Supplementary material

Supplementary Table 1. Relative number of sequenced DNA fragments mapped to each chromosome

Chromosome	**Percentage of total reads in reference samples**
1	8.97
2	8.48
3	6.45
4	5.52
5	5.59
6	5.49
7	5.21
8	4.81
9	4.40
10	4.88
11	4.94
12	4.48
13	2.81
14	3.12
15	2.90
16	3.58
17	3.40
18	2.46
19	2.82
20	2.49
21	1.26
22	1.58
X	3.81
Y	0.55

Supplementary Table 2. The final NGS protocol was a 10 step process with a 15 hour sample to data turnaround time

Step	Process	Hands on Time (mins)	Incubation Time (mins)
1	DNA quantitation and dilution	25	5
2	Fragmentation	10	15
3	Adaptor ligation	15	15
4	Size selection	30	15
5	Adaptor mediated amplification	15	40
6	Clean up	20	15
7	QC and quantification	10	40
8	OneTouch amplification and enrichment	30	270
9	Ion PGM 200bp chemistry sequencing	30	270
10	Data Analysis	15	15

Supplementary Table 3. Ultra-rapid isothermal NGS protocol can cut the time required to <8 hours

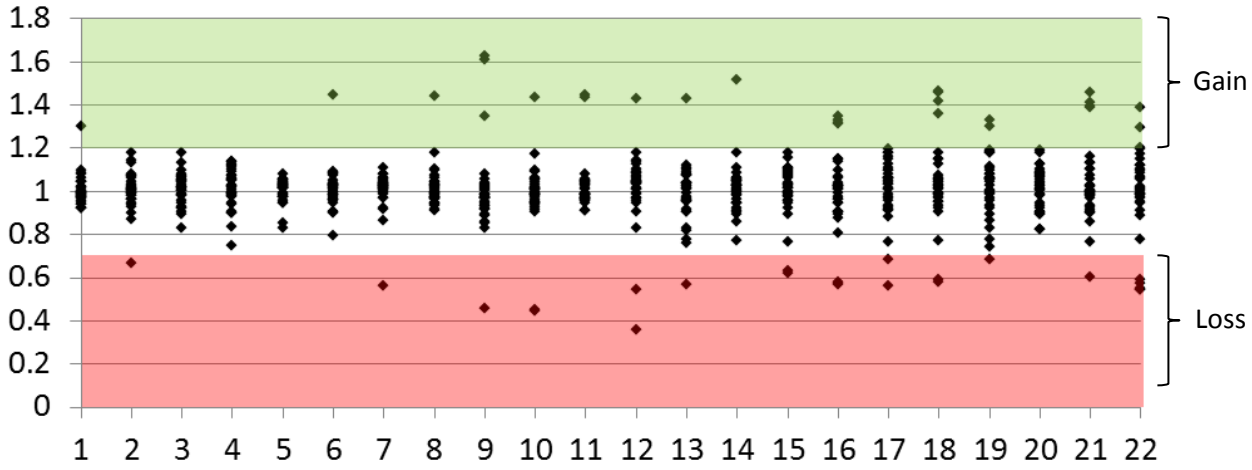
Step	Process	Hands on Time (mins)	Incubation Time (mins)
1	DNA Fragmentation	5	30
2	Adaptor ligation	10	15
3	Size selection	30	15
4	Clean up	20	15
5	QC and quantification	5	40
6	Isothermal amplification and enrichment	30	90
7	Sequencing	60	60
8	Data Analysis	15	15

Supplementary Table 4. Validation of an ultra-rapid NGS methodology using cells biopsied at the blastocyst stage. Two biopsies were taken from most of the embryos, one consisting of cells from the trophectoderm and another comprising part of the inner cell mass.

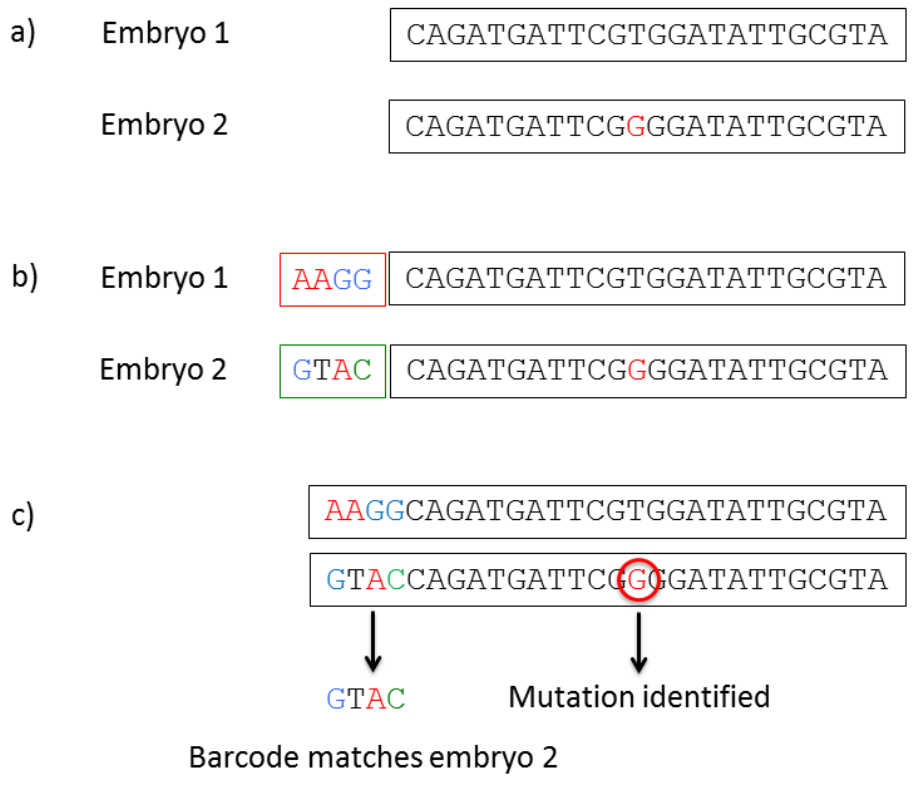
Sample number	Predicted karyotype based upon NGS result	Source of cells	Confirmatory result (aCGH)
62	47,XX,+1	Embryo (blastomere)	47,XX,+1
63	46,XY	Embryo (blastomere)	46,XY
64	46,XX	Embryo (blastomere)	46,XX
65	46,XY	Embryo (blastomere)	46,XY
66	46,XY	Embryo (blastomere)	46,XY
67	48,XY,+2,+16	Embryo (blastomere)	48,XY,+2,+16
68	Complex abnormal	Embryo (blastomere)	Complex abnormal
69	46,XY	Embryo (blastomere)	46,XY
70	46,XX	Embryo (blastomere)	46,XX
71	46,XY	Embryo (blastomere)	46,XY
72	Complex abnormal	Embryo (blastomere)	Complex abnormal
73	46,XX	Embryo (blastomere)	46,XX
74	48,XY,+2,+16	Embryo (blastomere)	46,XY,+2,+16
75	45,XY,-6	Embryo (blastomere)	45,XY,-6
76	46,XY	Embryo (blastomere)	46,XY
77	46,XX	Embryo (blastomere)	46,XX
78	45,XY,-6	Embryo (blastomere)	45,XY,-6

Supplementary Figure 1.

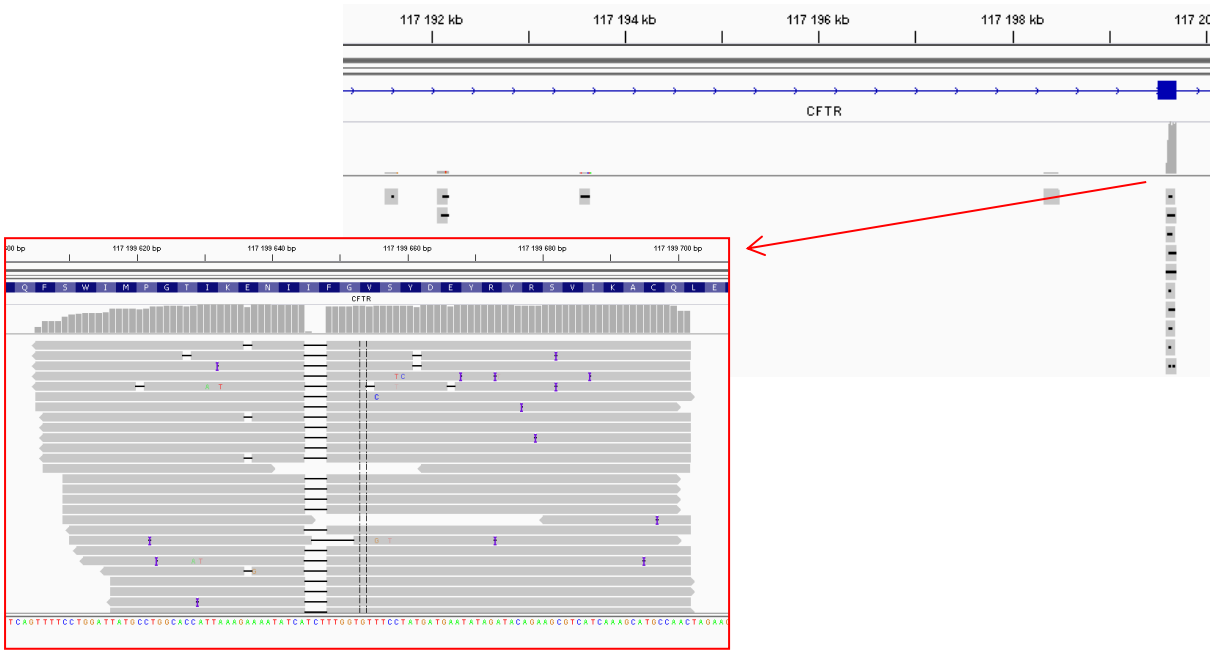
Detection of aneuploidy in cells biopsied from human preimplantation embryos. The percentage of reads mapping to each chromosome was divided by the average percentage for the same chromosome observed in a series of euploid samples. Chromosomes present in a disomic state displayed a test:reference ratio ranging from 0.7-1.2, whereas chromosomal gain (e.g. trisomy) and loss (e.g. monosomy) were associated with ratios >1.2 and <0.7, respectively. This figure summarizes the results from all of the samples tested.



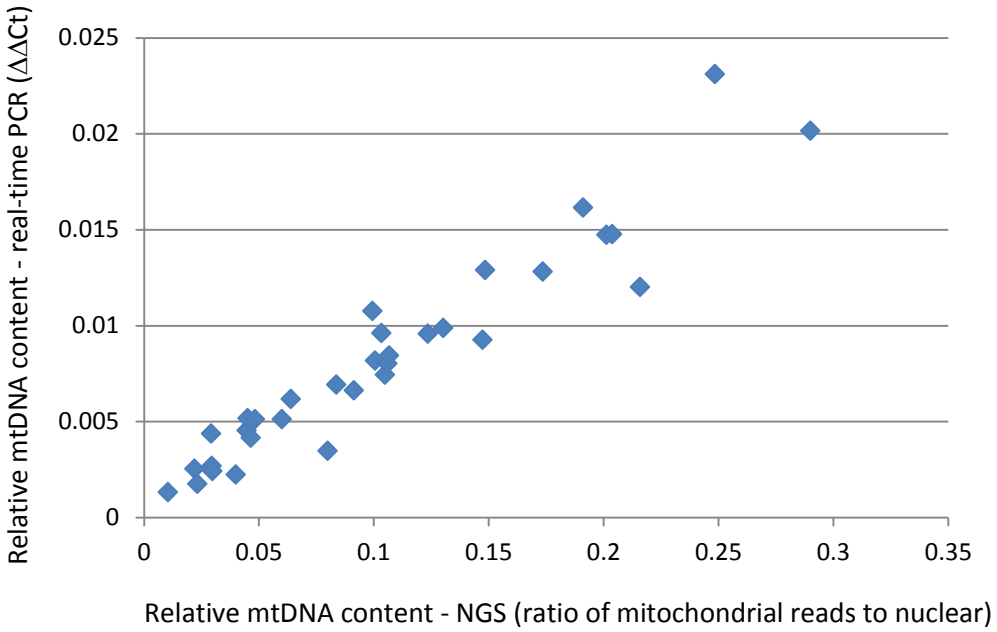
Supplementary Figure 2. The use of molecular 'barcodes' allows DNA samples from different sources to be simultaneously analyzed in a single next generation sequencing experiment. The amount of sequence data produced is divided across the samples tested (i.e. the total number of reads remains unchanged but the number of reads per sample is reduced). Importantly, the expense of analysis is also reduced proportionately (i.e. lower cost per sample). a) DNA samples derived from two or more sources (e.g. embryos) are collected. One may differ from another in terms of the amount of DNA from a specific chromosome (due to aneuploidy) or the DNA sequence at a specific site (due to mutation or polymorphism). b) After fragmentation of the DNA samples, molecular barcodes, short pieces of DNA of defined sequence, are ligated to each sample. In this example, the millions of DNA fragments in the sample from Embryo 1 now begin with sequence AAGG, whereas the millions of fragments from Embryo 2 all begin with GTAC (a four nucleotide barcode is just shown for illustrative purposes and would typically longer in reality). c) The DNA samples are then mixed and sequenced at the same time. If a mutation is detected (T>G in this example), the barcode at the beginning of the affected fragment can be interrogated to determine which embryo it was derived from (Embryo 2 in this case). This same principle can be used for quantitative applications such as aneuploidy detection.



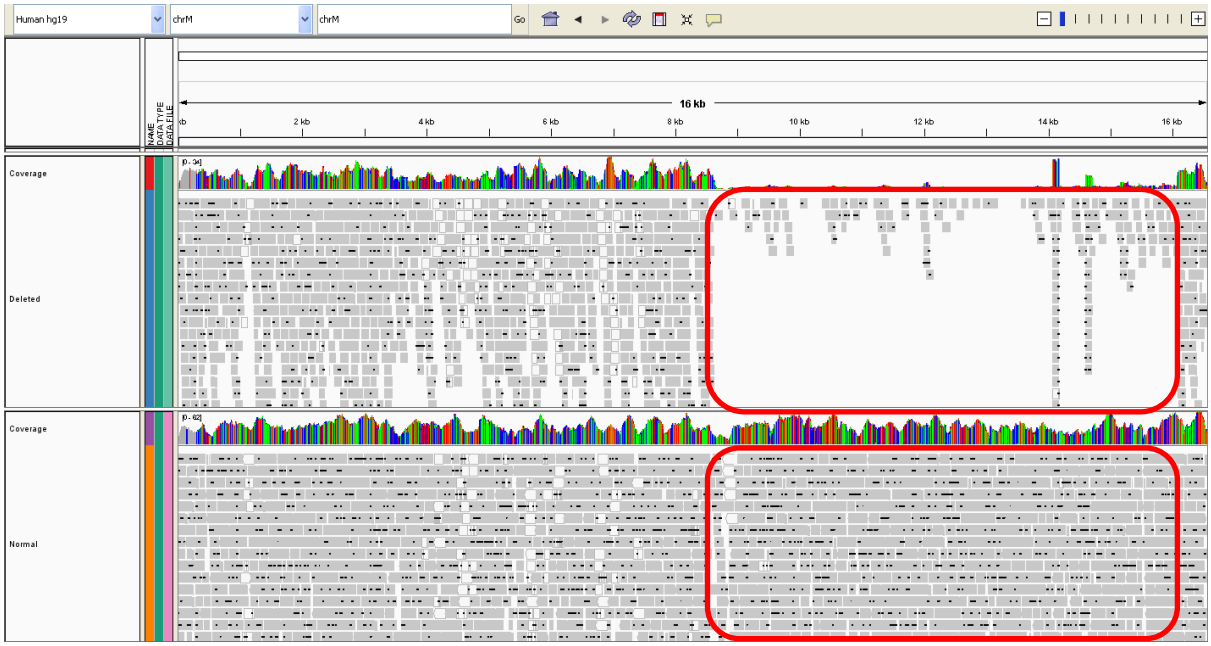
Supplementary Figure 3. Variant (*CFTR* ΔF508) detected in amplicon library spiked into WGA library (detail) – Integrative Genomics Viewer



Supplementary Figure 4. Confirmation of validity of NGS-based mtDNA quantification. Analysis of blastocyst stage embryos tested using NGS and real-time PCR demonstrated concordance between the two methods. In the case of quantitative PCR, mtDNA was referenced against an Alu repeat sequence present in numerous copies in the nuclear DNA. For NGS, the number of mtDNA sequence reads was divided by the total number of nuclear DNA sequences.



Supplementary Figure 5. Evidence of partial deletion of mitochondrial genome. Mapped reads to human genome reference (hg19) from sample with deleted mtDNA genome (top) and normal sample (bottom) – Integrative Genomics Viewer



Historical data confirming the accuracy of the aCGH method used for verification of NGS results during this investigation.

Supplementary Table 5. Results of blinded study assessing accuracy of aCGH (24Sure, Illumina formerly BlueGnome) applied to samples composed of 1, 3 or 5 cells.

Type of cell tested	aCGH results when applied to samples consisting of a single cell*	aCGH results when applied to samples consisting of 3 isolated cells*	aCGH results when applied to samples consisting of 5 isolated cells*	Original/alternative methods used for cytogenetic characterization
45,X Fibroblastoid cell line	45,X (14)	45,X (15)	45,X (15)	G-banding; FISH for chromosomes X & Y to assess mosaicism
47,XY+21 Fibroblastoid cell line	47,XY+21 (12) 47,XY,+13,+21 (1)	47,XY+21 (14)	47,XY+21 (15)	G-banding; FISH for chromosome 21 to assess mosaicism
47,XY,+18 Fibroblastoid cell line	47,XY,+18 (12)	47,XY,+18 (14)	47,XY,+18 (15)	G-banding; FISH for chromosome 18 to assess mosaicism
47,XY,+13 Fibroblastoid cell line	47,XY,+13 (13)	47,XY,+13 (14) 48,XY,+13,+19 (1)	47,XY,+13 (15)	G-banding; FISH for chromosome 13 to assess mosaicism
48,XXY,+16 Fibroblastoid cell line	48,XXY,+16 (14) 48,XX,+16 (1)	48,XXY,+16 (15)	48,XXY,+16 (14)	G-banding; FISH for chromosomes X, Y & 16 to assess mosaicism
47,XX,+22 Chorionic villus sample	47,XX,+22 (15)	47,XX,+22 (15)	47,XX,+22 (15)	G-banding; FISH for chromosome 22 to assess mosaicism
46,XY Buccal cell sample	46,XY (23)	46,XY (23)	46,XY (25)	G-banding
46,XX Buccal cell sample	46,XY (22)	46,XY (24)	46,XY (25)	G-banding
Total samples	127	135	139	
Total samples with correct cytogenetic diagnosis	125; 98.4%	134; 99.3%	139; 100%	
Total chromosomes assessed	3048	3240	3336	
Total chromosomes correctly enumerated	3046; >99.9%	3239; >99.9%	3336; 100%	

Figures in parentheses represent the number of samples that provided the result indicated.

Fibroblast cell lines were purchased from Corriel Medical Institute (http://ccr.coriell.org).

* Only samples that yielded a result are considered in this table. Approximately 5% of samples failed to give any result (9% failure rate for single blastomeres; 4% for samples composed of three cells; 1% for samples containing five cells).

Supplementary Table 6. Results of blinded study assessing accuracy of aCGH (24Sure, Illumina) applied to samples derived from preimplantation embryos.

Type of cell tested	Initial aCGH results	Blinded FISH analysis of remaining embryo cells*
Single blastomere	46,XY,-1,+20	46,XY,-1,+20 (11)
Single blastomere	47,XY,+19	47,XY,+19 (5)
Single blastomere	47,XY,+5,+6,-14	47,XY,+5,+6,-14 (5) 46,XY,+6,-14 (1)
Single blastomere	45,XX,+9,-21,-22	45,XX,+9,-21,-22 (8) 44,XX,+9,-13,-21,-22 (1)
Single blastomere	47,XX,+17	47,XX,+17 (12)
Single blastomere	47,XY,+22	47,XY,+22 (5) 46,X,+22 (1)
Single blastomere	48,XY,+18,+22	48,XY,+18,+22 (9)
Single blastomere	46,XXX,+2,-9,-21	46,XXX,+2,-9,-21 (6) 45,XX,+2,-9,-21 (1)
Trophectoderm biopsy	47,XX,+15	47,XX,+15 (15)
Trophectoderm biopsy	46,XX,+16,-18	46,XX,+16,-18 (33) 45,XX,-13,+16,-18 (1)
Trophectoderm biopsy	45,XX,-12	45,XX,-12 (17)
Trophectoderm biopsy	45,XY,-3	45,XY,-3 (26) 46,XY,-3,+22 (1)
Trophectoderm biopsy	48,XX,+8,+10	48,XX,+8,+10 (7)
Trophectoderm biopsy	47,XX,+21	47,XX,+21 (25)
Trophectoderm biopsy	47,XX,+4	47,XX,+4 (10)
Trophectoderm biopsy	45,XY,-7	45,XY,-7 (31)
Trophectoderm biopsy	47,XY,+11	47,XY,+11 (19) 45,XY,-11 (1)

Trophectoderm biopsy	44,X,-6	44,X,-6 (5)
Trophectoderm biopsy	69,XXY	69,XXY (21)
Trophectoderm biopsy	47,XY,+21	47,XY,+21 (13)
Trophectoderm biopsy	47,XX,+16	47,XX,+16 (49) 46,XX,+16,-18 (1)
Trophectoderm biopsy	45,XY,-4	46,XY (7) 45,XY,-4 (1) 45,XY,-18 (1)
Trophectoderm biopsy	45,XX,-22	45,XX,-22 (17)
Trophectoderm biopsy	48,XX,+10,+19	48,XX,+10,+19 (29) 47,XX,+10 (1)
Trophectoderm biopsy	47,XXY	47,XXY (9)
Trophectoderm biopsy	46,XY,+15,-22	46,XY,+15,-22 (22)
Trophectoderm biopsy	47,XX,+1	47,XX,+1 (16)
Trophectoderm biopsy	47,XX,+15	47,XX,+15 (9)
Total embryos assessed	28	
Embryo with a correct diagnosis	27; 96.4%	
Total chromosomes with confirmatory data available	176	
Total chromosomes correctly enumerated (considering the major cell line only)	175; 99.4%	

* After aCGH had been carried out, a portion of the remaining cells from each embryo were fixed and analyzed using FISH. This took place on either day-4, day-5 or day-6 post fertilization. The chromosomes tested were limited to those that aCGH had previously indicated to be abnormal (first round of FISH) followed by chromosomes 13, 16, 18, 21 and 22 (second round of FISH). After addition of the first set of FISH probes, the slides were coded in order to prevent the cytogeneticist from knowing which embryo (or indeed which chromosome) was being assessed. Results were later decoded by an independent scientist, revealing the chromosome(s) analyzed and the embryo from which the cells had been derived.

The following exclusions were applied: 1) Embryos with <5 cells yielding FISH results were not considered further and do not appear in the table. The accuracy of FISH applied to a single cell is questionable and consequently it was considered necessary to have FISH results from multiple cells in order to obtain a reliable assessment for each chromosome. In some cases relatively few cells were available for re-analysis, despite testing at the blastocyst stage, as other pieces of the embryo had been removed for other unrelated research purposes; 2) Embryos with mosaicism for the chromosomes tested using FISH were excluded. Mosaicism was defined as two or more cells having a different number of FISH signals compared to the other cells of the same embryo. This measure was intended to eliminate embryos with appreciable levels of mosaicism that might give aCGH and FISH results which were discordant, not due to technical error, but rather due to variation in the karyotype of individual cells. The purpose of this study was to evaluate the technical accuracy of aCGH and not to assess biological sources of error such as mosaicism. Embryos with single discordant cells possibly due to a FISH error or in some cases true low-level mosaicism were still included; 3) Tetraploid cells were not considered in this data-set, since the presence of such cells in embryos is hypothesized to be a normal feature of preimplantation development.