3. Amplification reaction

3.1 According to the number of reactions N, mix Amplification Buffer and Amp Enzyme Mix, shake to mix, and centrifuge briefly for later use.

Amplification Mix	Volume
Amplification Buffer	58 μL × N
Amp Enzyme Mix	2 μL × N
Total Volume	60 μL × N

3.2 Add 60 μL of amplification mixture to the 15 μL pre-amplification reaction product of the previous step and run the following procedure.

Number of Cycles	Temperature	Time
1	95°C	2 min
	95°C	15 s
14	65°C	1 min
	75°C	1 min
1	4°C	Hold

Note:

Note: The number of cycles can be adjusted as needed, and 14 cycles are recommended for single cell obtained by flow sorting and other methods.

Amplification Product Detection

1. Agarose Gel Electrophoresis
   Take 5 μL of the amplified product for agarose gel electrophoresis (1% agarose gel, 110 V, 25-35 minutes), and the size of the amplified product is 200-1500 bp.
2. Quantitative
   The amplified product was purified by magnetic beads or column, and the purified product was quantified using Qubit, and the final yield was 2-5 μg.

Version: 09/2023

Single Cell WGA Kit

Cat. No. : CW2844S (24 rxns)
CW2844M (96 rxns)

Shipping and Storage : Ship on dry ice, store all components at -20°C immediately upon receipt of the kit.In a constant temperature refrigerator, it can be stored for 12 months. For longer-term storage, please store below -70°C.

Components

Component	CW2844S (24 rxns)	CW2844M (96 rxns)
Cell Lysis Buffer	240 μL	960 μL
Cell Lysis Enzyme	16 μL	64 μL
Pre-Amp Buffer	120 μL	480 μL
Pre-Amp Enzyme Mix	7 μL	28 μL
Amplification Buffer	1.5 mL	4×1.5 mL
Amp Enzyme Mix	50 μL	200 μL

Principle

The single-cell whole-genome amplification kit can achieve whole-genome amplification using a single cell or a micro genomic DNA sample as a template. The single-cell amplification reaction time is short, and the total process is about 3 hours. After the reaction, 2-5 μg of genomic DNA can be obtained through the process of lysis, pre-amplification and amplification, and the size is about 200-1500 bp. The amplified product can be widely used in next-generation sequencing, large fragment copy number variation analysis, SNP typing, qPCR analysis, gene chip analysis, etc.

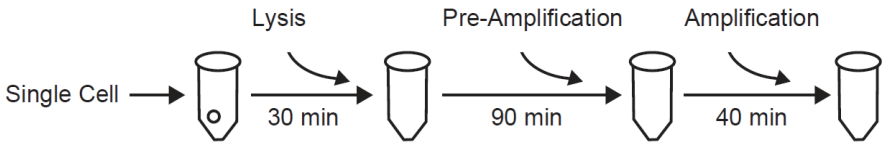
Self-provided reagents

1. PCR machine
2. Reaction tubes: low adsorption tubes are recommended
3. Pipette Tip: It is recommended to use a high-quality filter tip
4. Microcentrifuges, Vortex Mixers

Important Points Before Starting

The detection sensitivity of this product is extremely high. The experimental operation should be completed in a positive pressure ultra-clean workbench or in a clean environment. The concentration of the amplification reaction product is high, and isolation should be done to avoid aerosol pollution caused by the amplification product.

Schematic diagram of the operation process



Procedure

Preparation before experiment

Single cell is obtained by flow cytometry sorting, buffer dilution, micromanipulation, and laser microdissection. It is recommended to wash the cells before the experiment. The washing solution is 1×PBS solution without Mg2+ and Ca2+. Make sure that the volume of the PBS solution in subsequent experiments does not exceed 2 μL.

Note:

Since the whole experiment is carried out in the same PCR tube and the reaction volume is small, the pipette tip should not touch the liquid in the tube during the liquid addition operation, to avoid taking single cell or DNA out of the reaction system; when pipetting, please follow the tube wall. Add carefully, do not blow the liquid in the PCR tube; before the reaction, please perform a brief centrifugation to ensure that the liquid in the reaction system is evenly mixed thaw Cell Lysis Enzyme, Pre-Amp Enzyme and Amp Enzyme on ice before use.

1. Cell Lysis

1.1 According to the number of reactions N, mix the Cell Lysis Buffer and Cell Lysis Enzyme, shake to mix, and centrifuge briefly for later use.

Cell Lysis Mix	Volume
Cell Lysis Buffer	9.4 μL × N
Cell Lysis Enzyme	0.6 μL × N
Total Volume	10 μL × N

1.2 Mix the single cell with the cell lysis mix in a PCR tube and run the following procedure.

Number of Cycles	Temperature	Time
1	50°C	20 min
	95°C	10 min
	4°C	Hold

2. Preamplification Reaction

2.1 According to the number of reactions N, mix Pre-Amp Buffer and Pre-Amp Enzyme Mix, shake to mix, and centrifuge briefly for later use.

Preamplification Mix	Volume
Pre-Amp Buffer	4.75 μL × N
Pre-Amp Enzyme Mix	0.25 μL × N
Total Volume	5 μL × N

2.2 Add 5 μL of the pre-amplification mix to the 10 μL cleavage reaction product from the previous step and run the following procedure.

Number of Cycles	Temperature	Time
1	95°C	2 min
12	95°C	15 s
	15°C	50 s
	25°C	40 s
	35°C	30 s
	65°C	40 s
	75°C	40 s
1	4°C	Hold