Advancing PGT-A: Integrating SNP Panel Results for Trisomy Detection, Contamination Assessment, and Sample Mix-Up Verification

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Senior Manager Product Management,

May 2024

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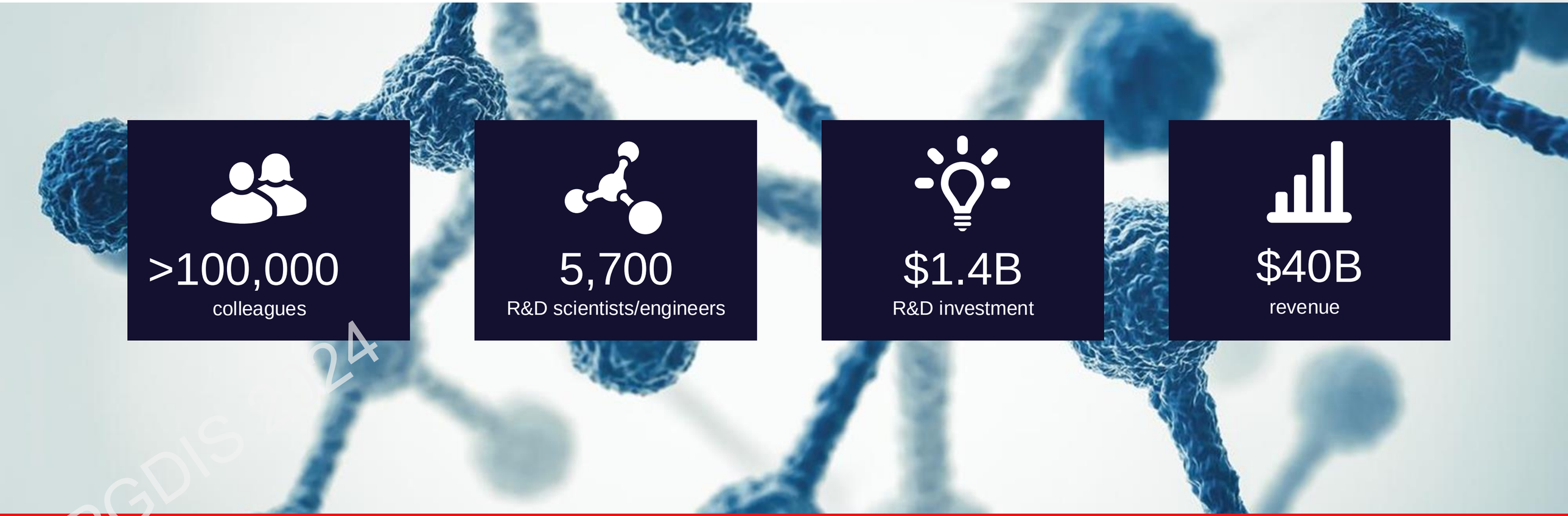

>100,000
colleagues

5,700
R&D scientists/engineers

$1.4B
R&D investment

$40B
revenue

We enable our customers to make the world healthier, cleaner and safer

Our Mission is our purpose

We enable our customers to make the world healthier, cleaner and safer

Complete solution for aneuploidy detection by low-pass genome sequencing

Starting from a biopsy sample of 1–10 cells

Library prep
Combined DNA extraction, whole-genome amplification (WGA), and sample barcoding

Template prep
Automated clonal amplification of libraries

Sequencing
Simple, cartridge-loaded reagents and user interface

Analysis
Automated Ion Reporter™ software

- **Rapid**—sample to answer typically in <10–13 hours
- **Flexible**—multiplexing of 16–96 samples/run
- **Reliable**—mosaicism detection and calling
- **Simple**—plug-and-play instrumentation

Ion ReproSeq PGS Kits for Ion GeneStudio S5 Systems

- Three kit configurations support sample scalability of 16, 24, and 96 samples per run
- Detection of whole-chromosome aneuploidies and chromosome-arm copy number events in as little as 10 hours
- Ion SingleSeq™ library kit includes reagents to extract, amplify, and prepare barcoded libraries from a single cell

Ion 510™ Chip
16 samples/run

Ion 520™ Chip
24 samples/run

Ion 530™ Chip
96 samples/run

Advancing Through Innovation:Premium PGT-A on Genexus™

ThermoFisher SCIENTIFIC

Scalability with improved performance and higher throughput

Ion ReproSeq PGS kits for Genexus™ Integrated Sequencer

- Two kit configurations enable **scalable sample processing:** 96 or 192 samples per run
- Flexible chip design to **support variable sample intake,** minimizing batching requirements
- **Expanded barcode** kits for extracting, amplifying, and preparing libraries of up to 384 samples, minimizing cross-contamination.

Maximum sample flexibility with Ion Torrent™ GX5™ Chip

- Four-lane design accommodates sample intake variability with ease
- Cost-effective runs achieved by simultaneous processing of 1-4 lanes
- Enables rapid results by minimizing the need for batching

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Analyze results with Genexus™ Software

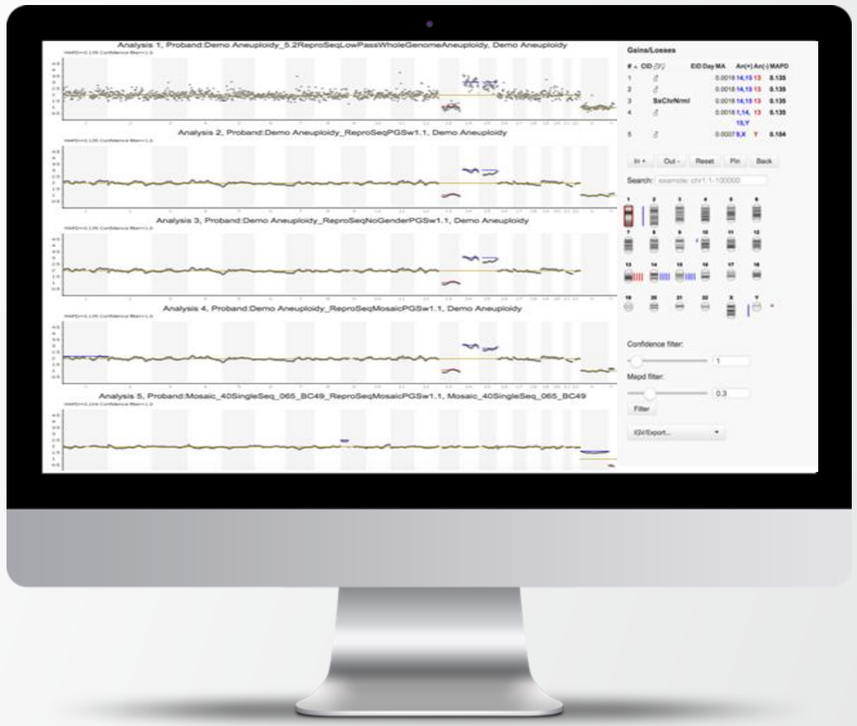
Achieve aneuploidy calls with just a few clicks

Integrated
Comprehensive PGT-A solution: seamlessly plan, monitor, track runs, analyze data, and generate personalized reports

Easy to use
Simplified user experience can reduce learning curve and human errors

Accurate
Tunable aneuploidy detection with preconfigured analysis workflows

Flexible
Option to choose between integrated analysis on instrument or analysis on another system through API



Ion ReproSeq™ PGS kit Sample Report

Test Address

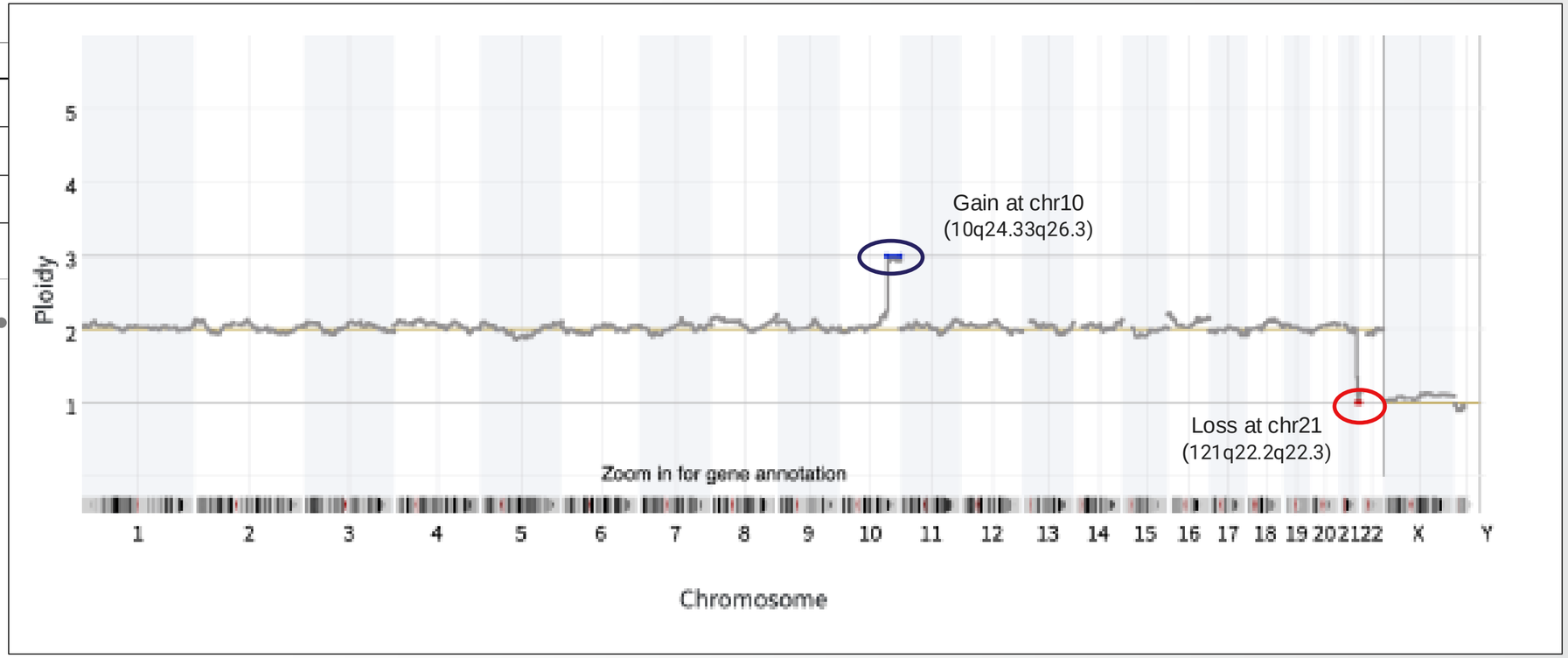
Reproseq Report
ReproSeq™ PGS - GX5 - w1.0.0 - v0.0.19

Date: 22 Jul 2022

Patient		Partner		Other	
Sample First Name:	**First1**	Partner First Name:	**Partner First1**	Consultant:	**Consultant123**
Sample Last Name:	**Laste1**	Partner Last Name:	**Partner Laste1**	Referring Center:	**CenterABC**

Summary

Embryo ID	Sample Name	Gender	AN(+)	AN(-)
reproSeq_embryo 4	reproSeq_sample _004	Male		11
reproSeq_embryo 3	reproSeq_sample _003	Female	14, 6	
reproSeq_embryo 2	reproSeq_sample _002	Female	14, 9	
reproSeq_embryo 1	reproSeq_sample _001	Male	10	21

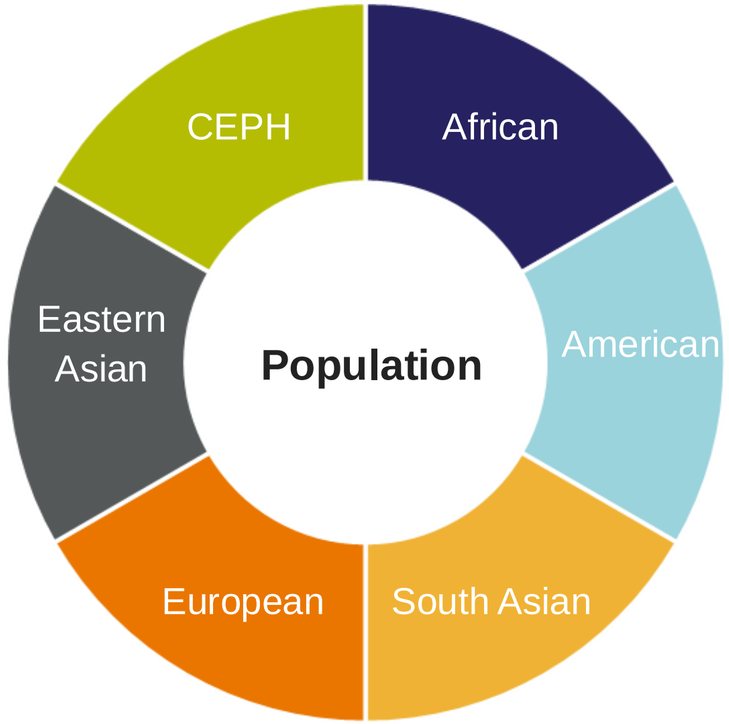


Unlocking Insights: SNP based Ion AmpliSeq™ Polyploidy Panel Kit

Unlock genomic variations unique to each embryo sample

- Enhance PGT-A analysis (using Ion ReproSeq PGS kits) with Polyploidy panel for **germline SNP detection**

- Single pool that provides comprehensive coverage of **>500 SNP sites**:
  - 74 microhaplotype amplicons for 222 SNP sites used in human identification
  - 368 single-SNP amplicons for population-wide representation based on minor allele frequencies in the 1000 Genomes Project [1] and screened for representation in Ion SingleSeq™ whole-genome amplification products



Minor allele frequencies in the 1000 Genomes Project

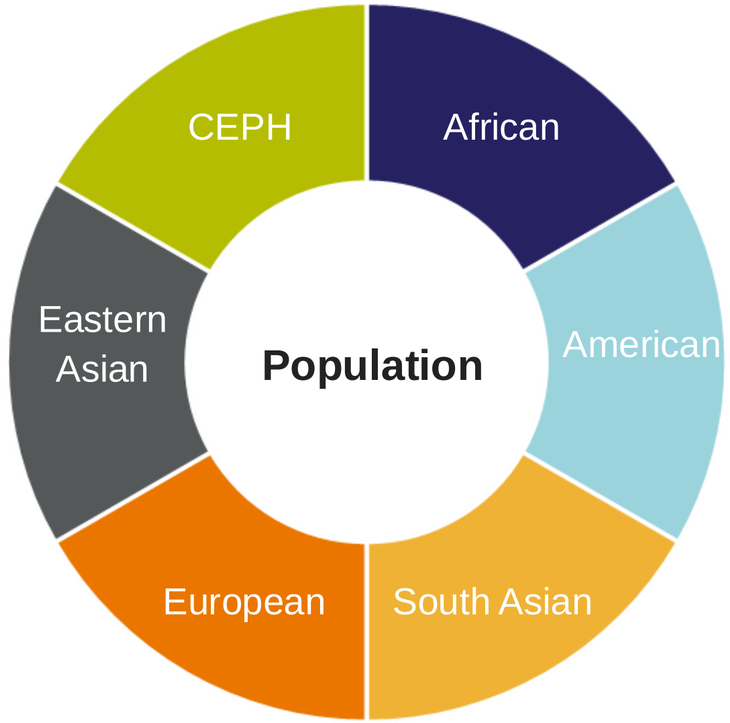
1. 1000 Genomes Project Consortium, Auton et al. A global reference for human genetic variation. *Nature*. 2015;526(7571):68-74. doi:10.1038/nature15393

Ion AmpliSeq™ Polyploidy Panel Kit

Polyploidy panel is compatible with both the Genexus™ and GeneStudio platforms

Genexus™

GeneStudio

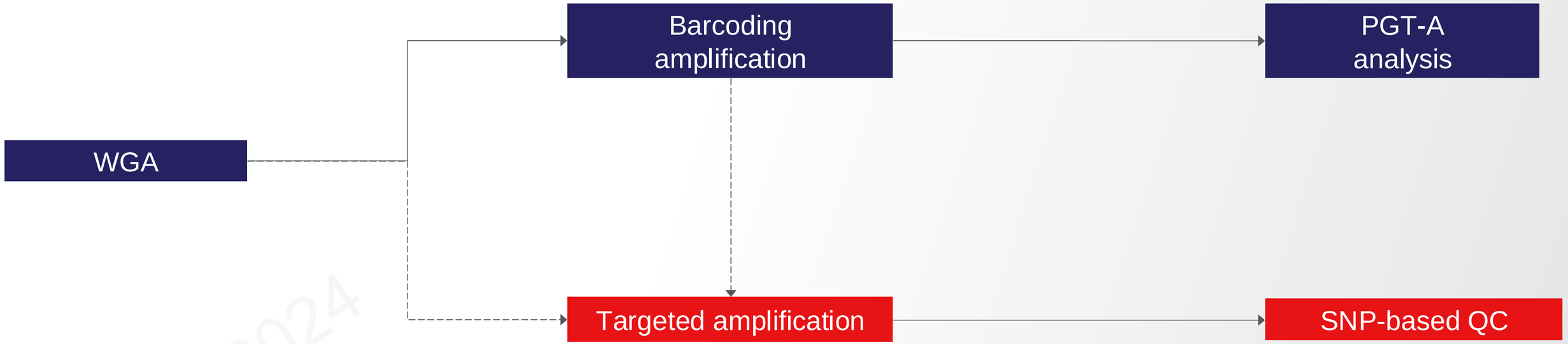


Minor allele frequencies in the 1000 Genomes Project

Library prep from a single sample

A single sample input for Ion ReproSeq PGS Kit and Ion AmpliSeq Polyploidy Panel Kit

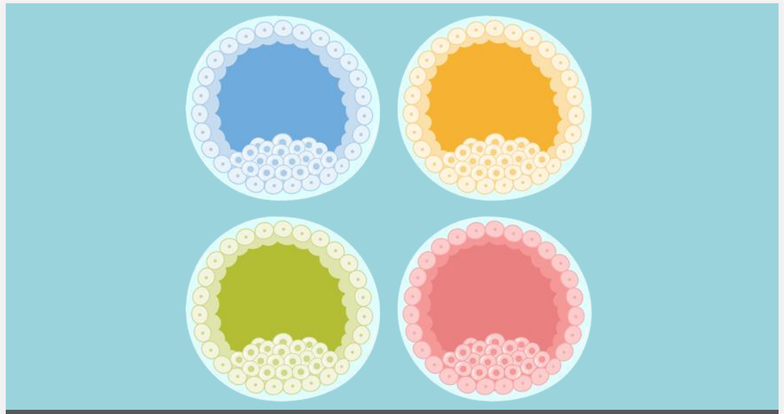
Workflow for streamlined WGA and library prep using Ion SingleSeq kit



Ion AmpliSeq sample workflow for SNP amplification

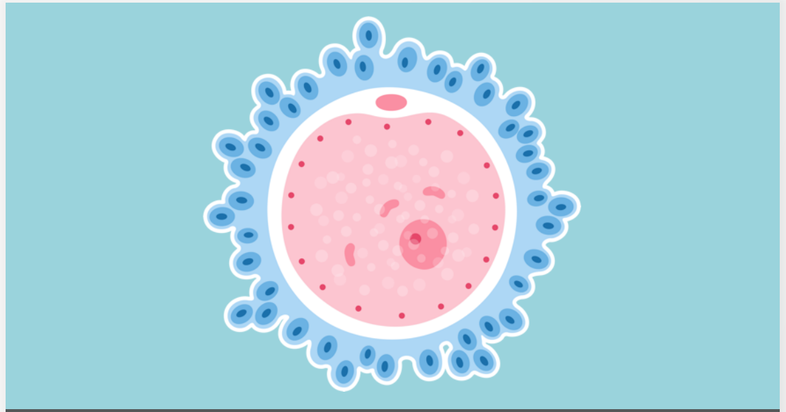
Raising the Bar: Delivering Premium PGT-A Analysis

Enhancing Quality Control: Optional SNP-Based Testing for Ion ReproSeq PGS Kits



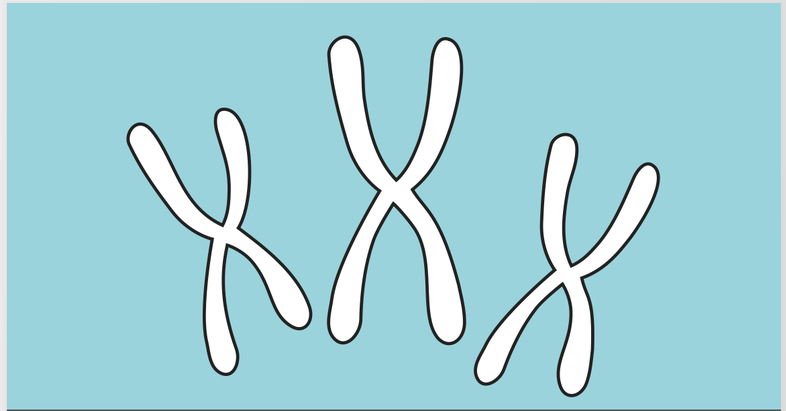
Prevent sample mix-up

- Accurate sample identification is crucial in PGT-A to prevent embryo mix-ups.
- Sibling QC can reduce the risk of sample mix-ups and improve the accuracy of PGT-A testing.



Detect maternal contamination

- Maternal cumulus cells can contaminate embryos and affect PGT-A results [2].
- To mitigate the risk, prioritize low-risk samples with maternal risk assessment.



Identify triploidy (69,XXX)

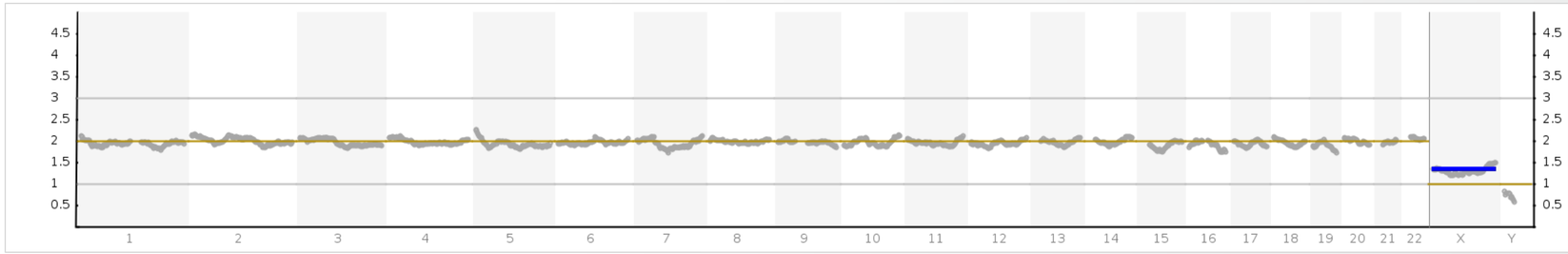
- PGT-A plots show 69,XXX females that are indistinguishable from 46,XX, making differentiation impossible [1].
- SNP-based analysis allows a reliable method to determine ploidy status.

1. Hammond et al. Characterizing nuclear and mitochondrial DNA in spent embryo culture media: genetic contamination identified. *Fertil Steril*. 2017 Jan;107(1):220-228.e5. doi: 10.1016/j.fertnstert.2016.10.015.
2. ESHRE PGT-SR/PGT-A Working Group; Coonen E, Rubio C, Christopikou D, Dimitriadou E, Gontar J, Goossens V, Maurer M, Spinella F, Vermeulen N, De Rycke M. ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations. *Hum Reprod Open*. 2020 May 29;2020(3):hoaa017. doi: 10.1093/hropen/hoaa017

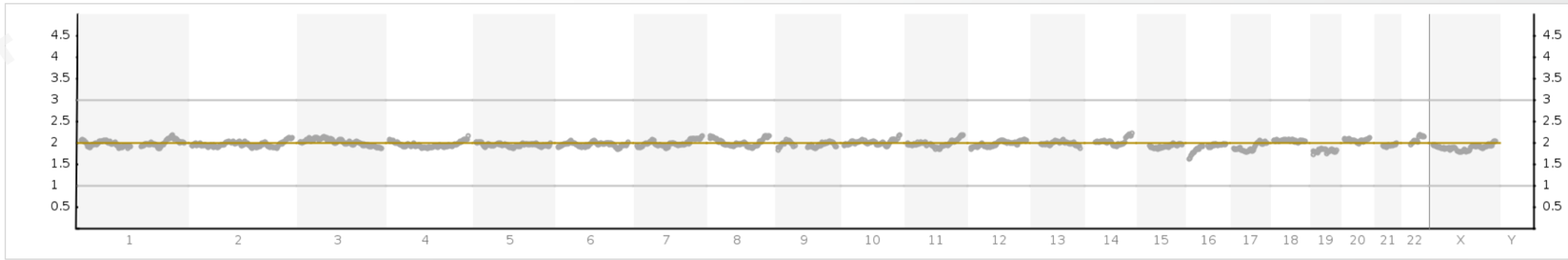
Information from SNP results

- Triploid Detection
  - 69,XXX females appear identical to 46,XX for PGT-A
- Maternal Contamination
  - Cumulus cells not stripped from the oocyte push PGT-A results toward normal
- Sibling QC
  - Cross-check for sample mix ups

69,XXY



69,XXX

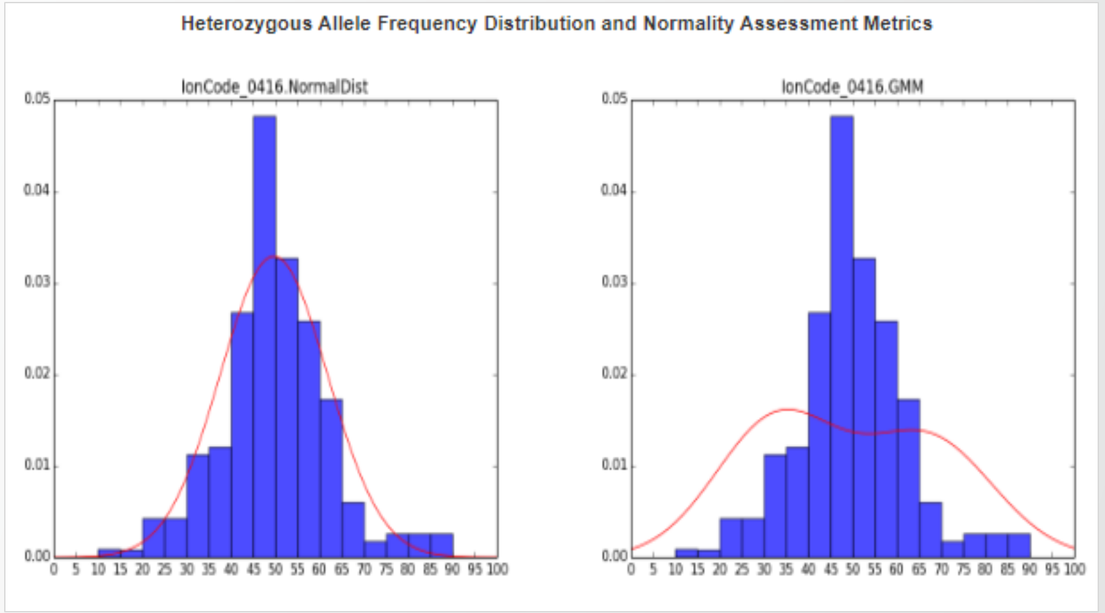


Triploid / diploid / haploid calling overview

- Allele frequencies of all heterozygous alleles are assessed
- Based on the similarity to bimodal vs. normal distribution, a call is made on the ploidy level.
  - Ploidies > 3 are not distinguished from triploid
- To be called Abnormal / Triploid, a sample must also have at least 3 triallelic sites and > 20% estimated contamination
- Haploids are called when a low proportion of heterozygous SNPs is detected

Polyploidy Report

Polyploidy Status	**Diploid**
Call confidence	High
Hom allele calls	576
Het allele calls used	232
Hets AF mean	49.66
Hets AF StdDev	12.15
Shapiro test	Not Normal
Normal vs. GMM	Diploid
Assignment probability	Diploid



Summary table – triploids and diploids

Overall summary call
Overrules to Normal when minimum 3rd alleles not met

Ploidy estimate based only on allele frequencies

ReproSeqSnpAnalysis

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SiblingQC Run Summary: 12 Family Groups Specified
All samples passed SiblingQC.

Barcode Name	Sample	Couple ID	SiblingQC	Status	Polyploidy	Contamination	Ploidy Call Confidence	Contamination Level	MHAP 3rd Alleles
IonCode_0101	T3_R01_NA01672_Triploid_O1_BC101_ori	NA01672	Pass	Abnormal	Triploid	High	High	35.86%	6
IonCode_0102	T3_R01_NA01672_Triploid_O1_BC102_ori	NA01672	Pass	Abnormal	Triploid	High	High	53.33%	5
IonCode_0103	T3_R01_NA01672_Triploid_O1_BC103_ori	NA01672	Pass	Abnormal	Triploid	High	Low	46.88%	5
IonCode_0104	T3_R01_NA01672_Triploid_O1_BC104_ori	NA01672	Pass	Abnormal	Triploid	High	High	40.68%	4
IonCode_0105	T3_R01_NA10013_Triploid_O1_BC105_ori	NA10013	Pass	Abnormal	Triploid	High	High	53.23%	10
IonCode_0106	T3_R01_NA10013_Triploid_O1_BC106_ori	NA10013	Pass	Abnormal	Triploid	High	Low	44.11%	10
IonCode_0107	T3_R01_NA10013_Triploid_O1_BC107_ori	NA10013	Pass	Abnormal	Triploid	High	High	48.51%	10
IonCode_0108	T3_R01_NA10013_Triploid_O1_BC108_ori	NA10013	Pass	Abnormal	Triploid	High	High	56.75%	10
IonCode_0109	T3_R01_NA13117_Diploid_O1_BC109_ori	Amish884	Pass	Normal	Diploid	None	High	0.00%	0
IonCode_0110	T3_R01_NA13118_Diploid_O1_BC110_ori	Amish884	Pass	Normal	Diploid	None	High	12.62%	2
IonCode_0111	T3_R01_NA13121_Diploid_O1_BC111_ori	Amish884	Pass	Normal	Diploid	None	High	10.94%	1
IonCode_0112	T3_R01_NA13122_Diploid_O1_BC112_ori	Amish884	Pass	Normal	Diploid	None	High	16.98%	1
IonCode_0113	T3_R01_NA12550_Diploid_O1_BC113_ori	French66	Pass	Normal	Diploid	None	High	13.11%	1
IonCode_0114	T3_R01_NA12551_Diploid_O1_BC114_ori	French66	Pass	Normal	Diploid	None	High	0.00%	0

True triploids appear also contaminated and should have ≥ 3 MHAP 3rd alleles and ≥ 20% contamination level

Maternal contamination overview

- Based only on tri-allelic microhaplotype sites
  - Two embryo alleles where heterozygous + one non-inherited maternal allele
  - Only microhaplotype sites have more than one variant allele in the hotspot file
- Minimum 3 sites must be tri-allelic (to distinguish from noise)
- Maternal contamination is estimated based on 2x mean of third allele frequencies
  - High contamination is ≥ 30%
  - Low contamination is ~5-29%
  - Very high contamination (> 90%) will look like low or no contamination
    - Running a maternal sample should reveal a high degree of relatedness with highly contaminated samples

Summary table – maternal contamination

20% and 30% contaminated samples compared to 0% and 100% (pure maternal)

ReproSeqSnpAnalysis

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SiblingQC Run Summary: 45 Family Groups Specified
All samples passed SiblingQC.

Contaminated samples often also appear triploid.

Contamination given by this column

Barcode Name	Sample	Couple ID	SiblingQC	Status	Polyploidy	Contamination	Ploidy Call Confidence	Contamination Level	MHAP 3rd Alleles
IonCode_0101	T4_R02_NA07018-NA06997_matcon-0pct_O4_BC101	T4_R02_NA07018-NA06997_matcon-0pct_O4_BC101	Pass	Normal	Diploid	None	High	0.00%	0
IonCode_0102	T4_R02_NA07018-NA06997_matcon-20pct_O4_BC102	T4_R02_NA07018-NA06997_matcon-20pct_O4_BC102	Pass	Abnormal	Triploid	High	Low	34.72%	9
IonCode_0103	T4_R02_NA07018-NA06997_matcon-30pct_O4_BC103	T4_R02_NA07018-NA06997_matcon-30pct_O4_BC103	Pass	Abnormal	Triploid	High	High	32.78%	13
IonCode_0104	T4_R02_NA07018-NA06997_matcon-100pct_O4_BC104	T4_R02_NA07018-NA06997_matcon-100pct_O4_BC104	Pass	Normal	Diploid	None	High	15.38%	1
IonCode_0105	T4_R02_NA13120-NA13114_matcon-0pct_O4_BC105	T4_R02_NA13120-NA13114_matcon-0pct_O4_BC105	Pass	Normal	Diploid	None	High	0.00%	0
IonCode_0106	T4_R02_NA13120-NA13114_matcon-20pct_O4_BC106	T4_R02_NA13120-NA13114_matcon-20pct_O4_BC106	Pass	Normal	Diploid	Low	High	19.51%	9
IonCode_0107	T4_R02_NA13120-NA13114_matcon-30pct_O4_BC107	T4_R02_NA13120-NA13114_matcon-30pct_O4_BC107	Pass	Contaminated	Diploid	High	High	29.83%	8
IonCode_0108	T4_R02_NA13120-NA13114_matcon-100pct_O4_BC108	T4_R02_NA13120-NA13114_matcon-100pct_O4_BC108	Pass	Normal	Diploid	None	High	0.00%	0
IonCode_0109	T4_R02_NA12565-NA12561_matcon-0pct_O4_BC109	T4_R02_NA12565-NA12561_matcon-0pct_O4_BC109	Pass	Normal	Diploid	None	High	0.00%	0
IonCode_0110	T4_R02_NA12565-NA12561_matcon-20pct_O4_BC110	T4_R02_NA12565-NA12561_matcon-20pct_O4_BC110	Pass	Normal	Diploid	Low	High	22.33%	10
IonCode_0111	T4_R02_NA12565-NA12561_matcon-30pct_O4_BC111	T4_R02_NA12565-NA12561_matcon-30pct_O4_BC111	Pass	Abnormal	Triploid	High	High	28.36%	11

Sibling QC overview

- SiblingQC uses CoupleID from sample attributes to determine number of expected families
- Clustering is performed based on allele similarities
  - Very poorly covered samples are excluded
- The clusters are divided into the same number of groups as the expected number of families
- Alignment of clusters and expected groups determines Pass / Fail scores for samples

Summary table - SiblingQC

ReproSeqSnpAnalysis

Executed At: 2023-04-12 23:26

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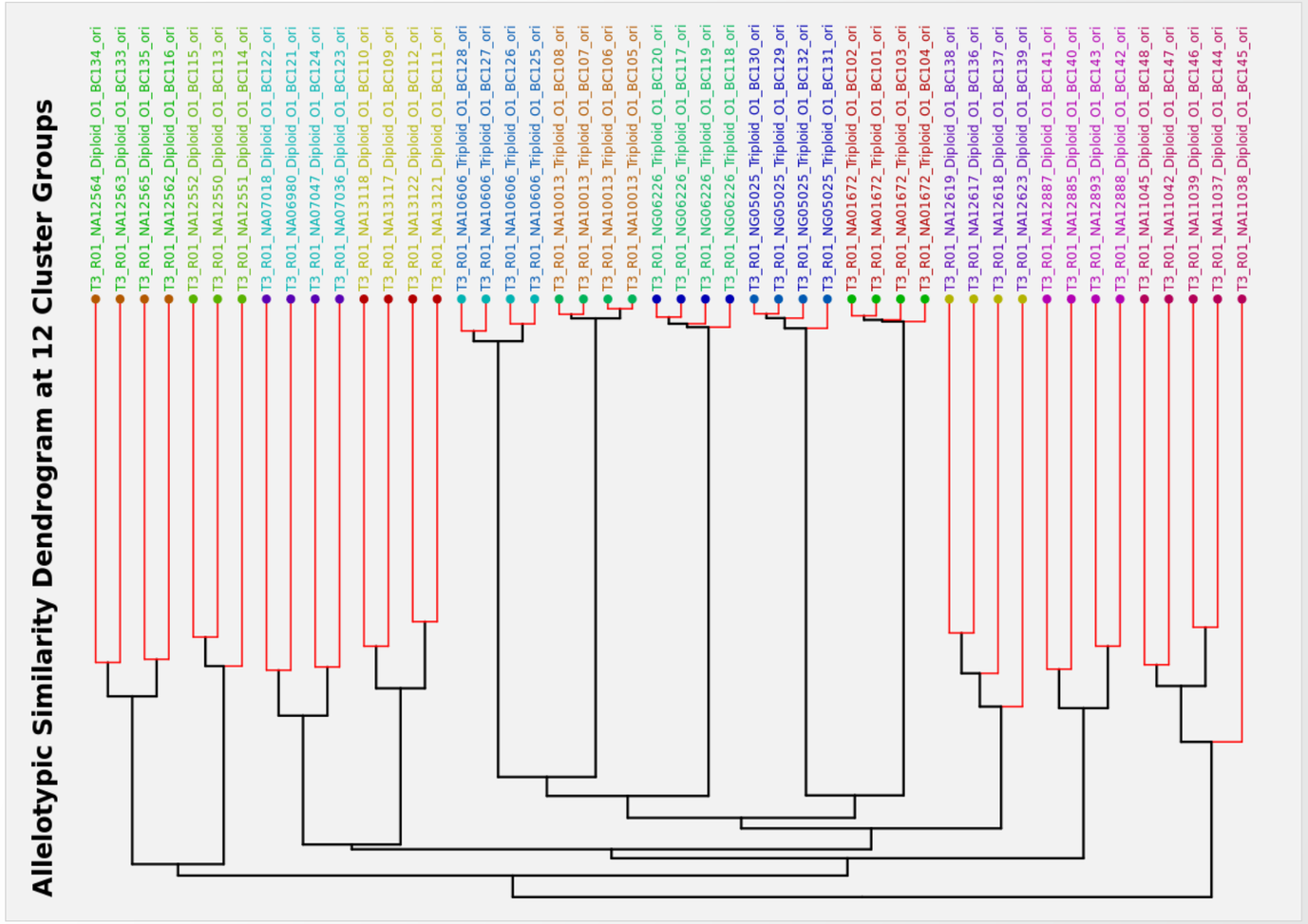
SiblingQC Run Summary: 12 Family Groups Specified
All samples passed SiblingQC.

Barcode Name	Sample	Couple ID	SiblingQC	Status	Polyploidy	Contamination	Ploidy Call Confidence	Contamination Level	MHAP 3rd Alleles
IonCode_0101	T3_R01_NA01672_Triploid_O1_BC101_ori	NA01672	Pass	Abnormal	Triploid	High	High	35.86%	6
IonCode_0102	T3_R01_NA01672_Triploid_O1_BC102_ori	NA01672	Pass	Abnormal	Triploid	High	High	53.33%	5
IonCode_0103	T3_R01_NA01672_Triploid_O1_BC103_ori	NA01672	Pass	Abnormal	Triploid	High	Low	46.88%	5
IonCode_0104	T3_R01_NA01672_Triploid_O1_BC104_ori	NA01672	Pass	Abnormal	Triploid	High	High	40.68%	4
IonCode_0105	T3_R01_NA10013_Triploid_O1_BC105_ori	NA10013	Pass	Abnormal	Triploid	High	High	53.23%	10
IonCode_0106	T3_R01_NA10013_Triploid_O1_BC106_ori	NA10013	Pass	Abnormal	Triploid	High	Low	44.11%	10
IonCode_0107	T3_R01_NA10013_Triploid_O1_BC107_ori	NA10013	Pass	Abnormal	Triploid	High	High	48.51%	10
IonCode_0108	T3_R01_NA10013_Triploid_O1_BC108_ori	NA10013	Pass	Abnormal	Triploid	High	High	56.75%	10
IonCode_0109	T3_R01_NA13117_Diploid_O1_BC109_ori	Amish884	Pass	Normal	Diploid	None	High	0.00%	0
IonCode_0110	T3_R01_NA13118_Diploid_O1_BC110_ori	Amish884	Pass	Normal	Diploid	None	High	12.62%	2
IonCode_0111	T3_R01_NA13121_Diploid_O1_BC111_ori	Amish884	Pass	Normal	Diploid	None	High	10.94%	1
IonCode_0112	T3_R01_NA13122_Diploid_O1_BC112_ori	Amish884	Pass	Normal	Diploid	None	High	16.98%	1
IonCode_0113	T3_R01_NA12550_Diploid_O1_BC113_ori	French66	Pass	Normal	Diploid	None	High	13.11%	1
IonCode_0114	T3_R01_NA12551_Diploid_O1_BC114_ori	French66	Pass	Normal	Diploid	None	High	0.00%	0

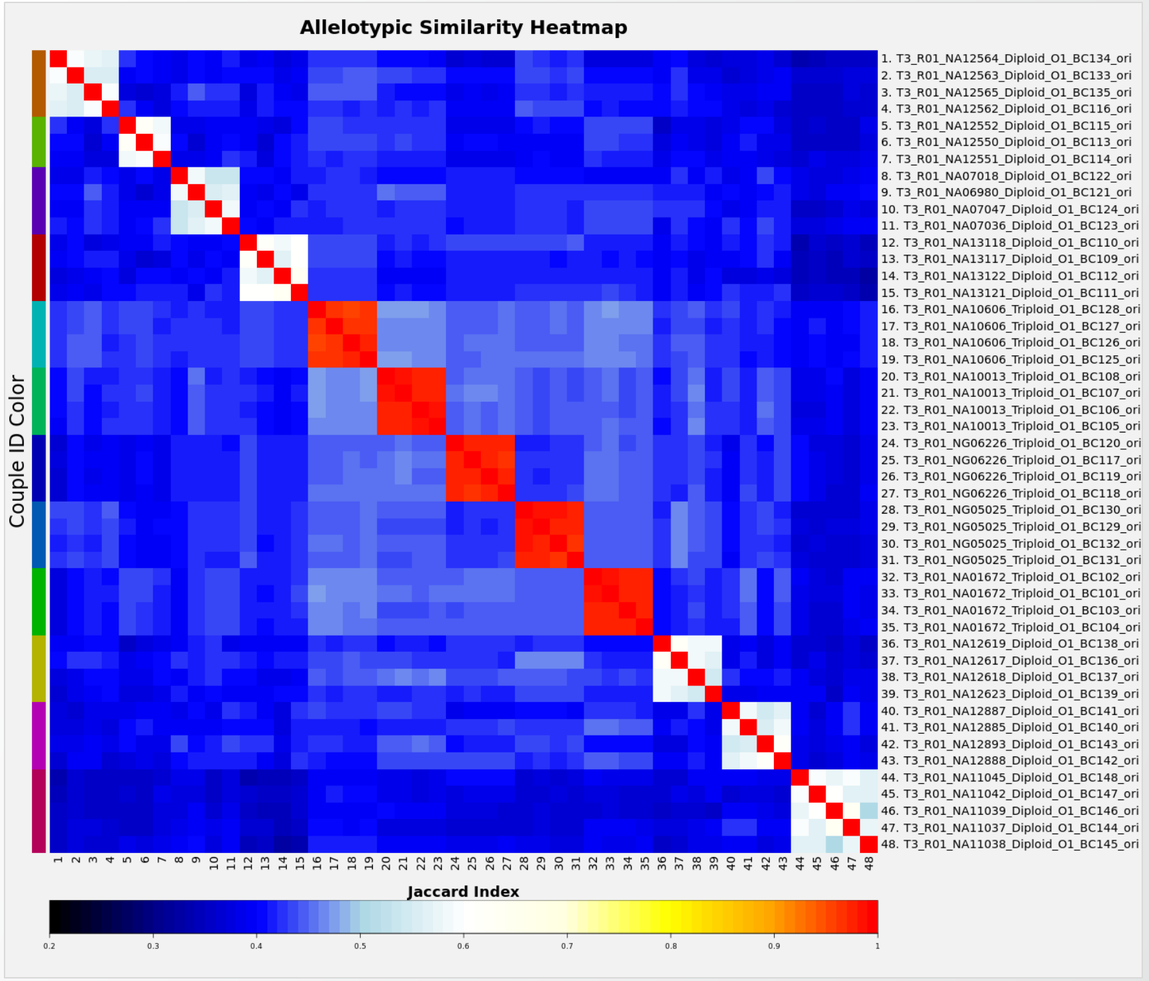
Sibling similarity dendrogram

- Couple ID values assigned to the samples define either sibling sets (diploid samples) or technical replicates (triploid samples)
- The 12 clusters generated based on the data match the expected groupings
- Note high similarity of technical replicates based on distance to node



Sibling similarity heatmap

- Degrees of similarity between all possible sample pairs are shown by a heatmap
- White (≈ siblings) or red (≈ identical) clusters align with Couple ID color assignments on the far left



Ion Torrent™ NGS systems for Ion ReproSeq™ PGS kits

Which system is the best fit for your lab?

Genexus™ Integrated Sequencer

- Analyze more PGT-A samples per run
- Leverage a simplified workflow that seamlessly integrates SNP-based QC
- Save space with an all-in-one instrument
- Readily expand beyond reproductive health

Simplify and scale your PGT-A workflow with an all-in-one NGS system

Ion GeneStudio™ S5 System

- Readily expand into reproductive health applications like combined PGT-A/PGT-M and expanded carrier screening
- Flexibly configure your own system with an Ion GeneStudio™ S5, Plus, or Prime System
- Easily add plug-ins for custom NGS panels

Deliver PGT-A and more with a flexible NGS system for reproductive health

Thank you

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