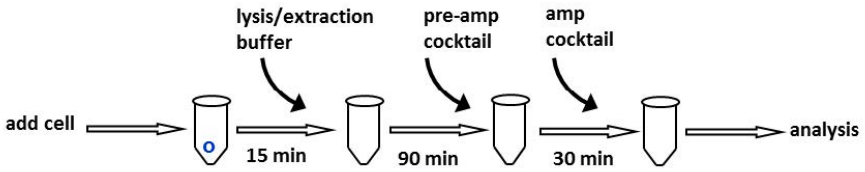
PicoPLEX® WGA Kit Protocol-At-A-Glance

Description

The one-tube, three-step, PicoPLEX WGA Kit was developed specifically for reproducible amplification of single human cells and picogram quantities of DNA. The cell lysis and thermal cycling library synthesis is followed by very low background amplification to yield 2–5 µg of product in under 3 hours:



Suitable applications for the PicoPLEX WGA Kit include:

- Copy number variation (CNV) analysis using oligonucleotide aCGH, BAC aCGH, or qPCR
- SNP genotyping
- Mutation detection

For more information, visit
http://www.takarabio.com/US/Products/cDNA_Synthesis_and_Library_Construction/Next_Gen_Sequencing_Kits/DNA-Seq_Kits/Single-Cell_Whole_Genome_Amplification

Storage and Handling

Store the PicoPLEX WGA Kit at –20°C. Transfer Cell Extraction Enzyme, Pre-Amp Enzyme, and Amplification Enzyme tubes to ice just before use. Thaw other components on ice and briefly vortex and quick-spin component tubes prior to use. Reagents should be stored, handled, and reaction setups performed following good laboratory practices for performing PCR.

Kit Contents

Name	Cap Color	Component Volume
Cell Extraction Buffer	Green	250 µl
Extraction Enzyme Dilution Buffer	Violet	240 µl
Cell Extraction Enzyme	Yellow	10 µl
Pre-Amp Buffer	Red	240 µl
Pre-Amp Enzyme	White	10 µl
Amplification Buffer	Orange	1250 µl
Amplification Enzyme	Blue	40 µl
Nuclease-Free Water	Clear	1710 µl

The volumes of components provided are sufficient for the preparation of up to 50 reactions.

Notes Before Starting

Additional materials and equipment needed: Hot-lid PCR thermal cycler (real-time instrument recommended), centrifuge, 96-well nuclease-free thin-wall PCR plates or PCR tubes, PCR plate seals, single-channel and multi-channel pipettes, low-binding filter pipette tips, phosphate-buffered saline (1X PBS free of Mg^{2+}, Ca^{2+}, and BSA), and single-donor reference DNA (positive control).

Starting material requirements:

- 1–10 human cells (e.g., blastomeres, polar bodies, trophoblastic cells, amniocytes, CTCs, cultured cells)
- 1,000–10,000 bacterial cells
- Isolated DNA (15–50 pg of human DNA)
- Sorted chromosomes
- Intact or fragmented, single- or double-stranded DNA
- Maximum sample volume of 5 µl

Cell-collection methods: Flow sorting, dilution, and micromanipulation are collection methods that are compatible with the PicoPLEX WGA Kit. Cell staining may negatively affect kit performance. Formalin fixation must be avoided to achieve optimum results.

Washing cells: Cell washing is strongly recommended to minimize non-cellular DNA contamination of the cell preparation. Mg^{2+}-free, Ca^{2+}-free, BSA-free PBS may be used for washing.

NOTE: Wash buffers containing Mg^{2+}, Ca^{2+}, or BSA must be avoided. **The PBS volume carried over with the cell sample into the protocol cannot exceed 2.5 µl**.

Using amplified control DNA as reference: Control DNA samples are useful references for some analytical platforms such as microarrays and qPCR. For the most accurate results, we strongly recommend that amplified samples are compared to amplified control DNA rather than un-amplified control DNA.

Control DNA samples must be prepared according to the sample preparation methods indicated below and amplified using the procedure specified in the protocol (next page). Best results will be obtained by pooling the products of multiple corresponding control DNA amplification reactions.

Purifying and quantifying WGA products: Many applications require purifying and quantifying WGA products before use. WGA products can be purified with spin columns or filter plates.

Quantify purified products by UV absorbance (OD_{260}=50 µg/ml). PicoGreen or other double-strand-specific measurements will not give reliable WGA product concentrations.

Store the purified WGA product at –20ºC.

Selecting appropriate reaction tubes/plates: Considerable (>5 µl) evaporation may occur during Step 6 of the protocol if the incubation is being performed in a PCR tube or plate without a tight seal, and such evaporation may reduce the robustness and reproducibility of the reaction.

Performing a mock incubation for Step 6 of the protocol using 15 µl of water is advised to confirm whether a selected tube or plate/seal combination can be used with minimal volume loss due to evaporation.

Sample preparation methods:

5-µl cell sample

1. Wash or dilute cells with PBS buffer according to the instructions provided above.
2. If collecting cells by flow sorting:
   Collect a single cell into 5 µl of Cell Extraction Buffer (green cap) in a PCR tube or well.

   If collecting cells by micromanipulation or dilution:
   Transfer a single cell in a minimal volume of PBS (<2.5 µl) to a PCR tube or well containing an appropriate volume of Cell Extraction Buffer (green cap) to achieve a total cell-sample volume of 5 µl.
3. Immediately freeze and store cells at –80ºC or proceed directly to the protocol (next section).

5-µl DNA sample

1. Prepare a 1 ng/µl purified DNA solution in a PCR tube or well by diluting a control DNA stock with 5 mM Tris-HCl (pH 8.0).
2. Vortex the 1 ng/µl DNA solution for 30 seconds.
3. Add 3 µl of the 1 ng/µl DNA solution to 197 µl of 5 mM Tris-HCl (pH 8.0) to prepare a 15 pg/µl DNA solution.
4. Vortex the 15 pg/µl DNA solution for 30 seconds.
5. Add 1 µl of the 15 pg/µl DNA solution to 4 µl of Cell Extraction Buffer (green cap) in a PCR tube or well.

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PicoPLEX WGA Protocol

1. Prepare the Cell Extraction Master Mix as indicated below for the chosen number of reactions plus 5% extra. Mix gently several times.

4.8 µl	Extraction Enzyme Dilution Buffer (violet cap)
0.2 µl	Cell Extraction Enzyme (yellow cap)
5 µl	Total volume per reaction

2. Add 5 µl of freshly prepared Cell Extraction Master Mix to each 5 µl of cell sample or DNA sample.
3. Place the plate or tube(s) in a thermal cycler and run the following program:

75°C	10 min
95°C	4 min
22°C	forever

4. Once the program is complete (after the cycler reaches 22ºC), remove the plate or tube(s) and centrifuge briefly.
5. Prepare a Pre-Amplification Master Mix on ice as indicated below for the chosen number of reactions plus 5% extra, mix gently several times, and keep on ice until used.

4.8 µl	Pre-Amp Buffer (red cap)
0.2 µl	Pre-Amp Enzyme (white cap)
5 µl	Total volume per reaction

6. Remove the seal on the plate or open the tube(s); add 5 µl of the Pre-Amplification Master Mix to each well.
7. Return the plate or tube(s) to the thermal cycler and run the following program:

95°C	2 min
12 cycles:	
95°C	15 sec
15°C	50 sec
25°C	40 sec
35°C	30 sec
65°C	40 sec
75°C	40 sec
4°C	forever

8. Remove the plate or tube(s) from the thermal cycler and centrifuge briefly; place the samples on ice.
9. Prepare Amplification Master Mix as indicated below for the chosen number of reactions plus 5% extra, mix gently several times, and keep on ice until used.

25 µl	Amplification Buffer (orange cap)
0.8 µl	Amplification Enzyme (blue cap)
34.2 µl	Nuclease-Free Water (clear cap)
60 µl	Total volume per reaction

NOTE: Sample amplification efficiency may be analyzed using a real-time thermal cycler by adding SYBR Green I dye (Thermo Fisher Scientific, Cat. #S7563) at 0.125X final concentration in the Amplification Cocktail (see Appendix A, below). Some instruments require additional dyes for signal normalization.

10. Remove the seal on the plate or open the tube(s); add 60 µl of the freshly prepared Amplification Master Mix to each well.
11. Place the plate or tube(s) in a thermal cycler and run the following program:

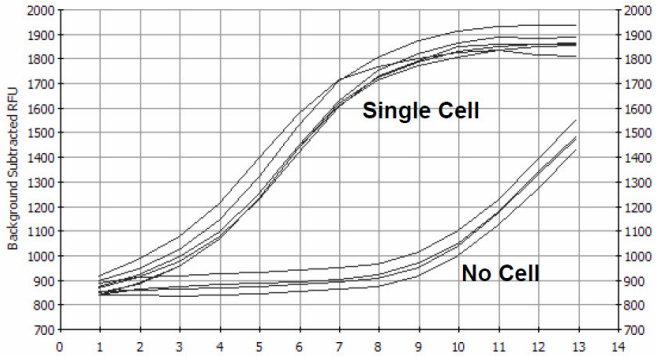
95°C	2 min
14 cycles*:	
95°C	15 sec
65°C	1 min
75°C	1 min
4°C	forever

*14 cycles is recommended based on testing performed with flow-sorted cultured cells. Some cell types may require additional cycles (up to 16) to obtain maximal results.

12. Immediately store the amplified WGA product at –20ºC or purify as described above.

Appendix A: Analyzing Amplification Efficiency

Sample amplification efficiencies can be analyzed by performing the amplification reactions with SYBR Green I in a real-time thermal cycler. During the amplification reaction, double-stranded amplified molecules are bound by the non-sequence-dependent SYBR Green I dye, and the accumulation of amplified product is detected as an increase in fluorescence by the real-time instrument. Data analysis should be performed on raw background-subtracted (not baseline cycle normalized) fluorescence, and the instrument/software should be set to the appropriate mode.



Example of background-subtracted RFU amplification curves for replicate single-cell and no-cell control WGA reactions that were monitored on a Bio-Rad I-Cycler iQ.

Amplification curves will have a similar appearance for all single-cell WGA reactions, with an immediate 8–9 cycle upward sloping phase, followed by a relatively flat "plateau" phase as shown in the example above. No-cell control amplification curves are delayed (right-shift) by at least 5 PCR cycles compared to single-cell amplification curves. A smaller delay of control curves may indicate DNA contamination introduced with the sample or during the WGA process.

Appendix B: Troubleshooting Guide

Problem	Potential Cause	Suggested Solutions
Single-cell amplification curve looks like no-cell control amplification curve or does not produce amplified product	Sample tube or well did not contain a cell	Confirm that cell collection method reproducibly results in single cell per tube or well
	Improper sample preparation	Follow instructions above
	Improper purification or quantification	Follow instructions above
Single-cell amplification curve reaches "plateau" phase earlier than 15-pg control DNA reaction	Greater than one cell in sample	Confirm that cell-collection method reproducibly results in single cell per tube or well
	Single cell sample is contaminated with extraneous DNA	Use fresh, BSA-free PBS
No-cell control amplification curve appears early or produces yield similar to single-cell reaction products	Control solution is contaminated with DNA	Use fresh control solution
	Work area is contaminated with DNA	Clean area thoroughly and use PCR-dedicated plastics and pipettes
	Kit has become contaminated with DNA	Use fresh kit

This document has been reviewed and approved by the Quality Department.

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