CHAPTER

20

Single cell genomics to study DNA and chromosome changes in human gametes and embryos

Robert C. Blanshard[*,†], **Chongyi Chen**[‡], **Xiaoliang Sunney Xie**[‡,§,1], **Eva R. Hoffmann**[*,¶,1]

**Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Brighton, United Kingdom*

†Clinical Genomics Group, Illumina Inc., Fulbourn, United Kingdom

‡Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, United States

§Beijing Advanced Innovation Center for Genomics, Beijing, China

¶Center for Chromosome Stability, University of Copenhagen, Copenhagen, Denmark

[1]Corresponding authors: e-mail address: xie@chemistry.harvard.edu; eva@sund.ku.dk

CHAPTER OUTLINE

Methods in Cell Biology, Volume 144, ISSN 0091-679X, https://doi.org/10.1016/bs.mcb.2018.03.037

Abstract

Genomic and chromosomal changes occur with a high rate in the germline and preimplantation embryos. To study such changes directly in the germline of mammals requires access to material as well as single cell genomics. Recent improvements in embryology and single-cell DNA amplification make it possible to study the genomic changes directly in human oocytes, sperm, and preimplantation embryos. This is particularly important for the study of chromosome segregation directly in human oocytes and preimplantation embryos. Here, we present a practical approach how to obtain high quality DNA sequences and genotypes from single cells, using manual handling of the material that makes it possible to detect genomic changes in meiosis and mitosis spanning the entire range from single nucleotide changes to whole chromosome aneuploidies.

1 INTRODUCTION

Genomic changes in the germline and somatic cells provide the genetic variability that drives immune diversity, tumor evolution, and germline evolution. Being able to study genome changes in single cells allows the deconvolution of ensemble population studies and therefore exploration of the true genetic states and heterogeneity of individual cells. The last decade has seen tremendous technological advances in probing DNA changes in single cells, referred to as single cell genomics (Gawad, Koh, & Quake, 2016; Huang, Ma, Chapman, Lu, & Xie, 2015; Zhang et al., 1992). Current technologies now make it possible to detect genomic changes ranging from single base mutations, to whole chromosome gains and losses (aneuploidy). With statistical methods to phase haplotypes, it is also possible to assess chromosome recombination and infer segregation patterns in meiosis, the specialized cell division process that generates gametes (Hou et al., 2013; Lu et al., 2012; Ottolini et al., 2015; Wang, Fan, Behr, & Quake, 2012).

Being able to explore genetic diversity of single cells together with advances in embryology has revealed new facets of chromosomal changes in meiosis that can explain genetic features of the human population, as well as those that impinge on reproductive health in women as they age (Capalbo, Hoffmann, Cimadomo, Maria Ubaldi, & Rienzi, 2017). Two studies of adult human oocytes have revealed the importance of recombination for chromosome segregation and thus prevention of aneuploidy (Hou et al., 2013; Ottolini et al., 2015). Detecting both sequence and copy number variation (CNV) in the same cell also allowed the identification of a new aberrant segregation pattern termed reverse segregation (Ottolini et al., 2015). Single cell technologies have also enabled de novo detection of complex chromosomal arrangements (chromothripsis) (Zhang et al., 2015), replication fork timing in individual cells (Chen et al., 2017), as well as mutation signatures.

In human preimplantation embryos, biopsies of one blastomere or 5–10 cells of the trophectoderm lineage, which gives rise to the placenta, are being used to assess chromosome loss or gain (aneuploidy) as well as mosaicism and structural gains and losses, to improve overall clinical outcomes for patients (Vermeesch, Voet, & Devriendt, 2016). However, this also provides us with an opportunity to assess genome changes and cellular fate in early embryos (Bolton et al., 2016). Common to single cell (or low input) genome sequencing is the overall requirement to distinguish real genetic changes from noise that is introduced by the technology, especially during the whole genome amplification (WGA) step. Technological and statistical advances continue to improve and push the limits of detection (Chen et al., 2017). Here, we discuss the considerations to be taken into account when designing a single cell genomics experiment, the use of linear amplification via transposon insertion (LIANTI), a novel WGA method, and human female meiosis as special focus.

2 METHODS

2.1 DESIGN OF A SINGLE CELL GENOMICS EXPERIMENT

The design of a single cell genomics experiment is critical and depends on the genomic changes that are being detected. In general, the work flow consists of four stages that influence subsequent processing: single cell isolation, WGA, sequencing, and data analysis (Fig. 1). Single cell sequencing for the purpose of detecting aneuploidy requires high reproducibility of WGA, but low coverage (0.01 ×) is sufficient to detect relative changes in chromosome numbers. In contrast, genotyping requires a high read depth for calling variants (30 × is preferred), which is expensive. An alternative to next-generation sequencing (NGS)-based variant calling, microarray approaches allow a cost-effective approach to detect highly characterized single-nucleotide polymorphisms (SNPs). However, the overall detection is limited by the number of features on the chip (e.g., 1.7 million on the Multi-Ethnic Global array (Illumina Inc.)). Both sequencing and microarrays have proven useful for detecting recombination events and inferring chromosome segregation patterns in human female meiosis (Hou et al., 2013; Ottolini et al., 2015) but require expert data

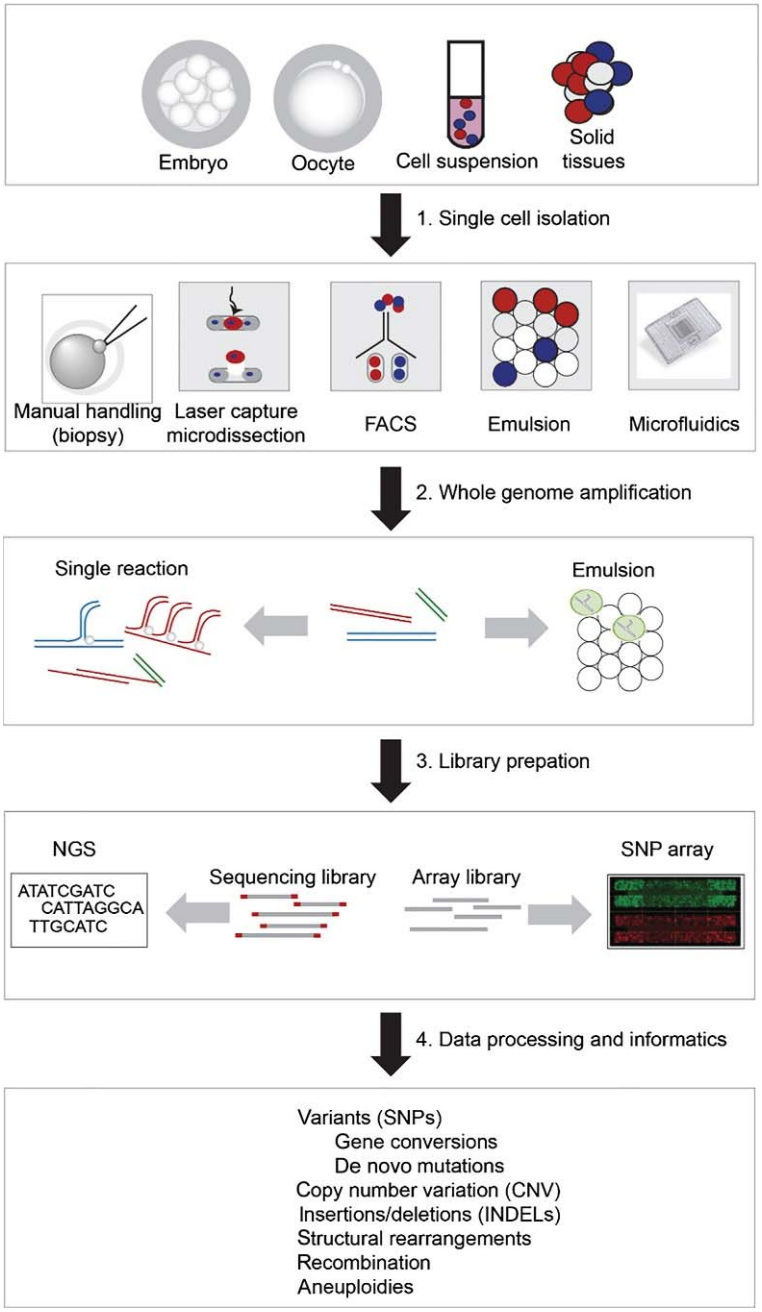

FIG. 1

Single cell genomics work flow.

analyses and informatics. Below, we focus on the two single cell pipelines used in our labs that utilize LIANTI and multiple displacement amplification (MDA)-based WGA, followed by NGS or microarray detection. We emphasize that other technologies can of course also be used and to illustrate this, we have made the methods described below as generic as possible.

Single cell isolation depends on the cell type and ranges from manual handling, such as for human oocytes and embryos, to microfluidics and emulsion approaches for cell suspensions (Fig. 1). Manual handling is time consuming and requires expert skills when obtaining single cells from rare material such as embryos and oocytes. In contrast, fluorescence-activated cell sorting (FACS), emulsion, and microfluidics approaches of cell suspensions are usually quick and relatively straightforward, allowing the parallel processing of thousands of cells. However, capturing multiple cells is a problem (doubles), and particularly the emulsion approach requires a large number of cells, because capture rates mostly follow poisson distributions (fewer than 10% of cells). "Dead" cells are another issue that influences the quality of the experiment, and we describe methods to exclude such cells both from the initial experiment and from the subsequent data analyses.

The original WGA method was PCR based (Zhang et al., 1992) and continues to provide a cost-effective solution to copy number detection, although genome coverage is limited (Blagodatskikh et al., 2017). Quasi-linear methods such as PicoPlex (Rubicon Genomics) or SurePlex (Illumina Inc.) and Multiple Annealing and Looping-Based Amplification Cycles (MALBAC; Yikon Genomics) have a "linear" phase, followed by a limited number of PCR-based cycles. Isothermal reactions such as MDA use relatively high fidelity polymerase (Φ29) with strand displacement activity, resulting in branched structures and high yields. When carried out in low volumes in microfluidics devices or in emulsion, MDA can detect de novo mutations with high precision and gives rise to an even coverage across the genome (Fu et al., 2015; Wang et al., 2012). MDA is widely used and has the advantage that the polymerase Φ29 has a low error rate and possesses strand displacement activity. Quasi-linear approaches such as PicoPlex or MALBAC are also available and used in several clinical applications, especially to detect CNV (Yan et al., 2015).

The most recent development in WGA is the use of a modified Tn5 transposon to fragment the single-cell genome and insert T7 promoters, from which T7 RNA polymerase can transcribe in vitro (LIANTI) (Chen et al., 2017). Transcription, not PCR or other DNA polymerases, thus amplify the genome in a linear fashion. LIANTI yields the best coverage yet in single-reaction amplification. Whereas LIANTI performs better than MDA and MALBAC, the yield is lower (3 μg compared to 25 μg), which may affect downstream processing. Quality control (QC) for WGA can be done directly after the reaction for MDA, where yields are high. For LIANTI, the QC is performed directly after the reaction, based on the yield, and again after library preparation based on quantitation of functional libraries by qPCR results, before loading to sequencers. Prior to sequencing or genotyping by microarrays, the amplified DNA must be processed to obtain an optimal length.

For NGS, adapters/linker sequences (known as indexes) are added for library multiplexing and flow cell attachment. There are several different technologies with some

that rely on mechanical sheering of the DNA (e.g., TruSeq DNA PCR-Free Library Preparation; Illumina Inc.) and others that use additional PCR-based amplification steps (e.g., Nextera DNA Library Preparation; Illumina Inc.). Microarray-based libraries are generated by enzymatic fragmentation. For both NGS and microarray-based genotyping, the data analyses associated with single-cell genome sequencing and genotyping are challenging since some algorithms need to be adapted for single cell applications. There are a variety of variant callers currently available, and the development in this area is moving very fast. Below, we give examples of two workflows from human female meiosis using microarray-based technologies and single cell sequencing using LIANTI to detect recombination and infer chromosome segregation.

2.2 WGA METHODS COMPARISON

Fig. 2 shows the comparison of single-cell amplification evenness between MDA, PicoPlex or MALBAC, degenerate oligonucleotide-primed (DOP)-PCR, LIANTI, and bulk sequencing. MDA relies on Φ29 DNA polymerase, which has high replication fidelity generating accurate single-cell single nucleotide variant (SNV) detection, but CNV detection is limited due to its exponential amplification noise. PicoPlex or MALBAC uses a quasi-linear amplification scheme to achieve better amplification evenness than MDA, which, together with normalization of the systematic genome-wide amplification noise, enables single-cell CNV detection with megabase resolution. DOP-PCR has a similar level of amplification noise, but at the cost of a very low genome coverage. The linear amplification scheme of LIANTI offers the highest amplification evenness and fidelity of the whole genome, enabling single-cell micro-CNV detection with kilobase resolution, as well as accurate single-cell SNV

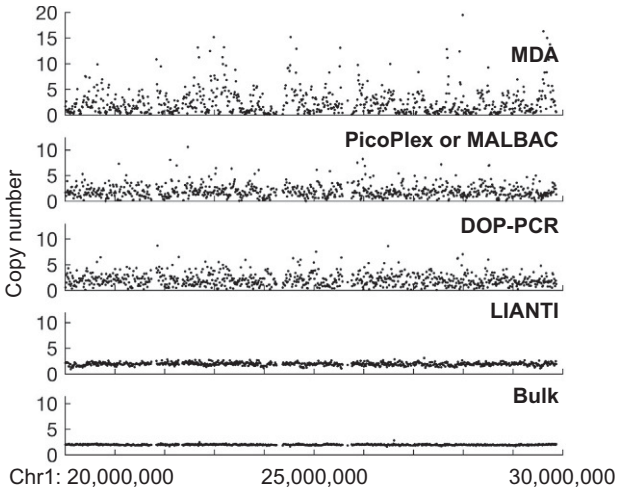

FIG. 2

Amplification evenness comparison between single-cell WGA methods. Plotted are read depths across the genome with 10-kb bin size (only a 10-Mb region in Chromosome 1 shown in the plot).

detection. LIANTI has been used to detect the stochastic DNA replication origin firing and replicon formation in single human cells in S-phase (Fig. 3) (Chen et al., 2017), and to detect micro-CNVs (microdeletions) and trisomy in human embryos with a variety of genetic disorders (Fig. 4) (Wapner, et al., 2012).

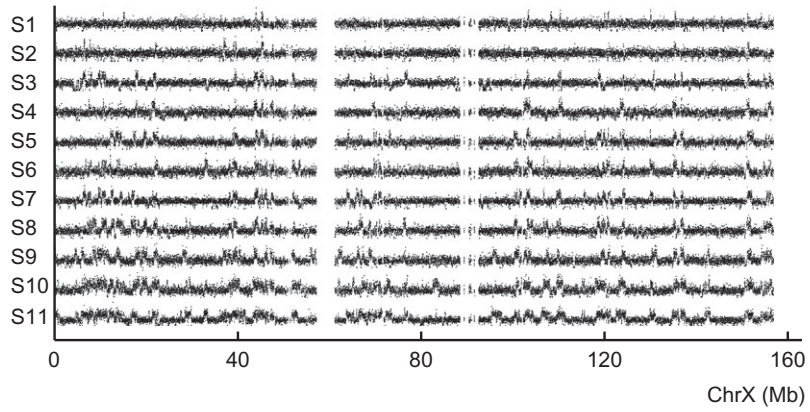

FIG. 3

DNA replication origin firing and replicon formation. Plotted are genome-wide CNVs detected by LIANTI in 11 single S-phase BJ cells with 10-kb bin size (only X chromosome shown in the plot).

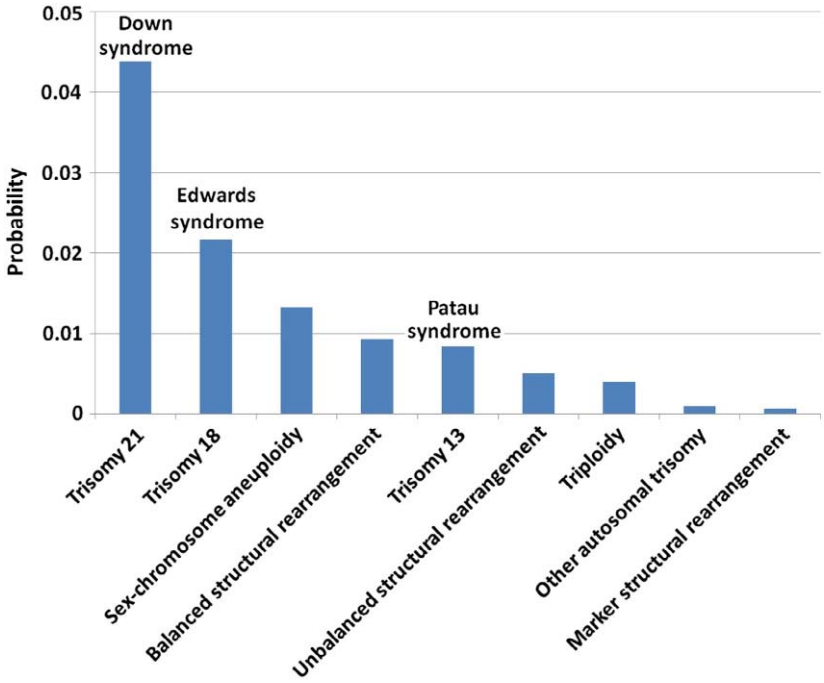

FIG. 4

Frequencies of microdeletions and trisomy in human embryos.

The needs of different applications are often met by different single-cell WGA methods. A comprehensive comparison of DOP-PCR, MDA, PicoPLex or MALBAC, and LIANTI has been provided (Table 1).

2.3 SAMPLE COLLECTION

2.3.1 Isolating single cells from cell lines

1. Cells may be isolated manually by micropipetting, FACS analysis, or other methods. Please note that the cell cycle stage may affect results, and synchronization may be needed. Make two buffers 1 × PBS + 0.1% PVP (Origio, 10905000) and 1 × PBS (Cell Signalling). Expose the buffers and the collection tubes (0.2-mL thin-walled PCR tubes with closed lids in a rack; handle with metal forceps) to UV-C for ≥30 min. For FACS, use a skirted 0.2-mL plate (UV treated) and ensure that wells are sealed promptly after collection (in a Class II Microbiological Safety Cabinet).
2. Harvest 1.0×10^6 cells and perform an Annexin V-based Dead Cell Removal (Miltenyi Biotech, 130-090-101) according to the manufacturer's instructions. Perform a viability assessment by staining with 0.4% Trypan blue. Viability of ≥98.0% is recommended. Centrifuge cell suspension at 100 × *g* in a microfuge for 5 min and wash pellet twice with 1 mL 1 × PBS + 0.1% PVP. The cells can be stored at 4°C for up to 5 h.
3. For manual isolation, use a stripper pipette (e.g., Origio) set at the WGA volume to handle individual cells and use a stereomicroscope in a hood. Use a sterile dish (e.g., Nunc, ICSI dish) and prepare 2–3 drops of 10 µL 1 × PBS + 0.1% PVP for diluting the cells. Isolate individual cells into 6 µL 1 × PBS + 0.1% PVP. Isolate 3–5 cells (positive control) and an empty drop (negative control). Wash the tip in a fresh drop of 1 × PBS between each cell. Include negative controls at several points and at the end. Check that all drops contain single cells and then remove cells in a fresh drop of 6 µL 1 × PBS + 0.1% PVP, pipetting the cell up and down. Dispense entire volume; move the tip away from cell; reverse pipette the transfer volume (0.5–1 µL, pending WGA); pick up the cell at the very tip. This allows efficient flushing of the cell when dispending into the WGA tube (e.g., the final volume for MDA is 4 µL). Dispense the cell *directly* into the solution into the collection tube without touching the sides. Rinse the tip under the microscope—no cell should be observed if the transfer is successful.
4. For FACS, wash the cell pellet in 1 × PBS and label the cells with Molecular Probes™ Dead Cell Apoptosis Kit (Fisher Scientific, 10257392) immediately prior to sorting to collect viable cells. Select appropriate gating for positive, negative, and single cell collection and prime the 0.2-mL tubes with the required volume of 1 × PBS. Determine the rate of two more cells if using a 96-well plate. These should not be used. The failure rate is about 10–15% (no cells).
5. Centrifuge the sample tubes/plates at 280 × *g* for 1 min, then proceed immediately to WGA. Alternatively, store samples at −80°C for no more than 24 h.

Table 1 Comparison Between Single-Cell WGA Methods

WGA Method	Genome Coverage (%)	SNV Accuracy	SNV FPR	CNV Accuracy	CNV Spatial Resolution	Amplification Principle	Amplification Enzyme	Kit Availability
DOP-PCR	45	Low	2×10^{-4}	High	Megabase	Exponential	PCR DNA polymerase	Yes
MDA	87	High	$(1–2) \times 10^{-5}$	Low	Chromosome level	Exponential	Phi29 DNA polymerase	Yes
PicoPlex or MALBAC	73	Low	$(1–4) \times 10^{-4}$	High	Megabase	Quasilinear	PCR DNA polymerase	Yes
LIANTI	95	High	$(2–5) \times 10^{-6}$	High	Kilobase	Linear	T7 RNA polymerase	No

2.3.2 Isolating all three products of female meiosis: Human oocytes, embryos, and polar bodies

To obtain the genetic information from female meiosis, human oocytes and matching polar bodies (PB) are biopsied (Ottolini et al., 2016, 2015). The PBs should be biopsied sequentially to ensure that PB1 and PB2 are distinguishable a priori, and to prevent or limit degradation of in the PB1 prior to DNA amplification. The PB1 is removed from the meiosis II (MII)-arrested oocyte using a laser-assisted microscope and immediately tubed. The MII oocytes are activated by artificial activation using 40 min exposure to 100 μM A23187, a Ca^{2+} ionophore that triggers the second meiotic division and extrusion of the PB2. A single female pronucleus should form. Alternatively, the MII oocytes can be fertilized by sperm using intracytoplasmic sperm injection (ICSI). This generates a zygote, and the female pronucleus can be removed (Hou et al., 2013). All of these procedures require extensive handling skills of embryo and oocytes, and should be carried out by trained embryologists under appropriate ethics permissions and informed consent.

The biopsied cells are transferred into an 0.2-mL tube for WGA using a stripper pipette into the appropriate volume of 1 × PBS. The PBs are very small and washing them in PBS or media does not improve performance; however, the volume of media transferred with the cells should be minimized.

2.4 WHOLE GENOME AMPLIFICATION

WGA reactions should be assembled in a clean, dedicated pre-PCR work area, preferably under HEPA-filtered air flow via a Class II Microbiological Safety Cabinet (https://www.labogene.com) to minimize the risk of external contamination. Clean the work area, pipettes, and tube racks with a nucleic acid decontamination reagent and allow to dry, prior to exposure of equipment and consumables to UV-C for ≥30 min. Alongside the cell isolation/biopsy controls, prepare replicates of 16–30 pg genomic DNA-positive controls in 1 × PBS, and 1 × PBS no template controls in 0.2-mL thin-walled PCR tubes, with a final volume matching that of the cell/biopsy collection. Immediately before use, remove the cell or biopsy samples from fresh or frozen storage and centrifuge at 300 × *g* for 3 min; immediate lysis is critical for the quality of the WGA. When adding reagents, it is critical to dispense the reagent above the sample droplet, onto the inner wall of the tube, to mitigate the risk of removing the sample cell or DNA when withdrawing the pipette tip. Change pipette tips between samples. Centrifuge sample and control tubes at 300 × *g* for 5 s to collect reagents at the bottom of the tube. Exponential amplification on the thermal cycler must be performed in a dedicated post-PCR area. Store amplified products at −20°C or −80°C. For best results, process samples within 1 week.

2.4.1 WGA by SureMDA

A number of MDA kits are commercially available. The SureMDA Amplification System (SureMDA; Illumina Inc.) has been QC tested by Illumina for the purpose of Karyomapping. We use a shortened amplification protocol than specified with

the REPLI-g® Single Cell Kit (QIAGEN) for our research. In brief, the reaction should be assembled according to the manufacturer's instructions with the following modifications.

1. Handle samples on liquid-sealed −20°C cold blocks. Do not use wet ice, as this increases the risk of external contamination.
2. Following cell lysis, addition of the stop solution should be performed swiftly. When processing large sample sizes, dispense 3 μL stop solution onto the inner wall of the tube or well, and tap the plate periodically to collect the reagents at the bottom of the tubes or wells, prior to the final centrifugation.
3. Critically, we reduce the amplification time at 30°C for 2 h with SureMDA. This generates a yield of 20–25 μg and reduces artifacts.

2.4.2 WGA by LIANTI

The LIANTI assay should be assembled according to published protocols (Chen et al., 2017). Several critical points worthy of note are:

1. Transposome is made by mixing equal volume of 1.5 μM annealed LIANTI transposon DNA and ~1 μM Tn5 transposase, which is prepared in house or purchased from Epicentre (Illumina Inc. via Lucigen Corp.).
2. LIANTI transposome is made freshly every time before use, since long time storage of transposome may lead to degradation.
3. The steps before in vitro transcription should be carried out in a dedicated clean setting, to minimize the risk of contamination.

2.4.3 QC by gel electrophoresis

Successful amplification can be determined by gel electrophoresis. The long fragments generated by MDA should be resolved on a 0.8% agarose gel against a 1-kb extension ladder. A bright smear should be seen around 3–7 kb for our MDA protocol. The shorter 100–1000 bp products generated by PicoPlex and LIANTI should be resolved on a 1.5% agarose gel against a 1-kb ladder.

2.4.4 QC by quantitation

Accurate quantitation of input DNA for NGS and microarray analysis is essential for achieving optimal fragment size distribution during library preparation. A fluorometric-based assessment of MDA products for duplex DNA should be performed (Quant-iT™ dsDNA High-Sensitivity (HS) Assay Kit; Invitrogen™). Perform a serial dilution of the WGA products in Molecular Biology Grade (MBG) dH_2O to within the specified range, using at least 5 μL DNA for each step of the dilution. Quantify the final diluted WGA products according to the manufacturers' instructions, using a microplate reader (FLUOstar® Omega; BMG Labtech) and appropriate software (MARS Data Analysis Software; BMG Labtech).

2.5 NGS AND MICROARRAY ANALYSIS

Selection of NGS vs microarray methodology should be based on output. This likewise determines the library preparation approach used. For high precision and variant calling, mechanical shearing and adaptor ligation is preferred, whereas CNV can readily be detected from low input volumes from PCR-based library preps. The hands-on time and required equipment needed to generate PCR-free libraries vs PCR-based libraries differ dramatically. High precision genotype calling at a lower density can be achieved by SNP microarrays at a much lower cost compared to the sequencing depth required for untargeted NGS-based genotyping.

2.5.1 NGS for CNV

CNV analysis can be performed on products from all WGA methods described here; however, the library preparation method for sequencing may vary depending on the input configuration. The main principles of library preparation for NGS comprise sample DNA fragmentation, library specific indexing for multiplexing, fragment size selection, library quantification, normalization, and pooling. CNV detection is performed by binning aligned sequence reads across the genome, and comparing the read counts against a euploid reference. Accurate copy number analysis requires a complete understanding of the noise and bias profiles of the WGA system. Amplification bias is well tolerated provided that it is systematic and not stochastic. There are several pipelines for CNV analysis (e.g., VeriSeq® from Illumina Inc. is a commercially available pipeline that uses SurePlex). For LIANTI, in addition to conventional CNV callers relying on read depth (e.g., Control-FREEC, BoevaLab), specific algorithms are also available to call single-cell CNV from LIANTI data based on the digital counting principle where sequencing reads with the same alignment positions can be grouped (e.g., the "LIANTI" toolkit from https://gitlab.com/lh3/lianti).

QC measures for NGS library preparations include quantitation of functional libraries by qPCR (e.g., KAPA Library Quantification Kit for Illumina® Platforms (KAPA Biosystems)) and fragment size distribution (e.g., 2100 Bioanalyzer (Agilent Technologies)).

2.5.2 NGS for variant calling

Whole-genome sequencing at high depth $>30\times$ is required for de novo mutation discovery and comprehensive genotyping. This may be an underestimate for de novo assembly. De novo mutation detection is sensitive to the fidelity of the polymerases used for NGS, and a PCR-free library preparation should be used. The advantage of PCR-based library preparation is a considerably shorter protocol, with input requirements as low as 1 ng DNA, where sequencing indexes are incorporated during an enzymatic fragmentation and reduced-cycle PCR reaction. In contrast, the PCR-free library preparation requires higher input (1–2 µg DNA depending on the desired average NGS insert size, which ranges from 350 to 550 bp), mechanical fragmentation, end repair, and adapter ligation prior to sequencing.

The NGS insert size determines the optimal read length, and therefore is crucial in assay design, for ensuring maximum genome coverage with paired-end sequencing.

2.5.3 Whole-genome genotyping by SNP array

The microarray workflow uses a unique on-array, two-color, single base extension (SBE) biochemistry to identify A/T and C/G base incorporation at targeted SNP loci (Infinium BeadChip arrays (Illumina Inc.)). Briefly, the sample genomic DNA or WGA product is further amplified, fragmented, and hybridized to the microarray overnight. Following SBE, the incorporated bases are detected by a two-dye fluorescent signal amplification step, prior to scanning and analysis of intensity data from both red and green channels. Genome coverage is determined by the number of features in the array design, ranging from ~300,000 (e.g., HumanCytoSNP-12; Illumina Inc.) to 1.7 million (e.g., Multi-Ethnic Global-8; Illumina Inc.). For single cell analysis using the HumanCytoSNP-12 array (Illumina Inc.), we follow the Infinium Karyomapping Protocol Guide (15052710; Illumina Inc.) without modification.

2.6 DATA PROCESSING AND INFORMATICS PIPELINES

The two major limitations for single cell genomics are the random nonamplification of one of the alleles (allele drop out) and mutation due to the technology used. To QC a specific single-cell method, we recommend using a cell line that is well characterized to obtain the noise parameters (Eberle et al., 2017). Below, we discuss different pipelines for data analysis.

2.6.1 Data analysis pipelines for NGS

Sequencing the whole human genome at high depth generates a significant amount of data, and therefore the requirement for data processing and informatics should be considered. For LIANTI, after alignment of Illumina sequencing reads using BWA-MEM, a dedicated pipeline ("lianti" toolkit) is available to carry out subsequent analysis (https://gitlab.com/lh3/lianti).

2.6.2 Data analysis pipelines for microarrays

SNPs are detected using laser scanning, and intensity data are normalized and reported using dedicated software (GenomeStudio, Genotyping module; Illumina Inc.). An additional set of control probes that are included in the Infinium assay (Ilumina Inc.) can be assessed for each stage of the process for QC and troubleshooting. The expected performance of each SNP locus is based on genomic DNA from a reference population with high heterogeneity (288 HapMap individuals). However, the limitation of such an approach is that it does not account for the noise within a single cell system; increasing the quality score in GenomeStudio improves precision, although at a cost of recall (Zamani Esteki et al., 2015).

2.6.3 Phasing algorithms

Once genotypes have been obtained, there are several phasing algorithms that do not rely on the information from three generations. We use the sibling approach that assumes that crossovers at the same position in different meioses are rare. Thus, when siblings share a specific crossover compared to an assumed reference (a 1N, 1C sample from an activated oocyte or PB2), the reference is likely to have experienced a crossover in that position (Hou et al., 2013; Ottolini et al., 2015). The assumed ancestor's chromosome can thus be rearranged according to the positions of common crossovers among its siblings and the genotype of the parent can be deduced. The sibling approach requires haploid products from at least three independent meioses. Genomic DNA from parents are required to resolve maternal and paternal SNPs, if using embryos. For oocytes, the positions of heterozygous SNPs can be inferred from multiple haploid, meiotic products, if maternal DNA is not available.

2.7 MATERIALS

2.7.1 Sources of human oocytes and embryos

Research with human oocytes and embryos is conducted only with appropriate ethics, licenses, and informed consent as well as personal data protection that covers extensive genome sequencing.

2.7.2 Single cell isolation

Dead Cell Removal Kit and MS Columns (Miltenyi Biotech, 130-090-101, 130-042-201)
Molecular Probes™ Dead Cell Apoptosis Kit (Thermo Fisher Scientific, 10257392)
The STRIPPER® micropipetter and 125 µm tips (Origio, MXL3-STR, MXL3-IND-125)
PVP Clinical Grade, without phenol red (Origio, 10905000)
Phosphate Buffered Saline 20× (Cell Signaling Technology, 9808)
Nunc™ IVF ICSI Dish 51 × 9 mm (Thermo Fisher Scientific, 150265)

2.7.3 WGA

SureMDA Amplification System (Illumina Inc., PR-40-405102-00)
REPLI-g® Single Cell Kit (QIAGEN, 150345)
SurePlex DNA Amplification System (Illumina Inc., PR-40-415101-00)
LIANTI (No commercial kit available at present)
Quant-iT™ dsDNA Assay Kit, high sensitivity (Invitrogen, Q33120)
FLUOstar® Omega microplate reader and MARS Data Analysis Software (BMG Labtech)

2.7.4 NGS library preparation

VeriSeq™ PGS Kit and BlueFuse® Multi Software (Illumina Inc., RH-101-1001)
Nextera DNA Library Prep Kit (Illumina Inc., FC-121-1031)
Nextera Index Kit (Illumina Inc., FC-121-1012)
TruSeq® DNA PCR-Free HT Library Prep Kit (Illumina Inc., 20015963)
TruSeq® DNA CD Indexes (Illumina Inc., 20015949)
KAPA Library Quantification Kit for Illumina® Platforms (KAPA Biosystems, KK4828)
2100 Bioanalyser High Sensitivity DNA Kit (Agilent, 5067-4626)

2.7.5 SNP microarray library preparation and arrays

HumanCytoSNP-12 v2.1 BeadChip Kit (Illumina Inc., WG-320-2103)
Multi-Ethnic Global-8 v1.0 BeadChip Kit (Illumina Inc., WG-316-1002)
GenomeStudio Software with Genotyping Module (Illumina Inc.)

3 CONCLUSIONS

We have discussed various considerations and methods for probing genome changes in single cells that allow new insights into meiosis and mitosis. We focused on two methods. LIANTI is a new WGA method that allows unprecedented coverage of the genome and resolution, which has allowed detection of replication origins in single cells. In contrast, MDA is an older generation WGA, but provides high fidelity and robustness, when studying female meiosis in precious material. Trouble shooting for mapping recombination and chromosome segregation in human female meiosis, termed MeioMapping, is further discussed elsewhere (Ottolini et al., 2016).

Single cell genomic technologies are rapidly evolving and successful integration into meiotic and mitotic studies require a combination of cellular approaches as well as computational skills. Several other single cell technologies have emerged, including sc-transcriptomics, sc-chromatin immunoprecipitation followed by sequencing, sc-methylation sequencing, and hybrid procedures that detect two or more features. Single cell proteomics is also available for human oocytes. Single cell genomics, however, remains one of the most challenging technologies due to the nature of detecting single copies at high precision and uniformly ("linear" amplification). Artifacts along the pipeline are amplified and may lead to erroneous conclusions, especially with regards to single variant calling (see Discussion in Chen et al., 2017).

The genome undergoes considerable changes, particularly in meiotic cells. Being able to explore these with high precision will enable future studies that allow new discoveries. The methods we have described are adaptable and should be useful for exploring genome changes.

ACKNOWLEDGMENTS

We thank our colleagues for technical discussions, in particular Alan Handyside, Antonio Capalbo, Sijia Lu, and Lei Huang. The authors receive research support from Illumina Inc., the Novo Nordisk Foundation (NNF15OC0016662), ERC (724718), MRC (G0902043, MR/M000664/1), the Danish National Research Foundation (DNRF115), NIH Director's Pioneer Award (5DP1CA186693) and National Cancer Institute Grant (5R33CA174560), Beijing Municipal Science and Technology Commission (Z131100005213006), National key Technology Research and Development Program (2016YFC0900100), National Basic Research Program of China (2015AA020407).

REFERENCES

Blagodatskikh, K. A., Kramarov, V. M., Barsova, E. V., Garkovenko, A. V., Shcherbo, D. S., Shelenkov, A. A., et al. (2017). Improved DOP-PCR (IDOP-PCR): A robust and simple WGA method for efficient amplification of low copy number genomic DNA. *PLoS One, 12,* E0184507.

Bolton, H., Graham, S. J., Van der Aa, N., Kumar, P., Theunis, K., Fernandez Gallardo, E., et al. (2016). Mouse model of chromosome mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental potential. *Nature Communications, 7,* 11165.

Capalbo, A., Hoffmann, E. R., Cimadomo, D., Maria Ubaldi, F., & Rienzi, L. (2017). Human female meiosis revised: New insights into the mechanisms of chromosome segregation and aneuploidies from advanced genomics and time-lapse imaging. *Human Reproduction Update*, *23*, 706–722.

Chen, C., Xing, D., Tan, L., Li, H., Zhou, G., Huang, L., et al. (2017). Single-cell whole-genome analyses by linear amplification via transposon insertion (Lianti). *Science*, *356*, 189–194.

Eberle, M. A., Fritzilas, E., Krusche, P., Kallberg, M., Moore, B. L., Bekritsky, M. A., et al. (2017). A reference data set of 5.4 million phased human variants validated by genetic inheritance from sequencing a three-generation 17-member pedigree. *Genome Research*, *27*, 157–164.

Fu, Y., Li, C., Lu, S., Zhou, W., Tang, F., Xie, X. S., et al. (2015). Uniform and accurate single-cell sequencing based on emulsion whole-genome amplification. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 11923–11928.

Gawad, C., Koh, W., & Quake, S. R. (2016). Single-cell genome sequencing: Current state of the science. *Nature Reviews. Genetics*, *17*, 175–188.

Hou, Y., Fan, W., Yan, L., Li, R., Lian, Y., Huang, J., et al. (2013). Genome analyses of single human oocytes. *Cell*, *155*, 1492–1506.

Huang, L., Ma, F., Chapman, A., Lu, S., & Xie, X. S. (2015). Single-cell whole-genome amplification and sequencing: Methodology and applications. *Annual Review of Genomics and Human Genetics*, *16*, 79–102.

Lu, S., Zong, C., Fan, W., Yang, M., Li, J., Chapman, A. R., et al. (2012). Probing meiotic recombination and aneuploidy of single sperm cells by whole-genome sequencing. *Science*, *338*, 1627–1630.

Ottolini, C. S., Capalbo, A., Newnham, L., Cimadomo, D., Natesan, S. A., Hoffmann, E. R., et al. (2016). Generation of meiomaps of genome-wide recombination and chromosome segregation in human oocytes. *Nature Protocols*, *11*, 1229–1243.

Ottolini, C. S., Newnham, L. J., Capalbo, A., Natesan, S. A., Joshi, H. A., Cimadomo, D., et al. (2015). Genome-wide maps of recombination and chromosome segregation in human oocytes and embryos show selection for maternal recombination rates. *Nature Genetics*, *47*, 727–735.

Vermeesch, J. R., Voet, T., & Devriendt, K. (2016). Prenatal and pre-implantation genetic diagnosis. *Nature Reviews. Genetics*, *17*, 643–656.

Wang, J., Fan, H. C., Behr, B., & Quake, S. R. (2012). Genome-wide single-cell analysis of recombination activity and de novo mutation rates in human sperm. *Cell*, *150*, 402–412.

Wapner, R. J., Martin, C. L., Levy, B., Ballif, B. C., Eng, C. M., Zachary, J. M., et al. (2012). Chromosomal microarray versus karyotyping for prenatal diagnosis. *N. Engl. J. Med*, *367*, 2175–2184.

Yan, L., Huang, L., Xu, L., Huang, J., Ma, F., Zhu, X., et al. (2015). Live births after simultaneous avoidance of monogenic diseases and chromosome abnormality by next-generation sequencing with linkage analyses. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 15964–15969.

Zamani Esteki, M., Dimitriadou, E., Mateiu, L., Melotte, C., Van der Aa, N., Kumar, P., et al. (2015). Concurrent whole-genome haplotyping and copy-number profiling of single cells. *American Journal of Human Genetics*, *96*, 894–912.

Zhang, L., Cui, X., Schmitt, K., Hubert, R., Navidi, W., & Arnheim, N. (1992). Whole genome amplification from a single cell: Implications for genetic analysis. *Proceedings of the National Academy of Sciences of the United States of America*, *89*, 5847–5851.

Zhang, C. Z., Spektor, A., Cornils, H., Francis, J. M., Jackson, E. K., Liu, S., et al. (2015). Chromothripsis from DNA damage in micronuclei. *Nature*, *522*, 179–184.