the enriched WGA and a total of 40 samples were subsequently multiplexed and sequenced on a MiSeq sequencer according to standard 2x75bp protocol (Illumina). The sequencing data was bioinformatically aligned to hg19 to determine aneuploidy status. In addition, ADO and depth of beta globin specific sequencing reads was evaluated.
Results: Correct aneuploidy diagnoses were achieved for all single and multi-cells with trisomy 21, and with abnormal sex chromosome numbers XXY (approximately 900,000 reads per sample). Enrichment of the beta globin gene sequences was confirmed by semi-quantitative gene specific PCR and NGS when compared to the control WGA DNA sample without enrichment. The NGS protocol used was typical for PGS but was not expected to yield the depth of reads required for PGD. The purpose of the analysis was to demonstrate a reduction in ADO rate through enrichment. No sequencing reads mapping to the beta globin gene were reported for control samples, compared to 11 reads mapping to beta globin (exon 3) and 76 reads mapping to beta globin (exon 1 and 2) in the enriched WGA samples. When the beta globin gene was sequenced independently, as would be done for PGD, read depths in excess of 300x for exons 1, 2 and 3 were achieved with 100% of the exons covered.
Conclusions: The targeted and concurrent enrichment of globin sequence during WGA reduced ADO and when separately indexed, provided a suitable depth of coverage for PGD without compromising PGS results. The enriched WGA products can be analysed using NGS or pre- established PGD workflows such as Sanger sequencing or size determination.

OC-03
Haplotyping and copy-number profiling of single cells by massive parallel sequencing

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Introduction: Genome-wide haplotyping (such as karyomapping or haplarithmisis) enables the reconstruction of genome-wide haplotypes and copy-number profiles and is able to determine the parental origin of haplotypes at the single cell level. This methodology is being implemented as a generic method for preimplantation genetic diagnosis (PGD) in many IVF laboratories. The method can be applied on blastomeres biopsied from cleavage embryos or trophectoderm sampled from blastocysts. This allows the diagnosis of disease alleles, numerical and structural chromosomal abnormalities genome-wide. Current methods use SNP arrays to deduce the haplotype. The resolution of SNP arrays is limited and the platform is costly. The ultimate genetic screen of a single cell would be whole genome sequencing. However, this is currently too expensive. To overcome these limitations, we developed a single cell based genotyping-by-sequencing (GBS) method.
Materials and methods: Proof-of-principle experiments were performed on cells from the HapMap cell lines. DNA is amplified by RepliG. Three different restriction enzymes were used to digest the DNA and reduced representation libraries were generated. The fragments were sequenced on Illumina HiSeq2500. Subsequent haplotyping was performed by haplarithmisis. To allow for sequencing based genotyping, the siCHILD computational workflow was amended.
Results: We first demonstrate that a median coverage of 20 allows for a genotyping accuracy of 99.9 % for bulk DNA. Across informative SNPs covered in both single cell and genomic DNA from the same individual we obtain a 70 % concordance for the single cell genotype compared to the bulk DNA genotype. Preliminary results show a confidence of 99% within a distance of 18 informative SNPs flanking a homologous recombination site for correct single cell haplotype inference compared to the haplotype derived from bulk DNA. Subsequently a validation study is performed by comparing the GBS based haplotypes with the SNP array based haplotypes which were derived on embryos from clinical PGD cycles. Results of this ongoing study will be presented.
Conclusions: Here, we present a novel methodology for genotyping single cells through next-generation sequencing. By applying the GBS protocol as a method for genome reduction and the advancing sequencing technologies, we foresee this methodology as a valuable alternative for genotyping using SNP arrays.

OC-04
From prenatal diagnosis of fetal abnormality to preimplantation genetic diagnosis for skeletal dysplasia using next-generation-sequencing technologies

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Introduction: Skeletal malformations and dysplasias involve more than 300 syndromic and non-syndromic disorders, characterized by considerable phenotypic and genetic heterogeneity. Regarding to Jeune syndrome, common signs and symptoms include a small chest and short ribs which restrict the growth and expansion of the lungs, often causing life-threatening breathing difficulties. In cases where chromosomal abnormalities are not detected along a pregnancy, Trio-WES (whole genome amplification) is revealed as a powerful technique for genetic testing of gene mutations associated with those malformations observed by ultrasound. Concurrently, if causative point mutations are detected, preimplantation genetic diagnosis (PGD) coupled with informativity testing is mandatory before *in vitro* fertilization (IVF) in order to transfer disease-free embryos successfully.
Material and Methods: We present the case study of a healthy couple with a previous history of two consecutive selected abortions due to skeletal dysplasia, evaluated by second-trimester ultrasound. A protocol for preconception assessment based on genetic diagnosis of the second affected abortion was developed, in order to prevent inheritance diseases in subsequent pregnancies. In the set-up phase, we performed genetic diagnosis on DNA isolated from both the malformed abortion material and parental blood samples by targeted Trio-WES, using Ion-AmpliSeq™ Exome Kit on Ion-Proton™ platform (Thermo Fisher Scientific). Afterwards, two IVF cycles were performed combining PGS (preimplantation genetic screening) and PGD between August and October 2016. After oocyte insemination and day-5 biopsy, DNA whole genome amplification was followed by PGS/PGD/informativity testing in all embryos. SNPs (single nucleotide polymorphism) detected through exome sequencing were used for the informativity test.
Results: After Trio-WES study, it was found that both members of the couple were genetic carriers of Jeune syndrome. Likewise, both mutations were detected in compound heterozygosity state in *DYNC2H1* gene (pathogenic p.(Thr2106fs*7), and likely pathogenic p.(Asp3015Gly)), after the analysis of fetal tissue. After 5-day embryo biopsy, a combined PGD/PGS cycle was carried out. Along informativity testing, SNP-genotyping linked to gene regions involved by mutations was performed according to original Trio-WES data, giving us an accurate genetic profiling of embryos before implantation. A total of 65 informative SNP markers were selected in 2000 Kb flanking region on both sides of *DYNC2H1* gene and Ion- AmpliSeq™ primer pools were designed. Targeted next-generation sequencing on Ion-PGM™ System along with Ion-AmpliSeq™ and Ion- ReproSeq™ workflow was performed. After both IVF cycles and among the seven embryos analyzed, 2 were euploid and 5 aneuploid. Both euploid ones were also healthy carriers for only one mutation, therefore they were suitable for transfer. This has not been performed yet due to gynecological issues in the patient. Presented framework will improve whole PGD/PGS in terms of turnaround-time, reliability and cost- effectiveness.
Conclusions: We applied Trio-WES results, combined with PGS/PGD and informativity testing in order to obtain healthy embryos for couples at risk for transmitting skeletal dysplasia. Beyond this, couples