Non-invasive prenatal diagnosis using cell-free fetal DNA in maternal plasma

Detection of subchromosomal abnormalities using massively parallel sequencing

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Introduction and objectives

cffDNA
- -Discovered in 1997, cell-free fetal DNA provides diagnostic material for non-invasive prenatal diagnosis.
- -Originated in the cytotrophoblast, has a fragmented nature (median of 150 bp) and only amounts to about 10% of the total DNA in plasma.
- -Can be detected from 4-5 weeks gestation and is rapidly cleared after delivery ➔ Allows diagnosis regardless of a previous pregnancy.
- -Sex determination and trisomy detection are clinically available.

MPS
- -Massively Parallel Sequencing (MPS) can detect variations in the amount of DNA (CNVs) if the right bioinformatic tools are used.
- -Illumina HiSeq 2000 is the most used platform in validation studies despite **inducing GC bias** (more GC content, more reads).

OBJECTIVES

-Review the use of MPS in the detection of subchromosomal abnormalities.

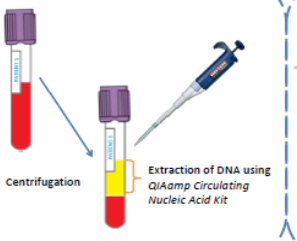
-Assess the clinical viability of this protocol.

WORK METHODOLOGIES

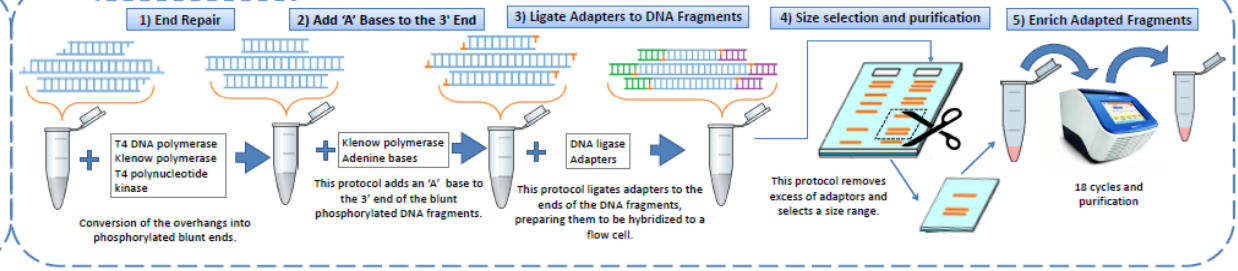
-Bibliographical search using NCBI search engine with cffDNA, NIPD and MPS as key words. Focusing on publications after 2012 and complementing with cited publications in order to understand the development of this field.

Methods

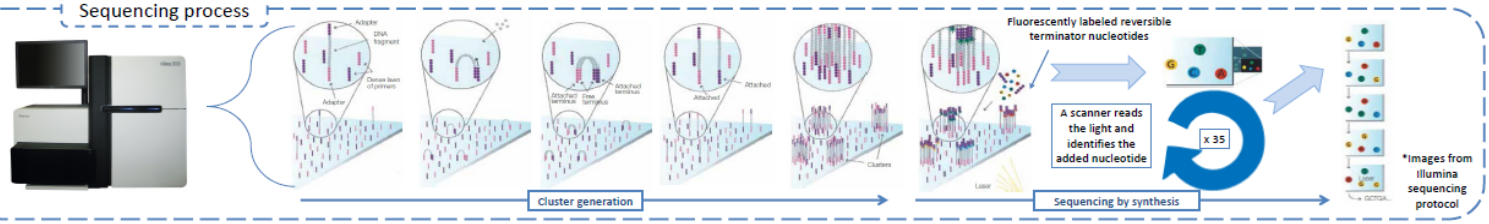
DNA extraction



Library preparation



Sequencing process



Data analysis

Genome divided into 1Mb and 100kb bins → Sequenced reads aligned to the Human Genome → Reads assigned into a single bin / GC% calculated for each bin (Ranked)

GC bias solved

$$BRV_{ij} = \frac{Tags_{ij}}{\sum Tags_{km}}$$

Calculation of z-values for each bin using **Bin Ratio Values**

$$z_{ij} = \frac{BRV_{ij} - BRV_{Medianij}}{1{,}4826 \cdot MAD_{ij}}$$

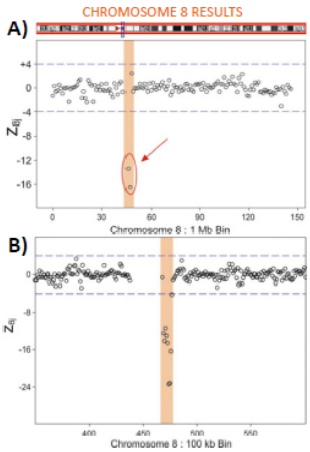
CNV found if:

Two or more 1Mb contiguous bins have z-values >±4

If there is only one above the threshold:
↳ Analyze 100kb bin data
If 2 or more > ±4 ➔ CNV

Results in a real case

*Images from *Srinivasan* et al.

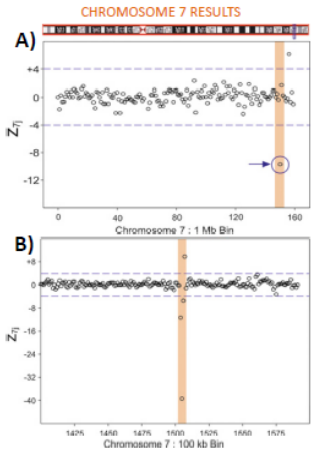


A) 1 Mb bins ➔ 2Mb deletion?

B) 100 kb bins ➔ 900 kb deletion

Fetal fraction calculated for the deleted region:

68,5% ➔ **MATERNAL IN ORIGIN**



A) 1 Mb bins ➔ Only 1 bin above ±4. ¿Deletion?

B) 100 kb bins ➔ 300 kb deletion

Fetal fraction calculated for the deleted region:

18,4% ➔ **FETUS WITH THE DELETION**

Discussion and conclusions

-Improvement of bioinformatic tools and statistical analysis are increasing the applications of sequencing. Detection of relatively small duplications and deletions is now feasible.

-GC bias is solved using statistical approaches.

-The methodologies used in this review are demonstrated to detect whole chromosome aneuploidies such as Down Syndrome, and deletions/duplications >200kb responsibles for some syndromes such as Cat-eye and Di George syndrome.

-The only difference between the described protocol and the already clinically available aneuploidy detection is the way data is analyzed. So, as a more evolved method this would be the natural surrogate to the recently implemented detection of trisomies.

-The cost is the only factor that limits this technology. To detect smaller CNV more reads are needed. This would increase even more the costs (nowadays a 1.000€), making impossible to make of this a globally used screening test.

-With the described protocol, is impossible to say if the fetus has the deletion when the mother is a carrier. Deeper sequencing and better bioinformatic tools were to be needed. A balance between capacity of detection and costs must be considered.

-It is clear that the end of invasive procedures is near; cell-free fetal DNA is the future of prenatal diagnosis. Still, it depends on our ability to make of this techniques a universal screening test offered to medium-risk or high-risk women on the basis of conventional prenatal screening.

Bibliography:

Srinivasan, A., Bianchi, D. W., Huang, H., Sehnert, A. J. & Rava, R. P. *Noninvasive detection of fetal subchromosome abnormalities via deep sequencing of maternal plasma*. Am. J. Hum. Genet. 92, 167–76 (2013).

Stumm, M. et al. *Noninvasive prenatal detection of chromosomal aneuploidies using different next generation sequencing strategies and algorithms. Prenatal Diagnostics* (2012).

Fan, H. C. & Quake, S. R. *Sensitivity of Noninvasive Prenatal Detection of Fetal Aneuploidy from Maternal Plasma Using Shotgun Sequencing Is Limited Only by Counting Statistics*. 5, *PLoS One* (2010).