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Accurate detection of somatic mutations in single cells by scNanoSeq

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Abstract

Accurately detecting somatic mutations in individual human cells poses a significant challenge for single-cell whole genome amplification methods. In this study, we introduce a novel single-cell genome amplification method based on the restriction enzyme-based NanoSeq chemistry, achieving an error rate of less than 10^{-10} in mutation calling (equivalent to 0.2 errors per human genome). We term this methodology as scNanoSeq. This precision is derived from theoretical error rate analyses within the duplexing sequencing scheme, which aligns with upper bound estimations derived from single-cell expansion experiments.

Protocol materials

- 1M KCl **bioworld** **Catalog #**40120947-1
- NP-40 Surfact-Amps™ Detergent Solution **Thermo Fisher Scientific** **Catalog #**85124
- 0.5M EDTA **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**E7889-100ML
- QIAGEN Protease (7.5 AU) **Qiagen** **Catalog #**19155
- Molecular grade water **Catalog #**BP561-1 1L
- 1M Tris-HCl, pH 8.0 **Thermo Fisher Scientific** **Catalog #**15568025
- 10% Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**93443-100ML
- rCutSmart Buffer **New England Biolabs** **Catalog #**B6004S
- HpyCH4V - 500 units **New England Biolabs** **Catalog #**R0620L
- Nuclease free water
- CutSmart® Buffer **New England Biolabs** **Catalog #**B7204S
- NEBuffer 4 - 5.0 ml **New England Biolabs** **Catalog #**B7004S
- Klenow Fragment (3'-5' exo-) - 1,000 units **New England Biolabs** **Catalog #**M0212L
- Adenosine 5-Triphosphate (ATP) **New England Biolabs** **Catalog #** P0756L
- 15 µM xGen CS adapter **Integrated DNA Technologies, Inc. (IDT)** **Catalog #**1080799
- T4 DNA Ligase **New England Biolabs** **Catalog #**M0202L
- Ampure XP beads **Beckman** **Catalog #**A63881
- iTaq Universal SYBR Green Supermix **Bio-Rad Laboratories** **Catalog #**172-5112
- NEBNext UltraII Q5 Master Mix **New England Biolabs** **Catalog #**M0544X

Troubleshooting

1. Single cell lysis

3h 55m

1 Prepare 1000 µL **Protease Lysis Mix**. 5m

30 µL of 1M Tris-HCl, pH 8.0 **Thermo Fisher Scientific Catalog #**15568025
4 µL of
0.5M EDTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7889-100ML
20 µL of 1M KCl **bioworld Catalog #**40120947-1
10 µL of
10% Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**93443-100ML
36 µL of
NP-40 Surfact-Amps™ Detergent Solution **Thermo Fisher Scientific Catalog #**85124
400 µL of [M] 20 mg/mL QIAGEN Protease (7.5 AU) **Qiagen Catalog #**19155
500 µL of Molecular grade water **Catalog #**BP561-1 1L

2 Aliquot 2.5 µL of **Protease Lysis Mix** in each low-binding PCR tubes. 20m

3 FACS sort individual cells/nuclei into PCR tubes containing **Protease Lysis Mix**. 1h

4 Brief centrifugation, and run the following cell lysis program on a thermo-cycler. 2h 30m

50 °C 02:00:00
75 °C 00:20:00
85 °C 00:05:00
4 °C Hold

5 Store the lysed cells at -80 °C .

Genome fragmentation

50m

6 Prepare the following **Fragmentation Mix** (2.5 uL per cell).

5m

0.5 µL of CutSmart® Buffer **New England Biolabs Catalog #**B7204S or rCutSmart Buffer **New England Biolabs Catalog #**B6004S

0.15 µL of HpyCH4V - 500 units **New England Biolabs Catalog #**R0620L

1.85 µL of Nuclease free water

7 Add 2.5 uL of **Fragmentation Mix** to each tube along the wall, tap to mix, and then centrifuge. 45m

Run the following program on a thermo-cycler.

37 °C 00:15:00

65 °C 00:20:00

End repair 45m

8 Prepare the following **End Repair Mix** (10 uL per cell). 5m

1 µL of NEBuffer 4 - 5.0 ml **New England Biolabs Catalog #**B7004S

7.35 µL of Nuclease free water

0.15 µL of

Klenow Fragment (3'-5' exo-) - 1,000 units **New England Biolabs Catalog #**M0212L

9 Add 10 uL of **End Repair Mix** to each tube along the wall, tap to mix, and then centrifuge. 40m

Run the following program on a thermo-cycler.

37 °C 00:30:00

Y-shape adapter ligation 1h 7m

10 Prepare the following **Ligation Mix** (22.4 uL per cell). 5m

2.24 µL of NEBuffer 4 - 5.0 ml **New England Biolabs Catalog #**B7004S

15.53 µL of Nuclease free water

3.74 µL of
Adenosine 5-Triphosphate (ATP) **New England Biolabs Catalog #** P0756L
0.33 µL of
15 µM xGen CS adapter **Integrated DNA Technologies, Inc. (IDT) Catalog #**1080799
0.56 µL of T4 DNA Ligase **New England Biolabs Catalog #**M0202L

11 Add 22.4 uL of **Ligation Mix** to each tube along the wall, tap to mix, and then centrifuge. 32m

Run the following program on a thermo-cycler.
20 °C 00:22:00

12 Immediately after ligation, purify the ligation product with 0.95X Ampure XP beads **Beckman Catalog #**A63881, and eluted in 15.5 µL of Nuclease free water. 30m

To avoid sample loss, flick the tubes for all the mixing steps instead of pipetting.

Library QC and amplification 4h

13 Use 0.5 µL of Purified ligation product for the following qPCR yield test. 2h

5 µL of
iTaq Universal SYBR Green Supermix **Bio-Rad Laboratories Catalog #**172-5112
0.25 µL of [M] 10 micromolar (µM) **Truseq5 primer (ACACTCTTTCCCTACACGAC)**
0.25 µL of [M] 10 micromolar (µM) **Truseq7 primer (GTGACTGGAGTTCAGACGTGT)**
4 µL of Nuclease free water
0.5 µL Purified ligation product

Run qPCR on a Roche LightCycler 96 machine following the program:
94 °C for 00:02:00
30 cycles of

- 94 °C for 00:00:20
- 58 °C for 00:00:20
- 72 °C for 00:01:00

Melting Curve

For a typical diploid cells, qPCR Ct value is expected to be 17.5~18.5.

14 For the cells with anticipated yield, use the remaining 15 µL of Purified ligation product for PCR amplification. 2h

25 µL of
NEBNext Ultrall Q5 Master Mix **New England Biolabs Catalog #**M0544X
5 µL of [M] 10 micromolar (µM) **P5_index5_Truseq5 primer**
(AATGATACGGCGACCACCGAGATCTACAC[8-base-index]ACACTCTTTCCCTACACGACGCTCTTCCGATCT**)**
5 µL of [M] 10 micromolar (µM) **P7_index7_Truseq7 primer**
(CAAGCAGAAGACGGCATACGAGAT[8-base-index]GTGACTGGAGTTCAG ACGTGTGCTCTTCCGATCT**)**
15 µL Purified ligation product

Run the following PCR program on a thermo-cycler:
98 °C for 00:00:30
17 cycles of
- 98 °C for 00:00:10
- 73 °C for 00:01:15

73 °C for 00:05:00

Purify the PCR product twice with 0.7X
Ampure XP beads **Beckman Catalog #**A63881, and eluted in 20 µL of Nuclease free water.

Pooled libraries are ready for sequencing on an Illumina NovaSeq 6000 / NovaSeq X / NovaSeq XPlus platform with 2×150 bp paired-end mode. The target depth is 5~6 reads per fragment based on pre-amplification qPCR quantification.

Protocol references

1. Niu, M., Zhang, Y., Luo, J., Sinson, J. C., Thompson, A. M., & Zong, C. (2023). Characterization of cancer evolution landscape based on accurate detection of somatic mutations in single tumor cells. bioRxiv, 2023-10.
2. Abascal, F., Harvey, L. M., Mitchell, E., Lawson, A. R., Lensing, S. V., Ellis, P., ... & Martincorena, I. (2021). Somatic mutation landscapes at single-molecule resolution. *Nature*, *593*(7859), 405-410.