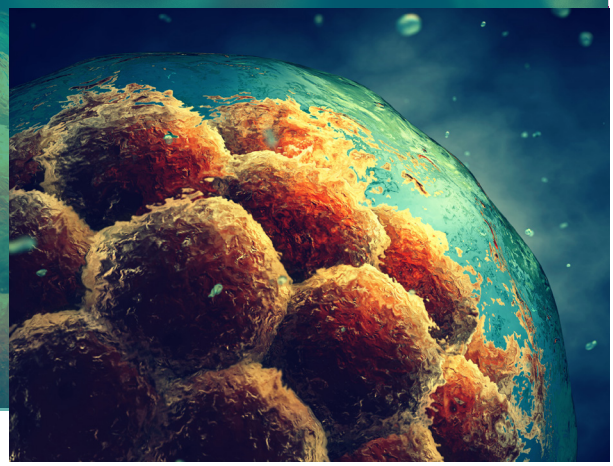
APPLIED GENOMICS

TECHNICAL NOTE

PG-Seq™ Kit 2.0 Validation Guidelines



Summary

Rapid advances in Next Generation Sequencing (NGS) technologies have made them the method of choice for genomic analysis, including in in vitro fertilization (IVF) embryo testing. Screening embryos prior to transfer into the uterus using PGT (preimplantation genetic testing) offers an additional selection tool to assess the chromosomal status of each embryo. Compared with array-based aneuploidy screening methodologies, NGS workflows offer potential improvements in accuracy, sensitivity and resolution for the detection of aneuploidy and copy number variants in embryos. PerkinElmer's PG-Seq™ kit 2.0 has been developed to accurately detect whole chromosome aneuploidy along with structural rearrangements such as unbalanced translocations and segmental errors using an efficient, streamlined workflow.

Here we provide laboratories with a recommended outline for performing an in-house validation of the PG-Seq™ kit 2.0, using a range of different cell types including cell lines, genomic DNA and/or embryo samples.

PG-SEQ™ KIT 2.0 CONTENTS:

Whole Genome Amplification (WGA) Reagents

The DOPlify® whole genome amplification technology was developed in PerkinElmer's laboratory in Adelaide, Australia and has been independently benchmarked as one of the leading WGA systems for accurate aneuploidy and copy number variant calling (Deleye et al, 2017). The WGA kit includes all the necessary reagents for cell lysis and amplification of the genome.

PG-Seq™ Kit 2.0 Library Preparation Reagents

The PG-Seq™ kit 2.0 library preparation technology was created in PerkinElmer's laboratories in Austin, Texas and is optimised for use with our WGA reagents. The protocol streamlines the library preparation procedure by combining DNA fragmentation, end-repair and A-tailing into a single reaction step to reduce both hands-on and overall library preparation time. The kit includes all necessary library preparation reagents, including a 96 well index plate and purification beads.

PG-Find™ Software

Software that is provided as part of the PG-Seq™ kit 2.0 allowing analysis of data from single or multiple cell samples from human embryos. The software allows for accurate detection of whole chromosome aneuploidy, structural rearrangements such as unbalanced translocations and segmental errors along with mosaicism detection in a highly automated software platform.

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Laboratory Set Up

Clean Laboratory/Pre-PCR Laboratory

Cell lysis and master mix set-up for WGA should be performed in a contained and dedicated clean/pre-PCR laboratory equipped with a laminar flow hood or PCR workstation, dedicated pipettes and a PCR thermocycler with a programmable ramp rate. It is imperative to maintain a clean, tidy work space with regular decontamination, limiting possible opportunities for DNA contamination. All PG-Seq™ kit 2.0 WGA reagents should be stored in a -20°C freezer located in the clean laboratory.

General/Post-PCR Laboratory

All PCR amplification and post PCR steps in the process, including agarose gel electrophoresis, handling of amplified DNA and all library preparation steps should be performed away from any WGA master mix set-up in a General/Post-PCR laboratory. PG-Seq™ kit 2.0 library preparation reagents (including adaptor plate) should be stored in a -20°C freezer and purification beads should be stored at 2-8°C.

Validation Study Design

The PG-Seq™ kit 2.0 is supplied in a 1 x 96 reaction kit. Each kit is supplied with the appropriate number of reagents for testing. The Cell Lysis and WGA steps are recommended to be performed in 0.2 or 0.5 mL PCR tubes (supplied by user; PerkinElmer uses Life Technologies Applied Biosystems, N801-0737). A cold block at a temperature of approximately 4°C should be used when preparing all master mixes and for storing samples during PCR and library preparation set up. Single blastomeres, polar bodies, trophoblastic cells, amniocytes, lymphocytes, as well as buccal cells, are suitable for amplification using the PG-Seq™ kit 2.0.

Please refer to the following table below as a rough guideline to follow for sample numbers to process for validation of PG-Seq™ kit 2.0:

	# of Samples/ Run	# of NGS Runs	Reagent kit for MiSeq®
Low throughput	16	2	V2 Micro kit (300 cycle)
Mid throughput	48	2	V3 (150 cycle)
High throughput	96	1	V3 (150 cycle)

The number of samples to process for Validation may depend on local regulatory guidelines, the number of samples available and individual laboratory preference.

Validation Options

It is recommended to utilise validation samples containing a range of different aneuploidies, including:

- Whole chromosome gains (trisomy).
- Whole chromosome losses (monosomies).
- Segmental gains and losses (partial chromosome gains and losses) of at least 5 Mb in size.
- Mosaic samples (recommended to perform dilution of two individual WGA products with different abnormalities together in varying percentages).

Customers have the option to amplify and process all samples in a single batch or split the validation samples into different batches to test reproducibility of the kit.

Validation Option 1: Genomic DNA and/or cultured cell lines

When validating using cell lines, flow sorting, dilution and micromanipulation are all collection methods that are compatible with the PG-Seq™ kit 2.0. When validating with genomic DNA, it is recommended that DNA is diluted to a final concentration of 30 pg/µl in 10mM Tris-HCl (pH 8.0) (no EDTA), with 1 µl of diluted DNA added to the Cell Lysis step.

Validation Option 2: Discarded day 3/5 embryos

Discarded embryos can include untested embryos and/or embryos classified as abnormal from previous biopsy and PGT-A testing that have been consented for use. Depending on the laboratory's PGT policies, either day 3 or day 5 embryos are suitable for PG-Seq™ kit 2.0 validation. Where an embryo has been rebiopsied for the validation study, the results from the PG-Seq™ kit 2.0 study should be compared to the original biopsy result to determine concordance/non-concordance. As mosaicism can clearly impact the concordance of results between biopsy samples from a blastocyst stage embryo, it is recommended to perform a third analysis of the remaining embryo tissue using the PG-Seq™ kit 2.0 if required.

Quality Control

PerkinElmer will support customers during the validation process and help to assess the performance of all recommended QC steps during the validation process to ensure successful completion of workflow procedures.

Please refer to the PG-Seq™ kit 2.0 kit insert for information on quality control parameters.

Result Analysis & Reporting

Once results have been generated, a table similar to the one below should be created (table 1). This chart will describe important test performance rates that each laboratory should understand and consider, including False Positive/ False Negative rates, No Call rate, and specificity/sensitivity.

Table 1. Example of the performance rates

Type of Error	Total
False Positive	# false pos./total
False Negative	# false neg./total
No Call Rate	# no result/# samples
Sensitivity (per embryo/cell)	# true pos./(true pos. + false neg.)
Sensitivity (per chromosome)	# true pos./(true pos. + false neg.)
Specificity (per embryo/cell)	# true neg./(true neg. + false pos.)
Specificity (per chromosome)	# true neg./(true neg. + false pos.)

Support & Training

PG-Seq™ kit 2.0 training and support is available from PerkinElmer. The format, location and duration of training will be dependent on the level of experience and expertise of the customer.

Remote training will involve the use of interactive web-based training sessions including webinars and video conferences held directly with PerkinElmer product specialists to train customers on the workflow. There is no cost for this training.

PerkinElmer onsite training may require the customer to travel to a PerkinElmer laboratory equipped with the necessary materials to train customers on the workflow.

Customer onsite training will involve a PerkinElmer application specialist travelling to the customers laboratory equipped with the necessary materials to train customers on the workflow.

Results Consultation

It is recommended that a period of time should be set aside to compare and/or discuss results from the validation with a PerkinElmer product specialist. Ideally customers will provide the following information for PerkinElmer to confirm successful use of the PG-Seq™ kit 2.0 and software or to assist with diagnosis of any cause of suboptimal kit performance including:

1. LabChip® GX Touch™ nucleic acid analyzer or agarose gel image of DOPlify® WGA products, including negative and positive controls
2. WGA quantification results
3. Library quantification results of each sample after library preparation
4. LabChip® GX Touch™ nucleic acid analyzer or agarose gel image of final libraries
5. Sequencing run metrics (cluster density, cluster PF, Reads, Reads PF, %>Q30) following sequencing
6. Sequencing sample metrics (indexing QC, number of reads)
7. PG-Find™ results vs previous expected result for re-tested samples including output graphs of each analysis method being compared.

PerkinElmer is committed to working closely with its customers to ensure acceptable QC metrics have been successfully achieved and results are satisfactory.

ORDERING INFORMATION

Please contact your local distributor or PerkinElmer representative for ordering information.

For technical support please contact **Support.AU@perkinelmer.com**

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

To learn more, visit www.perkinelmer-appliedgenomics.com

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