PicoPLEX™ DNA-seq Kit FAQs

Top 10 FAQs

1. What type of samples may be used with PicoPLEX™ DNA-seq Kit?

Samples that may be used with this kit include:

- Input of 1 - 10 mammalian 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

2. How rapid is PicoPLEX DNA-seq Kit?

PicoPLEX DNA-seq Kit lyses cells and prepares appropriately barcoded NGS ready library in < 3 hours.

3. What cell types have been successfully used by PicoPLEX DNA-seq?

Single blastomeres, trophectoderm cells, and cultured cells have been used successfully.

4. Which cell collection methods are compatible with PicoPLEX DNA-seq?

Flow sorting, dilution, and micromanipulation are compatible with PicoPLEX DNA-seq.

5. 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.

6. What is the sample input volume for PicoPLEX DNA-seq Kit?

This kit will accommodate a 5 µL sample input volume per reaction (with a maximum of 2.5 µL of cell & buffer carryover). If the cells are in 1x PBS, do not exceed 2.5 µL of PBS.

7. 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.

8. Can cell stained with surface antibodies be used?

Yes, surface antibody stained cells can be used if the cells are not fixed.

9. Do we have to separately purchase barcoded oligonucleotides?

No, single use barcoded oligonucleotides (in a 96 well format) are included with the kit.

10. How many bases should I trim for the analysis of the sequencing reads?

The first 11 cycles of each read will contain quasi-random bases introduced during the PicoPLEX DNA-seq library preparation. For sequence alignment, either trim the initial 14 bases from each read or begin calibration and data collection at base position 15.

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Additional FAQ's

Recommended PicoPLEX DNA-seq Applications

What type of samples may be used with PicoPLEX DNA-seq Kit?

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What applications are recommended for PicoPLEX DNA-seq Kit?

PicoPLEX™ DNA-seq Kit is used to amplify genomic DNA from single cells to reliably detect chromosomal aneuploidies, and copy number variations (CNV) by Next Generation Sequencing (NGS) on Illumina® platforms. Applications include pre-implantation genetic screening (PGS) using blastomeres and trophectoderm cells and analysis of chromosomal abnormalities in Circulating Tumor Cells (CTCs) in cancer research.

Can this PicoPLEX DNA-seq kit be used with Ion Torrent or Pac Bio platforms?

No, it is not compatible with Ion Torrent, or Pac Bio.

Can I prepare samples for both single- and paired end NGS sequencing?

Yes, Libraries prepared using PicoPLEX DNA-seq are compatible with both single end and paired end sequencing on Illumina platforms.

What are the strengths of this technology?

PicoPLEX DNA-seq offers robust and reproducible amplification of DNA from single cells to prepare NGS ready libraries for limited coverage sequencing analysis to study chromosomal anomalies such as Copy Number Variations (CNV).

- Based on this tendency of clustering process, it is important to appropriately adjust the DNA concentration used for clustering to achieve optimal cluster density on Illumina flow cell.
- With the libraries made from a single cell using PicoPLEX DNA-seq, typically a good starting point is to load 16pM considering the library size as 300 bp for calculation purposes, for MiSeq, v3.
- It is very important to add at least 5% PhiX DNA to the library prior to loading on the flow cell to achieve optimal diversity.

General & Sample Preparation Questions

How rapid is PicoPLEX DNA-seq Kit?

PicoPLEX DNA-seq Kit lyses cells and prepares appropriately barcoded NGS ready library in < 3 hours.

What cell types have been successfully used by PicoPLEX DNA-seq?

Single blastomeres, trophectoderm cells, and cultured cells have been used successfully.

Which cell collection methods are compatible with PicoPLEX DNA-seq?

Flow sorting, dilution, and micromanipulation are compatible with PicoPLEX DNA-seq.

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.

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What is the sample input volume for PicoPLEX DNA-seq Kit?

This kit will accommodate a 5 µL sample input volume per reaction (with a maximum of 2.5 µL of cell & buffer carryover). If the cells are in 1x PBS, do not exceed 2.5 µL of PBS.

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.

Can cell stained with surface antibodies be used?

Yes, surface antibody stained cells can be used if the cells are not fixed.

Do we have to separately purchase barcoded oligonucleotides?

No, single use barcoded oligonucleotides (in a 96 well format) are included with the kit.

Can we use barcodes designed in our laboratory?

No, these barcodes cannot be substituted with barcodes from other sources.

What is the size range of fragments expected with libraries prepared using PicoPLEX DNA-seq kit?

Libraries prepared using PicoPLEX DNA-seq kit results in a broad size range distribution of fragments, typically ranging from ~300 to 1000 bp total size (~200 to 900 bp insert size).

What are the storage conditions for this kit?

PicoPLEX DNA-seq 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.

PicoPLEX DNA-seq Protocol

Can PicoPLEX DNA-seq run on any thermal cycler?

- Use a thermal cycler that can accommodate 50-µL reaction volumes and equipped with a heated lid.
- Set the temperature of the heated lid to 100 - 105°C to avoid sample evaporation during incubation and cycling.

Can PicoPLEX DNA-seq kit be used without a real time PCR machine?

- A real time PCR cycler is used to monitor Amplification by adding of fluorescent dye (not provided with the kit) to the reaction.
- If a regular thermal cycler is used instead, there is no need to add the dye, substitute with appropriate amount of nuclease free water to adjust the volumes while preparing the Amplification reaction Master Mix.
- In the absence of real time cycler, use a regular thermal cycler, and library amplification can be analyzed by gel or by Bioanalyzer analysis using an aliquot of the library.

What is the recommended fluorescent dye to monitor Amplification using a real time PCR cycler?

- EvaGreen dye (Cat#31000-T, 20X EvaGreen dye in water, Biotium Inc.,) is recommended, due to its high sensitivity and low interference with amplification chemistry.
- Depending on the real time instrument used, a calibration dye may also be needed. Please refer to your Real Time PCR Instrument's user manual for additional information.

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How do I quantify the pooled purified library for loading on to Illumina sequencer?

- Quantify PicoPLEX DNA-seq libraries by real-time qPCR using the appropriate instrument-specific Illumina library quantification kit from KAPA Biosystems.
- Pooled purified libraries are diluted 50,000 to 500,000 fold and used as the template for quantification by real time PCR.
- It is recommended to use 300 bp as the size for calculating the library concentration.

What ratio of AMPure XP beads should I use for purifying the library prepared with this kit?

Mix the beads and the sample(s) at a 1:1 ratio, for most NGS based CNV detection purposes.

What is the volume of each barcode pair in the wells?

Each well contains sufficient volume of the dual index combination for a single use and is intended for high throughput applications. This plate is sealed with pierceable sealing foil. Use a multichannel pipette and pierce through the foil to collect required amount of the dual index to assemble the reactions.

NGS Using PicoPLEX DNA-Seq

Can I use this kit for whole genome de novo sequencing from a single cell?

No, PicoPLEX DNA-seq offers robust and reproducible amplification of DNA from single cells for limited coverage sequencing analysis for the detection of Copy Number Variations (CNV) and other chromosomal anomalies. This kit should not be used for high-coverage deep sequencing such as de novo sequencing and/or complete (whole genome) re-sequencing.

Does the PicoPLEX DNA-seq kit use single barcodes or dual barcodes?

PicoPLEX DNA-seq kit uses Illumina dual indexes (8nt barcodes) to avoid potential for sample misidentifications due to barcode switching. The kit comes with a 96-well single use dual index plate containing 48 combinations of (i5 and i7) indexes**.**

What pM amount of the purified library should I load on MiSeq, v3?

- With the libraries made from a single cell using PicoPLEX DNA-seq, typically a good starting point is to load 16pM considering the library size as 300 bp for calculation purposes, for MiSeq, v3.
- It is very important to add at least 5% PhiX DNA to the library prior to loading on the flow cell to achieve optimal diversity.

What size should I use for calculating the pM concentration of the library prepared using PicoPLEX DNA-seq?

It is recommended to use 300 bp as the size for calculating the library concentration.

How many bases should I trim for the analysis of the sequencing reads?

The first 11 cycles of each read will contain quasi-random bases introduced during the PicoPLEX DNA-seq library preparation. For sequence alignment, either trim the initial 14 bases from each read or begin calibration and data collection at base position **15.**

What concentration of PicoPLEX DNA-seq library should be loaded onto the Illumina MiSeq flow cell?

- With the libraries made from a single cell using PicoPLEX DNA-seq, typically a good starting point is to load 16pM considering the library size as 300 bp for calculation purposes, for MiSeq, v3.
- It is very important to add at least 5% PhiX DNA to the library prior to loading on the flow cell to achieve optimal diversity.

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Is it recommended to spike PhiX into the library prior to loading onto the flow cell of Illumina MiSeq?

It is very important to add at least 5% PhiX DNA to the library prior to loading on the flow cell to achieve optimal diversity.

Does PicoPLEX DNA-seq coverage span GC-rich regions?

GC region coverage: >90% of the reads fall between 25-70% GC.

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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