PicoPLEX® DNA-seq Kit Protocol-At-A-Glance

The PicoPLEX DNA-seq Kit (Cat. No. R300381, 48 reactions) is intended for amplifying genomic DNA from single cells to reliably detect chromosomal aneuploidies and copy number variations (CNV) on Illumina® next-generation sequencing (NGS) platforms. Applications include pre-implantation genetic screening (PGS) using blastomeres and trophectoderm cells. Other applications include NGS-based CNV detection in circulating tumor cells (CTCs) where single-cell amplifications are required.

Storage and Handling

Store the kit at –20˚C upon arrival. Prior to use, transfer enzymes to ice and centrifuge briefly. Thaw buffers, vortex briefly, and centrifuge prior to use. Keep all enzymes and buffers on ice until used.

Kit Contents

The PicoPLEX DNA-seq Kit is provided with one Dual Index Plate (DIP) and contains sufficient reagents (see Kit Contents table below) to accommodate 48 reactions at once or up to 4 uses of the plate as described in the PicoPLEX DNA-seq Kit User Manual.

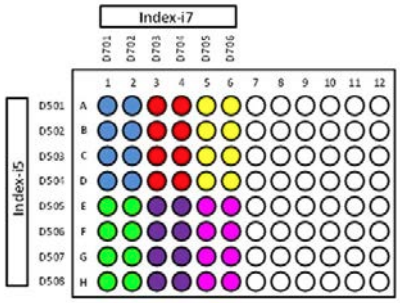
Kit Contents

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	230.4 µl
Pre-Amp Enzyme	White	9.6 µl
Amplification Buffer	Orange	1200 µl
Amplification Enzyme	Blue	24 µl
Nuclease-Free Water	Clear	1176 µl
Dual Index Plate		

PicoPLEX DNA-seq Dual Indexes

i5 Index	Sequence	i7 Index	Sequence
D501	TATAGCCT	D701	ATTACTCG
D502	ATAGAGGC	D702	TCCGGAGA
D503	CCTATCCT	D703	CGCTCATT
D504	GGCTCTGA	D704	GAGATTCC
D505	AGGCGAAG	D705	ATTCAGAA
D506	TAATCTTA	D706	GAATTCGT
D507	CAGGACGT		
D508	GTACTGAC		

Dual Index Plate



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), 80% ethanol (freshly made), single-donor reference DNA (positive control), Agencourt AMPure XP beads, and magnetic separation device.

Starting material requirements:

- Mammalian cells (1–10 cells) or isolated DNA (15–60 pg). Single mammalian cells from sources such as embryos, tumor cells, and cultured clonally expanded cells.
- Single cells obtained by micro-manipulation and flow-sorting as well as flow-sorted cells that are stained with surface antibodies are also suitable. **Avoid cell fixation for optimal results.**

Washing the cells: Wash the cells with sterile, nuclease-free 1X PBS, freshly prepared from a 10X PBS stock. **Carryover PBS volume must not exceed 2.5 µl**.

Selecting PCR plates/tubes: Select plates/tubes that are compatible with the thermal cyclers and/or real-time PCR instruments used. Ensure that there is no evaporation during the process by using proper seals/caps during cycling, as evaporation may reduce robustness and reproducibility.

Positive and negative controls: Including a positive control DNA (15 pg) and a no-template control (NTC) as a negative control in parallel is recommended to ensure that the reaction proceeds as expected.

Preparation of master mixes: Keep all enzymes and buffers on ice. Always prepare a master mix immediately prior to use and keep on ice while pipetting. Assembling the reactions on ice is recommended. We recommend preparing ~5% excess of each master mix to allow for pipetting losses.

Dual Index Plate (DIP): The PicoPLEX DNA-seq Kit is provided with a Dual Index Plate containing 48 Illumina-compatible dual indexes. Each well contains a unique combination of Illumina's eight-nt **TruSeq® HT i5 and i7 index sequences** (see table above, middle). The index combination at each well position is indicated by the column (i7) and row (i5) labels on the plate map (see figure above, right).

- Wells corresponding to columns 7–12 are empty.
- Each well contains a sufficient volume of a dual index combination for a single use. This plate is sealed with pierceable sealing foil. Refer to the plate handling instructions below for more information.

Low-level multiplexing: This kit is designed for high-throughput applications; however, it can also be used for low-level multiplexing of smaller numbers of samples. **The DIP can be frozen and thawed no more than four times.** Select appropriate dual-index combinations that meet Illumina recommended compatibility requirements. The well colors of the plate map (see figure above, right) illustrate one way to pool dual-index combinations for an eight-plex experiment; wells sharing the same color should be pooled together. For other ways to pool a low-plex (two- to 16-plex) experiment, please refer to Illumina's TruSeq Sample Preparation Pooling Guide (Illumina, Part # 15042173 Rev B, 2014).

Dual Index Plate handling instructions: Follow the instructions given below to avoid index cross-contamination.

- Thaw the index plate for 10 min on the benchtop prior to use. Once thawed, briefly centrifuge the plate to collect the contents to the bottom of each well.
- Thoroughly wipe the foil seal with 70% ethanol and allow it to dry completely.
- Pierce the seal above the specific index combination with a 200-µl filtered pipet tip; discard the tip.
- Use a new pipet tip to collect 5 µl of a specific index combination (i5 & i7) and add it to the reaction at the amplification step. If several reactions are being assembled, a multichannel pipette may be used. If indexes from the entire plate are not used at the same time, follow the instructions below to avoid contamination:
  - Cover any pierced index wells with scientific tape (e.g., VWR General Scientific Tape 0.5", Cat. No. 89097-920) to mark the index as used.
  - Once the dual index plate is used, wipe the seal with 70% ethanol and let it dry completely.
  - Replace the plastic lid, return the plate to its sleeve, and store at –20°C.

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Cell Lysis

1. Prepare a PCR plate (or tubes) in which each experimental well contains individual single cells or genomic DNA dilutions in 5 µl of buffer. (**Refer to the user manual for detailed instructions for preparing the single-cell or diluted DNA samples.**) Wells containing 5 µl of NTC negative control buffer sample(s) are also recommended.
2. Depending on the number of reactions, prepare the Cell Extraction Master Mix as described in the table 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

3. To each 5 µl of equilibrated sample from Step 1, add 5 µl of the Enzyme Extraction Master Mix, taking care not to disturb the cell.

NOTE: The final volume at this stage will be 10 µl.

4. Seal the PCR plate using proper sealing film or close the tube(s) tightly and centrifuge briefly to ensure that the entire volume of the reaction is collected at the bottom of each well.
5. Place the plate or tube(s) in a thermal cycler with a heated lid set to 101°C–105°C and perform the Lysis Reaction using the following program:

75°C	10 min
95°C	4 min
22°C	forever

6. At the end of the Lysis Reaction (after the cycler reaches 22ºC), remove the plate or tube(s) and centrifuge briefly. Proceed with Pre-Amplification (below) in the same plate or tube(s).

Pre-Amplification

1. 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

2. Remove the seal on the plate or open the tube(s); add 5 µl of the **Pre-Amplification Master Mix** to each well.

NOTE: The final volume at this stage will be 15 µl.

3. Seal the PCR plate using proper sealing film or tightly cap the tube(s) and centrifuge briefly to ensure that the entire volume of the reaction is collected at the bottom of each well.
4. Return the plate or tube(s) to the thermal cycler with a heated lid set to 101°C–105°C and perform the **Pre-Amplification Reaction** using the following cycling conditions:

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

5. Remove the plate or tube(s) from the thermal cycler and centrifuge briefly; continue with Library Amplification (below) in the same plate or tube(s).

Library Amplification

1. Prepare the 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)
0.5 µl	Amplification Enzyme (blue cap)
2.5 µl	Fluorescent Dyes*
2 µl or 4.5 µl	Nuclease-Free Water (clear cap)**
30 µl	Total volume per reaction

*Fluorescent dyes (for detection and optimal calibration) are added when monitoring the amplification in real time during cycling. Please refer to the real-time PCR instrument's user manual for calibration dye recommendations, and add an appropriate amount of the detection and calibration dye. If a regular thermal cycler is used, there is no need to add the dyes; instead, substitute with an appropriate amount of Nuclease-Free Water to adjust the final volumes.
**The amount of Nuclease-Free Water added per reaction will vary depending on the inclusion of fluorescent dye.

Example: EvaGreen/Fluorescein dye mix. Prepare by mixing a 9:1 v/v ratio of 20X EvaGreen dye (Biotium, Inc., Cat. No. 31000-T, 20X in water) and 1:500 diluted Fluorescein Calibration Dye (Bio-Rad Laboratories, Cat. No. 170-8780); add 2.5 µl of this mix per reaction.

2. Remove the seal on the PCR plate or open the tube(s) and add 30 µl of the **Amplification Master Mix** to each well.
3. Add 5 µl of the appropriate **index combination** from the DIP to each well and mix gently several times with a pipette, avoiding introducing excessive air bubbles. **Follow the DIP handling instructions (bottom of previous page) to avoid index cross-contamination.**

NOTE: The final volume at this stage will be 50 µl.

4. Seal the plate using proper sealing film or tightly cap the tube(s) and centrifuge briefly to ensure that the entire volume of the reaction is collected at the bottom of each well.
5. Return the plate or tube(s) to the thermal cycler with a heated lid set to 101°C–105°C and perform the Amplification Reaction using the following cycling conditions:

95°C	4 min
4 cycles:	
95°C	20 sec
63°C	25 sec
72°C	40 sec
7 cycles*:	
95°C	20 sec
72°C	55 sec
4°C	forever

*Acquire fluorescence data at this step if monitoring amplification in real time.

6. At the end of Library Amplification, remove the PCR plate or tube(s) from the thermal cycler and centrifuge briefly to collect the contents to the bottom of each well. At this stage, samples can be processed for library purification immediately or stored frozen at –20ºC for later processing.
7. Refer to the detailed user manual (available online) for recommendations on library purification, subsequent quantification, and next-generation sequencing (NGS).

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

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