25 °C 00:30:00 65 °C 00:10:00 .

12 Yield test. 3m 40s

Prepare qRT-PCR mix (10 uL per cell):

1 µL 10X ThermoPol Buffer **New England Biolabs**

0.25 µL [M] 10 micromolar (µM) T1ME

0.25 µL [M] 10 micromolar (µM) T2ME

0.2 µL

dNTP solution mix (10 mM each) **New England Biolabs Catalog #**N0447

0.15 µL

Deep Vent DNA Polymerase - 200 units **New England Biolabs Catalog #**M0258S

0.5 µL Evagreen dye, 20x in water **Biotium Catalog ##**31000

7.15 µL DEPC-treated water **Ambion Catalog #**AM9915G

0.5 µL Template.

Oligonucleotides sequences

T1ME: TCGTCGGCAGCGTC AGATGTGTATAAGAGACAG

T2ME: GTCTCGTGGGCTCGG AGATGTGTATAAGAGACAG

Perform the following program on a qPCR machine.

94 °C 00:02:00

26 cycles of

94 °C 00:00:20

68 °C 00:00:20

72 °C 00:01:00

13 Amplification step 1. 5m 10s

Add:

1 µL [M] 0.2 Molarity (M) EDTA

1.2 µL [M] 10 micromolar (µM) T2ME_ssDNA

2.5 µL [M] 10 micromolar (µM) Illumina Nextra i5 index

25 µL

NEBNext Ultra II Q5 Master Mix **New England Biolabs Catalog #**M0544X

PCR cycles:

98 °C 00:00:30

11 cycles of

98 °C 00:00:10

63 °C 00:00:30

72 °C 00:01:00

Cycle end

72 °C 00:03:00

Purify with 1.4 X Ampure XP beads **Beckman Catalog #**A63881 . Elute in

20 µL DEPC-treated water **Ambion Catalog #**AM9915G .

14 Amplification step 2. 5m 10s

Add the following reagents to 20 µL purified products.

2.5 µL [M] 10 micromolar (µM) Illumina Nextra i7 index

2.5 µL [M] 10 micromolar (µM) Illumina P5

25 µL

NEBNext Ultra II Q5 Master Mix **New England Biolabs Catalog #**M0544X

Oligo sequences:
Illumina P5: AATGATACGGCGACCACCGAGA

PCR cycles:

98 °C 00:00:30

5 cycles of

98 °C 00:00:10

63 °C 00:00:30

72 °C 00:01:00

Cycle end

72 °C 00:03:00

Purify with 1.4 X Ampure XP beads **Beckman Catalog #**A63881 . Elute in 20 µL DEPC-treated water **Ambion Catalog #**AM9915G .