Detection of aneuploidies, segmental aneuploidies, and mosaicism using Ion ReproSeq kits

Highlights

- Customizable aneuploidy analysis workflows in Ion Reporter™ Software v5.10 or later can be used to improve automated calling of mosaic and subchromosomal copy number variation (CNV) events
- Mosaic analysis workflows are capable of automatically calling whole-chromosome mosaicism of ≥20%
- Default high-sensitivity workflows can consistently call subchromosomal CNV events of ≥8 Mb, whereas CNV events of 2–8 Mb can be detected using adjustable custom sensitivity parameters for automated calling

Introduction

Mosaicism and segmental CNV events—variables in preimplantation genetic testing for aneuploidies

Preimplantation genetic testing for aneuploidies (PGT-A), previously known as preimplantation genetic screening or PGS, is a method designed to identify embryos with the correct complement of chromosomes, termed euploid. Transfer of euploid embryos is more likely to result in a successful pregnancy than transfer of embryos with the incorrect number of chromosomes, termed aneuploid.

From an embryo biopsy, PGT-A workflows such as with the Ion ReproSeq™ PGS Kits help categorize embryos as either euploid or aneuploid (whole chromosome and subchromosomal or segmental). However, more recently, a third embryo category, mosaic, has been defined for trophectoderm (TE) biopsies from blastocyst embryos. Mosaic embryos are mixtures of euploid and aneuploid cells, and the ratio of euploid to aneuploid cells can vary.

While euploid embryos are the obvious priority for implantation, the improved detection of mosaicism in biopsy samples by next-generation sequencing (NGS) methods has raised active debate and ongoing research into the suitability of mosaic embryos for transfer. Guidelines and recommendations exist [1,2], and a number of factors must be taken into account for embryos in this category, including the level of mosaicism, the chromosome involved, and whether mosaicism comprises the whole chromosome or just a segment.

A further requirement for PGT-A is the detection of unbalanced products from reciprocal translocations. Without prior knowledge, a member of a couple undergoing IVF could be a carrier of a balanced reciprocal translocation in which derivative chromosomes were formed, but the carrier is unaffected due to the maintenance of copy number at the whole-genome level. The impact on the resultant embryos is the potential for segmental unbalanced chromosomal aberrations that can be of various sizes and locations across all chromosomes. As a result, it is important to determine the analysis parameters and resolution limitation of PGT-A workflows to detect putative segmental unbalanced products from translocations and other segmental aneuploidies in preimplantation embryos.

Box 1: Customizable sensitivity and tile size

There are three default analysis workflows in Ion Reporter Software: (1) ReproSeq PGS w1.1, a workflow designed to detect and call CNV events with integer ploidy values (non-mosaic); (2) ReproSeq No Gender PGS w1.1, which is the same as the previous workflow but the gender of the biopsy is hidden from the user, and the ploidy of the sex chromosomes is reported as normal or abnormal; and (3) ReproSeq Mosaic PGS w1.1, a workflow designed to detect and call CNV events with non-integer or decimal ploidy values.

The default aneuploidy workflows can be customized by the user to increase or decrease sensitivity. There are two mechanisms for changing the CNV-calling sensitivity: (1) changing the tile size of the baseline, and (2) adjusting the transition penalty (TP); for example, the TP is –15 for the low-sensitivity setting, –8 (default) for the medium-sensitivity setting, and –3 for the high-sensitivity setting. The TP is an algorithmic deterrent to changing the copy number state for small segments and dictates the likelihood that the algorithm will call a different ploidy state between two adjacent tiles. A TP with lower (more negative) values results in the algorithm calling only larger CNV segments, or only segments with greater support for the changed state (greater difference in copy number).

As a consequence, lower-sensitivity TP values result in fewer false positives, but with the potential for more false negatives, while high-sensitivity TP values result in fewer false negatives, but with the potential for more false positives. A recommended starting value is –2.0 and –2.33 for non-mosaic workflows and mosaic workflows, respectively.

Further improvements in sensitivity to detect small segmental CNV events can be obtained by decreasing the tile size used to detect copy number changes across the genome. This adjustment must be accompanied by the selection or creation of a CNV baseline with the same corresponding smaller tile size. Prebuilt CNV baselines corresponding to smaller tile sizes are included in Ion Reporter™ Software v5.10 or later, including 1.0 Mb and 0.5 Mb tile sizes from the baseline dropdown list. Note that users can also define their own baseline and associated tile size in Ion Reporter Software.

To adequately account for the numerous factors under consideration for mosaicism and subchromosomal CNV event detection, a PGT-A software solution should be able to automatically call segmental aneuploidies and mosaicism while offering user customization to balance the tradeoffs in sensitivity and specificity. Here we describe parameters within the aneuploidy analysis workflows in Ion Reporter Software v5.10 or later that can be adjusted by users for improved mosaic and segmental CNV event calling.

Results

Mosaicism

Two research Coriell cell lines with known karyotypes (46, XY; 47, XX, +21) were combined in five cell number mixtures to mimic a typical biopsy sample, and the ratio between each cell line was used to define different levels of mosaicism in each pool. Selected under a dissection microscope, cells were pooled to create samples with the known mosaic profiles of 0%, 20%, 40%, 60%, 80%, and 100% for chromosome 21 (chr21) as well as the X chromosome (chrX) and the Y chromosome (chrY). Four replicates per combination were generated for a total of 24 samples processed using the Ion ReproSeq PGS Kits and sequenced on the Ion GeneStudio™ S5 System.

To evaluate the sensitivity of whole-chromosome mosaic calling, two transition penalty (TP) settings (–2.33 and –3) were used (see Box 1). The high-sensitivity TP setting (–3) produced the best result, with 94.2% sensitivity* and a positive predictive value (PPV) of 94.2%, with only one replicate not calling the 20% event on chrX. The event was filtered by the expected normal ploidy buffer (ENPB, see Box 2) and two replicates failing to produce an automated call for the 20% chrY mosaic event (Table 1). The predicted mosaic ploidy for the chrX event was below 1.15 (expected was 1.20) and was filtered by the ENPB. Decreasing the ENPB below 0.15 to capture the missed true positive introduced additional false positives.

In contrast to other aneuploidy software solutions that do not automatically call mosaic samples unless the level of mosaicism reaches 50%, the mosaicism aneuploidy workflows in Ion Reporter Software will automatically call any mosaic copy number variant segment with a gain or loss outside of the filter range of the ENPB. Examples of mosaic profiles of 0%, 20%, 40%, 60%, 80%, and 100% for chromosome 21 and the X and Y chromosomes are shown (Figure 1), illustrating how automated calling alleviates the need to manually call mosaic events at a threshold of <50%.

* Sensitivity = True positives/(True positives + False negatives)

Table 1. Whole-chromosome mosaic calling using five cell mixtures from 46, XY and 47, XX, +21 cell lines.*

Total calls	False negative	True positive	False positive	Sensitivity (%)	PPV (%)	False-negative events	False-positive events
52	3	49	3	94.2	94.2	1.8 chrX (n = 1); 0.2 chrY (n = 2)	2.2 chr16 (n = 1); 1.85 chr15 (n = 1); 1.85 chr2 (n = 1)

* Workflow parameters used were: type mosaic; TP –3; baseline tile size 2 Mb; ENPB 0.15.

Box 2: Mosaicism workflows and ENPB

Non-mosaic workflows only call copy number events with integer ploidy states, whereas mosaic workflows enable non-integer or decimal ploidy values with steps of 0.05x. One side effect of this enhanced ploidy calling is an increased frequency of false positives around normal ploidy, or 2N for autosomes and the X chromosome in females, and 1N for the X and Y chromosomes in males. To filter these putative false positives, you can create and customize the ENPB filter chain in Ion Reporter Software v5.10 and add it to any mosaic aneuploidy workflow.

In this study, the ENPB filter was set to filter out all copy number variant segments of gain or loss within 0.15 (15%) ploidy value of the expected normal. With this setting, ploidy changes between 2.15 and 1.85 on autosomes and female X chromosomes are filtered, as are events between 1.15 and 0.85 on male X and Y chromosomes.

Low-pass genome sequencing methods are not intended to detect polyploidies. However, mosaic workflows have the potential to call triploid male biopsies (69, XXY) since chrX and chrY should be called as mosaic. The three copies of autosomes will be normalized to 2N at a ratio of 1.5 (3/2 = 1.5), thus 2N chrX should be called 1.33 (2/1.5 = 1.33) and 1N chrY should be called 0.66 (1/1.5 = 0.66). Shown in Figure 2 is a confirmed 69, XXY, with the chrX called ploidy 1.3 and the chrY called ploidy 0.7.

Subchromosomal CNV

The sensitivity and PPV for different TP and baseline tile size parameters (Box 1) were investigated using a dataset consisting of a range of event sizes, including four whole-chromosome events (45, XX, –21; 47, XY, +18; 48, XXX, +21), 12 segmental CNV events ranging from 11 Mb to 99.1 Mb (Table 2), and two euploid cell lines (46, XY; 46, XX).

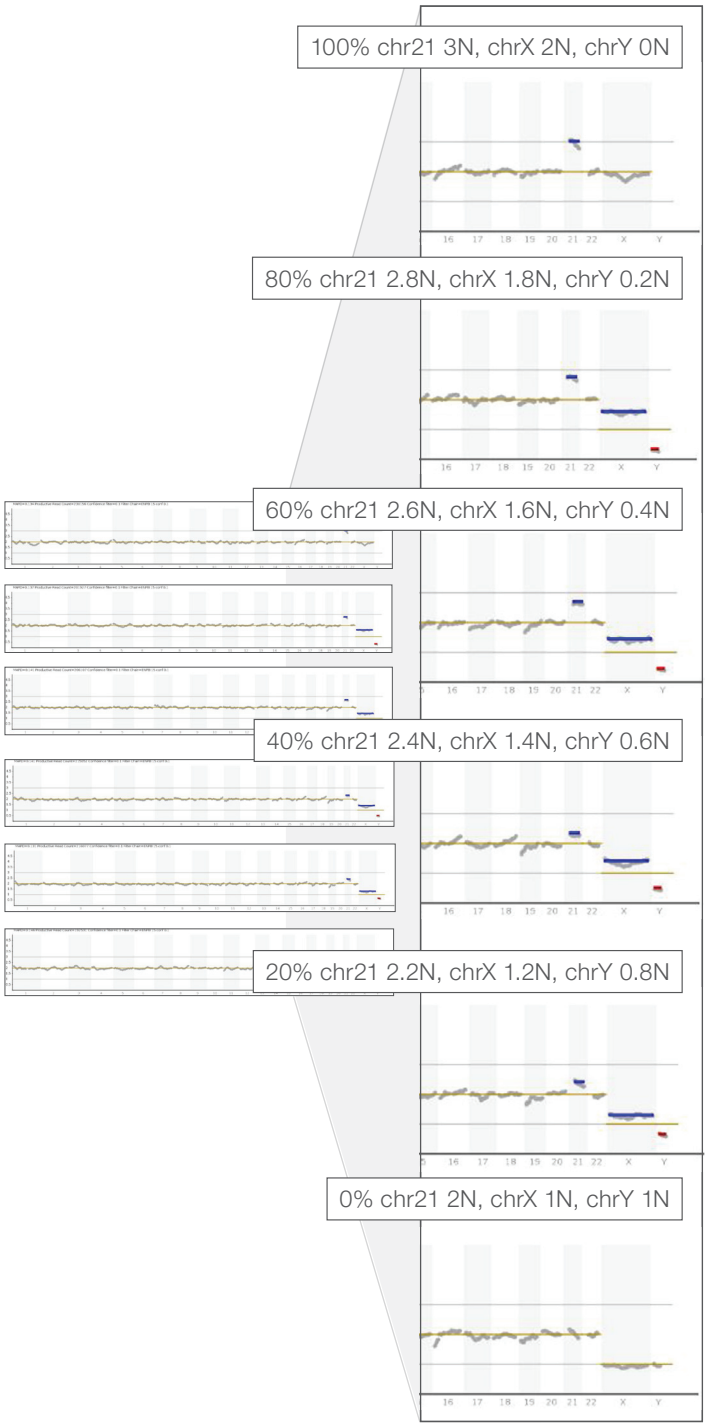

Figure 1. Results from two Coriell cell lines with known karyotypes (46, XY and 47, XX, +21) combined in five cell mixtures to generate mosaic profiles of 100%, 80%, 60%, 40%, 20%, and 0%. The expected non-integer ploidy calls are indicated for chr21 (blue line), chrX (blue line), and chrY (red line) for each mixture. Workflow parameters used were: type mosaic; TP –3; baseline tile size 2 Mb; ENPB 0.15; confidence filter 0.1.

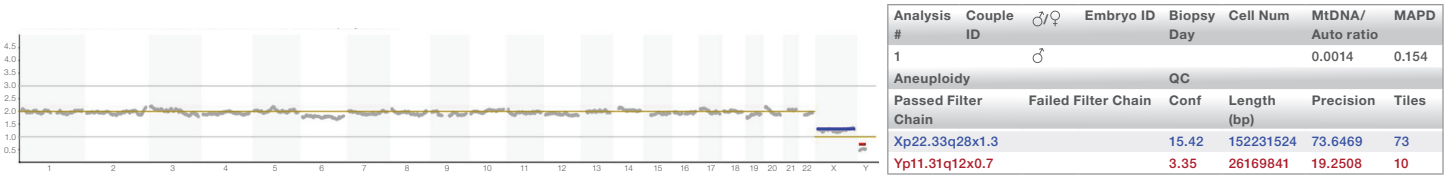

Figure 2. Detection of a confirmed triploidy (69, XXY) from a trophectoderm embryo biopsy. Automated mosaic calling estimated a non-integer ploidy for chrX of 1.3 (blue line) and for chrY of 0.7 (red line). Workflow parameters used were: type mosaic; TP –8 (medium sensitivity); baseline tile size 2 Mb; ENPB 0.2.

Table 2. Results for calling of >10 Mb subchromosomal CNV events* using the default high-sensitivity non-mosaic workflow.

Transition penalty	Baseline tile size (Mb)	Total calls	False negative	True positive	False positive	Sensitivity	PPV
–3	2	34	0	34	0	100%	100%

* Events investigated were: 11 Mb dup(9p); 14.8 Mb dup(22q); 20.97 Mb dup(6p); 26.9 Mb del(4p); 30.1 Mb del(5p); 32 Mb del(5p); 99.1 Mb dup(3q). Four whole-chromosome events (45, XX, –21; 47, XY, +18; 48, XXX, +21) were included as positive controls, and two euploid cell lines (46, XY; 46, XX) were included as negative controls.

Using 4–5 cell-line inputs selected under a microscope, three replicates per cell line were processed using the protocol for the Ion ReproSeq PGS Kits and the Ion GeneStudio S5 System. Detection of subchromosomal CNV events greater than 10 Mb using the default non-mosaic high-sensitivity workflow with 2 Mb baseline tile size called all events with sensitivity and PPV of 100% (Table 2).

To understand how different aneuploidy workflow settings perform across a variety of sample types and a larger size range of subchromosomal imbalances, a meta-analysis was performed reviewing 89 samples (consisting of seven cell-line gDNAs, 15 sorted cell-line cells, 26 trophectoderm embryo biopsies, and 41 reamplified whole-genome amplification (WGA) products from embryo biopsies) collected from five different laboratories. The CNV events ranged in size from 1.19 to 163 Mb, with seven samples <5 Mb, 10 samples 5–10 Mb, 19 samples 11–20 Mb, and 47 samples >20 Mb. Eight samples included whole-chromosome events.

Workflows with a TP of –3 (default high sensitivity) and a baseline tile size of 2 Mb consistently called subchromosomal CNV events to ≥8 Mb across all sample types examined (Table 3). Detection of smaller events between 2 and 8 Mb required adjustments in TP sensitivity and baseline tile size (Table 4).

The automatic calling of subchromosomal imbalances in other aneuploidy software solutions may require that the event size be greater than 50% of the size of the chromosome. With TP and baseline tile size sensitivity adjustments in Ion Reporter aneuploidy workflows, robust calling from trophectoderm embryo biopsies can be automated, as observed by the detection of potential segmental unbalanced chromosomal aberrations resulting from a suspected translocation between chr1 and chr15 (Figure 3).

Standard workflows can assist in the identification of previously unknown parental chromosome abnormalities, in particular those involving large chromosomal segments. Sensitivity improvements, using TP and baseline tile size adjustments, can be used for high-resolution detection of suspected subchromosomal CNV events or in the analysis of biopsies from embryos resulting from known carriers of balanced reciprocal translocations.

Table 3. Results summary by sample type for calling of subchromosomal CNV events using the default non-mosaic high-sensitivity workflow (TP: –3; baseline tile size: 2 Mb).

Sample type	No.	Size range (Mb)	Range called (Mb)
Cell-line gDNA	7	3.7—18.8	3.7—18.8
Sorted cell-line cells	15	1.19—100.4	11.1—100.4
Trophectoderm embryo biopsy	26	7.8—71.2	7.8—71.2
WGA product from an embryo biopsy	41	4.4—163	8—163

Table 4. High-resolution (<5 Mb) subchromosomal event calling using adjustments in TP, baseline tile size, or both.

Event	Sample type	TP	Tile size (Mb)
1.81 Mb del(9)	Sorted cell-line cells	–2	0.5
2.19 Mb dup(6)	Sorted cell-line cells	–2	0.5
2.53 Mb del(5)	Sorted cell-line cells	–3	0.5
3.36 Mb dup(16)	Sorted cell-line cells	–3	0.2
4.4 Mb dup(22)	WGA product from embryo biopsies	–3	0.5
4.5 Mb del(1)	Cell-line gDNA	–3	0.5

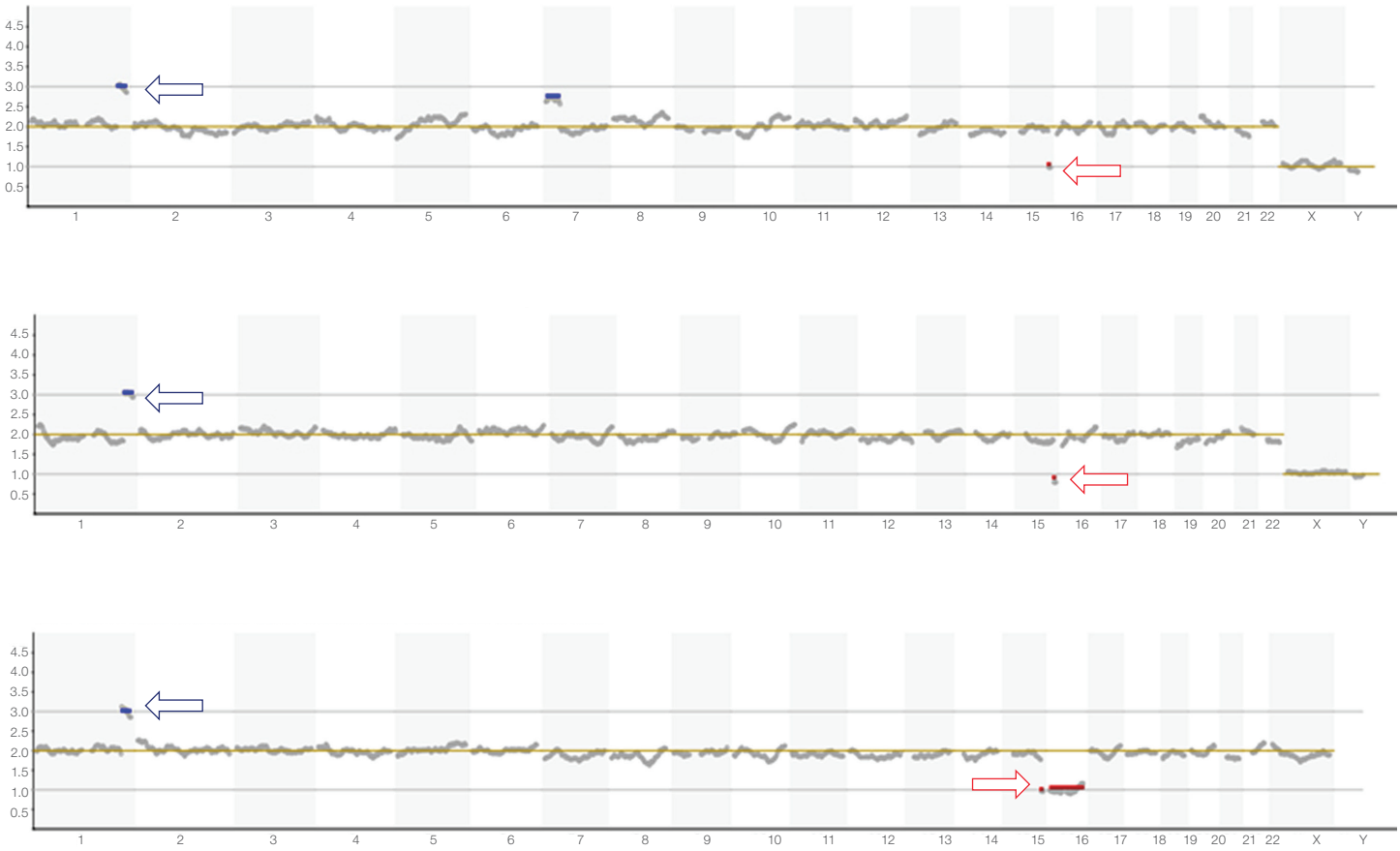

Figure 3. Detection of potential segmental unbalanced chromosomal aberrations from trophectoderm biopsy samples. Aberrations resulted from a suspected translocation between chr1 (blue arrow) and chr15 (red arrow) with predicted event sizes of 25 Mb and 8 Mb, respectively. Workflow parameters used were: type mosaic; TP –3; baseline tile size 2 Mb; ENPB 0.2.

Conclusions

PGT-A software solutions should be able to respond to recent developments in embryo biopsy categorization so that aneuploid and mosaic events will be automatically called. The mosaic workflows in Ion Reporter Software enable calling of non-integer ploidy values that—when used in combination with the ENPB—are capable of the sensitive and specific calling of whole-chromosome mosaicism down to 20% from five cell mixtures that mimic an embryo biopsy.

In addition to mosaicism calling, a PGT-A software should have adjustable algorithm parameters that can be used to increase the sensitivity and accuracy of subchromosomal CNV event calling. Meta-analysis of 89 diverse sample types with subchromosomal CNV events indicate that the default high-sensitivity (TP of –3) aneuploidy workflows (mosaic or non-mosaic) with a baseline tile size of 2 Mb can consistently call CNV events to ≥8 Mb.

Robust detection of smaller events of <8 Mb can require adjustments in sensitivity parameters for automated calling. TP parameter adjustment from –3 to –2 with a smaller baseline tile size (e.g., 0.5 Mb) can be used to automatically call events <8 Mb down to 2 Mb (Table 4). Customizable by the user, the TP and baseline tile size can be adjusted to balance the tradeoffs in sensitivity and specificity.

In conclusion, this study provides guidance on the workflows and the parameter settings needed to achieve robust mosaicism calling and detection of subchromosomal CNV events at particular size thresholds.

ion torrent

Acknowledgments

Genoma Group (Dr. Francesca Spinella), Jetanin Institute (Dr. Pornwaratt Niyomrattanakit), Igenomix (Dr. Carmen Rubio and Carmen Garcia), Sequence46 (Sophia Tormasi and Catherine Welch), Center for Reproductive Medicine of Weill Cornell Medical College (Dr. Jianwen Wei, Dr. Chenhui Zhang Xioa Qin, Dr. Zev Rosenwaks, and Dr. Kangpu Xu).

References

1. Preimplantation Genetic Diagnosis International Society (PGDIS). (2016) PGDIS position statement on chromosome mosaicism and preimplantation aneuploidy testing at the blastocyst stage. **http://pgdis.org/docs/newsletter_071816.html**
2. Controversies in Preconception, Preimplantation, and Prenatal Genetic Diagnosis (CoGEN). (2016) CoGEN position statement on chromosome mosaicism detected in preimplantation blastocyst biopsies. **http://www.ivf-worldwide.com/cogen/general/cogen-statement.html**

Ordering information

Product	Description	Quantity	Cat. No.
Ion ReproSeq PGS Kit with Ion 510 Chips	16 samples/run	64 samples	A34899
Ion ReproSeq PGS Kit with Ion 520 Chips	24 samples/run	96 samples	A34900
Ion ReproSeq PGS Kit with Ion 530 Chips	96 samples/run	384 samples	A34901
Instrument			
Ion Chef Instrument	Ion Chef Instrument for automated workflows	1 instrument	4484177
Ion GeneStudio S5 System	Ion GeneStudio S5 Sequencer	1 instrument	A38194
Ion GeneStudio S5 Plus System	Ion GeneStudio S5 Plus Sequencer	1 instrument	A38195
Ion GeneStudio S5 Prime System	Ion GeneStudio S5 Prime Sequencer and Ion S5 Torrent Server	1 system	A38196
Data analysis			
Ion Reporter Software	Cloud-based hosted data storage or on-site server options available	Find out more at **thermofisher.com/ionreporter**	
Support			
Priority Tech Support, Ion ReproSeq PGS Kit	Priority tech support is only available in Europe and North America at this time		ZGLPSCIONS5

Find out more at **thermofisher.com/reproseq**

ThermoFisher SCIENTIFIC