Héritas VISION: the challenge to develop the first Non-Invasive Prenatal Testing (NIPT) platform in Argentina

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Héritas
Medicina de precisión
CIBIC + INDEAR

Background

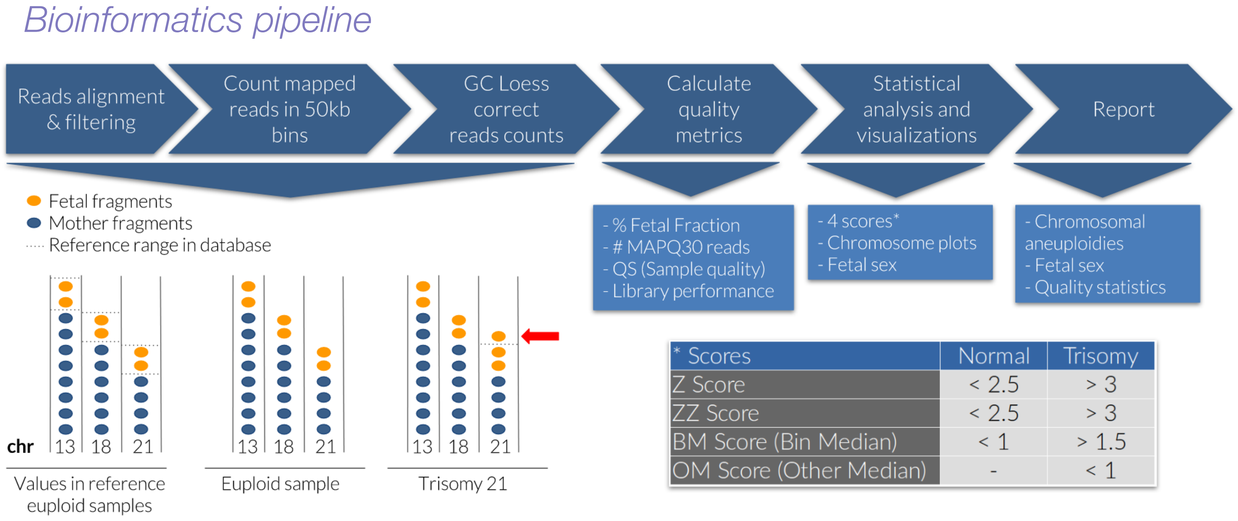
Circulating cell-free fetal DNA comprises 3-20% of cell-free DNA present in maternal plasma. Non-invasive prenatal testing (NIPT) has enabled early and secure testing for common fetal autosomal aneuplodies. NIPT using cell free DNA from maternal blood samples, can be performed any time after 9 weeks of pregnancy. By massively parallel sequencing and a sophisticated bioinformatics and statistical analysis platform, fetal aneuploidies and sex determination can be performed. NIPT gained quick acceptance abroad, and significantly reduced the number of invasive tests performed. Héritas Prenatal VISION is the first NIPT method developed entirely in Argentina.

Screening for fetal autosomal aneuploidies

Database development and clinical validation

Samples from 28 pregnant women were collected, pooled and sequenced on two sequencing runs in the Illumina NextSeq 500 platform 1x75. This cohort was employed for the construction of the reference euploid database. A validation cohort consisting of samples from 16 pregnancies, from pregnant women at high risk for aneuploidy was analized. Analysis reveleaded 2 cases positive for trisomy 21 and 14 samples were correctly classified as euploid samples. Additionaly 6 comercial controls T21 (8%, 4%, 2%), T13 (12%), T18 (12%) (Seraseq NGS for NIPT assays; Seracare) were tested (See left up table in Fig. 2).

Bioinformatics pipeline



* Scores	Normal	Trisomy
Z Score	< 2.5	> 3
ZZ Score	< 2.5	> 3
BM Score (Bin Median)	< 1	> 1.5
OM Score (Other Median)	-	< 1

Figure 1: Bioinformatics data analysis

Database improvement

In order to improve our detection thresholds at low fetal fractions we measured the coefficient of variation (CV) for each chromosome and generated a new database (HRv2) (See table left down and right image in Fig. 2).

seracare controls	Z Score	BM Score	Result
T21 – 1%	2.25	1.15	Not detected
T21 – 2%	2.32	1.44	Not detected
T21 – 4%	4.30	2.01	Detected
T21 – 8%	6.59	3.23	Detected
T13 – 12%	19.18	4.97	Detected
T13 – 12%	9.28	3.42	Detected

Coefficient of variation			
chromosome	HR v1	HR v2	Improvement
chr9	0.26	0.25	0.01
chr13	0.34	0.31	0.04
chr16	0.55	0.46	0.10
chr18	0.33	0.26	0.07
chr21	0.63	0.49	0.14
chr22	0.69	0.56	0.13

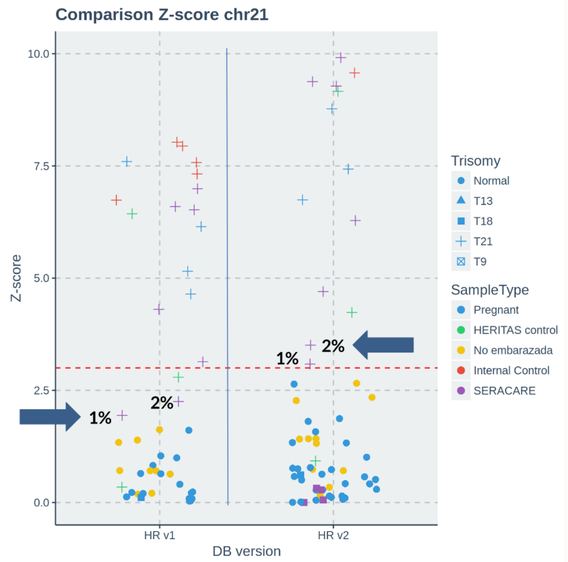


Figure 2: Left - up) Table shows the results for Seracare controls. Left - down) Table shows the coefficient of variation for selected chromosomes. HRv1: Héritas VISION database version 1. HRv2: Héritas VISION database version 2. Right) Comparison Z Scores of chr21 using Héritas VISION databases HRv1 and HRv2.

Support vector machine (SVM) for fetal sex determination

We calculated the $YHeritasProportion = ((GCcorrectedchrYreads/MAPQ30reads) * 1000)/FetalFraction$ and $YProportion = GCcorrectedchrYreads/rawchrYreads$ from samples in the euploid reference database with fetal sex determined by ultrasound. A SVM classifier was trained. A external cohort [Bayindir, 2015] with fetal sex confirmed at birth was used to validate our SVM model (Fig. 3).

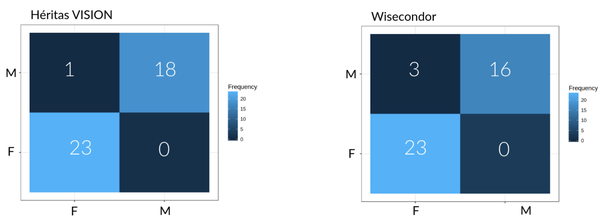


Figure 3: Confusion matrix comparing Héritas Vision (left) with Wisecondor (right).

NIPT for subchromosomal abnormalities

We implemented a workflow for detection of subchromosomal abnormalities applying a circular binary segmentation (CBS) algorithm [Venkatraman and Olshen, 2007]. This method uses the likelihood ratio statistic to search copy number regions without previous asumptions. A standar Z-score statistic is calculated in the break-points found by the CBS algorithm to confirm the event.

Data processing

Reads are aligned to the hg19 human reference genome and counted in 50kb bins. Counts are corrected and normalized for GC bias by LOESS regression (Fig. 4), followed by a principal components analysis to remove other artifacts. Then we used the PSCBS R package [Olshen et al., 2011] to apply the CBS algorithm to the normalized read bin counts. Potential CNV's are compared against a database of reference euploid samples using a Z-score.

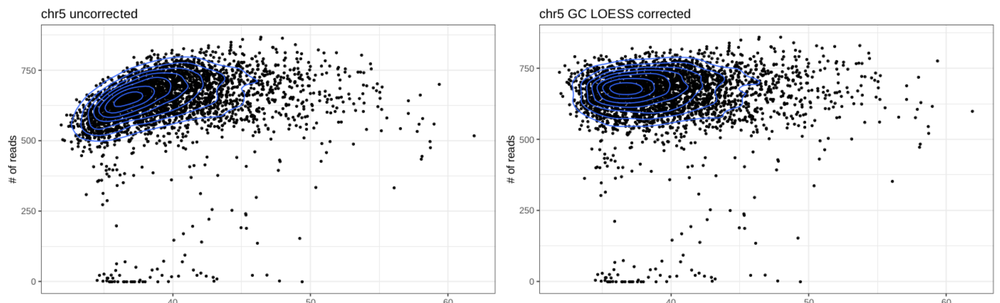


Figure 4: Chromosome 5 reads bin counts before and after GC LOESS correction

Case study 1 - chromosome 14q terminal deletion syndrome

Chromosome 14q deletion is a chromosome abnormality that occurs when there is a missing (deleted) copy of genetic material on the long arm (q) of chromosome 14. The severity of the condition and the signs and symptoms depend on the size and location of the deletion and which genes are involved.

We analyzed a pregnancy with an UNDETERMINED result for chromosome 14.

Case study 2 - Cri-Du-Chat syndrome (5p-)

Cri du chat syndrome is characterised by a high pitched, monotonous cry, microcephaly, a round face, hypertelorism, epicanthic folds, micrognathia, impaired growth and severe developmental delay and learning disability. Comparison of phenotype with detailed molecular cytogenetic analysis suggests that the cat-like cry maps to 5p13, whereas the facial dysmorphism, microcephaly and learning disability are located on 5p15.

We simulated an in-silico Cri-Du-Chat syndrome heterozygous deletion with 20% fetal fraction.

	Case Study 1	Case Study 2
Gestational Age	12	-
% Fetal Fraction	4,09	20
Z Score	-2.37	-3.01
ZZ Score	-5.38	-3.63
BM Score	-0.26	0.15
Héritas VISION result	undetermined	undetermined
CBS Ratio	0.5	0.9
CBS Z Score	-5.66	-14.09
CBS Result	maternal deletion	fetal deletion

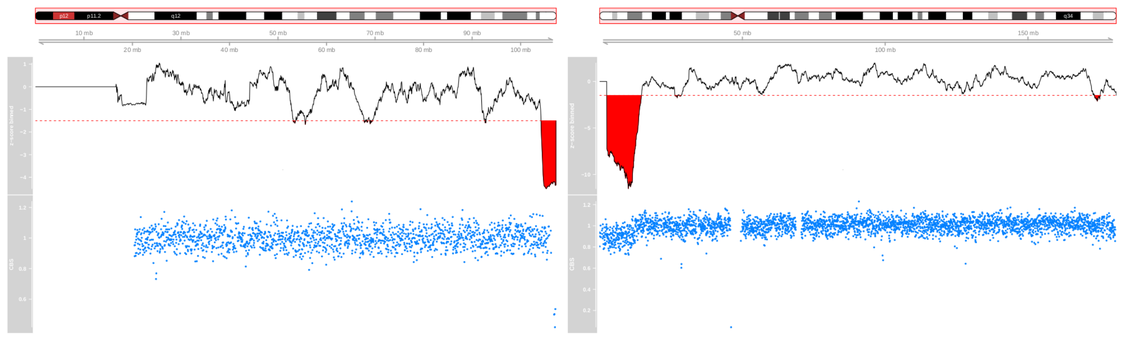


Figure 5: Up) Results for case studies 1-2. Both undetermined for trisomies. Down - left) Chromosome plot case study 1. Down - right) Chromosome plot case study 2.

Conclusions

We validated the first non-invasive prenatal assay in Argentina by developing our own proprietary algorithm using commercial international standard samples and several actual cases of aneuploidies in pregnant women. We also showed that our algorithm and platform can be extended for the detection of subchromosomal events.

Bibliography

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