YK001A/B

Single Cell Whole Genome Amplification Kit Handbook

Rapid, Reliable and Reproducible

Whole Genome Amplification for Single Cells

Cat:YK001A/B
Version: 1302.1

Yikon Genomics
亿康基因

Contents

Kit Components

The Single Cell WGA Kit provides a sufficient supply of DNA extraction and genome amplification reagents to perform 10 whole genome amplification reactions (Cat: YK001A) or 50 whole genome amplification reactions (Cat: YK001B).

Components	YK001A (10 rxns)	YK001B (50 rxns)
Cell Lysis Buffer	100 µL	300 µL
Cell Lysis Enzyme	10 µL	30 µL
Pre-Amp Buffer	300 µL	750µL x 2
Pre-Amp Enzyme Mix	10 µL	50 µL
Amplification Buffer	300 µL	750µL x 2
Amp Enzyme Mix	8 µL	40 µL

Storage Information

Store all components at -20℃ for up to 12 months. The expiration date can be found on the box label. Transfer the Cell Lysis Enzyme, Pre-Amp Enzyme Mix and Amp Enzyme Mix tubes to ice just before use. All other components can be thawed on ice prior to use. Please aliquot reagents for multiple usages and avoid freeze-thaw cycles.

Intended Use

The Single Cell WGA kit is intended to be used for research purposes only. The Single Cell WGA kit may not be transferred to third parties, resold, modified for resale, used to provide commercial services or used to manufacture commercial products without written approval of Yikon Genomics Co. Ltd.

Safety Information

Always wear suitable lab coat, disposable gloves and protective goggles when using the single cell WGA kit. For more information please refer to the appropriate online material safety data sheets (MSDSs).

Required Materials Not Included

PCR thermal cycler
PCR tubes
Dedicated pipette set
Filter pipette tips
Low Binding Barrier
Ice
Micro centrifuge
-20°C Storage Freezer
Vortex
Spectrophotometer or equivalent to quantify DNA
Nuclease-Free Water
1XPBS buffer

Introduction

The Single Cell WGA kit efficiently amplifies genome DNA from single cells or equivalent DNA amount to produce 2-4 micrograms of amplified DNA in about four hours. The kit can also be used when the input amount of DNA is low (e.g. hundreds of cells or sub-ng DNA). With most cell types such as single blastomere, polar bodies, single cells from culture lines, single sperm, the kit is able to achieve >95% amplification success rate, and achieves consistent amplification efficiency in both AT-

and GC-rich regions. The kit generates reproducible loci representation, achieving >0.9 correlation coefficient for qPCR Ct values from replicate single cell reactions. More than 90% of the loci can be amplified and analyzed by various platforms, such as real-time PCR (qPCR) and next-generation sequencing.

Product Features

- A typical yield of 2 - 4 µg amplified DNA from single mammalian cells
- Single-tube, 3-step, 4-hr process, No intermediate purifications
- >95% amplification success rate with flow sorted cells, or >0.5pg of genome DNA
- Achieves reproducible locus representation and consistent amplification efficiency in both AT- and GC-rich regions
- <10% locus drop-out, <10% allele drop-out

Basic Principle

The Single Cell WGA kit generates highly uniform amplification across the entire genome. The method is based on the MALBAC (Multiple Annealing and Looping Based Amplification Cycles) technology (1), which carries out close-to-linear pre-amplification cycles of the entire genome using a mixture of highly-processive DNA polymerases with strand displacement activity, followed by an exponential amplification by PCR to a sufficient amount for various downstream analyses.

Application

DNA amplified with the Single Cell WGA Kit can be used in a variety of downstream analytical platforms:

- Detection of mutations
- SNP genotyping
- CNV profiling and aneuploidy detection
- Whole genome and exome sequencing
- Real-time PCR
- Molecular cloning
- Microarray CGH

Source Material and Research Area

The Single Cell WGA Kit is an innovative tool for whole genome amplification from various starting materials including genomic DNA, fresh or dry blood, fresh or frozen tissue, such as:

Human and animals

- Biomarker discovery (CNVs, SNVs)
- IVF pre-implantation genetic screening
- Genotyping of transgenic animals
- Embryo and stem cell research
- Single sperm genotyping

Tumor

- Somatic Variation
- Tumor development and evolution
- Cancer stem cells
- Circulating tumor cells

Bacteria

- Metagenomics research
- Microorganism genotyping

Sample Specifications

Sample amount

The Single Cell WGA kit is uniquely suitable for single cell genome amplification. The kit is also suitable for a wide range of starting amount, from 0.5pg or a single chromosome to ~1ng genome DNA.

Collection Methods

Flow sorting, dilution, and micromanipulation are collection methods that are compatible with the Single Cell WGA Kit. Although best results are obtained with live cells, the Single Cell WGA Kit is also suitable for paraformaldehyde-fixed or laser-guided micro-dissected samples.

Pre-treatment of samples

Cell washing is strongly recommended to minimize non-cellular DNA contamination. Mg^{2+}-free, Ca^{2+}-free, PBS may be used for washing at a 1X concentration. Wash buffers containing Mg^{2+}, Ca^{2+} must be avoided.

The PBS volume carried over with the cell sample into the Amplification Protocol should not exceed 1 µL.

Recommended Lab Practice

1. Dedicated area for pre-amplification sample preparations

- To avoid DNA contamination from external sources or from the amplified DNA product, sample preparation steps before amplification

should be performed in a separate and dedicated room (or at least in a dedicated pre-amplification working space). Dedicated experimental materials such as pipette, pipette tips, PCR tubes, 1.5 ml micro centrifuge tubes, tube racks, lab coats, etc. should be used solely for setting up single cell WGA reactions.

- The amplified DNA product should be stored separately from the pre-amplification reagents. All downstream treatment such as DNA purification, sequencing preparations should be done in a separate room or lab space.

2. Pipetting Tips

All reactions described in the Single Cell WGA procedure take place in the same tube in which the single cell has been isolated and lysed. To avoid accidentally removal of the cell (or parts of the cellular genome), carefully add the appropriate volume of reaction mix onto the wall of the tube, without disturbing the liquid, followed by a brief centrifuge spin.

3. Control DNA Sample (5 µL)

It is recommended to prepare control DNA samples as positive controls using 30pg of genome DNA.

- Dilute the control DNA stock to 30 pg/µL DNA with nuclease-free water.
- Add 1 µL of the 30 pg/µL DNA solution to 4 µL of Cell Lysis Buffer in a PCR tube or well.

Procedure

One tube, 3-step, 4-hour procedure

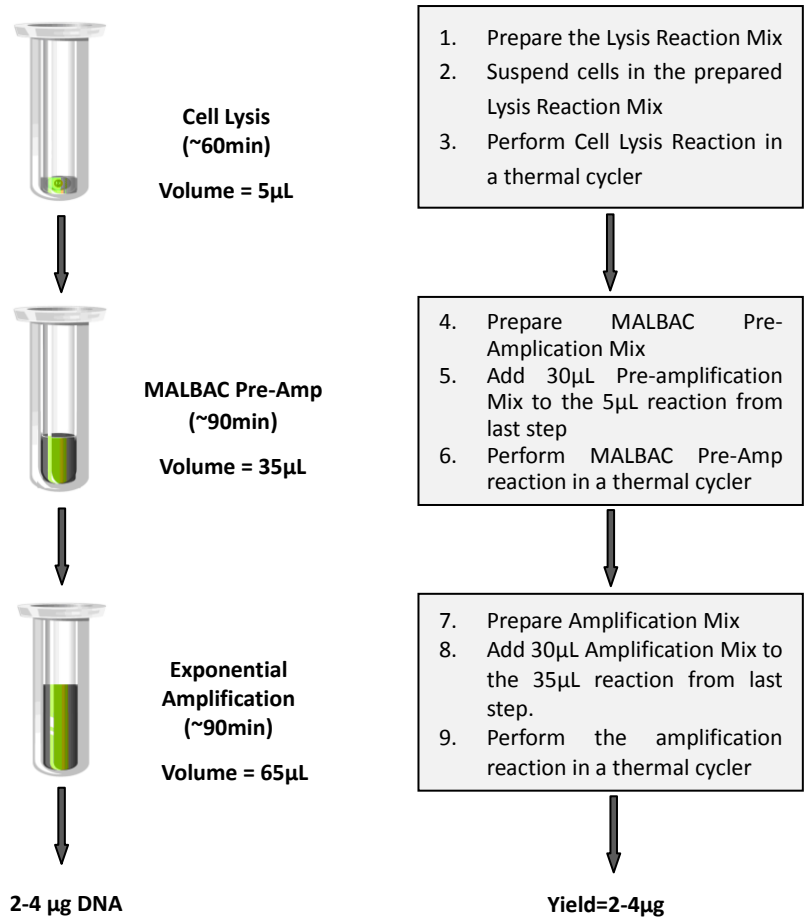

Figure 1: Workflow of a single cell WGA reaction

Detailed Protocol

Cell Lysis

1. Prepare the Cell Lysis Reaction Mix by combining the following components and mix well (N= number of reactions).

Lysis Reaction Mix	Volume
Cell Lysis Buffer (blue cap)	5µL x N
Cell Lysis Enzyme (blue cap)	0.1 µL x N
Total Volume	5.1 µL x N

2. Collect a single cell into 5µL of the prepared Cell Lysis Reaction Mix in a PCR tube or well. (The PBS volume carried over with the cell sample should not exceed 1µL.)
3. Incubate the sample in a thermal cycler with heated lid using the following temperature steps.

Cycles	Temp	Time
1	50℃	50min
	80℃	10min
	4℃	forever

MALBAC Pre-Amplification

4. Prepare the MALBAC Pre-amplification Reaction Mix by combining the following components and mix well (N=number of reactions):

Pre-amplification Mix	Volume
Pre-Amp Buffer (green cap)	30 µL x N
Pre-Amp Enzyme Mix (green cap)	1 µL x N
Total Volume	31 µL x N

5. Add 30 µL of freshly-prepared Pre-amplification Mix to each 5 µL cell sample or control DNA sample.
6. Incubate in a thermal cycler:

Cycles	Temp	Time
1	94℃	3 min
8	20℃	40s
	30℃	40s
	40℃	30s
	50℃	30s
	60℃	30s
	70℃	4min
	95℃	20s
	58℃	10s
1	4℃	forever

7. Proceed immediately to the next step.

Exponential Amplification

8. Prepare the Amplification Reaction Mix by combining the following components and mix well (N=number of reactions):

Amplification Mix	Volume
Amplification Buffer (red cap)	30 µL x N
Amp Enzyme Mix (red cap)	0.8 µL x N
Total Volume	30.8 µL x N

9. Mix 30 µL of freshly-prepared Amplification Reaction Mix with the 35µL Pre-Amp product and mix gently by pipet.
10. Incubate the samples in a thermal cycler.

Cycles	Temp	Time
1	94℃	30s
17*	94℃	20s
	58℃	30s
	72℃	3min
1	4℃	forever

*****We recommend using 14 cycles for 100pg gDNA, 17x for a single flow-sorted mammalian cell; 19-21x for a single chromosome. Number of cycles may need to be optimized with other cell types or using other sample preparation procedure.

11. Purify the amplification DNA product and store the purified amplification product at –20°C.

Amplification Yield

With optimized number of amplification cycles, each Single Cell WGA reaction consistently generates 2- 4 μg of amplified DNA per 65 μL reaction from a single mammalian cell (or equivalent high-quality genomic DNA), with a size distribution of 300-2000 bp.

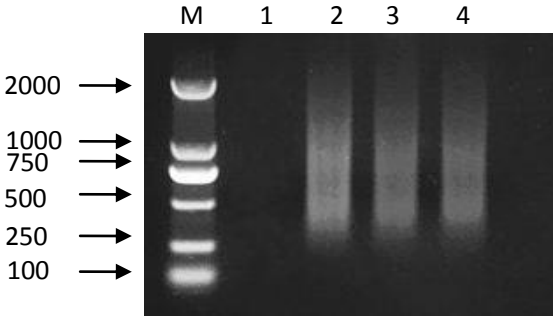

Figure 2. The Single Cell WGA Kit produces consistent yield and size range (300-2000bp). lane M: DM 2000 DNA ladder (CWBiotech). Lane 1: Negative control, Lane 2: Positive control using 30pg human gDNA, Lane 3: Single Cell WGA product, Lane 4: single cell WGA product (repeat).

Reference

1 C. Zong*, S. Lu*, A.R. Chapman*, X.S. Xie. Genome-Wide Detection of Single Nucleotide and Copy Number Variations of a Single Human Cell. ***Science.*** 338, 1622-1626 (2012)

2 S. Lu*, C. Zong*, W.Fan*, M. Yang*, et al., Probing Meiotic Recombination and Aneuploidy of Single Sperm Cells by Whole Genome Sequencing using MALBAC. ***Science .***338,1627-1630 (2012)

Troubleshooting Guide

No amplified product	
Sample loss during cell collection	Redo the cell collection process, avoid accidentally removal of the genetic material
Polymerase inhibitors	Polymerase inhibitors carried over from the starting material can cause failure of the WGA reaction, please refer to page 7 for pre-treatment of samples
Inactive Enzyme	All components should be stored at -20°C. All Enzyme Mix tubes should be transfered to ice just before use

Low amplification yield	
Sample contains polymerase inhibitors	Wash the sample before cell capture, please refer to page 7 for pre-treatment of samples
Degradation of genome DNA	Avoid inappropriate storage of cells or template preparation processes that potentially degrade DNA

The negative controls produces DNA yields similar to single cell WGA products	
Reagents have been contaminated by external DNA	Keep the kit reagents and the amplified DNA in separate storage space and use dedicated experimental materials for setting up the amplification reaction, please refer to page 8 for recommended lab practices
Work area is contaminated by external DNA	Decontaminate the work space thoroughly by DNA and RNA removing reagents
Control solution is contaminated by external DNA	Use fresh control solution

Technical Assistance

For technical assistance and additional information, please contact Yikon Genomics Co.,Ltd

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