PicoPLEX™ WGA Kit FAQs

Top 10 FAQs

1. What applications are recommended for PicoPLEX™ WGA Kit?

This kit is used for:

- Copy number variation (CNV) analysis: superior for oligonucleotide and BAC aCGH
- SNP genotyping
- Mutation detection by PCR

2. Is there a non-stick wash buffer that can be used for single cell wash prior to using PicoPLEX WGA Kit?

This has not been validated at Rubicon Genomics, but some customers have successfully used PVA (Polyvinyl Alcohol; e.g. 0.1%) in PBS to wash/collect their cells. Additionally, your washing/collecting buffer (PBS) must be also Ca- and Mg-free.

3. Are there special requirements for flow sorting?

Yes, we strongly recommend not fixing, and using light scattering or phase contrast to sort or collect samples. Microscopic/visual confirmation of successfully sorted cells can be used to optimize sorting conditions.

4. What is the sample input volume for PicoPLEX WGA Kit?

This kit will accommodate a 5 µl sample input volume per reaction (with a maximum of 2.5 µl of cell & buffer carryover).

5. Can the product of PicoPLEX WGA Kit be taken straight to NGS?

No.

6. Does Rubicon Genomics have an NGS-ready kit for single cell applications?

No. Although, there is currently a kit in development, please inquiry about the availability.

7. Sigma-Aldrich sells a GenomePlex WGA kit, is it the same technology as PicoPLEX WGA?

No.

8. What is the expected genomic locus representation and Loss of Heterozygosity (LOH) for PicoPLEX WGA products?

For reference purposes, see the data obtained from a third party in the table below.

SNP Genotyping Method	Single-Cell Amplification Success Rate	SNP Call Rates	LOH
PCR	95%	> 95%	< 10%
Illumina Infinium SNP array	95%	50% - 60%	7% - 12%

9. Is the product of this technology single- or double-stranded?

The end product of PicoPLEX WGA is a mixture of single- and double-stranded DNA.

10. How rapid is PicoPLEX WGA Kit?

PicoPLEX WGA Kit lyses cells and amplifies the DNA to an end-point of $2-5$ µg in < 3 hours.

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Additional FAQ's

General Requirements

What are the sample requirements for the PicoPLEX WGA Kit?

- Samples that can be used with this kit include:
- Single- or double-stranded DNA
- Intact or fragmented
- Input size over 500 bases
- Input of 1 - 10 human cells or 1,000 - 10,000 bacterial cells
- Isolated DNA (15 pg - 50 pg of human DNA)
- Higher than 50 pg inputs are possible, but require cycle optimization at Steps 6 & 10
- Sorted chromosomes

What cell types have been successfully amplified by PicoPLEX WGA?

Single blastomeres, polar bodies, trophoblasts, amniocytes, and cultured cells have been amplified successfully.

Should cells be washed before collection?

Yes, cells should be washed to minimize extracellular DNA or growth media contaminants. We recommend washing in PBS (Ca-free, Mg-free, and BSA-free) and limiting carryover of wash buffer to less than 2.5 µl.

Is there a non-stick wash buffer that can be used for single cell wash prior to using PicoPLEX WGA Kit?

This has not been validated at Rubicon Genomics, but some customers have successfully used PVA (Polyvinyl Alcohol; e.g. 0.1%) in PBS to wash/collect their cells. Additionally, your washing/collecting buffer (PBS) must be also Ca- and Mg-free.

Which cell collection methods are compatible with PicoPLEX WGA?

Flow sorting, dilution, and micromanipulation are compatible with PicoPLEX WGA.

Are there special requirements for flow sorting?

Yes, we strongly recommend not fixing, and using light scattering or phase contrast to sort or collect samples. Microscopic/visual confirmation of successfully sorted cells can be used to optimize sorting conditions.

What is the sample input volume for PicoPLEX WGA Kit?

This kit will accommodate a 5 µl sample input volume per reaction (with a maximum of 2.5 µl of cell & buffer carryover).

What are the storage conditions for this kit?

PicoPLEX WGA kit should be stored at -20°C.

What is the shelf life for this kit?

This kit has a shelf life of 1 year from the date of the manufacturing.

Overall Scope of the Technology

What are the strengths of this technology?

The greatest PicoPLEX advantage over the other WGA technologies is the reproducibility obtained from cell to cell, while using single cells. This technology also yields a very low background.

Sigma–Aldrich sells a GenomePlex WGA kit, is it the same technology as PicoPLEX WGA?

No.

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How rapid is PicoPLEX WGA Kit?

PicoPLEX WGA lyses cells and amplifies the DNA to an end-point of $2 - 5$ μg in < 3 hours.

How robust is the PicoPLEX WGA process?

PicoPLEX WGA has about 90% amplification success rate with flow-sorted tissue culture cells, limited by the uncertainties of sorting rather than amplification. Single cells always give a high, reproducible yield of amplified genomic DNA. Buffer controls should always give virtually no background.

Does PicoPLEX WGA reproducibly amplify genomic loci?

Yes, with about 90% correlation coefficient for qPCR Ct data from replicate single-cell reactions.

What is the expected genomic locus representation and Loss of Heterozygosity (LOH) for PicoPLEX WGA products?

For reference purposes, see the data obtained from a third party in the table below.

SNP Genotyping Method	Single-Cell Amplification Success Rate	SNP Call Rates	LOH
PCR	95%	> 95%	< 10%
Illumina Infinium SNP array	95%	50% - 60%	7% - 12%

Can buffer control reactions be distinguished from single cell reactions?

Yes, PicoPLEX WGA amplifies with single-copy sensitivity and high specificity.

Is the product of this technology single- or double-stranded?

The end product of PicoPLEX WGA is a mixture of single- and double-stranded DNA.

Applications for PicoPLEX WGA

What applications are recommended for PicoPLEX WGA Kit?

This kit is used for:

- Copy number variation (CNV) analysis: superior for oligonucleotide and BAC aCGH
- SNP genotyping
- Mutation detection by PCR

Can PicoPLEX WGA be used for SNP genotyping?

Yes, PicoPLEX WGA is well suited for SNP genotyping, because the same loci are reproducibly amplified between different cells, and SNPs contained within well-represented regions can be accurately detected without stochastic dropouts and noise associated with other WGA methods.

Can PicoPLEX WGA be used for gel-based STR genotyping?

PicoPLEX WGA has not been optimized for gel-based STR genotyping.

Can PicoPLEX WGA be used for probe-based qPCR, and sequencing assays?

Yes, as long as the amplimers are less than 250 bp.

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How do I obtain successful BAC and oligonucleotide arrays?

We strongly recommend amplifying both the experimental and the reference DNA using PicoPLEX WGA, to normalize any locus-specific bias introduced by PicoPLEX.

How can I label PicoPLEX WGA products for microarrays?

Random-prime labeling and chemical labeling.

How much amplified DNA do I need for array and qPCR assays?

For microarrays, we recommend starting with the amount recommended in the instructions from the assay manufacturer, and titrating if necessary to improve results even further. For PCR assays, we recommend using 5 - 200 ng of amplified PicoPLEX WGA library.

PicoPLEX WGA and NGS

Does Rubicon Genomics have an NGS-ready kit for single cell applications?

No. Although, there is currently a kit in development, please inquiry about the availability.

Can the product of PicoPLEX WGA Kit be taken straight to NGS?

No.

What changes need to be made to my PicoPLEX WGA libraries to make them NGS-ready?

Rubicon Genomics does not support this application, but for references please check the following: What DNA fragmentation size do you recommend?

Yang et al. (2011) PNAS **108**(1):12-17	NGS analysis of PicoPLEX WGA-amplified single-copy sorted chromosomes to infer the haplotype of individual human chromosomes. Detailed protocol provided in the method section.
Leung et al. (2012) *PNAS* **109**(20):7665-7670	NGS analysis of single bacteria cells using PicoPLEX WGA amplification on microfluidic device. Results provided with no method revealed.

What NGS application(s) can PicoPLEX WGA be used for?

PicoPLEX WGA would work best for copy number variation analysis.

Note:

If the above FAQs did not address your specific question(s) please email us at support@rubicongenomics.com or call at **734-677-4845.**

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