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🌐 Accurate detection of somatic mutations in single cells by scNanoSeq

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We use this protocol and it's working

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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 μ L **Protease Lysis Mix.**

5m

 30 μ L of  1M Tris-HCl, pH 8.0 Thermo Fisher Scientific Catalog #15568025

 4 μ L of

 0.5M EDTA Merck MilliporeSigma (Sigma-Aldrich) Catalog #E7889-100ML

 20 μ L of  1M KCl bioworld Catalog #40120947-1

 10 μ L of

 10% Triton X-100 Merck MilliporeSigma (Sigma-Aldrich) Catalog #93443-100ML

 36 μ L of

 NP-40 Surfact-Amps™ Detergent Solution Thermo Fisher Scientific Catalog #85124

 400 μ L of  20 mg/mL  QIAGEN Protease (7.5 AU) Qiagen Catalog #19155

 500 μ L of  Molecular grade water Catalog #BP561-1 1L

2 Aliquot  2.5 μ L 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 μ L per cell).

5m

pipette 0.5 μ L of  CutSmart® Buffer New England Biolabs Catalog #B7204S or

 rCutSmart Buffer New England Biolabs Catalog #B6004S

pipette 0.15 μ L of  HpyCH4V - 500 units New England Biolabs Catalog #R0620L

pipette 1.85 μ L of  Nuclease free water

7 Add 2.5 μ L 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 μ L per cell).

5m

pipette 1 μ L of  NEBuffer 4 - 5.0 ml New England Biolabs Catalog #B7004S

pipette 7.35 μ L of  Nuclease free water

pipette 0.15 μ L of

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

9 Add 10 μ L 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 μ L per cell).

5m

pipette 2.24 μ L of  NEBuffer 4 - 5.0 ml New England Biolabs Catalog #B7004S

pipette 15.53 μ L of  Nuclease free water

pipette 3.74 μ L of

Adenosine 5-Triphosphate (ATP) New England Biolabs Catalog # P0756L

pipette 0.33 μ L of

15 μ M xGen CS adapter Integrated DNA Technologies, Inc.
(IDT) Catalog #1080799

pipette 0.56 μ L of T4 DNA Ligase New England Biolabs Catalog #M0202L

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

Run the following program on a thermo-cycler.

thermocycler 20 °C

thermocycler 00:22:00

12 Immediately after ligation, purify the ligation product with 0.95X 30m

Ampure XP beads Beckman Catalog #A63881, and eluted in

pipette 15.5 μ L of

Nuclease free water.

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

Library QC and amplification 4h

13 Use pipette 0.5 μ L of **Purified ligation product** for the following qPCR yield test. 2h

pipette 5 μ L of

iTaq Universal SYBR Green Supermix Bio-Rad Laboratories Catalog #172-5112

pipette 0.25 μ L of [M] 10 micromolar (μ M) **Truseq5 primer**

(ACACTCTTCCCTACACGAC)

pipette 0.25 μ L of [M] 10 micromolar (μ M) **Truseq7 primer**

(GTGACTGGAGTTCAGACGTGT)

pipette 4 μ L of Nuclease free water

pipette 0.5 μ L of **Purified ligation product**

Run qPCR on a Roche LightCycler 96 machine following the program:

thermocycler 94 °C for

thermocycler 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 UltraII Q5 Master Mix New England Biolabs Catalog #M0544X

 5 μL of  [M] 10 micromolar (μM) **P5_index5_Truseq5 primer**

(AATGATACGGCGACCACCGAGATCTACAC[8-base-index]ACACTCTTCCCTACACGACGCTCTCCGATCT)

 5 μL of  [M] 10 micromolar (μM) **P7_index7_Truseq7 primer**

(CAAGCAGAAGACGGCATACGAGAT[8-base-index]GTGACTGGAGTTAGACGTGTGCTCTCCGATCT)

 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.