Article Title:

First clinical validation of whole genome screening on standard trophectoderm biopsies of preimplantation embryos

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Abstract

Whole Genome Sequencing (WGS) is used in healthcare and in the clinic, with the notable exception of preimplantation genetic testing (PGT). In PGT, only a few cells are available for sequencing, requiring DNA amplification which reduces data quality, sequence fidelity and sharply limits subsequent clinical impact. Here we demonstrate the first clinical validation of WGS on embryo biopsies using our lab development protocol, opening the door to broad use of WGS in fertility. We find that amplified DNA with comparable sensitivity and specificity to genomic DNA when performing whole genome sequencing assays. DNA amplification on cell lines and donated human embryos had an amplification success rate of >99.9% and 98.2% respectively and accuracy on both was >99.9% on aneuploidy status. GIAB samples (Genome in the Bottle reference NA12878) showed that our amplified DNA was broadly comparable to genomic DNA (99.99% accuracy, 99.99% specificity, 98.0% sensitivity and 98.1% precision). Using our assay, we were able to call variants, detect mitochondrial heteroplasmy, perform high precision screening without access to parental genomes, detect compound heterozygous variants, and score microdups/dels and uniparental disomies (to reduce risk of diseases such as DiGeorge syndrome and Prader-Willi syndrome). Our clinical study suggests that the full spectrum of traditional clinical genome bioinformatics, so far reserved to large samples, can now be performed on embryos before implantation.

Keywords

Preimplantation genetic testing, Whole genome screening, In vitro fertilization, Next generation sequencing, Clinical validation

Manuscript

Introduction

Preimplantation genetic testing (PGT) reduces the number of cycles needed to achieve a successful pregnancy by improving fetal viability rates per embryo transfer(1). However, current methods can only assess ploidy status or screen for specific mendelian variants for which parents are known to be carriers, in which case DNA samples from both parents are required. However, for cases where donor eggs or donor sperm are used, it is difficult or impossible to gain access to donor DNA to evaluate carrier status(2). In addition, small copy number variations (CNV) such as DiGeorge are not screened in regular PGT-A because the deletion size falls below the sensitivity threshold (~2Mb). Uniparental disomy (UPD) (such as Prader-Willi and Angelman) is also not regularly screened in PGT-A as low-pass next generation sequencing (NGS) data cannot accurately capture beta-allele frequency. To our knowledge, no other clinical whole genome sequencing is currently performed due to poor coverage and high allelic dropout rates(3). Here, we present a validation study on our latest whole-genome screening assay on embryos. We sought to measure the performance of this new assay against the current clinical standard in ploidy analysis, variant detection via WGS, and screening for mitochondrial genetic disorders, microduplications/deletions, and UPD from a single biopsy.

Materials and Methods

Sample collection

Cell lines were obtained from Coriell. Embryo samples were all donated to research and provided from the HRC Fertility Center (wIRB #20215134). All embryos were created using Intracytoplasmic sperm injection (ICSI). Embryos from day 5/6/7 were biopsied following standard SOP from clinics. No special SOP is needed. Each biopsy contained approximately 5 cells and was collected in a 200ul PCR tube with 3ul of cell buffer. Clinical case study was done under wIRB # 2022264.

Next generation sequencing

Samples were processed in the Orchid lab (certified: CAP # 9234146 & CLIA # 34D2260214). DNA or biopsies were amplified using lab-developed protocol. DNA sizes after WGA were first confirmed by running 1-2% Agarose E-Gel (Invitrogen). After size determination, 250-500ng of DNA was used for library preparation with KAPA HyperPlus kit per manufacturer's instructions. Dual Index UMI adapters (Integrated DNA Technologies) were used in the ligation. Library concentration was quantified using the Qubit 4 dsDNA HS. Library sizes were measured through Agilent 4150 Tapestation Genomic ScreenTape assay (Agilent Technologies). Sequencing runs were performed on a MiniSeq for low-pass aneuploidy screening and NovaSeq6000 for 30X WGS per manufacturer's instructions.

Genomic Data Analyses

NGS data were processed via a combination of Gencove Sentieon-based human-genome pipeline and an in-house pipeline following GATK best practices(4). Gvcfs were generated for each sample and jointly called as part of a larger cohort. GATK VariantRecalibrator (VQSR) was run on the resulting callset. Mitochondrial DNA was analyzed using GATK without VQSR. Sensitivity, precision, etc were calculated through RTG packages. NxClinical and Ginkgo were used for CNV calling using default parameters as the

package is designed for amplified DNA. All data were aligned to GRCh37 with a bin size of 500 kbp. Embryos were excluded from the analysis based on the following criteria: 1) the whole embryo was deemed not viable or, 2) both the whole embryo and biopsies were inconsistent with previous PGT-A results.

Results

Validation on aneuploidy screening via cell lines and human embryos

Ploidy analysis was initially validated by assessing the accuracy of aneuploidy screening. A total of 73 samples from Coriell cell lines were used first to validate our aneuploidy screening (Figure S1). These cell lines covered common karyotypes seen in PGT-A, including euploid males and females and autosomal and sex chromosome aneuploidy. We diluted the DNA to ~20-50 pg (picogram), which is equivalent to the amount of DNA in embryo trophectoderm biopsies. Whole-genome amplification was then performed, followed by low pass sequencing (~0.01 - 0.05X) for aneuploidy evaluation with a bin size of 500 kbp on the genome in the analysis. Overall, we achieved >99.9% successful amplification and >99.9% accuracy (Figure S1B). Notably, we obtained >1500 ng of DNA for all samples, which is higher and more robust than most WGA approaches.

Next, we extended our PGT-A validation on donated human embryos with a larger variety of karyotypes (Figure 1, Table S1). We obtained 2-3 samples per embryo, on which we performed WGA and ploidy analysis. We first evaluated the consistency of these samples by comparing our CNV analysis to the previous clinical PGT-A reports. Among the samples, we observed a mosaic embryo with two samples being deletion of the short arm of chromosome 2 (-2p) and one sample being monosomy 2 (-2) (#55-57); and a mosaic embryo with one sample being mosaic trisomy 19 (+19) and one sample being trisomy 19 (+19). (#105-106). Considering the previous PGT-A results reported the same chromosome and the unknown accuracy of the original 2017 PGT-A testing technology, our PGT-A results are acceptably consistent. Two samples (marked indeterminate, Table S1) have more than 8% mitochondrial reads in the NGS data, negatively impacting our CNV calling; however, as high levels of mitochondrial reads often suggest embryo viability issues, the clinical impact of processing samples contaminated by high levels of mitochondrial DNA may be limited(5). Viability is generally higher with fr7esh embryos compared to banked embryos because banked embryos suffer from multiple freeze-thaw cycles and additional rounds of biopsies. Regardless, we achieved >99.9% accuracy overall with 1.8% indeterminate among 110 embryo samples.

Low-level mosaicism in embryos has been reported to be compatible with a successful pregnancy and live birth, especially when the aneuploidy mosaicism level is less than 40%(6). Therefore, our algorithm was designed to capture mosaicism above 30%. We performed a dilution series from 50% to 10% mosaicism, generated by mixing aneuploid (47, XY, +18) and euploid (46, XX) DNA. As seen in Figure S2B-Batch1, mosaic samples above 30% could be visualized and were captured successfully by the algorithm. To confirm the results, we performed another mosaicism dilution using a mix of NA09288 (47, XY, +9) and euploid (46, XX) DNA and found the same result (Figure S2B-Batch2). Additionally, 24 cell line mixtures with 30-35% aneuploidy mosaicism were tested and 100% of them were successfully marked during analysis (Figure S2B-Batch3).

Validation of whole genome sequencing using cell lines and human embryos

Although multiple clinical labs have demonstrated good performance in PGT-A(7), to our knowledge none have attempted validation on PGT-whole genome sequencing. DNA derived from whole genome amplification to date has poor quality, and lowered sequence fidelity when compared to genomic DNA and has not been used for embryo screening under clinical conditions(8). Our results using lab-developed WGA protocol show that our assay achieves comparable sensitivity and specificity to genomic DNA.

We used the NIST Genome in a Bottle – NA12878 and its variants as the data analysis reference. Both genomic DNA and amplified DNA from NA12878 were analyzed and compared to benchmark calls from GIAB (NIST v4.2.1). As shown in Table S2 and Table S3, accuracy and specificity at whole genome level are 99.99% for both amplified DNA and genomic DNA. We observed an average of 99.8% genomic coverage in amplified DNA and the same in gDNA (Table S2 and Table S3). The precision of amplified DNA and gDNA are 98.1% and 97.8%, respectively, suggesting our amplified DNA does not generate more false positive calls than gDNA (Table S2). Moreover, sensitivity reaches 98.0% in amplified DNA as compared to 98.9% in gDNA, indicating false negative calls in data are similar in magnitude as well (Table S2). Therefore, our WGS on amplified DNA was validated against the variant list from NIST and its sequencing outcome is comparable to gDNA.

To validate performance on biopsies of clinical embryos, we compared the results of the biopsies to their corresponding embryos. Using the whole embryo as the reference, we observed an average of 99.6% genomic coverage, 99.9% accuracy, 99.9% specificity, 98.0% precision, 98.1% sensitivity (Table 1 & Table S4). The demonstrated concordance allows us to treat WGS of an embryo biopsy as an accurate reflection of the entire embryo's genome.

Validation on mitochondrial DNA

A high proportion of pathogenic heteroplasmy has been correlated to mitochondrial diseases including early onset metabolic and degenerative diseases. Our validation on mitochondrial DNA focused on mitochondrial genome coverage, sensitivity, and precision by comparing biopsies to their embryos. Setting 15% as our reportable heteroplasmy threshold, we observed an average of 100% mitochondria DNA coverage, >99.9% accuracy, >99.9% specificity, 99.0% sensitivity and >99.9% precision (Table S5). Since mitochondrial DNA is known to be exclusively inherited from the mother, our assay has the potential to be used to perform maternity confirmation.

Detection of specific variants through WGS without parental samples

We performed WGS on biopsies from embryos that previously underwent PGT-M, as well as on Coriell cell lines that carry specific pathogenic variants. Following our protocol with 30X sequencing, all variants detected by previous PGT-M assays were detected successfully in our WGS results without the use of parental genomes (Table 2). To further confirm our ability to capture pathogenic variants in WGS, Coriell cell lines were selected to represent common genetic disorders. Amplified DNA from picogram-level of Coriell cell line followed by 30X sequencing further confirmed our PGT-WGS assay has ability to detect all reported pathogenic variants regardless of the number of variants in one sample (Table 3).

We demonstrate the value of this screening via a clinical research case (Table S6). Here, both parents carry different HOGA1 variants as determined by carrier screening. The carrier screening included analysis for pathogenic variants on ~1300 genes related to neurodevelopmental disorders, birth defects and hereditary cancers based on ACMG panels, as well as the HOGA1 variants identified on previous carrier analysis. Embryo HOGA1 variants were screened from WGS data directly without use of parental genomes. From this batch of embryos we were able to identify a euploid non-carrier embryo available for implantation without parental genotypes or custom probes.

Common microduplications/deletions, triploid and uniparental disomy screening

Besides variants, we also validated the screening for microduplications, microdeletions, and uniparental disomy etc that conventional PGT-A is unable to detect. Amplified DNA from picogram-level of Coriell cell lines were used as a substitute since availability of research embryos with the above genotypes are extremely rare. We covered a variety of copy number variation related diseases including but not limited to Cri-du-chat, Williams-Beuren, DiGeorge and Prader-Willi syndrome (Table S7). Based on our limit of detection study, we were able to reproducibly detect copy number variation greater than 400 Kb and UPD greater than 20 Mb at a sequence depth of 30X WGS. Examples are shown in Figure S3 and Table S7.

Discussion

We investigated the performance of a new protocol for amplifying extremely limited amounts of DNA obtained from embryo biopsies. We considered PGT-A, PGT-whole genome sequencing, mitochondrial DNA, microduplication, microdeletions and uniparental disomy using our latest lab developed assay. Remarkably, the DNA genomic coverage, sensitivity, and specificity from amplified material was broadly equivalent to assays performed on the current clinical gold standard, unamplified genomic DNA. Based on equivalent coverage, sensitivity, and specificity, this technique should make it possible to screen embryos before implantation for chromosomal and genetic disorders. Potential benefits of the new technique may include increasing the likelihood of a successful pregnancy (based on the fraction of mitochondrial reads) and reducing the likelihood of birth defects or pediatric or adult developmental disorders.

To ensure accurate and relevant identification of genetic causes of severe, highly penetrant monogenic diseases, we scored 1,300 genes associated with well-studied conditions(9). These gene selections are based on ACMG panels for applicable embryo screening and are grounded in extensive research spanning decades, including familial analysis and cohort studies(10). For many of these genes, functional investigations have established strong links between them and specific disorders. Considering that humans possess over 20,000 genes, our approach takes a conservative stance by concentrating on the 1,300 genes that have undergone comprehensive review and have been unequivocally linked to severe disease. This targeted gene selection ensures that we prioritize clinically meaningful implications for patients when making decisions about embryo transfer. Traditional carrier screening, for example, requires genes to have a confirmed relationship between the detected mutations and a well-defined disorder to provide valuable information to patients to allow reproductive decision making. It is crucial to strike a balance between the amount of data provided and the complexity it presents, as an excess of

information can pose challenges when patients are asked to make reproductive decisions. We similarly prioritize diagnostic precision, giving high accuracy in identifying the genetic causes of severe monogenic diseases.

Polygenic risk scores are also easily computable with our method due to high genomic coverage and accuracy in variants. Some polygenic risk scores utilize 6 - 7 million SNPs in total(11), which is difficult to achieve on SNP array chips without relying heavily on statistical imputation. The theoretical limits of genome imputation as applied to rare variant detection is a topic of open research(12).

Conclusion

To our knowledge, this is the first clinical validation of whole genome embryo screening. In this study, we demonstrated high accuracy for aneuploidy calls (>99.9%) and genetic variants (99.99%), even in the absence of parental genomes. This assay demonstrates advancements in genomic screening and an extended scope for testing capabilities in the realm of preimplantation genetic testing.

Author Contributions

YX, JS designed experiments. BB, NRS, NS, XD, JK, RB, EU contributed key materials, methods, and discussion. YX, DC, EB, BP, MH, QZ, JK, EB, JC performed and analyzed experiments. YX, WC, MK wrote the manuscript.

Conflict of Interests

Orchid health is a clinical PGT lab. BB is a scientific advisor to Orchid.

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Legends

Figure 1. Summary of PGT-A on embryo samples. A) Examples of copy number results on embryo samples.

Figure S1. Summary of PGT-A on cell lines. A) Examples of copy number results on cell lines. B) List of all 73 samples with Coriell cell lines.

Figure S2. Mosaicism is reported at 30%. A) Mosaicism was reported at 30% level. B) For both batch 1 & 2, no samples were reported at < 30% mosaicism, but >99.9% samples were reported at >=30%. Batch 3 has 24 samples from 30% to 35% mosaicism, and >99.9% were reported.

Figure S3. Plot of beta-allele frequency on the whole genome indicates UPD on chromosome 8. Chromosome X and Y had no beta-allele frequency. This is because male has one X and one Y so that beta-allele frequency is zero.

Table 1. Comparison of biopsies and their embryos in terms of genomic coverage, total SNV called, accuracy, specificity, precision, and sensitivity. The whole embryos were used as references.

Table 2. Variants in embryo samples that underwent PGT-M previously. PGT-WGS captured all reported variants in positive samples and was clean on negative samples.

Table 3. Variants in Coriell cell lines that possess known variants. PGT-WGS captured all reported variants in positive samples.

Table S1. List of embryo samples in PGT-A validation.

Table S2. Comparison of gDNA and amplified DNA from NA12878 in terms of genomic coverage, total SNV called, accuracy, specificity, precision, and sensitivity. Variants list of NA12878 from NIST was used as the reference.

Table S3. Tabulated numbers for true positive, false positive, true negative and false negative counts from NA12878 samples. These numbers were used to compute Table S2.

Table S4. Tabulated numbers for true positive, false positive, true negative and false negative counts from embryo samples. These numbers were used to compute Table 1.

Table S5. Analysis of mitochondrial variants from PGT-WGS data. Mitochondrial variants from the whole embryos were used as references.

Table S6. Biopsy screening results on HOGA1 variants.

Table S7. Validation on additional chromosomal abnormalities including microduplication, microdeletion, triploid and uniparental disomy.

Reference

1. Vermeesch JR, Voet T, Devriendt K. Prenatal and pre-implantation genetic diagnosis. Nat Rev Genet 2016;17(10):643–56.

2. Sacchi L, Albani E, Cesana A, Smeraldi A, Parini V, Fabiani M, et al. Preimplantation Genetic Testing for Aneuploidy Improves Clinical, Gestational, and Neonatal Outcomes in Advanced Maternal Age Patients Without Compromising Cumulative Live-Birth Rate. J Assist Reprod Genet 2019;36(12):2493–504.

3. Zheng Y, Wang N, Li L, Jin F. Whole genome amplification in preimplantation genetic diagnosis. J Zhejiang Univ Sci B 2011;12(1):1–11.

4. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, del Angel G, Levy-Moonshine A, et al. From FastQ Data to High-Confidence Variant Calls: The Genome Analysis Toolkit Best Practices Pipeline. Curr Protoc Bioinformatics 2013;43(1):11.10.1-11.10.33.

5. Dickson D, Tao T, Qin W. Exploring mitochondrial DNA content as a novel biomarker to improve embryo implantation potential: A review. Biomedical Technology 2024;5:82–6.

6. Abhari S, Kawwass JF. Pregnancy and Neonatal Outcomes after Transfer of Mosaic Embryos: A Review. J Clin Med 2021;10(7).

7. Treff NR, Zimmerman R, Bechor E, Hsu J, Rana B, Jensen J, et al. Validation of concurrent preimplantation genetic testing for polygenic and monogenic disorders, structural rearrangements, and whole and segmental chromosome aneuploidy with a single universal platform. Eur J Med Genet 2019;62(8):103647.

8. Huang L, Ma F, Chapman A, Lu S, Xie XS. Single-Cell Whole-Genome Amplification and Sequencing: Methodology and Applications. Annu Rev Genomics Hum Genet 2015;16(1):79–102.

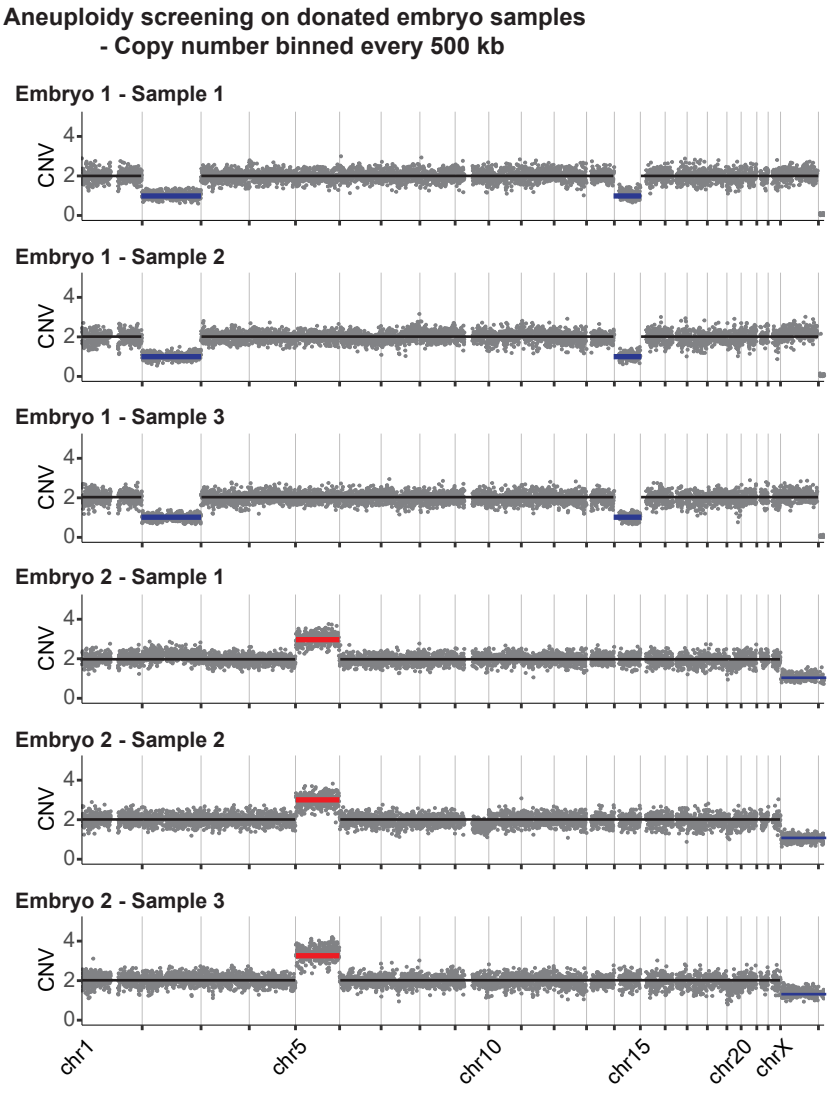
9. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in Medicine 2015;17(5):405–23.

10. Miller DT, Lee K, Chung WK, Gordon AS, Herman GE, Klein TE, et al. ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genetics in Medicine 2021;23(8):1381–90.

11. Khera A V, Chaffin M, Aragam KG, Haas ME, Roselli C, Choi SH, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. Nat Genet 2018;50(9):1219–24.

12. Si Y, Vanderwerff B, Zöllner S. Why are rare variants hard to impute? Coalescent models reveal theoretical limits in existing algorithms. Genetics 2021;217(4):iyab011.

Figure 1


Aneuploidy screening on donated embryo samples
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Embryo 1 - Sample 1
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Embryo 1 - Sample 3
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Embryo 2 - Sample 2
Embryo 2 - Sample 3
CNV
0
2
4
chr1
chr5
chr10
chr15
chr20
chrX

Table 1

Sample	Genomic Coverage	Total SNV	Accuracy	Specificity	Precision	Sensitivity
1	99.7%	3343804	99.995%	99.997%	97.8%	98.4%
2	99.6%	3327631	99.995%	99.997%	98.0%	98.1%
3	99.6%	3312139	99.995%	99.997%	97.9%	98.1%
4	99.4%	3279468	99.993%	99.997%	97.8%	97.1%
5	99.6%	3404155	99.995%	99.998%	98.4%	98.1%
6	99.7%	3414277	99.996%	99.998%	98.4%	98.4%
7	99.6%	3360682	99.995%	99.998%	98.4%	98.1%
8	99.6%	3163591	99.995%	99.998%	98.2%	98.2%
9	99.6%	3160113	99.995%	99.998%	98.3%	98.2%
10	99.6%	3304310	99.994%	99.996%	97.2%	98.0%
11	99.7%	3301716	99.994%	99.997%	97.5%	98.2%
12	99.7%	3368834	99.996%	99.998%	98.6%	98.2%
13	99.7%	3340483	99.995%	99.998%	98.8%	97.5%
14	99.6%	3270893	99.995%	99.997%	97.5%	98.4%
15	99.5%	3262566	99.995%	99.997%	97.6%	98.3%
16	99.6%	3274334	99.995%	99.997%	97.8%	98.2%
17	99.6%	3275227	99.995%	99.997%	97.7%	98.2%
18	99.6%	3254447	99.995%	99.998%	98.1%	98.4%
19	99.4%	3234717	99.995%	99.998%	98.2%	97.9%
20	99.7%	3274002	99.995%	99.997%	97.9%	98.5%
21	99.5%	3257361	99.995%	99.998%	98.1%	98.2%
22	99.6%	3270572	99.995%	99.998%	98.2%	97.9%
23	99.7%	3284833	99.995%	99.998%	98.2%	98.3%
24	99.6%	3237713	99.993%	99.997%	97.9%	97.0%
25	99.7%	3382545	99.996%	99.998%	98.3%	98.6%
Average	**99.6%**	**3294417**	**99.995%**	**99.997%**	**98.0%**	**98.1%**

Table 2

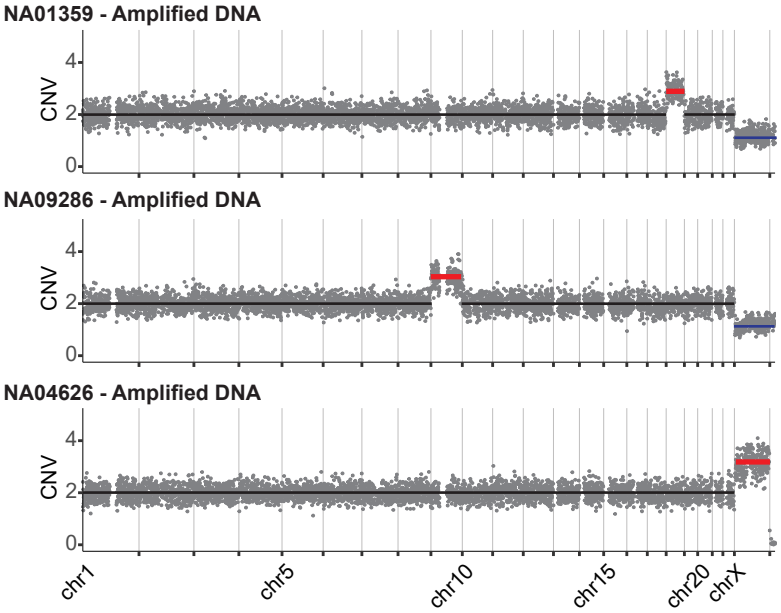
Family	Sample	Previous PGT-M Reports	Gene	Disease	Detected in Orchid WGS?
Family 1	Sample 1	c.2279G>A	TGM1	Lamellar Ichthyosis	Yes
	Sample 2	c.2279G>A	TGM1	Lamellar Ichthyosis	Yes
	Sample 3	c.2279G>A	TGM1	Lamellar Ichthyosis	Yes
	Sample 4	c.2279G>A	TGM1	Lamellar Ichthyosis	Yes
	Sample 5	c.2279G>A	TGM1	Lamellar Ichthyosis	Yes
	Sample 6	c.2279G>A	TGM1	Lamellar Ichthyosis	Yes
	Sample 7	Negative			No
	Sample 8	Negative			No
	Sample 9	Negative			No
Family 2	Sample 10	c.901-1G>A	ABCD1	X-linked Adrenoleukodystrophy	Yes
	Sample 11	c.901-1G>A	ABCD1	X-linked Adrenoleukodystrophy	Yes
	Sample 12	c.901-1G>A	ABCD1	X-linked Adrenoleukodystrophy	Yes
	Sample 13	c.901-1G>A	ABCD1	X-linked Adrenoleukodystrophy	Yes
	Sample 14	c.901-1G>A	ABCD1	X-linked Adrenoleukodystrophy	Yes
	Sample 15	Negative			No
	Sample 16	Negative			No
	Sample 17	c.901-1G>A	ABCD1	X-linked Adrenoleukodystrophy	Yes
	Sample 18	c.901-1G>A	ABCD1	X-linked Adrenoleukodystrophy	Yes
	Sample 19	c.901-1G>A	ABCD1	X-linked Adrenoleukodystrophy	Yes
	Sample 20	c.901-1G>A	ABCD1	X-linked Adrenoleukodystrophy	Yes
	Sample 21	c.901-1G>A	ABCD1	X-linked Adrenoleukodystrophy	Yes

Table 3

Sample	Variants Reported in Coriell	Gene	Disease	Detected in Orchid WGS?
NA09301	NM_003640.5:c.2204+6T>C	ELP1	Dysautonomia	Yes
NA19977	NM_022455.5:c.6450dup(p.Lys2151GlnfsTer15)	NSD1	Sotos Syndrome	Yes
NA07500	NM_000038.6:c.4612_4613del(p.Glu1538IlefsTer5)	APC	Familial Adenomatous Polyposis	Yes
NA14090	NM_007300.4:c.68_69del(p.Glu23ValfsTer17)	BRCA1	Hereditary Breast and Ovarian Cancer	Yes
NA14170	NM_000059.4:c.5946del(p.Ser1982ArgfsTer22)	BRCA2	Hereditary Breast and Ovarian Cancer	Yes
NA13715	NM_007300.4:c.5329dup(p.Gln1777ProfsTer74)	BRCA1	Hereditary Breast and Ovarian Cancer	Yes
NA00998	NM_000492.4:c.1521_1523del(p.Phe508del)	CFTR	Cystic Fibrosis	Yes
NA13591	NM_000492.4:c.1521_1523del(p.Phe508del) NM_000492.4:c.350G>A(p.Arg117His) NM_000410.4:c.187C>G(p.His63Asp) NM_005957.5:c.665C>T(p.Ala222Val)	CFTR CFTR HFE MTHFR	Cystic Fibrosis, Hereditary Hemochromotosis, Homocystinuria (MTHFR related)	Yes
NA10080	NM_000314.8:c.781C>T(p.Gln261Ter)	PTEN	PTEN hamartoma tumor syndrome	Yes
NA13326	NM_000051.4:c.1179_1180del(p.Trp393Ter) NM_000051.4:c.4396C>T(p.Arg1466Ter) NM_024675.4:c.3508C>T(p.His1170Tyr) NM_000535.7:c.88C>A(p.Gln30Lys)	ATM ATM PALB2 PMS2	Ataxia Telangiectasia, Lynch Syndrome	Yes
NA12932	NM_001079804.3:c.1441T>C(p.Trp481Arg) NM_001079804.3:c.1326+1G>A	GAA GAA	Glycogen storage disease Type 2	Yes
NA14646	NM_000410.4:c.845G>A(p.Cys282Tyr)	HFE	Hereditary Hemochromotosis	Yes
NA22752	NM_000090.4:c.636+5G>A	COL3A1	Ehlers-Danlos syndrome	Yes
NA00325	NM_000402.4:c.653C>T(p.Ser218Phe)	G6PD	G6PD Deficiency	Yes
NA12878	NM_000769.4:c.681G>A(p.Pro227=)	CYP2C19	Clopidogrel resistance	Yes
NA11319	NM_001127328.2:c.178G>C(p.Ala60Pro)	ACADM	Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCAD)	Yes
NA07859	NM_000492.4:c.3302T>A(p.Met1101Lys)	CFTR	Cystic Fibrosis	Yes
NA07415	NM_000368.5:c.993_994insA(p.Ser332IlefsTer9)	TSC1	Tuberous Sclerosis Complex	Yes
NA21976	NM_000138.5:c.3444dup(p.Asn1149GlnfsTer10)	FBN1	Marfan Syndrome	Yes
NA11906	MT:8344 A⇒G		MT-TK (myoclonic epilepsy with ragged-red fibers)	Yes
NA04368	ENST00000361381.2:c.1019G>A(p.Arg340His)	MT-ND4	Leber Hereditary Optic Neuropathy	Yes
NA13741	ENST00000361899.2:c.467T>G(p.Leu156Arg)	MT-ATP6	Mitochdonrial Complex V Deficiency	Yes
NA10742	ENST00000361381.2:c.1019G>A(p.Arg340His)	MT-ND4	Leber Hereditary Optic Neuropathy	Yes

Figure S1

A) **Aneuploidy screening on cell lines**
- Copy number binned every 500 kb

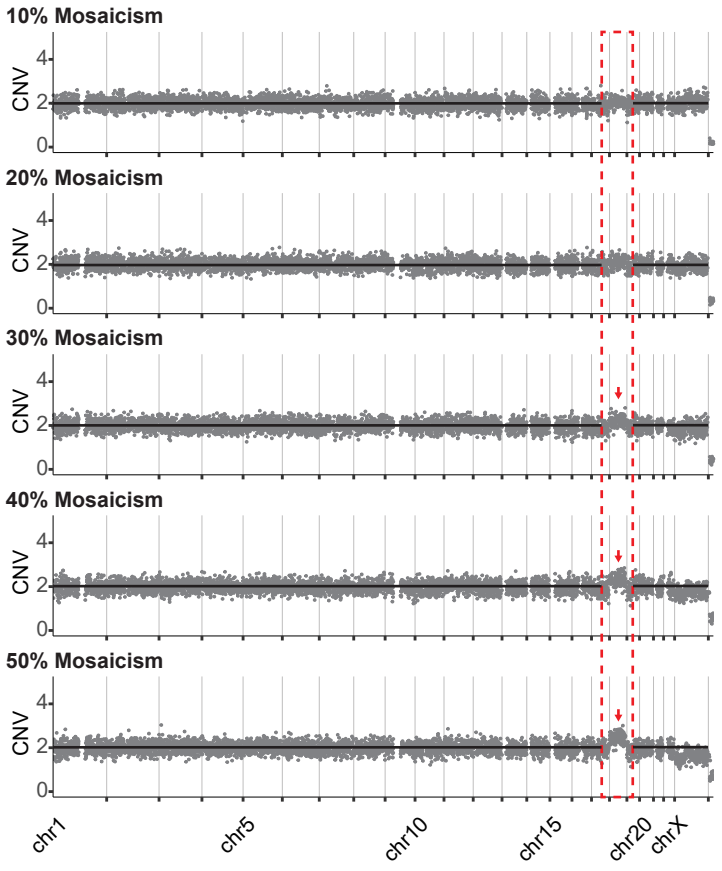


B)

Coriell Cell line ID	# tested	# with result	Coriell Karyotype	Accuracy
NA12878	15	15	46XX	100%
NA24385	5	5	46XY	100%
NA19240	7	7	46XX	100%
NA01359	13	13	47XY,+18	100%
NA09286	13	13	47XY,+9	100%
NA04626	6	6	47XXX	100%
NA24149	10	10	46XY	100%
NA24146	10	10	46XX	100%
Total	73	73		>99.9%

Figure S2

A) Aneuploidy screening on mosaic samples
- Copy number binned every 500 kb



B)

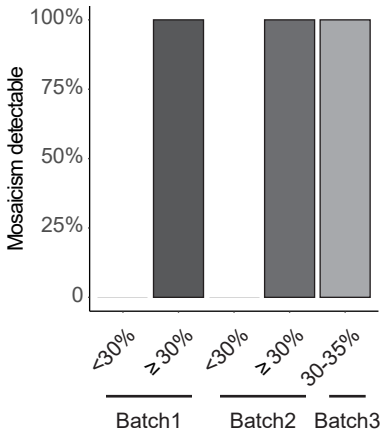


Figure S3

UPD of chr8 in a male sample
Whole genome screening on amplied DNA

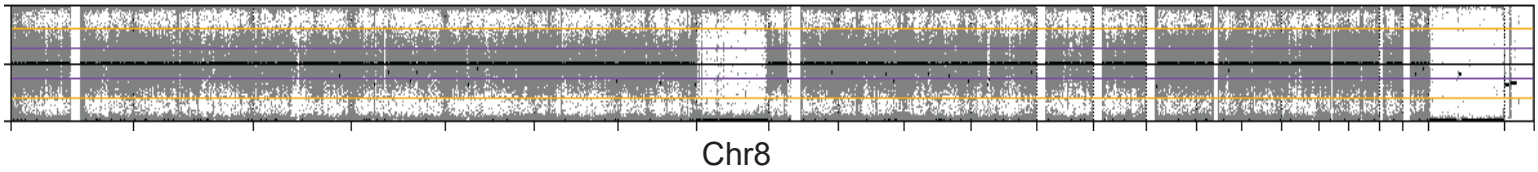


Table S1

Sample ID	Original Report Date	Original PGT results	Orchid Collection Date	Orchid Blind Call	Sample ID	riginal Report Dat	Original PGT results	chid Collection Da	Orchid Blind Call
1	2/20/2016	XY, +8	11/11/2021	XY, +8	56	6/22/2017	XX, -2	1/10/2022	XX, -2p
2	2/20/2016	XY, +8	11/11/2021	XY, +8	57	6/22/2017	XX, -2	1/10/2022	XX, -2
3	2/20/2016	XY, +8	11/11/2021	XY, +8	58	7/23/2018	XY, -18, +22	2/2/2022	XY, -18, +22
4	2/20/2016	XX, +4, -10, -18	11/11/2021	XX, +4, -10, -18	59	7/23/2018	XY, -18, +22	2/2/2022	XY, -18, +22
5	2/20/2016	XX, +4, -10, -18	11/11/2021	XX, +4, -10, -18	60	12/18/2017	XX, -2, -14	2/2/2022	XX, -2, -14
6	2/20/2016	XX, +4, -10, -18	11/11/2021	XX, +4, -10, -18	61	12/18/2017	XX, -2, -14	2/2/2022	XX, -2, -14
7	2/20/2016	XY, +15	11/11/2021	XY, +15	62	12/18/2017	XX, -2, -14	2/2/2022	XX, -2, -14
8	2/20/2016	XY, +15	11/11/2021	XY, +15	63	12/18/2017	XY, +5	2/2/2022	XY, +5
9	2/20/2016	XY, +15	11/11/2021	XY, +15	64	12/18/2017	XY, +5	2/2/2022	XY, +5
10	2/20/2016	XX, -20	11/11/2021	XX, -20	65	12/18/2017	XY, +5	2/2/2022	XY, +5
11	2/20/2016	XX, -20	11/11/2021	Indeterminate	66	12/17/2015	XX	2/2/2022	XX
12	2/20/2016	XX, -20	11/11/2021	XX, -20	67	12/17/2015	XX	2/2/2022	XX
13	2/20/2016	XY, -13, -16	11/11/2021	XY, -13, -16	68	12/17/2015	XX	2/2/2022	XX
14	2/20/2016	XY, -13, -16	11/11/2021	XY, -13, -16	69	12/17/2015	XX, -16	2/2/2022	XX, -16
15	2/20/2016	XY, -13, -16	11/11/2021	XY, -13, -16	70	12/17/2015	XX, -16	2/2/2022	XX, -16
16	2/20/2016	XX, -21	11/11/2021	XX, -21	71	12/17/2015	XX, -16	2/2/2022	XX, -16
17	2/20/2016	XX, -21	11/11/2021	XX, -21	72	6/8/16	XY, -16	2/14/2022	XY, -16
18	2/20/2016	XX, -21	11/11/2021	XX, -21	73	6/8/16	XY, -16	2/14/2022	XY, -16
19	2/20/2016	XY, +7, -15	11/11/2021	XY, +7, -15	74	6/8/16	XY, -16	2/14/2022	XY, -16
20	2/20/2016	XY, +7, -15	11/11/2021	XY, +7, -15	75	10/18/21	XX	2/14/2022	XX
21	2/20/2016	XY, +7, -15	11/11/2021	XY, +7, -15	76	10/19/21	XX	2/14/2022	XX
22	7/4/2013	XY	12/6/2021	XY	77	10/20/21	XX	2/14/2022	XX
23	7/4/2013	XY	12/6/2021	XY	78	11/9/2018	46, No sex reported	8/10/2022	XX
24	7/4/2013	XY	12/6/2021	XY	79	11/9/2018	46, No sex reported	8/10/2022	XX
25	7/4/2013	XX	12/6/2021	XX	80	11/9/2018	46, No sex reported	8/10/2022	XX
26	7/4/2013	XX	12/6/2021	Indeterminate	81	11/9/2018	46, No sex reported	8/10/2022	XY
27	7/4/2013	XX	12/6/2021	XX	82	11/9/2018	46, No sex reported	8/10/2022	XY
28	7/4/2013	XY, -5, +18	12/6/2021	XY, -5	83	11/9/2018	46, No sex reported	8/10/2022	XY
29	7/4/2013	XY, -5, +18	12/6/2021	XY, -5	84	11/9/2018	46, No sex reported	8/10/2022	XX
30	7/4/2013	XY, -5, +18	12/6/2021	XY, -5	85	11/9/2018	46, No sex reported	8/10/2022	XX
31	7/4/2013	XY, +2, +22	12/6/2021	XY, -2, +22	86	11/9/2018	46, No sex reported	8/10/2022	XX
32	7/4/2013	XY, +2, +22	12/6/2021	XY, -2, +22	87	11/9/2018	46, No sex reported	8/10/2022	XX
33	7/4/2013	XY, +2, +22	12/6/2021	XY, -2, +22	88	11/9/2018	46, No sex reported	8/10/2022	XX
34	7/4/2013	XX, +9, +13, +21	12/6/2021	XY, +9, +13, +21	89	11/9/2018	46, No sex reported	8/10/2022	XX
35	7/4/2013	XX, +9, +13, +21	12/6/2021	XY, +9, +13, +21	90	11/9/2018	46, No sex reported	8/10/2022	XY
36	7/4/2013	XX, +9, +13, +21	12/6/2021	XY, +9, +13, +21	91	11/9/2018	46, No sex reported	8/10/2022	XY
37	7/4/2013	XY	12/6/2021	XY	92	11/9/2018	46, No sex reported	8/10/2022	XY
38	7/4/2013	XY	12/6/2021	XY	93	11/9/2018	46, No sex reported	8/10/2022	XX
39	7/4/2013	XY	12/6/2021	XY	94	11/9/2018	46, No sex reported	8/10/2022	XX
40	9/26/2016	XX	12/6/2021	XX	95	11/9/2018	46, No sex reported	8/10/2022	XX
41	9/26/2016	XX	12/6/2021	XX	96	11/17/2017	XY	8/10/2022	XY
42	9/26/2016	XX	12/6/2021	XX	97	11/17/2017	XY	8/10/2022	XY
43	10/5/2020	XX, -3, +15, +20, +21, +22	12/6/2021	XX, -3, +15, +20, 21, 22	98	11/17/2017	XY	8/10/2022	XY
44	10/5/2020	XX, -3, +15, +20, +21, +22	12/6/2021	XX, -3, +15, +20, 21, 22	99	11/17/2017	XY	8/10/2022	XY
45	10/5/2020	XX, -3, +15, +20, +21, +22	12/6/2021	XX, -3, +15, +20, 21, 22	100	11/17/2017	XY	8/10/2022	XY
46	4/25/2017	XY	1/10/2022	XY	101	11/17/2017	XY	8/10/2022	XY
47	4/25/2017	XY	1/10/2022	XY	102	11/17/2017	XX, +22	8/10/2022	XX, +22
48	4/25/2017	XY	1/10/2022	XY	103	11/17/2017	XX, +22	8/10/2022	XX, +22
49	4/25/2017	XY	1/10/2022	XY	104	9/6/2017	XX, +19	8/10/2022	XX, +19 mosaic
50	4/25/2017	XY	1/10/2022	XY	105	9/6/2017	XX, +19	8/10/2022	XX, +19
51	4/25/2017	XY	1/10/2022	XY	106	10/12/2022	XX, -18	12/8/2022	XX, -18
52	6/22/2017	XX, -16	1/10/2022	XX, -16	107	10/12/2022	XX, -18	12/8/2022	XX, -18
53	6/22/2017	XX, -16	1/10/2022	XX, -16	108	10/12/2022	XX, -18	12/8/2022	XX, -18
54	6/22/2017	XX, -16	1/10/2022	XX, -16	109	12/8/2020	XY	12/8/2022	XY
55	6/22/2017	XX, -2	1/10/2022	XX, -2p	110	12/8/2020	XY	12/8/2022	XY

Table S2

Sample	Genomic Coverage	Total SNV	Accuracy	Specificity	Precision	Sensitivity
NA12878 gDNA Sample 1	99.8%	3286979	99.996%	99.997%	97.8%	98.8%
NA12878 gDNA Sample 2	99.9%	3290927	99.996%	99.997%	97.8%	99.0%
NA12878 gDNA Sample 3	99.8%	3286497	99.996%	99.997%	97.8%	98.8%
NA12878 gDNA Sample 4	99.9%	3287953	99.996%	99.997%	97.9%	99.0%
NA12878 gDNA Sample 5	99.8%	3277286	99.996%	99.997%	98.0%	98.8%
Average	**99.8%**	**3285928**	**99.996%**	**99.997%**	**97.8%**	**98.9%**
NA12878 Amplified DNA Sample 1	99.7%	3238978	99.994%	99.997%	97.9%	97.6%
NA12878 Amplified DNA Sample 2	99.8%	3263126	99.995%	99.997%	98.0%	98.4%
NA12878 Amplified DNA Sample 3	99.8%	3252094	99.995%	99.998%	98.2%	98.3%
NA12878 Amplified DNA Sample 4	99.8%	3258943	99.996%	99.998%	98.2%	98.4%
NA12878 Amplified DNA Sample 5	99.7%	3229761	99.994%	99.997%	97.9%	97.3%
Average	**99.8%**	**3248580**	**99.995%**	**99.997%**	**98.1%**	**98.0%**

Table S3

Sample	True Positives	False Positives	True Negatives	False Negatives
NA12878 gDNA Sample 1	3214156	73257	2498498635	37667
NA12878 gDNA Sample 2	3218277	73072	2498498820	33546
NA12878 gDNA Sample 3	3214192	72753	2498499139	37631
NA12878 gDNA Sample 4	3218242	70121	2498501771	33581
NA12878 gDNA Sample 5	3212093	65599	2498506293	39730
Average	**3215392**	**70960**	**2498500932**	**36431**
NA12878 Amplified DNA Sample 1	3172496	67132	2498504760	79327
NA12878 Amplified DNA Sample 2	3199885	63958	2498507934	51938
NA12878 Amplified DNA Sample 3	3194879	57880	2498514012	56944
NA12878 Amplified DNA Sample 4	3200575	59098	2498512794	51248
NA12878 Amplified DNA Sample 5	3162468	67937	2498503955	89355
Average	**3186060**	**63201**	**2498508691**	**65763**

Table S4

Sample	True Positives	False Positives	True Negatives	False Negatives
1	3270826	72978	2498427158	52753
2	3260902	66729	2498433445	62639
3	3241275	70864	2498447239	64337
4	3208229	71239	2498446853	97394
5	3348398	55757	2498354672	64888
6	3358076	56201	2498354258	55180
7	3307540	53142	2498399224	63809
8	3106492	57099	2498602671	57453
9	3105403	54710	2498605123	58479
10	3213236	91074	2498453268	66137
11	3218889	82827	2498461470	60529
12	3322745	46089	2498393320	61561
13	3299943	40540	2498398825	84407
14	3187551	83342	2498499668	53154
15	3184474	78092	2498504866	56283
16	3201676	72658	2498492003	57378
17	3201352	73875	2498490706	57782
18	3192477	61970	2498516295	52973
19	3175772	58945	2498519275	69723
20	3205470	68532	2498499144	50569
21	3196791	60570	2498507126	59228
22	3212393	58179	2498482967	70176
23	3225625	59208	2498481928	56954
24	3169496	68217	2498487962	98040
25	3324367	58178	2498394241	46929
Average	**3229576**	**64841**	**2498466148**	**63150**

Table S5

Sample	Total Bases	True Positive	True Negative	False Positive	False Negative	Accuracy	Specificity	Sensitivity	Precision
1	16569	19	16550	0	0	100.000%	100.0%	100.0%	100.0%
2	16569	19	16550	0	0	100.000%	100.0%	100.0%	100.0%
3	16569	21	16548	0	0	100.000%	100.0%	100.0%	100.0%
4	16569	21	16548	0	0	100.000%	100.0%	100.0%	100.0%
5	16569	32	16537	0	0	100.000%	100.0%	100.0%	100.0%
6	16569	32	16537	0	0	100.000%	100.0%	100.0%	100.0%
7	16569	32	16537	0	0	100.000%	100.0%	100.0%	100.0%
8	16569	25	16544	0	0	100.000%	100.0%	100.0%	100.0%
9	16569	21	16548	0	4	99.976%	100.0%	84.0%	100.0%
10	16569	25	16544	0	0	100.000%	100.0%	100.0%	100.0%
11	16569	25	16544	0	0	100.000%	100.0%	100.0%	100.0%
12	16569	51	16518	0	0	100.000%	100.0%	100.0%	100.0%
13	16569	50	16519	0	1	99.994%	100.0%	98.0%	100.0%
14	16569	6	16563	0	0	100.000%	100.0%	100.0%	100.0%
15	16569	6	16563	0	0	100.000%	100.0%	100.0%	100.0%
16	16569	6	16563	0	0	100.000%	100.0%	100.0%	100.0%
17	16569	6	16563	0	0	100.000%	100.0%	100.0%	100.0%
18	16569	6	16563	0	0	100.000%	100.0%	100.0%	100.0%
19	16569	6	16563	0	0	100.000%	100.0%	100.0%	100.0%
20	16569	25	16544	0	0	100.000%	100.0%	100.0%	100.0%
21	16569	25	16544	0	0	100.000%	100.0%	100.0%	100.0%
22	16569	24	16545	0	1	99.994%	100.0%	96.0%	100.0%
23	16569	25	16544	0	0	100.000%	100.0%	100.0%	100.0%
24	16569	29	16540	0	1	99.994%	100.0%	96.7%	100.0%
25	16569	32	16537	0	0	100.000%	100.0%	100.0%	100.0%
Average	16569	22.09	16,546.91	0.00	0.26	99.998%	>99.99%	99.0%	>99.99%

Table S6

Sample	Chromosome Abornalities	Father Carrier: HOGA1 c.700+5G>T	Mother Carrier: HOGA1 c.110G>T	Genetic disease status	Available for Transfer
Sample 1	Not detected	Positive	Positive	Compount Het.	No
Sample 2	Not detected	Positive	Positive	Compount Het.	No
Sample 3	6p25.3p22.3(1-20560000)X3	Positive	Negative	Carrier	No
Sample 4	Not detected	Negative	Negative	Non-carrier	Yes

Table S7

Sample	Coriell Reports	Category	Size	Disease	Detectable in Orchid WGS?
NA05875	16p12.1p11.2(27,203,030-32,180,964)x1	Segmental	5Mb		Yes
NA06228	11q23.3q25(116188703-134449982)x3 22q11.1q11.21(14435170-18676540)x3	Segmental	19Mb 4Mb		Yes Yes
NA07736	11p14.1p13(28229986-34925688)x1	Segmental	7Mb		Yes
NA10013	69,XXX	Triploid	>20Mb		Yes
NA10995	1q31.3q44(192686117-247190999)x3	Segmental	>20Mb		Yes
NA14043	2p25.3p25.1(5702-9484791)x2 hmz 2q22.3q32.3(144864227-195289707)x2 hmz 12q14.1q14.3(56656199-63469126)x2 hmz	UPD	>20Mb		Yes Yes Yes
NA14117	5p15.33p14.3(68519-22367289)x1	Segmental	20Mb	Cri du chat syndrome	Yes
NA14184	7q11.23(72297530-73780028)x1	Segmental	1.5Mb	Williams-Beuren syndrome	Yes
NA15603	8p23.3p23.1(161,221-6,815,328)hmz 8p23.1p11.21(8,087,143-42,858,299)hmz	UPD	>20Mb		Yes
NA17942	22q11.21(18748427-21611337)x1	Segmental	2Mb	DiGeorge syndrome	Yes
NA20201	15q11.1q13.3(18276328-30630402)x4	Segmental	10Mb		Yes
NA20408	Prader-Willi syndrome (Region not specified)	UPD	>20Mb	Prader-Willi syndrome	Yes
NA22397	15q11.2q13.1(21245026-26500067)x3	Segmental	6Mb		Yes
NA22569	1p36.33p36.31(615320-6255256)x1	Segmental	6Mb	1p36 deletion syndrome	Yes