Automation of bioinformatic analysis to improve efficiency of Non-Invasive Prenatal Diagnosis for monogenic disorders.

Helena Ahlfors
Senior Bioinformatician, NE Thames Regional Genetics Service
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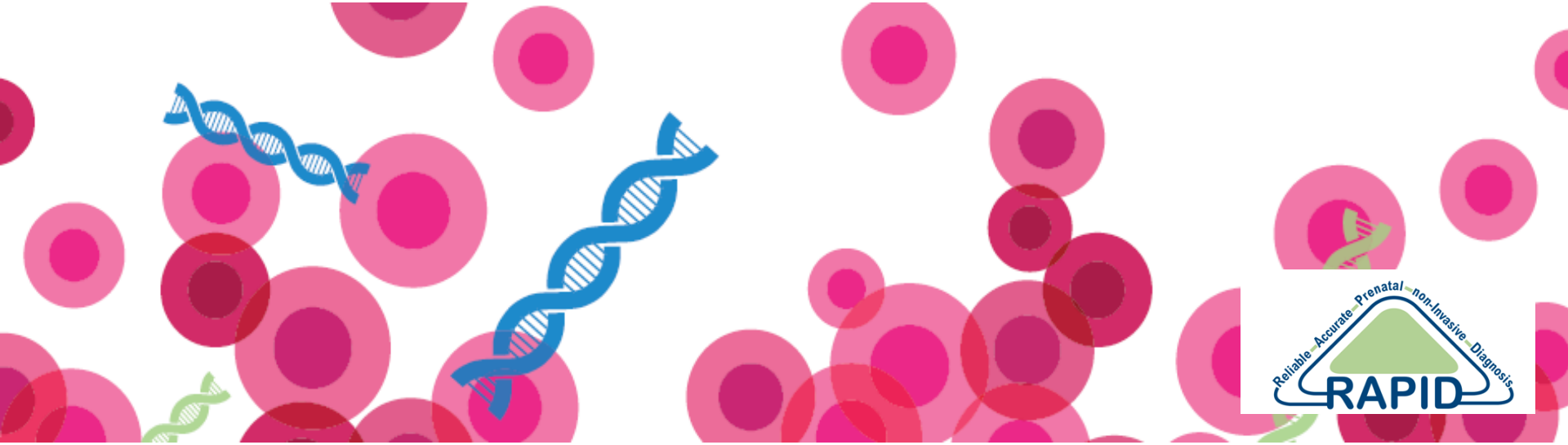
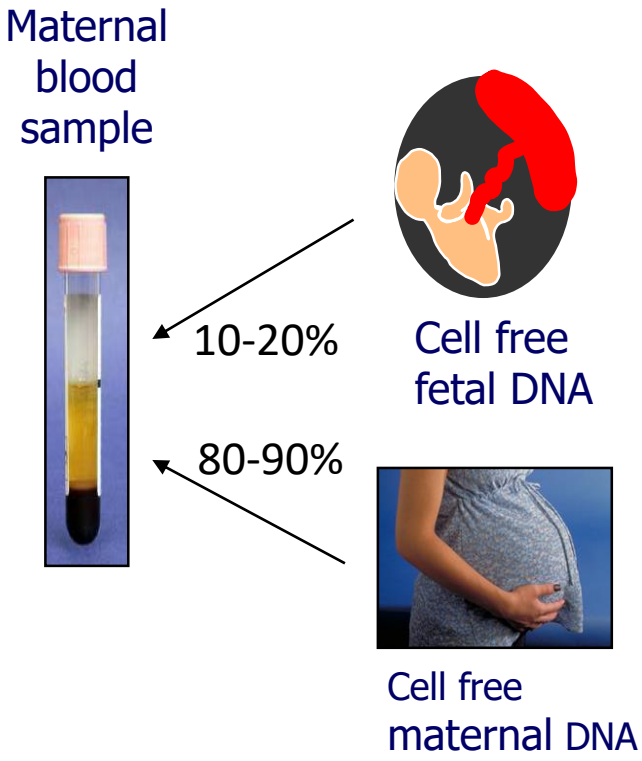


Cell-free fetal DNA (cffDNA)



cffDNA:

Originates from placenta.

Represents whole fetal genome.

Detectable from 4 weeks.

Proportion increases with gestation.

Cleared from circulation within 30 min of delivery

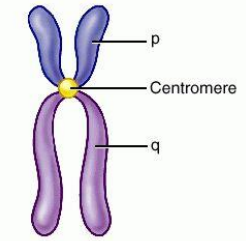
Non-Invasive Prenatal methods

NIPT = a screening test:

Used for aneuploidy screening.

Requires invasive testing for confirmation.

NIPD = diagnosis:



Applications:

- Fetal sex determination.
- Testing for risk of Rhesus disease (HDN).
- Some paternally inherited or de novo single gene disorders.

Does not require invasive test for confirmation.

NIPD for Cystic Fibrosis

Paternal exclusion method

Only applicable to couples who are known carriers of **different** CF mutations AND

The paternal mutation is one of the 10 most common mutations (i.e. p.(Phe508del))

Analysis

Blood samples are analysed by targeted sequencing of 5 CFTR amplicons using Illumina MiSeq.

Counting mutant and wild-type reads.

Excel spreadsheet with macros.

NIPD for Cystic fibrosis UKGTN approval 2014

Hill et al. Prenat Diagn 2015 Feb epub

Limitations of the paternal exclusion method

Biological challenges

Current method able to detect only paternal alleles.

Estimating fetal fraction difficult

- High background from the maternal DNA.
- Concentration of cffDNA varies with gestation and between samples.

For CF, only selected common paternal mutations applicable.

Parents need to be carriers of different mutations.

Technical/bioinformatics challenges

Slow analysis method relying on Excel macros on Windows machines.

No Bioinformatic QC.

Only preselected mutations can be analysed.

Improvements – part1

Replacing the excel macro with a bash script

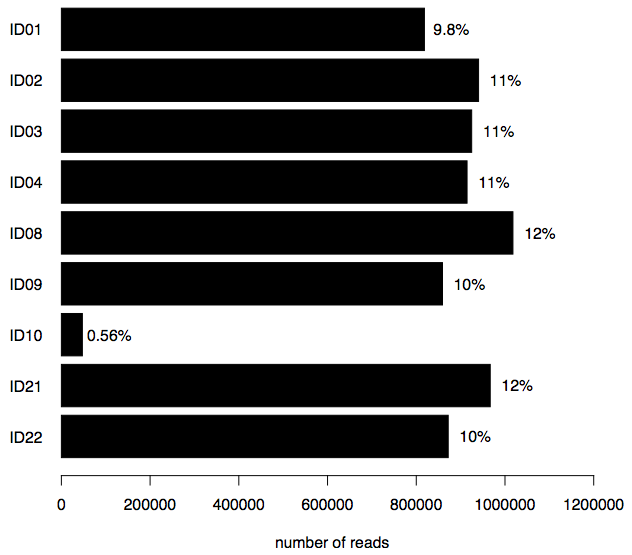
Bash script generates counts for wild type and mutant sequences.

Analysis time decreased from 3-4 hours to 15 min.

Improved TAT

Automated analysis

Reduces the risk of manual error.

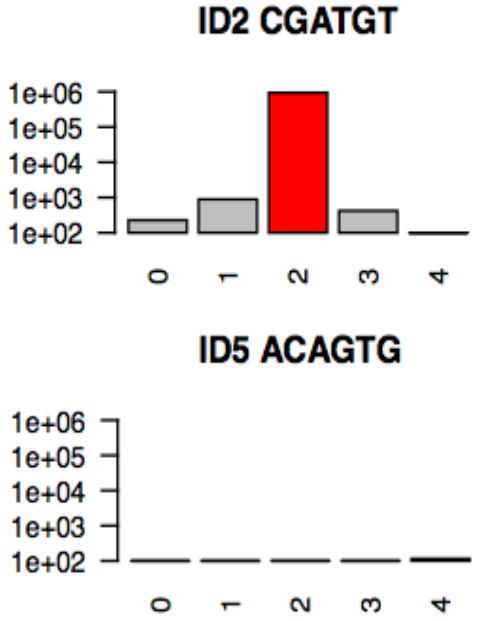


Incorporating data QC

Filtering out poor quality reads.

QC plots generated

Detecting sample contaminations or potential mixups by monitoring sample indexes.



Improvements – part2

Relative Haplotype Dosage Analysis (RHDO)

Involves analysis of multiple SNPs which flank the disease gene / locus of interest.

If the phase of the mutation carried by both parents is known then RHDO can assess whether the maternal mutant or wild-type allele has been transmitted to the fetus.

Provides definite diagnosis for recessive conditions

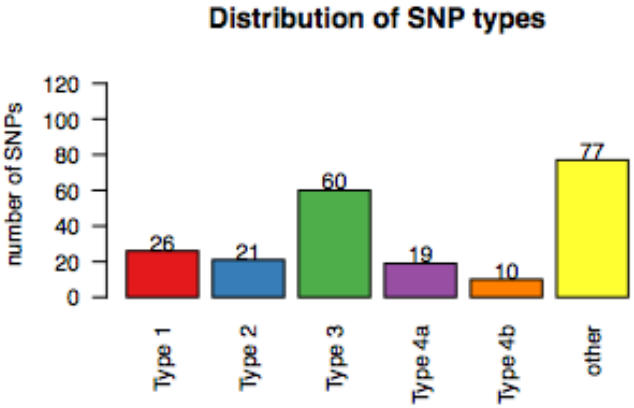
Analysis

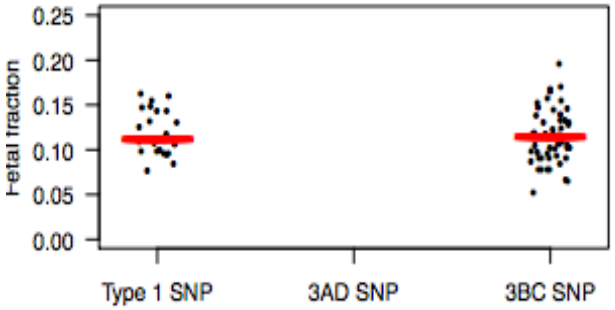
Automated R script instead of manual data processing in excel.

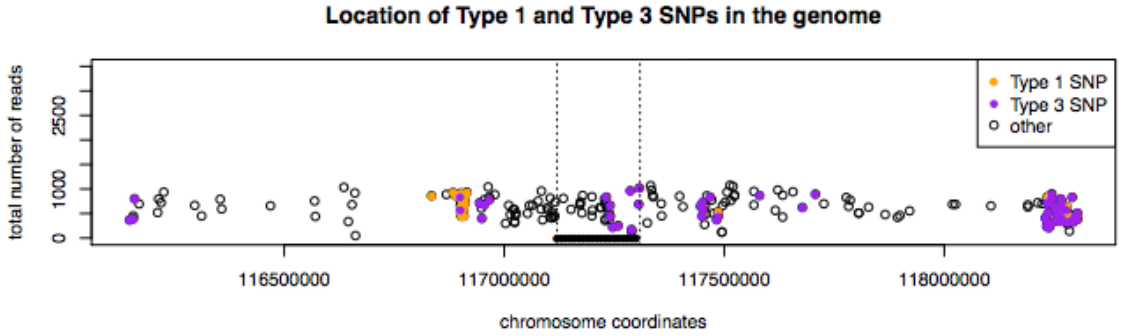
Data QC

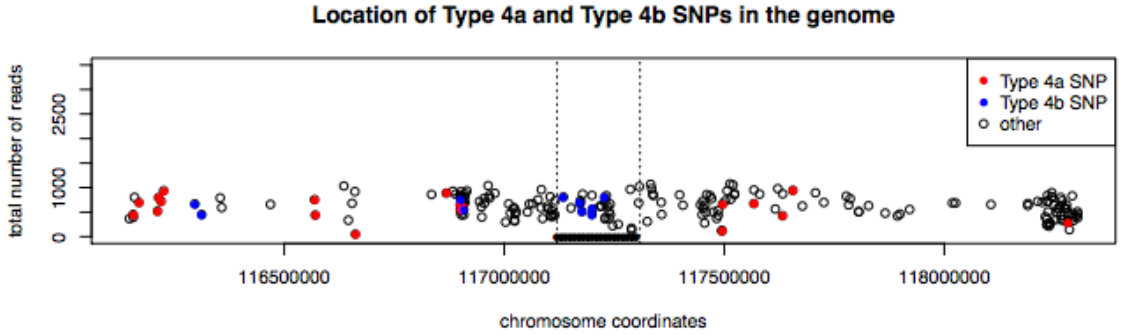
Excel spreadsheet generated containing the detailed results.

RHDO analysis report

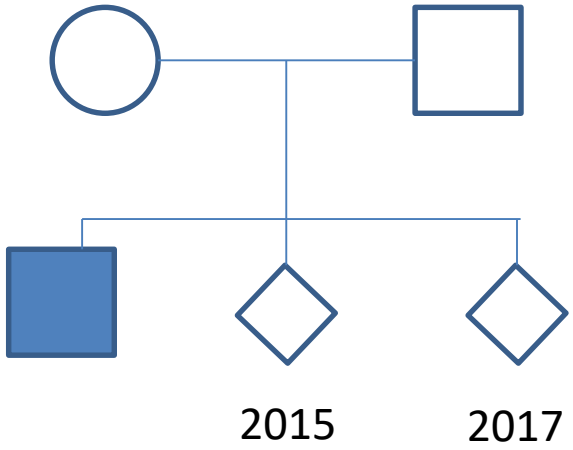








Case study G129789



Maternal mutation c.1521_1523delCTT p.(Phe508del)

Paternal mutation c.489+1G>T

Affected son

2015 – NIPD paternal mutation exclusion

no mutation detected, invasive not required

2017 – NIPD RHDO

paternal high risk, maternal low risk alleles detected

Invasive not required (would have been required if only paternal mutation exclusion had been available)

RHDO – Cystic Fibrosis Service

Case	Gestation	Fetal Fraction	Outcome
1	10+3	8%	Paternal high risk Maternal high risk
2	11	4.8%	Paternal high risk Maternal low risk
3	9+1	15.4%	Paternal low risk Maternal low risk
4	12+2	14.6%	Paternal low risk Maternal high risk
5	9+4	11%	Paternal high risk Maternal high risk
6	9+0	6.4%	Paternal high risk Maternal low risk
7	9+0	11.6%	Paternal low risk Maternal low risk

ISO15189 accredited NIPD service provided

Paternal exclusion to detect the presence of a paternal or *de novo* allele:

FGFR3-related skeletal dysplasia including achondroplasia and thanatophoric dysplasia.

FGFR2-related craniosynostosis syndromes e.g. Apert and Crouzon.

10 common cystic fibrosis mutations.

Bespoke NIPD for a variety of conditions.

RHDO

NIPD for Cystic Fibrosis in service from 3rd October 2016

NIPD for Spinal Muscular Atrophy (SMA) and Congenital Adrenal Hyperplasia (CAH) under development.

30% of all our molecular prenatal diagnosis since 2015 have been non-invasive

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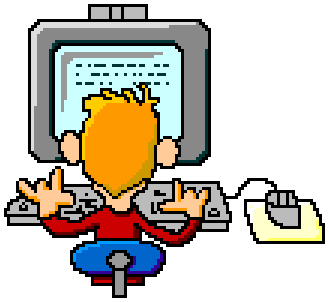
Conclusions

Uptake is high; patients have welcomed the availability of our bespoke NIPD service

Important to incorporate various bioinformatics quality control methods as well as monitoring and reviewing data over time.

Automation of the pipelines reduces manual error and speeds up data processing.

These improvements will also help us to widen the panel of tests we can provide.



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Reliable Accurate Prenatal non-Invasive Diagnosis

Great Ormond Street Hospital for Children
NHS Foundation Trust