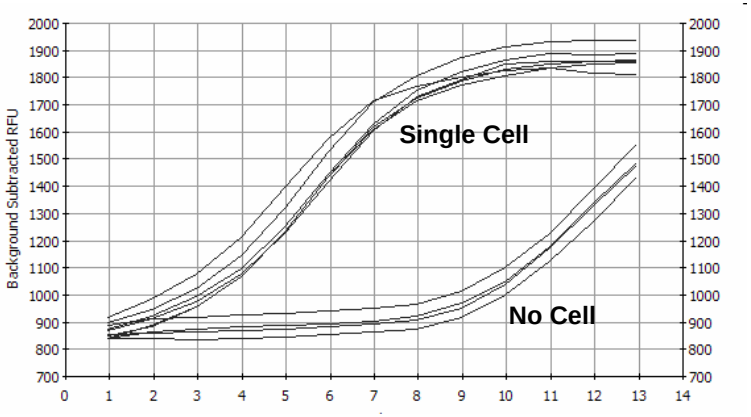
Appendix A: Analyzing Amplification Efficiency

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 control no-cell WGA reactions that were monitored on a Bio-Rad I-Cycler iQ.

Amplification curves will have a similar appearance for all single-cell PicoPLEX 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 control no-cell 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 in (Section F)
	Improper purification or quantification	Follow instructions in (Section H)
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
Control no-cell 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

Notice to Purchaser

PicoPLEX WGA Kit is to be used for research purposes only. It may not be used for any other purpose, including but not limited to, use in diagnostics, forensics, therapeutics, or in humans. PicoPLEX WGA Kit may not be transferred to third parties, resold, modified for resale, used to manufacture commercial products or to provide a service to third parties without written approval of Rubicon Genomics, Inc. PicoPLEX WGA Kit is protected by US Patent 8,206,913; and pending

For information and technical support, contact: support@rubicongenomics.com, 734-677-4845

(MG00181, QAM-105-007)

RUBICON GENOMICS

Product Number: R30050
Amount: 50 reactions
Storage: -20 °C
FOR RESEARCH USE ONLY

PicoPLEX™ WGA Kit

For Single-Cell Whole Genome Amplification

A. Kit Components

Component Name	Part Number	Cap Color
Cell Extraction Buffer	BF00051	Green
Extraction Enzyme Dilution Buffer	BF00052	Violet
Cell Extraction Enzyme	EN00053	Yellow
Pre-Amp Buffer	BF00054	Red
Pre-Amp Enzyme	EN00055	White
Amplification Buffer	BF00056	Orange
Amplification Enzyme	EN00057	Blue
Nuclease-Free Water	BF00058	Clear
User Manual	MG00181	

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

B. Storage and Handling

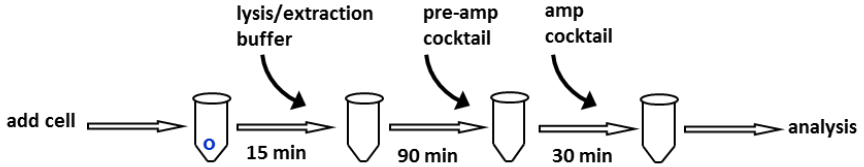
Store the PicoPLEX WGA Kit at -20 °C. Transfer Cell Extraction Enzyme, Pre-Amp Enzyme, and Amplification Enzyme tubes to ice just before use. Thaw other components on ice and briefly vortex and quick-spin component tubes prior to use. Reagents should be stored, handled, and reaction setups performed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

C. Additional Required Materials

- Thermal cycler (Real-time instrument recommended)
- PCR tubes or 96-well PCR plate (see Section I)
- PCR plate seals (see Section I)
- Low-binding barrier tips
- PBS Buffer (see Section F)

D. Product Description

The one tube, 3-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 micrograms of product in under 3 hours.



Sample 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 pg - 50 pg of human DNA)
- Sorted Chromosomes
- Intact or fragmented, single- or double-stranded DNA
- Maximum Sample Volume of 5 µL

E. Applications for PicoPLEX WGA Products

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

Rubicon Genomics, Inc., 4743 Venture Drive, Ann Arbor, MI 48108 USA
www.rubicongenomics.com

F. Collecting Cells for PicoPLEX WGA

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, and 1x PBS prepared from the following stock solutions is recommended:
> 10x PBS (Affymetrix, Inc, Catalog# 75889)
> 20x PBS (Cell Signaling Technology, Inc., Catalog# 9808)

Wash buffers containing Mg^{2+}, Ca^{2+}, or BSA must be avoided. The PBS volume carried over with the cell sample into the PicoPLEX Protocol cannot exceed 2.5 µL.

G. 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, Rubicon strongly recommends that PicoPLEX-amplified samples are compared to PicoPLEX-amplified control DNA rather than un-amplified control DNA.

Control DNA samples must be prepared according to Sample Preparation Methods (Section J) and amplified using the procedure specified in the PicoPLEX protocol (Section K). Best results will be obtained by pooling the products of multiple corresponding control DNA amplification reactions.

H. Purifying and Quantifying PicoPLEX Products

Many applications require purifying and quantifying WGA products before use. PicoPLEX products can be purified with spin columns or filter plates. Rubicon has validated the following purification systems:
> DNA Clean & Concentrator™-5 Kit (Zymo Research, Catalog# D4014)
> MultiScreen PCR_{96} plate (Millipore, Catalog# MSNU03050)
> QIAquick PCR Purification Kit (Qiagen, Catalog# 28104)

Quantify purified products by UV absorbance (1 OD260 = 50 µg/mL). PicoGreen™ or other double-strand specific measurements will not give reliable PicoPLEX product concentrations.

Store the purified, amplified PicoPLEX product at -20 °C.

I. Selecting Appropriate Reaction Tubes/Plates

Considerable (>5 µL) evaporation may occur during PicoPLEX Protocol Step 6 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 PicoPLEX WGA.

A mock PicoPLEX Protocol Step 6 incubation 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.

J. Sample Preparation Methods

5 µL Cell Sample

1. **Wash or dilute cells with PBS buffer, according to instructions in Section F.**
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 minimal PBS volume (<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 -80 °C or proceed directly to the PicoPLEX Protocol (Section K).**

5 µL DNA Sample (single cell simulation)

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.**

K. PicoPLEX Protocol

1. **Combine Extraction Cocktail components and mix well.**

Extraction Cocktail Component	Cap Color	Volume Per 5 samples
Extraction Enzyme Dilution Buffer	Violet	24 µL
Cell Extraction Enzyme	Yellow	1 µL
Total Volume		**25 µL**

2. **Add 5 µL of freshly-prepared Extraction Cocktail to each 5 µL Cell Sample or DNA Sample prepared in Section J.**
3. **Incubate sample in a thermal cycler as follows:**

1 cycle	**75 °C**	**10 min**
1 cycle	**95 °C**	**4 min**
1 cycle	**Room Temp**	**Hold**

4. **Briefly centrifuge the tube or plate.**
5. **Combine Pre-Amp Cocktail components and mix well by vortex.**

Pre-Amp Cocktail Component	Cap Color	Volume Per 5 samples
Pre-Amp Buffer	Red	24 µL
Pre-Amp Enzyme	White	1 µL
Total Volume		**25 µL**

6. **Add 5 µL of Pre-Amp Cocktail to each cell or DNA sample.**
7. **Incubate sample according to thermal cycler program below:**

1 cycle	**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**
1 cycle	**4 °C**	**hold**

8. **Briefly centrifuge sample and place Pre-Amp incubation product on ice.**
9. **Combine the following Amplification Cocktail components and mix well.**

Amplification Cocktail Component	Cap Color	Volume Per 5 samples
Amplification Buffer	Orange	125 µL
Amplification Enzyme	Blue	4 µL
Nuclease-Free Water	Clear	171 µL
Total Volume		**300 µL**

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

10. **Mix 60 µL of the freshly prepared Amplification Cocktail with the 15 µL Pre-Amp incubation product and mix by pipet.**
11. **Amplify sample according to thermal cycler program below:**

1 cycle	**95 °C**	**2 min**
14 cycles	**95 °C**	**15 sec**
	65 °C	**1 min**
	75 °C	**1 min**
1 cycle	**4 °C**	**hold**

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

12. **Immediately store the amplified PicoPLEX product at -20 °C or purify (see Section H)**