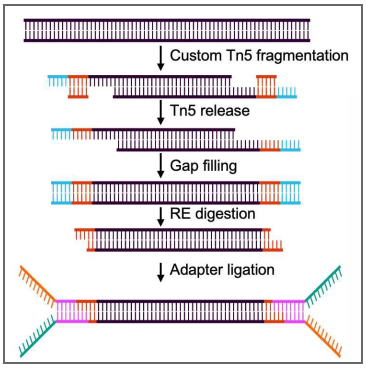

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Protocol status: Working
We use this protocol and it's working

CompDuplex: Accurate detection of somatic mutations by duplex-seq with comprehensive genome coverage

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DISCLAIMER

Patent application covering CompDuplex chemistry has been filed by Baylor College of Medicine.

ABSTRACT

Somatic mutations continuously accumulate in the human genome, posing vulnerabilities towards aging and increased risk of various diseases. However, accurate detection of somatic mutations at the whole genome scale is still challenging. By tagging and independently sequencing the two complementary strands of DNA, the recent development of duplex-sequencing methods has greatly improved the detection accuracy, however, the limited genome coverage and the compromised compatibility with existing sequencing platforms have constrained the broad applications of these methods.

To overcome these technical challenges, here we developed a duplex sequencing method with comprehensive genome coverage, which we refer to as CompDuplex-seq. The streamlined chemistry of CompDuplex assay allows efficient generation of libraries readily compatible with standard Illumina 2x150 paired-end sequencing. In addition, we validated the accuracy of somatic mutation calling and comprehensive genome coverage of CompDuplex by profiling a single-cell expanded clone. To summarize, CompDuplex chemistry supports genome-wide coverage while maintaining high accuracy, which we believe will facilitate the whole genome characterization of somatic mosaicism in various biological systems.

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PROTOCOL MATERIALS

NEBNext Ultrall Q5 Master Mix **New England Biolabs Catalog #M0544X** In 2 steps
0.5M EDTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E7889-100ML** Step 8
T4 Polynucleotide Kinase Reaction Buffer **New England Biolabs Catalog #B0201S** Step 9
1M Tris-HCl, pH 8.0 **Invitrogen - Thermo Fisher Catalog #15568025** In 2 steps
iTaq Universal SYBR Green Supermix **Bio-Rad Laboratories Catalog #172-5112** Step 11
Glycerol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #G5516** Step 3
Water **Invitrogen - Thermo Fisher Catalog #46-2224** Step 5
BtgZI - 500 units **New England Biolabs Catalog #R0703L** Step 7
10% Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #93443-100ML** Step 8
5M NaCl **Invitrogen - Thermo Fisher Catalog #AM9760G** Step 1
Tn5 transposase, unloaded **Diagenode Catalog #C01070010-20** Step 3
Tagmentation Buffer 1 **Illumina, Inc. Catalog #20015171** Step 5
rCutSmart Buffer **New England Biolabs Catalog #B6004S** Step 7
StickTogether DNA Ligase Buffer **New England Biolabs Catalog #B0535S** Step 10
Ampure XP beads **Beckman Catalog #A63881** In 5 steps
Protease **Qiagen Catalog #19155** Step 8
T7 DNA Ligase - 750,000 units **New England Biolabs Catalog #M0318L** Step 10
Water **Invitrogen - Thermo Fisher Catalog #46-2224** Step 1

Custom Tn5 transposase assembly

1h

1

2m

Prepare 500 µL of **2X Annealing Buffer**.
40 µL 1M Tris-HCl, pH 8.0 **Invitrogen - Thermo Fisher Catalog #15568025**
10 µL 5M NaCl **Invitrogen - Thermo Fisher Catalog #AM9760G**
450 µL Water **Invitrogen - Thermo Fisher Catalog #46-2224**

2 **Transposon annealing.** 27m

Prepare the following mix in a PCR tube.

20 µL **2X Annealing Buffer**
10 µL [M] 200 micromolar (µM) ME_REV
10 µL [M] 200 micromolar (µM) T_BtgZ1_ME

Oligonucleotides sequences
ME_REV: /5Phos/CTGTCTCTTATACACATCT
T_BtgZ1_ME: ATGTGTGGAGCGATG AGATGTGTATAAGAGACAG

Mix up the reaction, spin down, and perform the following reactions on a thermal cycler.
95 °C 5 min
65 °C 5 min, Ramp rate 0.1°C/s
4 °C Hold, Ramp rate 0.1°C/s

This results in a [M] 50 micromolar (µM) transposon mix .

3 **Custom Tn5 assembly.** 31m

Prepare the following mix in a 1.5 mL tube.
20 µL Tn5 transposase, unloaded **Diagenode Catalog #C01070010-20**
20 µL [M] 50 micromolar (µM) transposon mix

Gently pipet to mix, and incubate at 23 °C for 30 min.

Add 20 µL Glycerol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #G5516** to the tube, mix well, aliquot, label as "**Tn5_BtgZ1**", and store at -20 °C .

CompDuplex Procedure

4 **1. Re-purification of genomic DNA**

Dilute 20 ng Sample to 10 µL nucleus-free water in a PCR tube. Add 5 µL Ampure XP beads **Beckman Catalog #A63881** (0.5X) to the tube, mix well, and incubate at Room temperature for 5 min.

Put the tube on the magnetic stand. When the aqueous phase becomes transparent, remove the supernatant, wash twice with 120 µL [M] 80 % volume Ethanol. Air-dry the beads.

5 2. On-bead custom Tn5 tagmentation

Prepare the following **tagmentation mix**.

2 µL Tagmentation Buffer 1 **Illumina, Inc. Catalog #20015171**
0.48 µL 1:30 diluted **Tn5_BtgZ1**
7.52 µL Water **Invitrogen - Thermo Fisher Catalog #46-2224**

Resuspend the air-dried beads with 10 µL **tagmentation mix**.
Incubate on a thermal cycler using the following program: 20 °C for 5 min, 55 °C for 15 min.

Quench the reaction by adding 3 µL of 0.2M EDTA.
Incubate on a thermal cycler at 50 °C for 30 min.

6 3. Gap filling

Add 3 µL of 0.2M $MgCl_2$ to the tube.
Add 16 µL NEBNext UltraII Q5 Master Mix **New England Biolabs Catalog #M0544X** to the tube.
Incubate on a thermal cycler at 65 °C for 3 min.
Quench the reaction by adding 2 µL of 0.5M EDTA.
Perform double size selection with 0.48X/0.75X Ampure XP beads **Beckman Catalog #A63881**, elute in 7.5 µL water.

7 4. Restriction enzyme digestion

Prepare the following restriction enzyme digestion mix (Total 15 µL).

7.5 µL Gap filling product
1.5 µL rCutSmart Buffer **New England Biolabs Catalog #B6004S**
0.7 µL BtgZI - 500 units **New England Biolabs Catalog #R0703L**
5.3 µL Water

Incubate on a thermal cycler with the following program: 25 °C for 10 min, 37 °C for 3 h, 10 °C for 12 h.

8 5. Restriction enzyme release

Prepare the following **restriction enzyme release mix** (Total 500 µL).

15 µL 1M Tris-HCl, pH 8.0 **Invitrogen - Thermo Fisher Catalog #15568025**
12 µL 0.5M EDTA **Merck MilliporeSigma (Sigma-Aldrich) Catalog #E7889-100ML**
10 µL 1M KCl
5 µL 10% Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #93443-100ML**
18 µL 10% NP40
420 µL Water
20 µL [M] 20 mg/mL Protease **Qiagen Catalog #19155**

Add 15 µL **restriction enzyme release mix** to the sample.
Incubate on a thermal cycler with the following program: 50 °C for 45 min, 25 °C for 2 h.
Purify with 0.85X Ampure XP beads **Beckman Catalog #A63881** , elute in 10 µL water.

9 6. Y-shape ligation adapter annealing

Prepare the following [M] 15 micromolar (µM) T1T2_TCTT ligation adapter .

1 µL T4 Polynucleotide Kinase Reaction Buffer **New England Biolabs Catalog #B0201S**
10.5 µL [M] 100 micromolar (µM) **T1_BtgZ1_ME**

7.5 µL [M] 100 micromolar (µM) **Rev_T2_BtgZ1**
31 µL Water.

Oligonucleotides sequences
T1_BtgZ1_ME: TCGTCGGCAGCGTC AGATGTGTAT
Rev_T2_BtgZ1: /5Phos/ TCTT ATACACATCT CCGAGCCCACGAGAC

Mix up the reaction, spin down, and perform the following reactions on a thermal cycler.
65 °C 5 min
20 °C Hold, Ramp rate 0.1°C/s

10 7. Y-shape ligation adapter ligation

Prepare the following ligation mix (Total 50 µL).
10 µL Sample from Step 8
25 µL StickTogether DNA Ligase Buffer **New England Biolabs Catalog #B0535S**
3 µL [M] 15 micromolar (µM) T1T2_TCTT ligation adapter
3 µL T7 DNA Ligase - 750,000 units **New England Biolabs Catalog #M0318L**
9 µL Water

Incubate at 25 °C for 30 min.
Purify with 0.8X Ampure XP beads **Beckman Catalog #A63881** , elute in 51 µL water.

11 8. Quantification of library complexity

Dilute 1 µL of ligation product with 9 µL water. Use the 1:10 diluted product to quantify library complexity using qPCR. Any Illumina Nextera libraries with known concentration can be used as a standard. An example qPCR procedure is shown below.

5 µL iTaq Universal SYBR Green Supermix **Bio-Rad Laboratories Catalog #172-5112**
0.25 µL [M] 10 micromolar (µM) **T1ME**
0.25 µL [M] 10 micromolar (µM) **T2ME**
3.5 µL water

1 µL template

Oligonucleotides sequences
T1ME: TCGTCGGCAGCGTC AGATGTGTATAAGAGACAG
T2ME: GTCTCGTGGGCTCGG AGATGTGTATAAGAGACAG

94 °C 2 min
30 cycles of
94 °C 20s
68 °C 20s
72 °C 1 min, fluorescence scanning
Melting curve

12 **9. Library amplification**

We recommend to amplify 14 cycles for a library with 20 million DNA fragments.

25 µL NEBNext UltraII Q5 Master Mix **New England Biolabs Catalog #M0544X**
2.5 µL [M] 10 micromolar (µM) **Illumina Nextera N5XX index primer**
2.5 µL [M] 10 micromolar (µM) **Illumina Nextera N7XX index primer**
20 µL ligation product and water

Oligonucleotides sequences
Illumina Nextera N5XX index primer: AATGATACGGCGACCACCGAGATCTACAC NNNNNNNN TCGTCGGCAGCGTC
Illumina Nextera N7XX index primer: CAAGCAGAAGACGGCATACGAGAT NNNNNNNN AGATGTGTATAAGAGACAG

PCR cycles:
98 °C 30s
14 cycles of
98 °C 10s
63 °C 30s

72 °C 1 min

72 °C 3 min

Purify with 0.8X Ampure XP beads **Beckman Catalog #A63881**, elute in 20 µL water.