Supplementary Information

Clinical-grade whole genome sequencing-based haplarithmisis enables all forms of preimplantation genetic testing

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Supplementary Note

Mendelian inconsistency

Mendelian inconsistencies can be defined as the variant calls that are inconsistent with Mendelian inheritance rules. Mendelian inconsistency could reflect true genomic variation as a result from de novo mutations. However, it is more likely to be caused by amplification errors, sequencing errors, or genotype calling errors. The Mendelian inconsistency rate is the proportion of variant calls that violate mendelian inheritance patterns of the total number of variant calls. Rules of Mendelian inheritance violation patterns are shown in the table below. A refers to the referent allele, B refers to the alternative allele, NC: No Call

Embryo	Father	Mother
BB	AB or BA	AA
AA	AB or BA	BB
BB	AA	AB or BA
AA	BB	AB or BA
AA or BB	AA	BB
AA or BB	BB	AA
AA	NC	BB
BB	NC	AA
AA	BB	NC
BB	AA	NC
Is not AA, or is not NC	AA	AA
Is not BB, or is not NC	BB	BB

As we described in the manuscript, we used ≥ 80 informative SNPs upstream and ≥ 80 informative SNPs downstream of the ROI to perform diagnosis. With a 0.04 chance of misdiagnosis per SNP-call, the probability of observing a misdiagnosis in 80% of the informative SNP-calls (which is 64 out of 80) using the binomial distribution is very close to zero ($P = 4.77 \times 10^{-74}$). Specially, the binomial distribution counts the number of successes in a fixed number of independent Bernoulli trials, where each trial has only two possible outcomes (informative SNP-calls supporting the normal haplotype or informative SNP-calls supporting the affected haplotype).

The binomial probability formula is:

$$P(X = k) = \binom{n}{k} p^k (1-p)^{n-k}$$

Where:

- n is the number of trials (80 informative SNP-calls);
- k is the number of successes, which is 80% of 80 (= 64);

- p is the probability of success on a single trial (~0.04).

Detailed description of Parents-only haplarithmisis for PGT-AO

Haplarithmisis is a conceptual workflow that enables simultaneous genome wide haplotyping and copy number typing using genotyping information from offspring (embryos), parents and a close-relative such as grandparents or a sibling[1]. To validate parents-only haplotyping and its determination of segregational origin, (i) the genotype of close-relatives was used as a seed for phasing and the segregational origin of copy number gains was determined, (ii) the genotype of the embryo itself was used as a seed for phasing and segregational origin of the same aberrations was determined (Fig. 3a).

Using conventional close-relative phasing, the segregational origin of aberrations can be determined by interpreting the parity and reciprocity of features for detection of segregational origin as described previously in detail[1] (Fig. 3a). When a copy number increase involves a combination of different homologous chromosomes of one parent, this constitutes a meiotic error. If the centromeric regions originate from different homologues (e.g. maternal H1 and maternal H2), this indicates a meiotic I error. Alternatively, if the centromeric regions indicate the same homologue (e.g. maternal H1 and maternal H1), this indicates a meiotic II error. In contrast to meiotic errors, mitotic errors consist of an exact duplication of a single homologue. Distinguishing between meiotic II and mitotic error is only possible when a crossover event has occurred in the aberrant chromosome. A paternal trisomy involves two paternal and one maternal chromosome, while a maternal trisomy has two maternal and one paternal chromosome. Conversely, a paternal monosomy retains the paternal chromosome and expels the maternal one, and a maternal monosomy retains the maternal chromosome while expelling the paternal one.

For parents-only haplotyping, the same ruleset applies. However, the phasing is now limited to only include parental inheritance (paternal or maternal), instead of specific parental homologue inheritance (Paternal H1 or H2, maternal H1 or H2). Therefore, haplarithmisis categorises the parental chromosomal constitution (i.e., $1_{paternal}$:$2_{maternal}$ allelic ratio) but cannot further categorise P1, P2, M1, and M2 B-allele frequencies (BAFs) as the specific parental homologue information is missing. Thus, in case of a maternal meiotic trisomy the segmented M1 BAFs (blue lines maternal haplarithm) will remain at a value of 0 instead of a value of 0.33 as compared to close-relative phasing. The segmented M2 BAFs (red lines maternal haplarithm) are consistent with segmented M2 BAFs of close-relative phasing. In case of a maternal mitotic trisomy, a crossover event is not recognised in parents-only phasing, placing the segmented M1 and M2 BAFs at 0 and 0.33 respectively. Importantly, the inconsistencies of segmented M1 and P1 BAFs between parents-only and close-relative phasing do not hamper determination of segregational origin, since segmented M2 and P2 BAFs are sufficient for this purpose.

Supplementary Tables

Supplementary Table 1 | Genetic Indications of families

Family	PGT Type	Indication	Inheritance Pattern	Carrier	Genetic Interval a	Genetic Interval b	Speciality
Family 1	PGT-M	TGM1	AR	both	chr14:24259813-24259814		Double indication (PGT-M + PGT-SR)
	PGT-SR	t(8;16)(p21.3;q24)	NA	father			
Family 2	PGT-M	MEFV	AR	both	chr16:3243407-3243408	chr16:3243310-3243311	Double indication (PGT-M + PGT-M), consanguineous
	PGT-M	DMD	X-R	both	chrX:31657989-31836820		
Family 3	PGT-M	ARSA			chr22:50626228-50626229		Consanguineous, telomere
Family 4	PGT-M	BRCA1	AD		chr17:43094569-43094570		Double indication (PGT-M + PGT-M)
	PGT-M	OCA2	AR	both	chr15:28081711-28081712	chr15:27985101-27985102	
Family 5	PGT-M	FSHD	AD		chr4:189518883-189519279		Telomeric and homology to region on chr10
Family 6	PGT-M	D2HGDH	AR	both	chr2:241743754-241743755		Consanguineous
Family 7	PGT-M	PLN	AD		chr6:118558961-118558963		Double indication (PGT-M + PGT-M)
	PGT-M	DMD	X-R	both	chrX:32644132-32645152		
Family 8	PGT-M	GNAS	AD		chr20:58910751-58910752		Recombination in maternal haplotype
Family 9	PGT-M	BSCL2	AD		chr11:62702493-62702494		Double indication (PGT-M + PGT-SR)
	PGT-SR	t(2;11)(q35;q23)	NA	mother			
Family 10	PGT-M	BOR1	AD		chr8:71215510-71215511		Recombination in maternal haplotype in embryo
Family 11	PGT-M	SCN9A	AD		chr2:166195187-166375987		Paternal mitotic trisomy in chromosome of interest for one selected embryo
Family 12	PGT-M	NF1	AD		chr17:31261761-31261762		Direct mutation possible?
Family 13	PGT-M	TMEM67	AR	both	chr8:93786253-93786254	chr8:93755039-93755044	Recombination in paternal haplotype in embryo
Family 14	PGT-M	TSC2	AD		chr16:2080365-2080366		Meiotic trisomy in selected embryo
Family 15	PGT-M	HemA	X-R	both	chrX:155022473-155022474		Telomeric
Family 16	PGT-M	GJB2	X-R	both	chr13:20223037-20565037	chr13:20189547-20189548	Recombination in maternal and paternal haplotype
Family 17	PGT-M	SLS	AR	both	chr17:19543703-19751473		Centromeric
Family 18	PGT-M	SHFM3	AD		chr10:101227249-101624830		Mitotic trisomy in selected embryo
Family 19	PGT-SR	t(3;13)(q27;q33)	-	father			Double indication (PGT-SR + PGT-SR), without close relative
	PGT-SR	ins(10;7)(p11.2;q32.1q36.2)	-	father			
Family 20	PGT-MT	MELAS	-	mother	chrMT: m.3243 A > G		mitochondrial indication
Family 21	PGT-MT	MELAS	-	mother	chrMT: m.3243 A > G		mitochondrial indication

Supplementary Table 2 | Assessment criteria for key parameters for GBS-PGT and WGS-PGT.

Since the data generated with WGS has a higher resolution, the assessment criteria thresholds are more stringent than for GBS. The assessment criteria are subdivided for a preparatory analysis and an embryo analysis. A preparatory test is performed to predict whether the sequencing data from parents and close relative(s) have sufficient quality to make a diagnosis. If the preparatory test passes the threshold, the couple can proceed with PGT. The ROI is defined as 2Mb up- and downstream of the genomic location of the indication. WGS: whole genome sequencing; GBS: genotyping-by-sequencing; PGT: preimplantation genetic testing; ROI: region of interest.

Guideline	**GBS-PGT**	**WGS-PGT**
Preparatory analysis		
Total number of reads	≥ 26.000.000*	≥ 160.000.000*
Mendelian inconsistency rate		≤ 2%
Informative SNPs		
Number of genome-wide informative SNP positions	≥ 45.000	≥ 600.000
Density at ROI	≥ 4/Mb	≥ 40/Mb
Embryo analysis		
Total number of reads embryo		≥ 90.000.000
Mendelian inconsistency rate	≤ 15%	≤ 6%
Distance ROI to homologous recombination event	≥ 1 Mb	
Haplotyping criteria		
Number and fraction concordant SNPs between ROI and homologous recombination	≥ 10 AB SNP's ≥ 90% Conc.	≥ 10 AB SNP's ≥ 90% Conc.
Concordant SNPs in the region of ROI ± 2 Mb	≥ 80%	≥ 80%
Concordant SNPs in the region of ROI + 2Mb	≥ 70%, **and** ± 2 Mb ≥ 80%	≥ 70%, **and** ± 2 Mb ≥ 80%
Concordant SNPs in the region of ROI – 2Mb	≥ 70%, **and** ± 2 Mb ≥ 80%	≥ 70%, **and** ± 2 Mb ≥ 80%
Number of consecutive correct or discordant SNPs < 10 concordant SNPs away from the ROI	≤ 5 SNPs and ≤ 100 kb	

*if the number of reads in the preparatory preimplantation genetic test does not meet the specified assessment criteria thresholds, and is lower than the recommended number of 160,000,000 but the SNP density within ±2MB around ROI meets the criteria of ≥40/Mb, then the preparatory test can still be approved. Moreover, close-relatives

should include both grandparents, the gene should not be located in a telomeric/centromeric region, and the gene should not be located in a “difficult-to-analyse” region such as homologous region or a region with pseudogenes.

Supplementary Table 3 | Chromosomal abnormalities with parental and segregational origin and degree of mosaicism (%) determined by haplarithmisis

Family	Embryo	Aberration*	chr	Parental origin	Segregational origin	Degree of mosaicism
Family 1	1	Trisomy	2	Paternal	Mitosis	30%
	4	Monosomy	19	Maternal	ND	100%
Family 4	11	Monosomy	18	Maternal	ND	45%
Family 7	18	Trisomy	10	Maternal	ND	25%
Family 8	22	Trisomy	16	Maternal	Meiosis I	90%
		Trisomy	21	Maternal	Meiosis II	100%
Family 9	25	Monosomy	2	Paternal	ND	100%
		Trisomy	11	Maternal	Meiosis II	100%
Family 11	28	Trisomy	2	Paternal	Mitosis	100%
		Monosomy	8	Maternal	ND	100%
		Trisomy	14	Paternal	Meiosis I	100%
Family 12	30	Trisomy	16	Maternal	Meiosis I	100%
Family 14	32	Trisomy	4	Maternal	Meiosis I	80%
		Trisomy	16	Maternal	Meiosis I	100%
Family 17	35	Monosomy	19	Paternal	ND	100%
Family 18	37	Triploidy	All (except 15)	Maternal	Meiosis II	100%
		Disomy/trisomy	15	-	-	50%

Supplementary Table 4 | PGT-MT number of reads at the MELAS (m.3243A>G) indication at different depths of coverage

Numbers shown represent the total number of reads at the site and the number of reads with the mutation in brackets. Embryos 45-48 from family 20 are shown.

PGT: preimplantation genetic testing; MT: mitochondrial DNA disorders; TE: trophectoderm; MELAS: mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes.

Depth	Embryo 45		Embryo 46		Embryo 47		Embryo 48	
	TE biopsy	Surplus embryo	TE biopsy	Surplus embryo	TE biopsy	Surplus embryo	TE biopsy	Surplus embryo
Full	11123 (5521)	17205 (8517)	11559 (6411)	9287 (6287)	8160 (6169)	6952 (5303)	6789 (2773)	11559 (4464)
30X	4210 (2106)	4634 (2283)	4176 (2833)	3996 (2743)	3920 (2955)	6952 (5303)	3656 (1467)	4333 (1742)
20X	3602 (1792)	4191 (2076)	3575 (2383)	3346 (2301)	3180 (2399)	2956 (2228)	2952 (1194)	3776 (1473)
10X	2373 (1178)	2965 (1481)	2415 (1626)	2231 (1509)	2134 (1612)	1972 (1518)	1879 (771)	2472 (974)
5X	1366 (675)	1831 (910)	1478 (977)	1288 (885)	1250 (936)	1135 (872)	1058 (436)	1488 (586)

Supplementary Table 5 | PGT-MT heteroplasmy levels for the MELAS (m.3243A>G) mutation at different depths of coverage

Embryos 45-48 from family 20 are shown.

PGT: preimplantation genetic testing; MT: mitochondrial DNA disorders; TE: trophectoderm; MELAS: mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes.

Depth	Embryo 45		Embryo 46		Embryo 47		Embryo 48	
	TE biopsy	Surplus embryo	TE biopsy	Surplus embryo	TE biopsy	Surplus embryo	TE biopsy	Surplus embryo
Full	49.6%	49.5%	67.1%	67.7%	75.6%	76.3%	40.8%	38.6%
30X	50.0%	49.3%	67.8%	68.6%	75.4%	76.3%	40.2%	40.2%
20X	49.8%	49.6%	66.7%	68.85	75.4%	75.6%	40.4%	39.0%
10X	49.6%	49.9%	67.3%	67.6%	75.5%	77.0%	41.0%	39.4%
5X	49.5%	49.7%	66.1%	68.7%	74.9%	76.9%	41.2%	39.4%

Supplementary table 6 | Extended PGT-MT results for family 21 - mutation: MELAS (m.3243A>G)

TE PCR = RFLP-PCR applied directly to TE biopsy material, TE MDA-PCR = RFLP-PCR applied to WGAed TE biopsy material. WGS coverage shows number of reads at the indication site and number of reads with the mutation in brackets.

PGT: preimplantation genetic testing; MT: mitochondrial DNA disorders; TE: trophectoderm; MELAS: mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; WGS: whole genome sequencing; MDA: multiple displacement amplification; WGAed: whole genome amplified; RFLP: restriction fragment length polymorphism; PCR: polymerase chain reaction.

Embryo	Sample type	TE PCR	TE WGA-PCR	WGS coverage
49	TE biopsy	23%	21%	1424 (281)
	Surplus embryo	-	-	5473 (1174)
50	TE biopsy	77%	76%	1304 (777)
	Surplus embryo	-	-	985 (1017)

Supplementary Table 7 | Comparison of WGS-PGT with the existing sequencing-based PGT methods

WGS-PGT: whole genome sequencing preimplantation genetic testing; HMM: Hidden Markov Model; ETS-PGT: embryo tracking system preimplantation genetic testing; PGT-M: preimplantation genetic testing for monogenic disorders; PGT-SR: preimplantation genetic testing for structural rearrangements; PGT-A: PGT for aneuploidies; PGT-MT: PGT for mitochondrial DNA disorders; PGT-AO: PGT for aneuploidy origin; SNV: single nucleotide variant; CNVs: copy-number variation; UPhDs: uniparental heterodisomy; UPiDs: uniparental isodisomy; PRS: polygenic risk score; PS: parental support; ADO: allele drop-out; ADI: allele drop-in; LOH: loss of heterozygosity; CBS: circular binary segmentation; TSS: transcription start site.

PGT method	Description	Similarities	Differences (~) / Limitations (-)		Novelties of WGS-PGT over an existing method	
			Technical	Conceptual	Technical	Conceptual
SHaploseek (Backenroth et al. 2023, PMID: 37865712)	**o** Haplotyping of embryos using a Viterbi path returned by HMM on low-pass sequencing data; **o** Copy-number profiling using low-pass sequencing data from embryos, reference and parents; **o** SHaploseek is similar to the original Haploseek but instead of combination of sequencing and SNP array, it is completely based on sequencing; **o** Tested 31 embryos.	**o** Copy number profiling using read depth analysis; **o** Differentiation between balanced translocation carriers and chromosomally normal embryos based on haplotyping; **o** Aneuploidy detection using copy number profiles; **o** Higher diagnostic yield at sub-telomeric regions; **o** Phasing ability of consanguineous families; **o** Clinically validated.	**-** Low-pass sequencing of the parents (≤5X) and ultra-low-pass sequencing of embryos (0.2X-0.4X) and therefore no accurate SNV detection; **-** Missing SNVs are imputed instead of direct detection; **-** Aneuploidy origin cannot be detected; **~** Makes use of an HMM for haplotype prediction (similar to PMID: 24810687); **~** CNVs are deduced by an HMM;	**-** Meiotic/mitotic origin is not determined; **-** Inherent to (ultra) low-pass sequencing level of mosaicism of chromosomal aberrations cannot accurately be determined; **-** Mitochondrial mutation cannot be determined; - *De novo* SNVs cannot be identified; - Breakpoints of translocations cannot be accurately determined.	**+** Whole-genome sequencing with validated sufficient depth-of-coverage (10X); + Substantially lower Mendelian inconsistency rate; + Ability to directly detect breakpoints of translocations using aberrantly mapped read pairs; + Equipped with 1D median filtering for discrete (SNV-calls) haplotyping; + Equipped with haplarithmisis for continuous (BAF-values) haplotyping;	**+** Detection of *de novo* SNVs; **+** Detection of meiotic and mitotic origin of trisomies and UPhDs and UPiDs (using a parents-only approach), i.e. PGT-AO; + Detection of mitochondrial mutations (heteroplasmy levels); + Determination of level of mosaicism of chromosomal aberration; + Clinically implemented.

			- Lack of DNA barcoding system to detect sample switching.		+ Equipped with a DNA barcoding system, called ETS-PGT (PMID: 36149256), which detects sample switching; + Enabled robotizing with liquid handlers; **+** Clinically implemented.	
GBS-PGT **GENType/Hopla** (de Witte et al. 2022, PMID: 35552408)	**o** Reduced representation sequencing after restriction enzyme digestion; **o** Hopla was used that is based on Merlin algorithm (PMID: 11731797) and corrects raw haplotypes using weighted window voting; **o** CNV calling was carried out by read-depth analysis using WisecondorX (PMID: 30566647) and Vivar (PMID: 25503062); **o** Demonstrated parents-only haplotyping;	**o** Haplotyping of embryo using genotype information from parents and relative(s), or using one single parent; **o** Copy number profiling using read depth analysis; **o** Meiotic and mitotic errors can be distinguished when one recombination site is present; **o** Clinically validated and implemented.	~ Parents only PGT-M for third-party reproduction setting (donor), requiring prior diagnosis of a reference embryo via direct mutation analysis with an independent technology. - Only discrete genotype calls are used for haplotype calling; ~ Equipped with Merlin (PMID: 11731797); ~ CNV calling using WisecondorX (PMID: 30566647) and Vivar (PMID: 25503062);	- Restriction enzyme-based sequencing, covering only a fraction of the genome; - Detection of meiotic/mitotic origin of aneuploidies using discrete SNP calls is challenging; - Level of mosaicism of chromosomal aberration cannot accurately be determined; - For the single-parent analysis, still a close relative is required.	**+** Whole-genome sequencing with validated sufficient depth-of-coverage (10X); + Substantially lower Mendelian inconsistency rate; + Ability to directly detect breakpoints of translocations using aberrantly mapped read pairs; + Equipped with a DNA barcoding system, called ETS-PGT (PMID: 36149256), which detects sample switching; + Enabled automation/robotizing with liquid handlers.	**+** Detection of *de novo* SNVs; **+** Detection of meiotic and mitotic origin of trisomies and UPhDs and UPiDs; + Detection of mitochondrial mutations (heteroplasmy levels); + Determination of level of mosaicism of chromosomal aberration.

	o Clinically validated on 119 embryos from 31 families; **o** Clinical implementation (104 embryos reported).		- Lack of DNA barcoding system to detect sample switching.			
scGBS (Masset et al. 2019 and 2023, PMIDs: 31348829 and 35212381)	**o** Reduced representation sequencing PGT method for PGT-M, PGT-SR, and PGT-A	**o** Haplotyping of embryo using genotype information from parents and relative(s), or using one single parent using haplarithmisis. **o** Copy number profiling using read depth analysis via QDNAseq; **o** Discerning embryos with balanced translocation from normal ones using flanking haplotypes; **o** Meiotic and mitotic errors can be distinguished when one recombination site is present; **o** Clinically validated and implemented.	- Restriction enzyme-based sequencing, covering only a fraction of the genome; - Paired-end information could not be used for the detection of structural rearrangement; - Lack of DNA barcoding system to detect sample switching.	- High mendelian inconsistency rates; - Balanced translocation carriers can only be detected using haplotypes (i.e. indirectly); - Ambiguity at times for the detection of SNVs in the proximity of centromeres and telomeres; - Mitochondrial genome is not effectively covered; - Requires a close-relative to detect monogenic disorders;	**+** Whole-genome sequencing with validated sufficient depth-of-coverage (10X); + Substantially lower Mendelian inconsistency rate; + Ability to directly detect breakpoints of translocations using aberrantly mapped read pairs; + Equipped with a DNA barcoding system, called ETS-PGT (PMID: 36149256), which detects sample switching; + Enabled automation/robotizing with liquid handlers.	**+** Detection of *de novo* SNVs; + Detection of translocation breakpoints at base resolution; **+** Detection of meiotic and mitotic origin of trisomies and UPhDs and UPiDs using only parents; + Detection of mitochondrial mutations (heteroplasmy levels); + Determination of level of mosaicism of chromosomal aberration.

PGT using parental support (PS) algorithm (Kumar et al. 2022, PMID: 35314819)	**o** PGT approach that combines whole genome sequencing of parental genomes and genotyping of embryos to predict haplotypes and calculate PRS; **o** 110 embryos were tested using PS.	**o** Haplotypes of embryo using genotype information from parents.	~ Parents are phased using PS method, a statistical model to determine maximum likelihood by using both recombination frequencies from reference panels and SNP measurements from sibling embryos; - Reconstructed embryo genomes used for PRS calculation; - SNP array used for genotyping embryo biopsies; Whole genome sequencing (30X) used for parents and born children's DNA. Long-read sequencing used to capture the phase in case of rare variants in parents; + Results from embryo are compared to genomic samples in 10 born children;	- Preclinical research study; + Combination of rare variants and PRS to predict odds ratio across sibling embryos; - Copy number variation not profiled.	**+** Whole-genome sequencing with validated sufficient depth-of-coverage (10X); + Copy number profiling using read depth; + Ability to directly detect breakpoints of translocations using aberrantly mapped read pairs; + Equipped with a DNA barcoding system, called ETS-PGT (PMID: 36149256), which detects sample switching; + Enabled robotizing with liquid handlers.	**+** Detection of *de novo* SNVs; + Differentiation between balanced structural rearrangement carrier embryos and normal embryos based on haplotypes; + Detection of translocation breakpoints at base resolution; **+** Detection of meiotic and mitotic origin of trisomies and UPhDs and UPiDs (using a parents-only approach); + Detection of mitochondrial mutations (heteroplasmy levels); + Determination of level of mosaicism of chromosomal aberration; + Clinically implemented.

			- Lack of DNA barcoding system to detect sample switching.			
HaploPGT (Xie et al. 2022, PMID: 36066440)	**o** GBS-based and haplotyping-based PGT for PGT-M, PGT-SR and PGT-A; **o** 188 blastocyst-stage embryos were tested.	**o** PGT-M using a close relative; **o** MDA is used as a WGA method; **o** Copy number profiling using read depth analysis; **o** Discerning embryos with balanced translocation from normal ones using flanking haplotypes; **o** Determining parental origin of CNVs; **o** Determining level of mosaicism in aneuploidies using BAF-values; **o** Detection of triploids, haploids and LOH.	- Restriction enzyme-based sequencing, covering only a fraction of the genome; - ≥2 informative loci per Mb; ~ Triploid and LOH detected via CBS algorithm and Z-test. - Copy-number profiling and CNV breakpoints were determined by CBS algorithm and Z-test. - Lack of DNA barcoding system to detect sample switching; - Detection of triploids, haploids and LOH uses CBS algorithm and Z-test.	- Requires a close-relative to detect monogenic disorders; - Requires a translocation carrying embryo to discern embryos carrying a balanced translocation from normal embryo; - SNVs cannot be detected at base level; - Direct detection of translocation breakpoints is not possible in the parents and embryos in base resolution.	**+** Whole-genome sequencing with validated sufficient depth-of-coverage (10X); + Ability to detect *de novo* SNVs; + Ability to directly detect breakpoints of translocations using aberrantly mapped read pairs; + Ability to detect meiotic and mitotic origin of aberrations; + Equipped with a DNA barcoding system, called ETS-PGT (PMID: 36149256), which detects sample switching; + Enabled automation/robotizing with liquid handlers.	**+** Detection of *de novo* SNVs; + Differentiation between balanced structural rearrangement carrier embryos and normal embryos based on haplotypes; + Detection of translocation breakpoints at base resolution; **+** Detection of meiotic and mitotic origin of trisomies and UPhDs and UPiDs (using a parents-only approach); + Detection of mitochondrial mutations (heteroplasmy levels); + Determination of level of mosaicism of chromosomal aberration; + Clinically validated and implemented.
Preimplantation genetic testing for de novo	**o** PGT for translocations or de novo mutations from parents;	**o** Direct mutation detection by haplotyping	~ *De novo* mutation is detected by Sanger sequencing;	- Still requires family and locus-specific designs.	**+** Whole-genome sequencing with validated	**+** Detection of *de novo* SNVs via a generic wet-lab approach;

mutations. (Yuan, et al. 2020, PMID: 32783137)	**o** Whole genome sequencing in embryos (2X) and family members (10X); **o** Tested on TE cells and polar bodies from the same embryo of two families.	using information on risk haplotype; **o** Copy-number profiling using read depth; **o** No additional family member needed to directly detect the mutation site; **o** SNP markers that are homozygous in one parent and heterozygous in the other parents are used for haplotyping.	- Different assays are needed, specifically: WGS and Sanger sequencing are used to reliably perform diagnosis; ~ WGS of > 2X in embryos and > 10X in parents; - Lack of DNA barcoding system to detect sample switching.		sufficient depth-of-coverage (10X); + Ability to directly detect breakpoints of translocations using aberrantly mapped read pairs; + Ability to detect meiotic and mitotic origin of aberrations; + Equipped with a DNA barcoding system, called ETS-PGT (PMID: 36149256), which detects sample switching; + Enabled automation/robotizing with liquid handlers.	+ Differentiation between balanced structural rearrangement carrier embryos and normal embryos based on haplotypes; + Detection of translocation breakpoints at base resolution; **+** Detection of meiotic and mitotic origin of trisomies and UPhDs and UPiDs (using a parents-only approach); + Detection of mitochondrial mutations (heteroplasmy levels); + Determination of level of mosaicism of chromosomal aberration; + Clinically validated and implemented.
Genome sequencing for pathogenic variation screening (Murphy, et al. 2020, PMID: 32123222)	**o** A pilot study in high-risk couples to detect pathogenic variants in embryos at depth of > 45X; **o** Variant annotation databases and existing functional prediction algorithms were used to	**o** Ability to detect *de novo* SNVs; **o** Ability to assess variants of unknown significance and screen for pathogenic variants not previously described in the parents.	~ Sequencing at 48.2X in embryos and 46.1X in parents; **~** Embryos had previously undergone clinical PGT for a familial disease via Karyomapping (PMID: 24810687);	~ Screening for early fatal genetic conditions that affected quality of life of individual and families; - Method does not test for familial variants;	**+** Whole-genome sequencing with validated sufficient depth-of-coverage (10X); + Ability to directly detect breakpoints of translocations using aberrantly mapped read pairs;	+ Differentiation between balanced structural rearrangement carrier embryos and normal embryos based on haplotypes. + Detection of translocation breakpoints at base resolution;

	detect transmission or introduction of pathogenic mutations; **o** Tested on 11 embryos from 5 couples.	**o** Copy number profiling using read depth analysis; **o** MDA was used for whole-genome amplification; **o** Genome-wide coverage.	**~** Variants were classified on pathogenicity using ClinVar (PMID: 29165669) archive; **~** For variants of unknown significance, prediction algorithms were used; **~** CNVs were called using CNVnator (PMID: 21324876); **~** Structural variants were called using Breakdancer (PMID: 19668202) and CREST (PMID: 21666668); - Lack of DNA barcoding system to detect sample switching.	- Meiotic and mitotic errors cannot be distinguished; - Mendelian consistencies are not reported; - Level of mosaicism of chromosomal aberration cannot accurately be determined.	+ Ability to detect meiotic and mitotic origin of aberrations; + Equipped with a DNA barcoding system, called ETS-PGT (PMID: 36149256), which detects sample switching; + Enabled automation/robotizing with liquid handlers.	**+** Detection of meiotic and mitotic origin of trisomies and UPhDs and UPiDs (using a parents-only approach), i.e. enabling PGT-AO; + Detection of mitochondrial mutations (heteroplasmy levels); + Determination of level of mosaicism of chromosomal aberration; + Clinically validated and implemented.
Comprehensive PGT (Chen et al. 2021, PMID: 33306794)	**o** A PGT approach without the use of additional family members, which requires a priori detection of a mutation in an embryo; **o** Not all the embryos could be diagnosed with	**o** Haplotyping using parents-only (proband-independent) by using embryo carrying the mutation as a reference; **o** Copy number profiling using read depth analysis;	**~** Sequencing at 10X coverage for parents and 4X coverage for embryos; **-** Direct genotyping of reference embryo could fail due to ADO, which may be due to relatively	**-** 6 out of 8 families could be helped with parents-only (proband-independent) haplotyping; **-** Breakpoints of translocations were not detected directly in	+ Lower Mendelian inconsistency rates (both ADI and ADO): 4.6% on average + Ability to directly detect breakpoints of translocations using	+ Differentiation between balanced structural rearrangement carrier embryos and normal embryos based on haplotypes. + Detection of translocation breakpoints at base resolution;

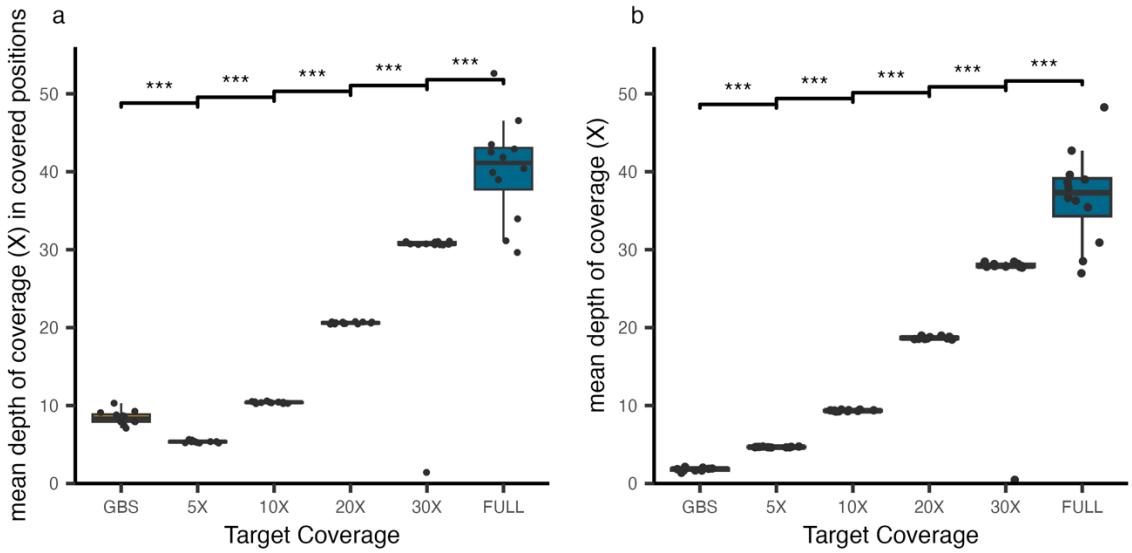
	proband-independent approach; **o** Tested on 53 embryos from 10 families.	**o** Differentiation between balanced translocation carriers and normal embryos based on haplotyping; **o** Ability to detect meiotic and mitotic origin of chromosomal aberrations; **o** Possibility of direct and indirect detection of pathogenic SNVs. Although 6 out of 8 families could be helped with the direct approach of this study.	low coverage of embryos (4X); ~ Informative SNPs in the 500 kb up and down-stream region are analysed; ~ Copy-number profiling and CNV breakpoints were determined by binary segmentation algorithm (PMID: 23372689); - ADO rate is ~10% for blastocysts; - If a sibling embryo with an unbalanced translocation is not available, parents-only PGT-SR is not possible; - Lack of DNA barcoding system to detect sample switching.	parents/embryos and in base resolution, they were rather inferred from an embryo with unbalanced translocation; - Detection of mitochondrial mutations is not demonstrated.	aberrantly mapped read pairs; + Equipped with a DNA barcoding system, called ETS-PGT (PMID: 36149256), which detects sample switching; + Enabled automation/robotizing with liquid handlers.	**+** Detection of meiotic and mitotic origin of trisomies and UPhDs and UPiDs using a parents-only approach, i.e. enabling PGT-AO; + Detection of mitochondrial mutations (heteroplasmy levels); + Determination of level of mosaicism of chromosomal aberration; + Clinically validated and implemented.
MaReCs (Xu et al. 2017, PMID: 28973897)	**o** PGT method for PGT-SR and PGT-A; **o** Tested on 108 embryos.	**o** Differentiation between balanced translocation carriers and normal embryos based on (local) haplotyping of reference embryo;	~ Comprehensive chromosome screening via CGH or NGS with 200 kb resolution; **~** Subsequent targeted NGS at breakpoints to	- Family- or breakpoint-specific strategy for targeted sequencing; **-** Inherent to (ultra) low-pass sequencing level of mosaicism of chromosomal	**+** Whole-genome sequencing with validated sufficient depth-of-coverage (10X); + Ability to directly detect breakpoints of translocations using	**+** Detection of monogenic disorders in embryos; **+** Detection of *de novo* SNVs; + Detection of translocation breakpoints at base resolution;

		o Copy number profiling using read depth analysis.	obtain SNPs around breakpoints; - Two methods are needed: shallow NGS/CGH and targeted sequencing; - Lack of DNA barcoding system to detect sample switching.	aberration cannot accurately be determined; - Meiotic/mitotic origin of aneuploidy is not determined.	aberrantly mapped read pairs; + Ability to detect meiotic and mitotic origin of aberrations; + Equipped with a DNA barcoding system, called ETS-PGT (PMID: 36149256), which detects sample switching; + Enabled automation/robotizing with liquid handlers.	**+** Detection of meiotic and mitotic origin of trisomies and UPhDs and UPiDs (using a parents-only approach); + Detection of mitochondrial mutations (heteroplasmy levels); + Determination of level of mosaicism of chromosomal aberration; + Clinically validated and implemented.
MARSALA (Yan et al. 2015, PMID: 26712022)	**o** PGT method for PGT-M using linkage analysis and for aneuploidy; **o** PCR primers targeting SNV of interest are used. PCR product is mixed with WGA product and sequenced at sequencing depth (0.1-2X).	**o** Local haplotype of embryo using genotype information from parents; **o** Copy number profiling using read depth.	- Specific primer design for allele of interest is needed; - Lack of DNA barcoding system to detect sample switching.	- Requires a disease-carrying close-relative to detect monogenic disorders; - Family- or mutation-specific strategy for targeted sequencing; **-** Inherent to (ultra) low-pass sequencing level of mosaicism of chromosomal aberration cannot accurately be determined;	**+** Whole-genome sequencing with validated sufficient depth-of-coverage (10X); + Ability to directly detect breakpoints of translocations using aberrantly mapped read pairs; + Ability to detect meiotic and mitotic origin of aberrations; + Equipped with a DNA barcoding system, called ETS-PGT (PMID:	**+** Detection of *de novo* SNVs; + Differentiation between balanced structural rearrangement carrier embryos and normal embryos based on haplotypes; + Detection of translocation breakpoints at base resolution; **+** Detection of meiotic and mitotic origin of trisomies and UPhDs and UPiDs (using a parents-only approach);

				- Meiotic/mitotic origin of aneuploidy is not determined.	36149256), which detects sample switching; + Enabled robotizing with liquid handlers.	+ Detection of mitochondrial mutations (heteroplasmy levels); + Determination of level of mosaicism of chromosomal aberration.
PGT using long-fragment read (LFR) technology (Peters et al. 2015, PMID: 25672852)	**o** PGT for *de novo* single-nucleotide and short indel mutations using long fragment read technology (as in PMID: 22785314)	**o** MDA is used as WGA method; **o** Potential use for a genome-wide PGT-M; **o** Mendelian inconsistencies are used to determine accuracy; **o** Mitochondrial genome read coverage; **o** Detection of UPhDs (using a parents-only approach);	- Not high throughput as denatured DNA from ≥10 cells is aliquoted across 384 wells, barcoded and sequenced; - if the number of cells is less than 10 (~66 pg DNA), the accuracy drops substantially; - Lack of DNA barcoding system to detect sample switching; **o** Loss of 15% sensitivity as compared to standard WGS; - 82% of *de novo* SNVs could be called; - parental DNA was used to impute SNVs that are not called, i.e.	- Meiotic/mitotic origin of aneuploidy is not determined + Parental haplotypes are directly generated using fuzzy logic operations on the shared aliquot matrix; - Clinically not validated; - Concurrent haplotyping and copy-number typing is not described; - PGT-A, PGT-SR and PG-MT are not demonstrated.	+ Besides PGT-M and PGT for *de novo* mutations, WGS-PGT enables PGT-SR, PGT-MT and a new form of PGT-A, called PGT-AO; **+** Whole-genome sequencing with validated sufficient depth-of-coverage (10X); + Ability to directly detect breakpoints of translocations using aberrantly mapped read pairs; + Ability to detect meiotic and mitotic origin of aberrations; + Equipped with a DNA barcoding system, called ETS-PGT (PMID: 36149256), which detects sample switching;	**+** PGT method for familial pathogenic variants; + Differentiation between balanced structural rearrangement carrier embryos and normal embryos based on haplotypes; + Detection of translocation breakpoints at base resolution; + Detection of mitochondrial mutations (heteroplasmy levels); + Determination of level of mosaicism of chromosomal aberration; + Clinically validated and implemented.

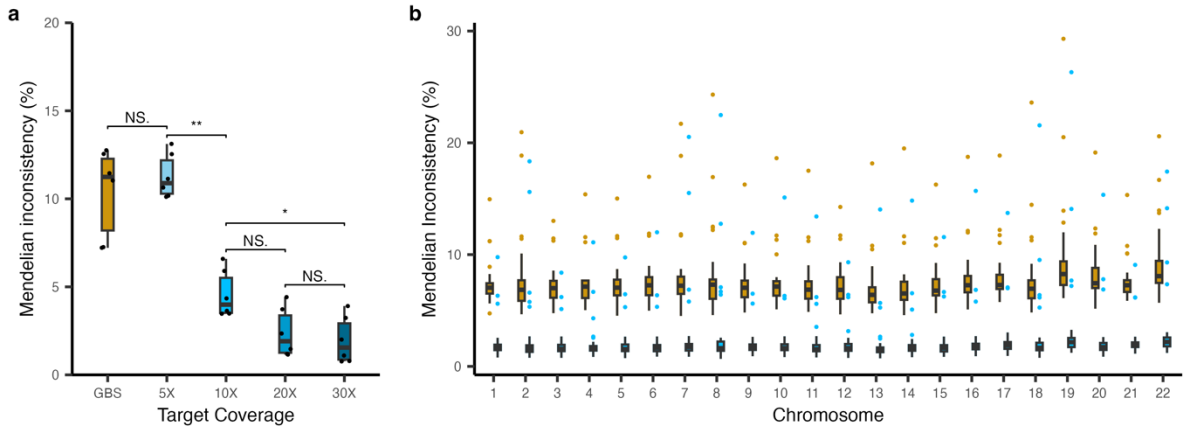
			de novo mutations can be missed; ~ Detrimental variants are categorized into two subcategories affecting amino acid sequence or intron/exon structure and changes in TSS or regulation.		+ Enabled automation/robotizing with liquid handlers.	

Supplementary Figures



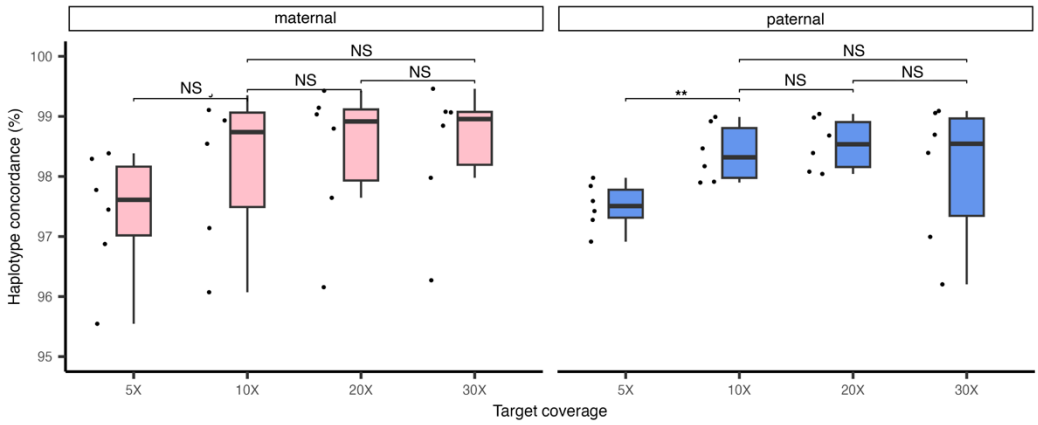
Supplementary Figure 1 | Depth of coverage in the pilot study.

Depth of coverage for bulk DNA and WGAed embryo samples whole genome sequenced at an original sequencing depth (FULL: 30-40X) and corresponding *in silico* subsampled sequencing data at target coverages of 5X, 10X, 20X, 30X and GBS samples. The target depths of coverage (x-axis), and corresponding mean depth of coverage per sample (y-axis) are shown. **a,** The mean depth of coverage is calculated by dividing the number of bases of aligned reads by genomic positions that are covered by at least one read. **b,** the mean depth of coverage is calculated by dividing the number of bases of aligned reads by all genomic positions. The horizontal lines of the boxplots represent the 25th percentile, median and 75th percentile and the whiskers extend to 1.5 * IQR. The dots represent individual samples and WGS-PGT and GBS-PGT data is shown in blue and gold, respectively. P < 0.05 (*), P < 0.01 (**), P < 0.001 (***), two sided Wilcoxon's rank sum. WGS: whole genome sequencing; GBS: genotyping-by-sequencing; IQR: interquartile range; PGT: preimplantation genetic testing.



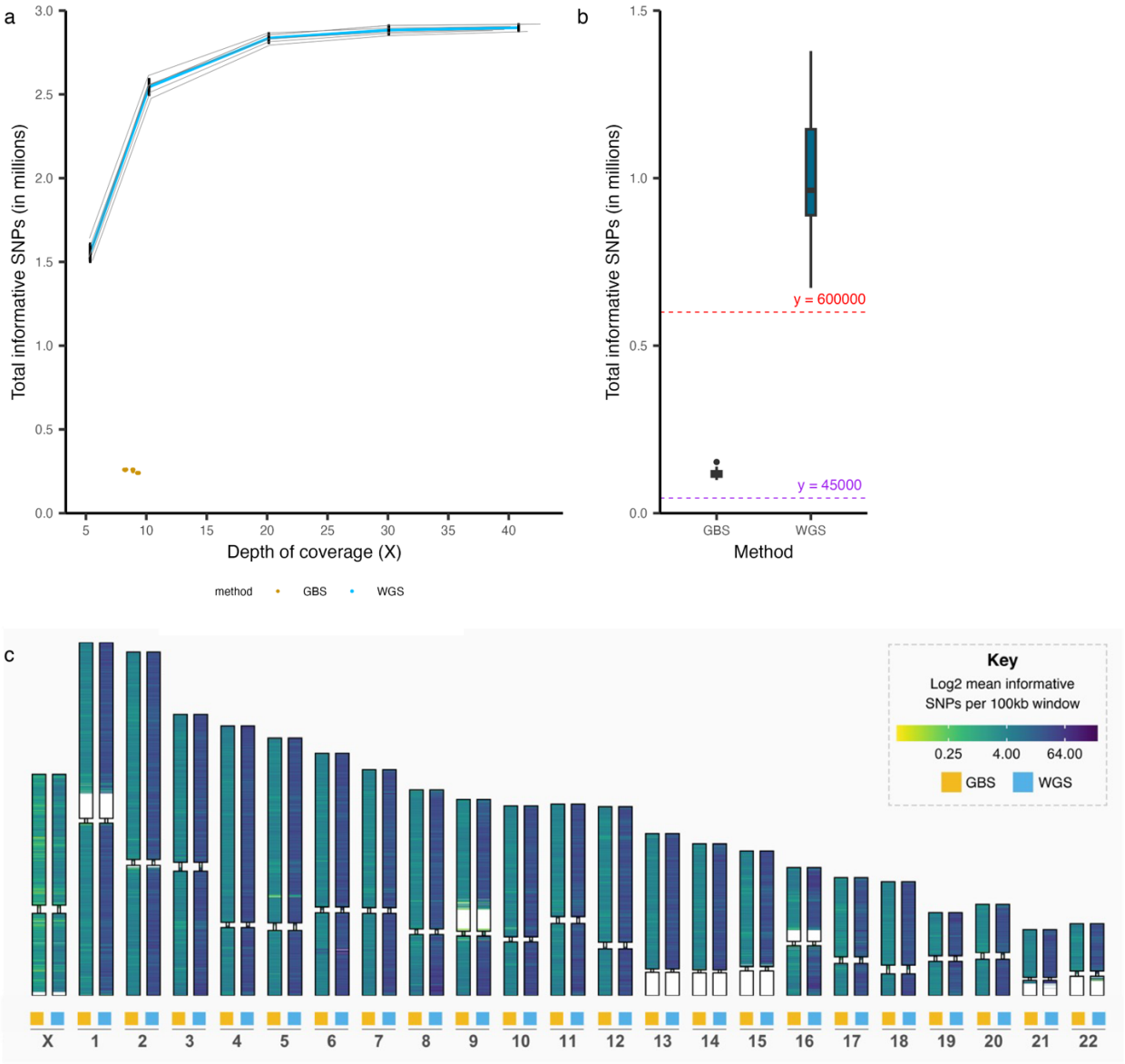
Supplementary Figure 2 | Mendelian inconsistency.

Mendelian inconsistency rates represent the proportion of genotypes in the embryo that violate Mendelian inheritance patterns from the total number of genotypes analysed. **a,** Mendelian inconsistency rates for the autosomes from *in silico* subsampled WGS-PGT data and corresponding GBS-PGT data (*n* = 6). The target depths of coverage (x-axis), and Mendelian inconsistency rates (y-axis) are shown. **b,** Mendelian inconsistency rates from WGS-PGT data sequenced at 10X and corresponding GBS data (*n* = 31) shown per autosomal chromosome (chr1-22). The horizontal lines of the boxplots represent the 25th percentile, median and 75th percentile and the whiskers extend to 1.5 * IQR. The dots represent individual samples and GBS-PGT and WGS-PGT data are shown in gold and blue, respectively. P < 0.05 (*), P < 0.01 (**), P < 0.001 (***), two-sided Wilcoxon's rank sum. WGS: whole genome sequencing; PGT: preimplantation genetic testing; GBS: genotyping-by-sequencing; IQR: interquartile range.



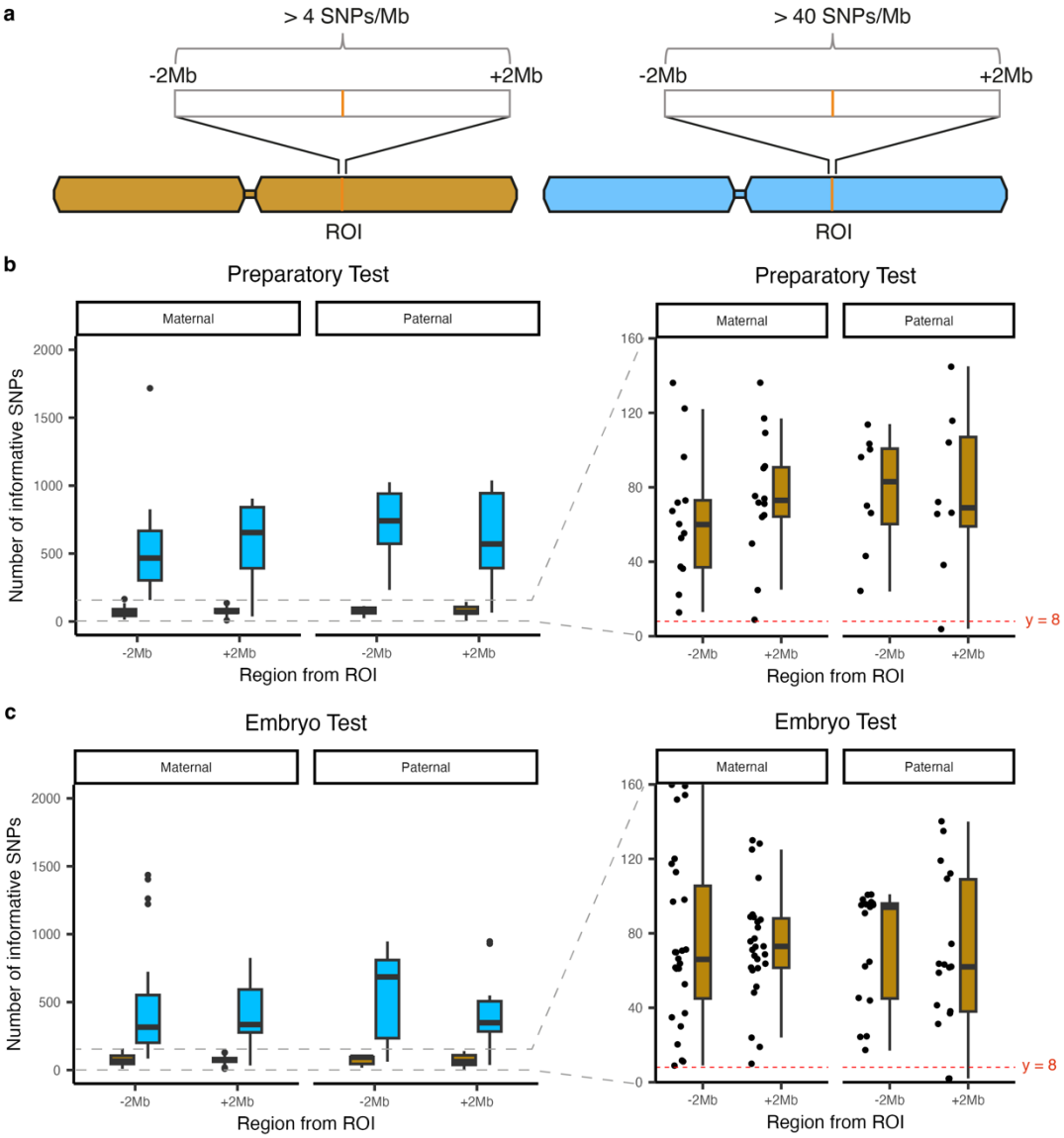
Supplementary Figure 3 | Haplotype concordance.

Haplotype concordance between WGS-PGT data and GBS-PGT data at different *in silico* subsampled depth of coverages (*n* = 6) for maternal (pink) and paternal (blue) haplotypes. The horizontal lines of the boxplots represent the 25th percentile, median and 75th percentile and the whiskers extend to 1.5 * IQR. The dots represent individual samples and maternal and paternal haplotype concordance rates are shown in pink and blue, respectively. P < 0.05 (*), P < 0.01 (**), P < 0.001 (***), two-sided Wilcoxon's rank sum. WGS: whole genome sequencing; PGT: preimplantation genetic testing; GBS: genotyping-by-sequencing; IQR: interquartile range.



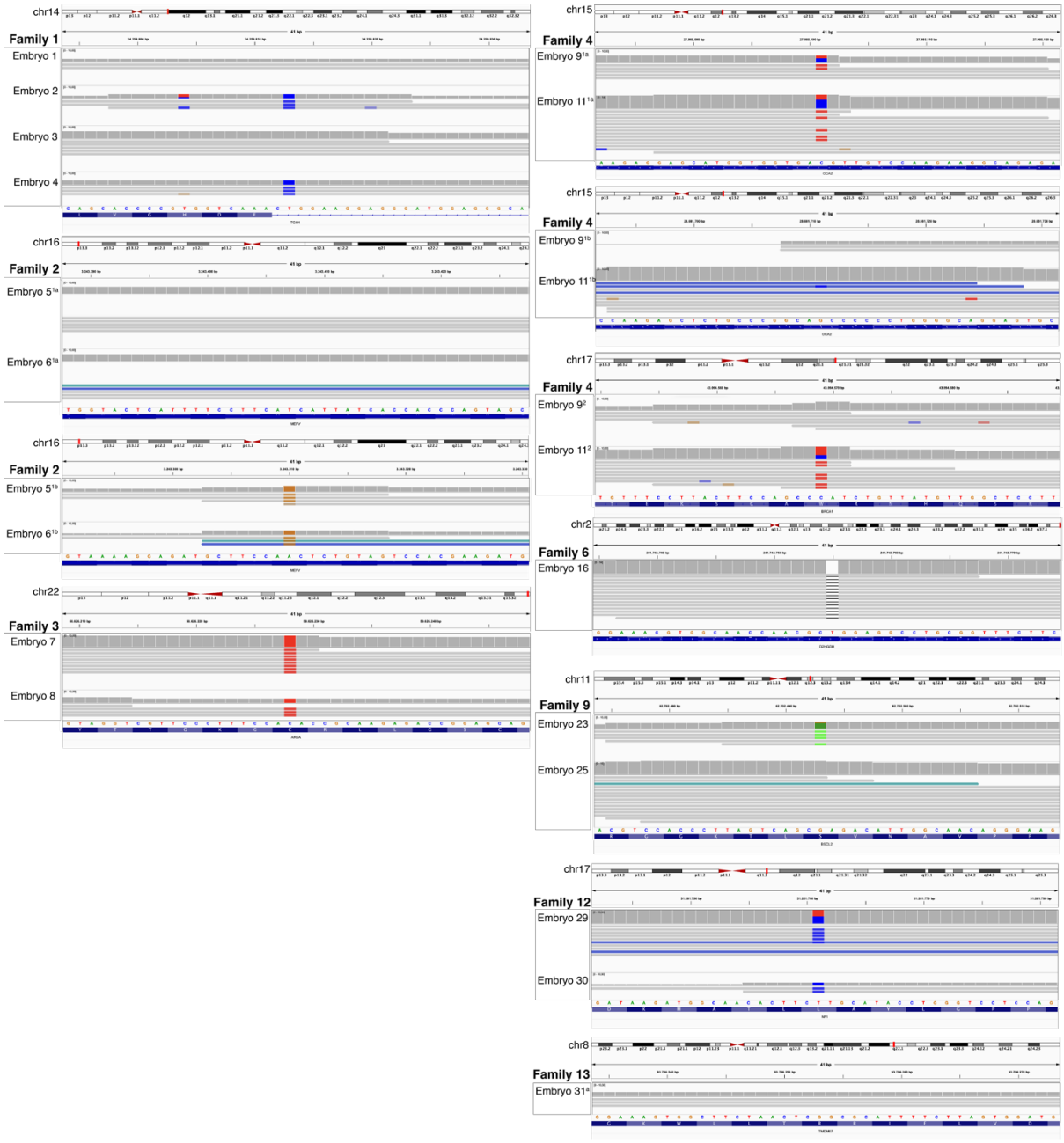
Supplementary Figure 4 | Informative SNPs.

a, Total number of informative SNPs per embryo (*n* = 6) at different *in silico* subsampled depth of coverage from WGS data (lines: blue) and the corresponding GBS data (dots: gold). **b,** Total number of informative SNPs for GBS-PGT (gold) and WGS-PGT (blue). The dashed horizontal lines indicate the minimum required number of informative SNPs according to the quality control criteria for GBS (45.000 informative SNPs, purple) and WGS (600.000 informative SNPs, red). The horizontal lines of the boxplots represent the 25th percentile, median and 75th percentile and the whiskers extend to 1.5 * IQR. **c,** Heatmap showing the mean number of informative SNPs per phased parental haplotype in 100kb bins across chromosomes in data obtained by GBS or WGS. The mean number of informative SNPs was calculated from 51 phased parental (maternal or paternal) haplotypes from 31 embryos. SNP: single nucleotide polymorphism; WGS: whole genome sequencing; PGT: preimplantation genetic testing; GBS: genotyping-by-sequencing; IQR: interquartile range.



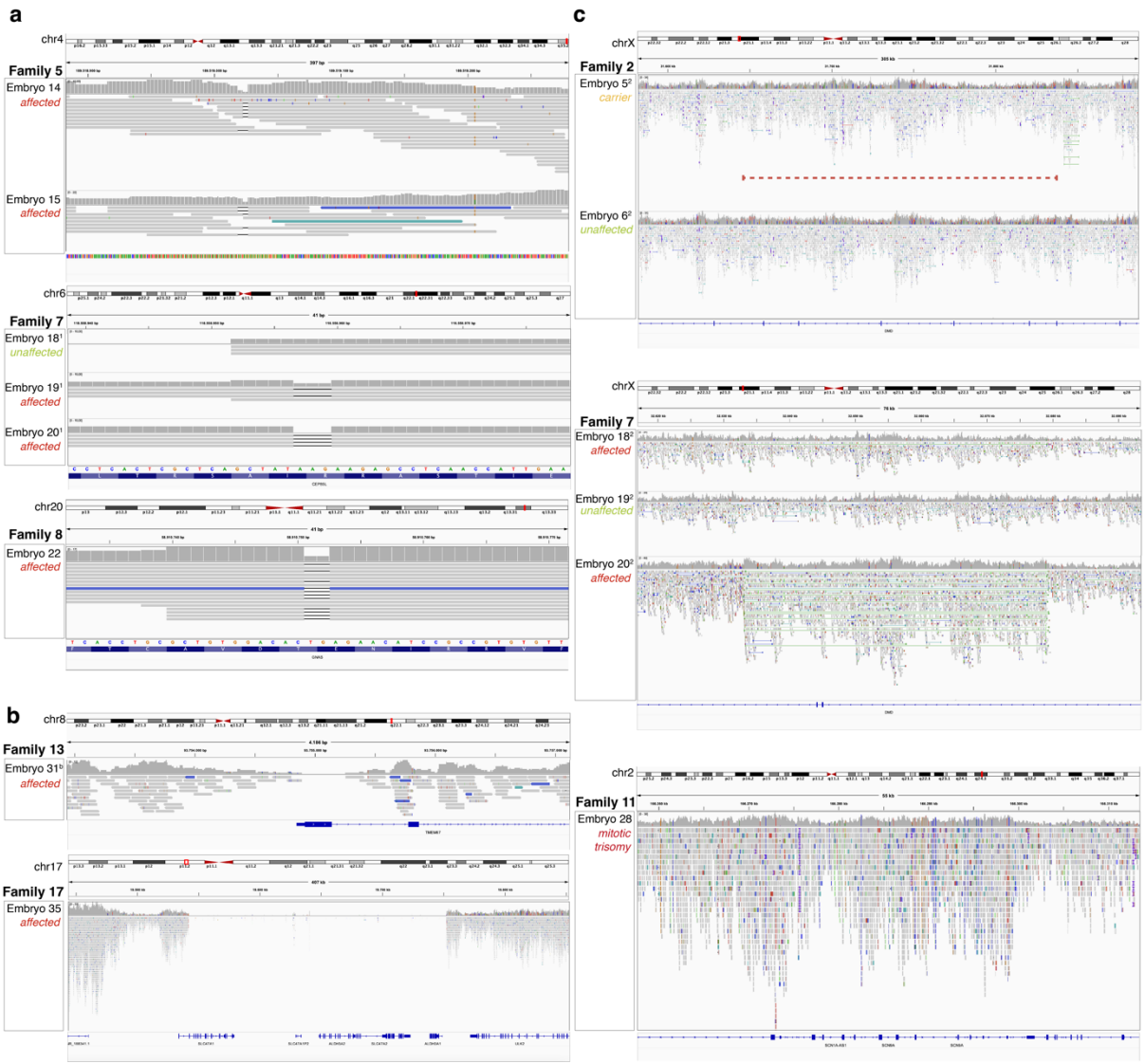
Supplementary Figure 5 | Number of informative SNPs at ROI.

The number of informative SNPs 2 Mb up- and downstream of the ROI for WGS data (blue) and GBS (gold) data. **a**, chromosome ideogram showing 2 Mb up- and down-stream of the ROI (orange) for GBS (gold) and WGS (blue) with corresponding thresholds. Informative SNPs are categorised into maternal and paternal categories. Right panels: zoom-in on the number of informative SNPs for GBS-PGT data, the red dashed horizontal line represents the assessment criteria threshold for the minimum number of informative SNPs required for the region 2 Mb up- and downstream of the ROI. The horizontal lines of the boxplots represent the 25th percentile, median and 75th percentile and the whiskers extend to 1.5 * IQR. Dots represent number of informative SNPs for a single embryo. **b,** the number of informative SNPs for the preparatory test. **c,** presents the number of informative SNPs for the embryo test. SNP: single nucleotide polymorphism; WGS: whole genome sequencing; GBS: genotyping-by-sequencing; ROI: region of interest; IQR: interquartile range; PGT: preimplantation genetic testing.



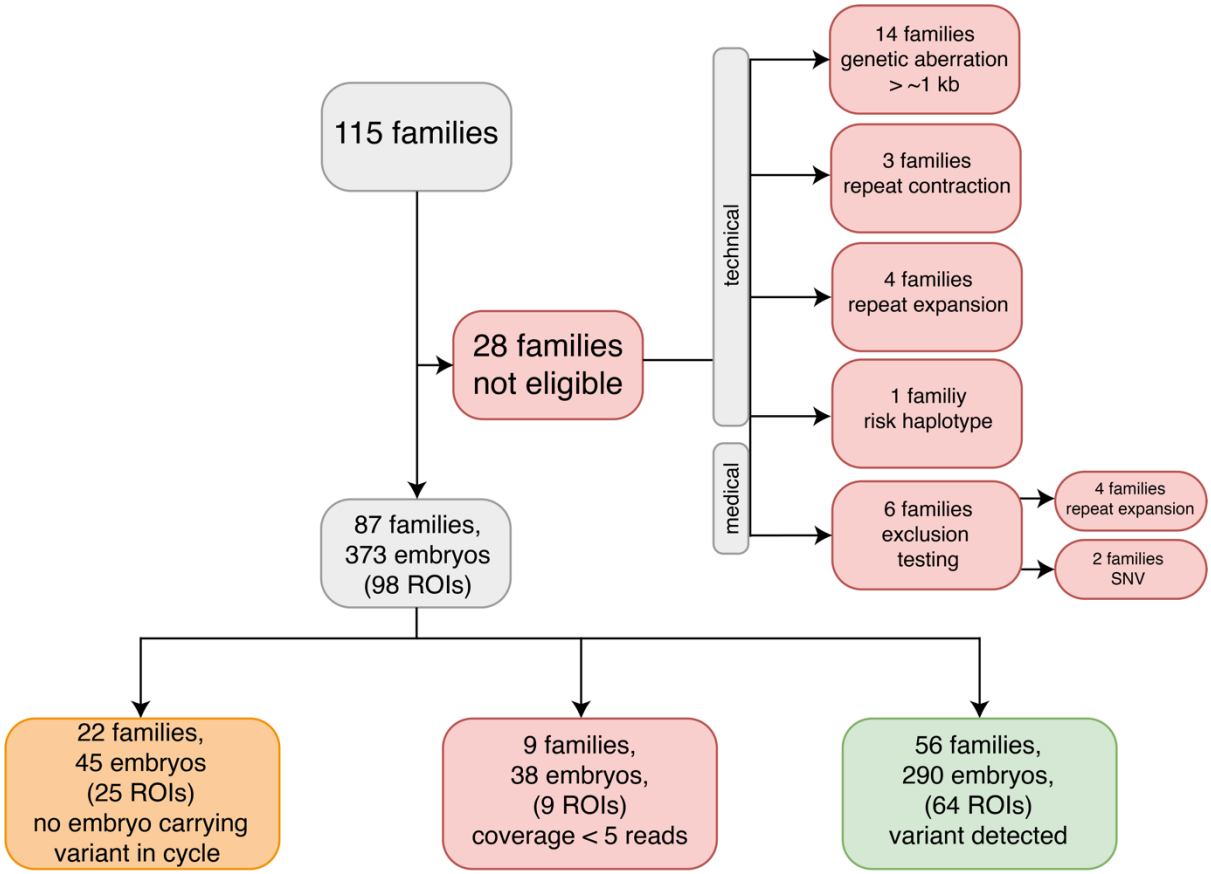
Supplementary Figure 6 | IGV sessions for single nucleotide direct mutation visualization

Each panel provides a comprehensive view of the genetic locus per family displaying single base pair substitutions and deletions. The family number and embryo numbers are indicated on the left side. At the top of each panel, an ideogram of the relevant chromosome is depicted. The precise location of the analysed window on chromosome scale is marked in red. A horizontal span arrow illustrates the width of this window, with the specific number of base pairs indicated. Within each panel, two tracks are featured for every embryo: the upper track serves as a coverage track, the lower track displays the reads. Nucleotides that match the reference genome are depicted as grey reads. Positions that do not match the reference genome are color-coded (A: green, C: blue, G: orange, T: red or deletion: short black horizontal line). The reference genome track, utilising the hg38 reference genome is positioned at the bottom for reference. Shown is WGS-PGT data at ~10X coverage. IGV: integrative genomics viewer; WGS: whole genome sequencing; PGT: preimplantation genetic testing.



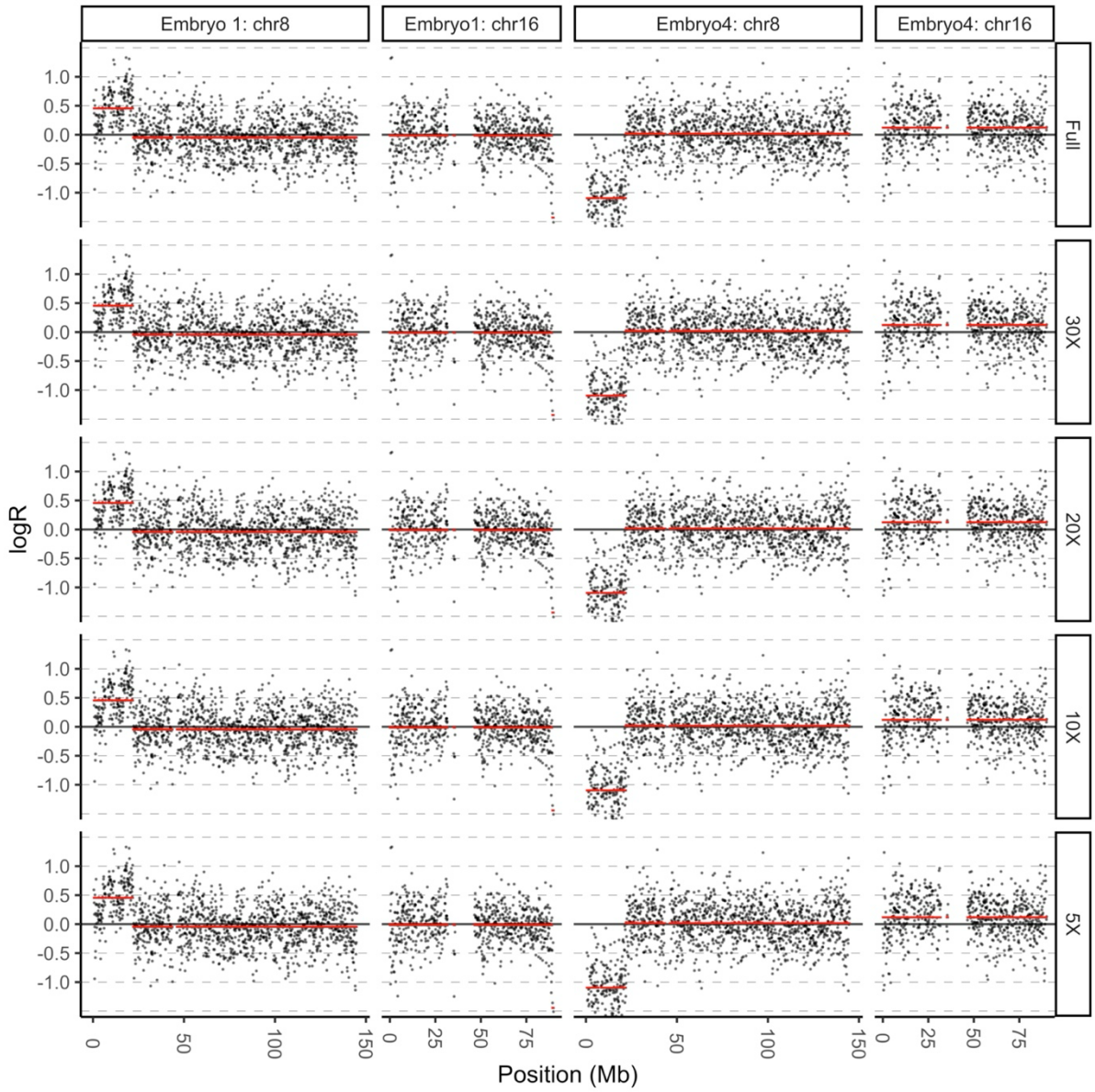
Supplementary Figure 7 | IGV sessions for direct deletion visualization.

Each panel provides a comprehensive view of the genetic locus per family displaying deletions greater than 1 base pair. The family number and embryo numbers are indicated on the left side. Moreover, the haplarithmisis diagnostic result that belongs to this ROI of the embryo is presented along. At the top of each panel, an ideogram of the relevant chromosome is depicted. The precise location of the analysed window on chromosome scale is marked in red. A horizontal span arrow illustrates the width of this window, with the specific number of base pairs indicated. Within each panel, two tracks are featured for every embryo; the upper track serves as the coverage track, the lower track displays the reads. **a,** panels for embryos from families with a deletion ranging 2-3 base pairs. Deletions are visualised as short black horizontal line. **b,** panels for embryos from families with an autosomal recessive deletion. Deletions are visualised as loss of coverage. **c,** panels for embryos from families with autosomal dominant or x-linked deletions. Deletions are visualised using option view as pairs, where the coloured horizontal line connects the read pairs and is spanning the size of the deletion. The dashed red line indicates the size of the (undetected) putative deletion in embryo 5 from family 2.
IGV: Integrated genomics viewer; ROI: region of interest.



Supplementary Figure 8 | Flow diagram for direct SNV detection in embryos following clinical implementation of WGS-PGT

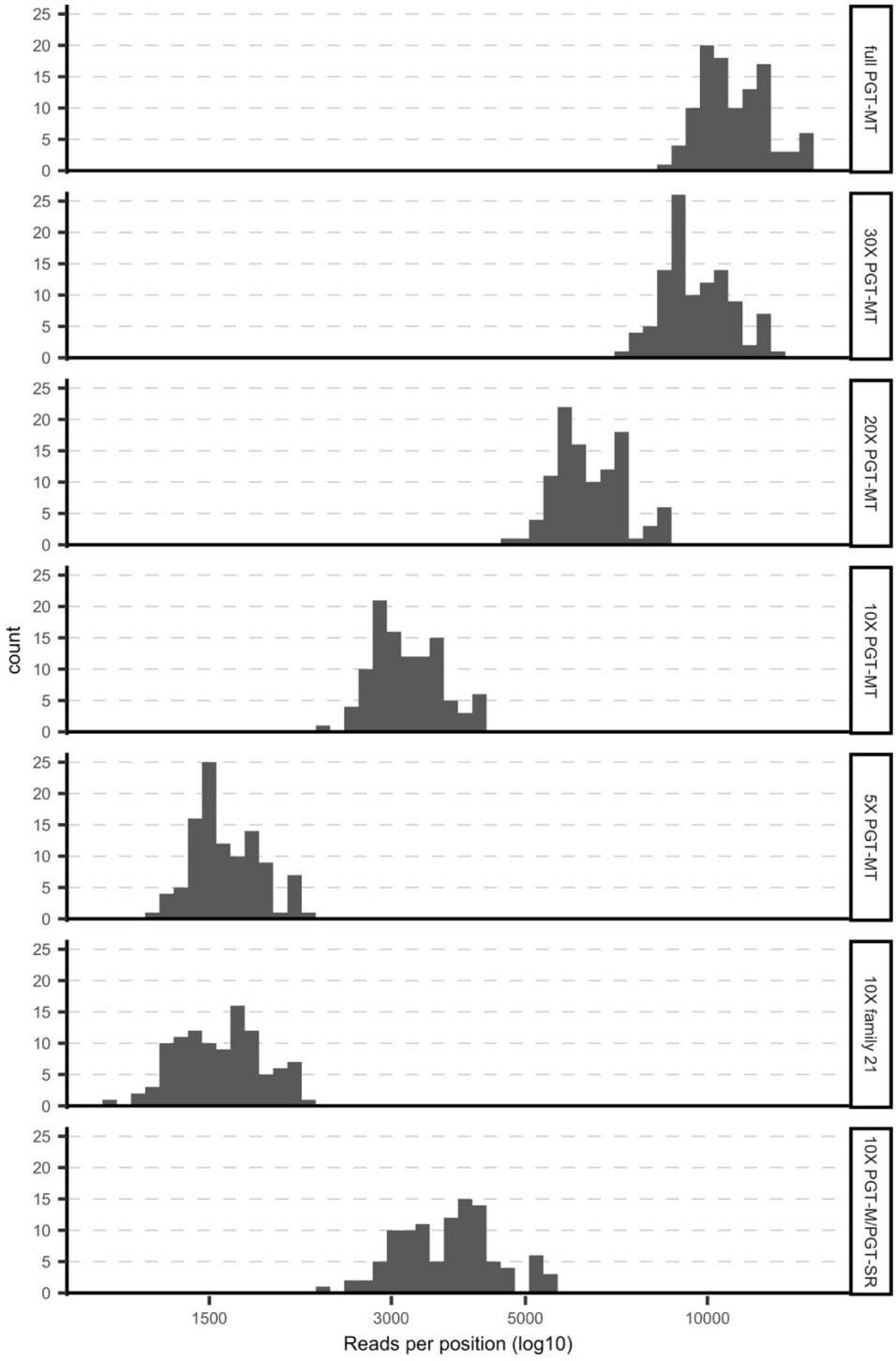
WGS-PGT has been clinically performed for 115 families. 28 families were not eligible for direct SNV detection, due to genetic indication that is bigger than > 1 bp (n = 14), genetic indication that is a repeat contraction (n = 3), a repeat expansion (n = 4), or an indication of a risk haplotype (n = 1). Six families were excluded because the family requested exclusion testing of haplotype of affected grandparent. In 87 families, direct SNV detection was attempted, encompassing a total of 98 ROIs. Of these, 22 families had no affected embryo within the cycle carrying the variant, precluding the possibility to detect the SNV. Nine families had insufficient coverage (< 5X) at the ROI, rendering SNV detection unfeasible. In 56 families the pathogenic SNV could directly be detected and was corresponding to the indirect diagnosis. bp: basepair; ROI: region of interest.



Supplementary Figure 9 | PGT-SR segmental deletion and duplication detection at different depths of coverage

Raw logR values (black dots) and segmented logR values (red) derived from *in silico* subsampled sequencing data obtained from the original WGS-PGT data (FULL; ~30-40X). Two representative embryos (embryos 1 and 4) from a family with a paternal translocation of chromosomes 8 and 16 (family 1) are shown.

PGT: preimplantation genetic testing; SR: structural rearrangement; WGS: whole genome sequencing.



Supplementary Figure 10 | PGT-MT depth of coverage at mitochondrial pathological mutation sites at different depths of coverage

Histogram showing the mean number of reads per position of pathogenic mtDNA variants (*n* = 105) at different *in silico* subsampled depths of coverage as indicated (right side labels). The mean is calculated from four PGT-MT TE-biopsy samples (rows 1-5), two PGT-MT TE-biopsy samples (for 6) or 23 embryos sequenced at 10X for PGT-M / PGT-SR indications (row 7). Data from WGS-PGT is shown.

PGT: preimplantation genetic testing; MT: mitochondrial DNA disorders; TE: trophectoderm.