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Single-Cell Multiple Displacement Amplification (SCMDA)

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Abstract

Single-Cell Multiple Displacement Amplification (SCMDA) is a protocol developed for accurate single-nucleotide variant detection in single cells after whole-genome amplification. This protocol accompanies Dong et al (Nature Methods, publishing online 20 March, 2017; 10.038/nmeth.4227); it was added to the manuscript after formal peer review, as an aid to users

Reagents

PBS, Ph7.4 1x (Gibco, Cat No. 10010-023)

Exo-resistant random primers (500 µM,Thermo Scientific Cat No.SO108)

KOH[2N] (G-Biosciences Cat No.R008)

DTT (REPLI-g Ultrafast Mini Kit, Qiagen, Cat No.150035)

EDTA (Fluka Analytical, REF 03690-100ml)

HCl[2N] (Fisher Scientific, SA431-500)

Tris-HCl solution (1M, pH7.5, Fisher BioReagent)

MDA reaction buffer (REPLI-g Ultrafast Mini Kit, Qiagen, Cat No.150035)

Phi29 polymerase (REPLI-g Ultrafast Mini Kit, Qiagen, Cat No.150035)

AMPureXP-beads (Beckman Coulter, REF A63881)

Ethanol 200 proof (Decon Laboratories)

Nuclease-free water

Qubit High Sensitivity dsDNA Kit (Invitrogen, REF Q32854)

Agarose (Sigma)

TrackIt 1Kb DNA Ladder (Invitrogen, Cat No.10488085)

TBE (10X, Fisher Scientific)

Fast Sybr green master mix (Applied Biosystems, REF 4385612)

Locus-specific primer (Supplementary Table 2, Dong et al., Nature Methods in press; from IDT, 1 µM)

Equipment

0.2-ml PCR tubes

Mini centrifuge (Benchmark)

PCR workstation (CBS Scientific, Mode P-036-02)

PCR machine (Bio-Rad)

DynaMag-2 magnet (Invitrogen)

Qubit Fluorometer (Thermo Scientific)

Qubit tube (Molecular Probed by Life, Q32856)

Agarose gel electrophoresis unit (Thermo Scientific)

FAS Digi Gel Imaging System (Bulldog Bio)

96-well plate (Applied Biosystems)

Optical Adhesive Covers (Applied Biosystems, REF 4360954)

Centrifuge (Eppendorf, 5430R)

qPCR machine (Applied Biosystems)

Procedure

1. Single cell preparation:

1) Isolate single cells (multiple single cell isolation methods are available, for example, the CellRaft system, which was used here) and deposit into 0.2-ml PCR tubes containing 2.5 µl PBS.

2) Immediately place the tubes on dry ice and store at -80°C.

2. Whole genome amplification:

Carry out the following Step 1) to 4) in a PCR workstation.

1) Add 1 µl exo-resistant random primers and 3 µl lysis buffer containing 400 mM KOH, 100 mM DTT, 10 mM EDTA solution, into the PCR tube containing a single cell.

2) Flick the mixture several times, spin down quickly and keep it on ice for 10 min.

3) Add 3 µl stop buffer containing 400 mM HCl and 600 mM Tris-HCl solution (1 M, pH7.5) to neutralize the lysis buffer. Keep it on ice for 2 min.

4) Add 32 µl of master mix containing 30 µl MDA reaction buffer and 2 µl Phi29 polymerase. Mix by pipetting gently.

5) Incubate at 30°C for 1.5 hr and 65°C for 3 min.

3. Amplified product purification:

1) Purify amplified product with 75.6 µl AMPureXP-beads and mix it by pipetting.

2) Leave the mixture at room temperature for 5 min and put it on a magnetic separator until the solution is clear.

3) Remove the clear liquid by pipetting and wash beads twice with fresh 80% ethanol.

4) Open lid of the tube to air-dry beads for about 5 min. (Avoid beads cracking)

5) Take out the tube from magnetic separator and elute beads using 32 µl nuclease-free water.

6) Put back the tube to magnetic separator and keep it at room temperature for 5 min until the solution is clear and a pellet is formed again.

7) Transfer 30 µl clear liquid from the tube to a clean tube.

8) Store the purified product at 4°C for short-term storage or -20°C for long-term storage.

4. Concentration and size measurement:

1) Dilute the purified product 10-20 times and quantify the samples using Qubit. Usually, the yield of the purified product is 10-12 µg.

2) Check the product size by agarose gel electrophoresis. The following conditions are recommended: 1.0% agarose gel; 1.0% TBE solution; 100V for 40 min. A bright band (with a little smear) over 10 kb in a gel picture is desirable.

5. Estimation of amplification uniformity:

1) Dilute the purified product and unamplified genomic DNA as a control to 1 ng/µl using nuclease-free water.

2) Prepare 96-well plate and the PCR mixture for each well including 5 µl Fast Sybr green master mix, 1 µl locus-specific primers, 2 µl nuclease-free water and 2 µl diluted DNA.

3) Seal the plate and spin down.

4) Incubate under the following qPCR program: 95°C for 20s, thermocycling (40 cycles) at 95°C for 3 s, and 60°C for 30 s.

5) Calculate the relative uniformity values (RUVs) for 8 loci (equation (1) in Supplementary Notes, Dong et al., Nature Methods in press).

6) Select the samples with RUV values for at least 6 of the 8 loci of between 0.25-4 for library construction.

Accurate identification of single-nucleotide variants in whole-genome-amplified single cells

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