Cost effective screening of the *PKD1* and *PKD2* genes in patients with autosomal dominant polycystic kidney disease (ADPKD)

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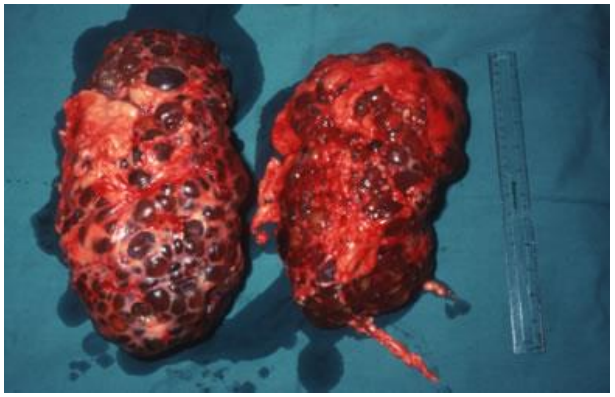
The Problem

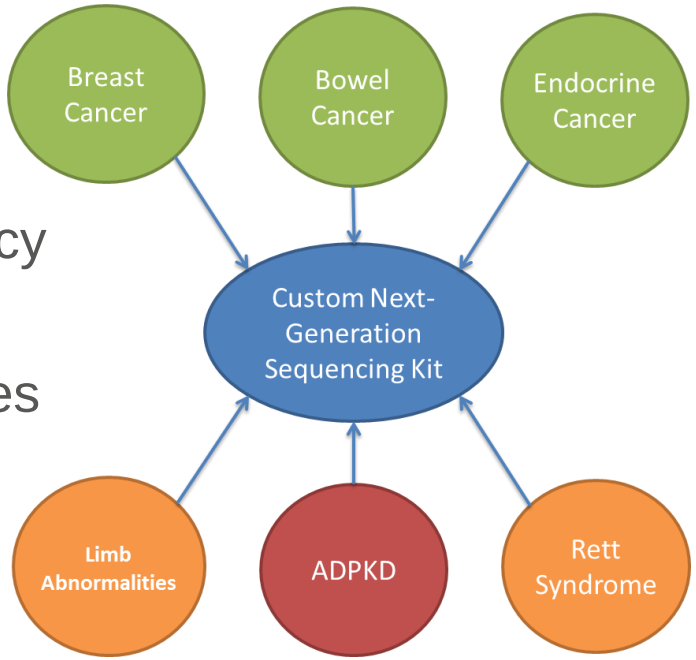


- Low clinical utility
- Relatively expensive test:
  - Referral rate too low for efficient batching
  - Multiple *PKD1* **pseudogenes**
  - **Large size**
  - **GC rich** exons
  - Requirement for copy number variant (**CNV**) analysis
- Tolvaptan treatment option required a more cost-effective test
  - **Cheaper**
  - Require **no additional staff**
  - Require **no additional equipment**
  - Meet national reporting time guidelines
  - Circumvent the problems associated with ADPKD genetic testing

We Listen
We Care

The Solution

- Custom NGS panel from SOPHiA GENETICS – 38 genes
- High cancer referral rate promotes efficiency in other services
- Single, simple workflow for multiple services



- SOPHiA DDM bioinformatics:
  - **Fast**
  - **Free**
  - **Accurate**
  - **User friendly**
  - **CNV analysis**
  - Bioinformatic differentiation of *PKD1* from its pseudogenes

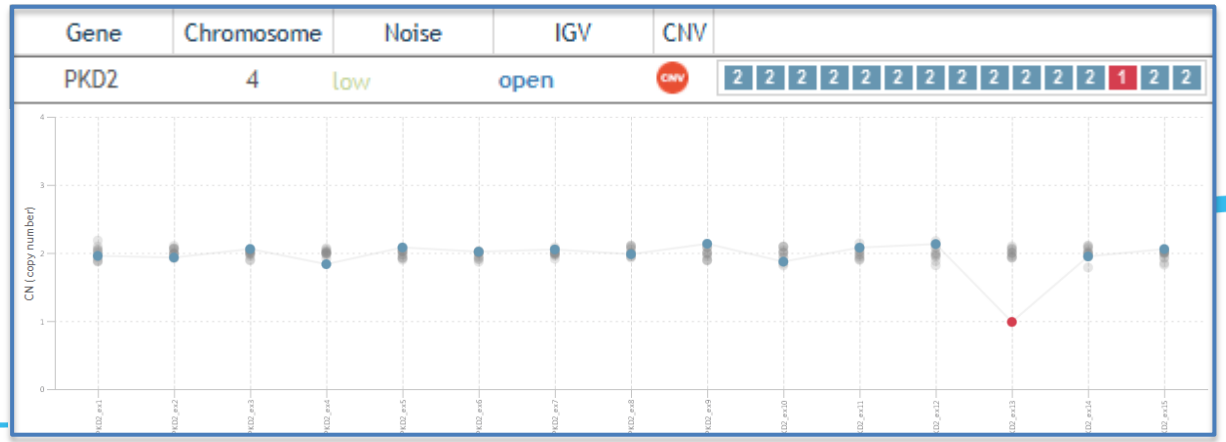
We Listen
We Care

The Results

- MLPA use decreased by over **95%**
- Sanger sequencing reduced
- Over **900 hours** of staff time saved per year
- Cost of ADPKD testing significantly reduced
- Greater laboratory **efficiency**
- **Faster Turnaround time** for all services

Reports	Class 4/5	CNV
51	29	2

- Including a case with a *PKD2* exon 13 deletion that would not have been picked up by MLPA



We Listen
We Care

Focused exome pool-seq of parent offspring trios in previously negative cases

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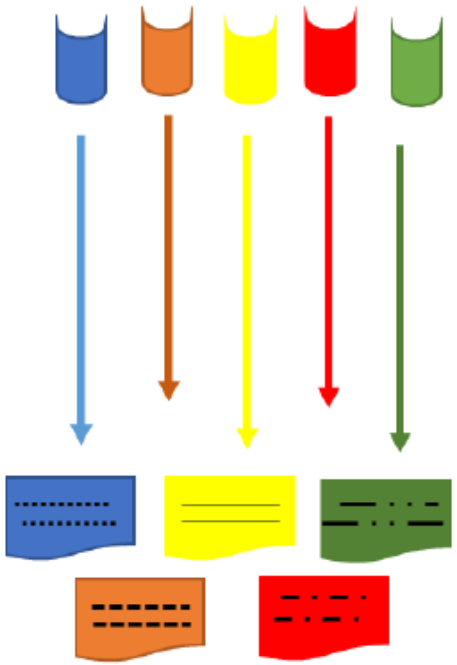
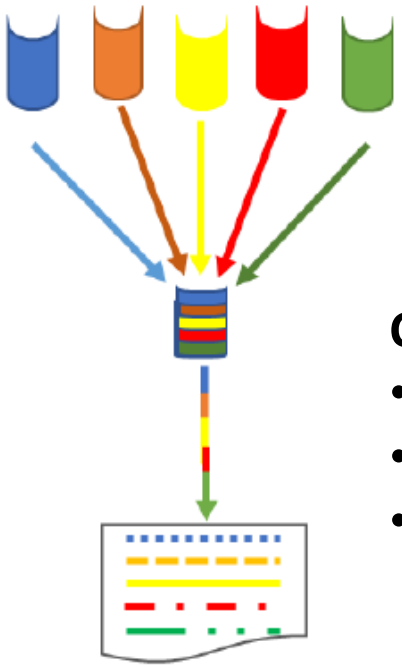
What is Pool-Seq?

Manchester University
NHS Foundation Trust



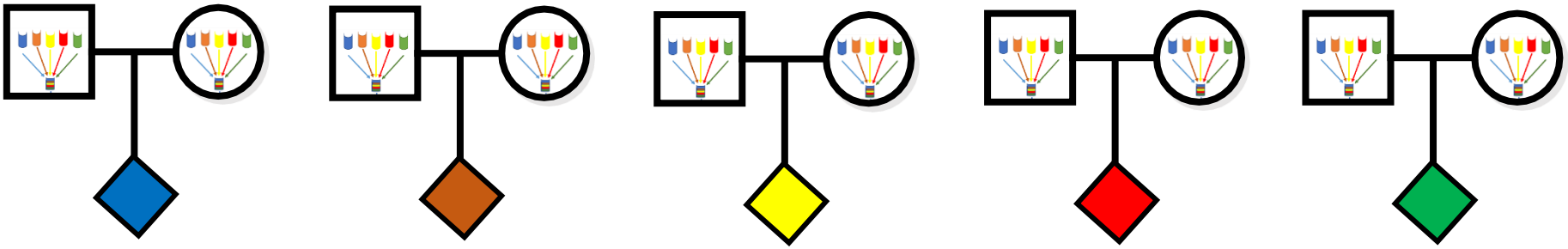
Singleton Seq
(proband)



Pool-Seq
(x5 maternal, x5 paternal)

Challenges?

- Estimation of allele frequency
- Variant calling
- Technical artefacts



Pool-Seq allele frequency estimation

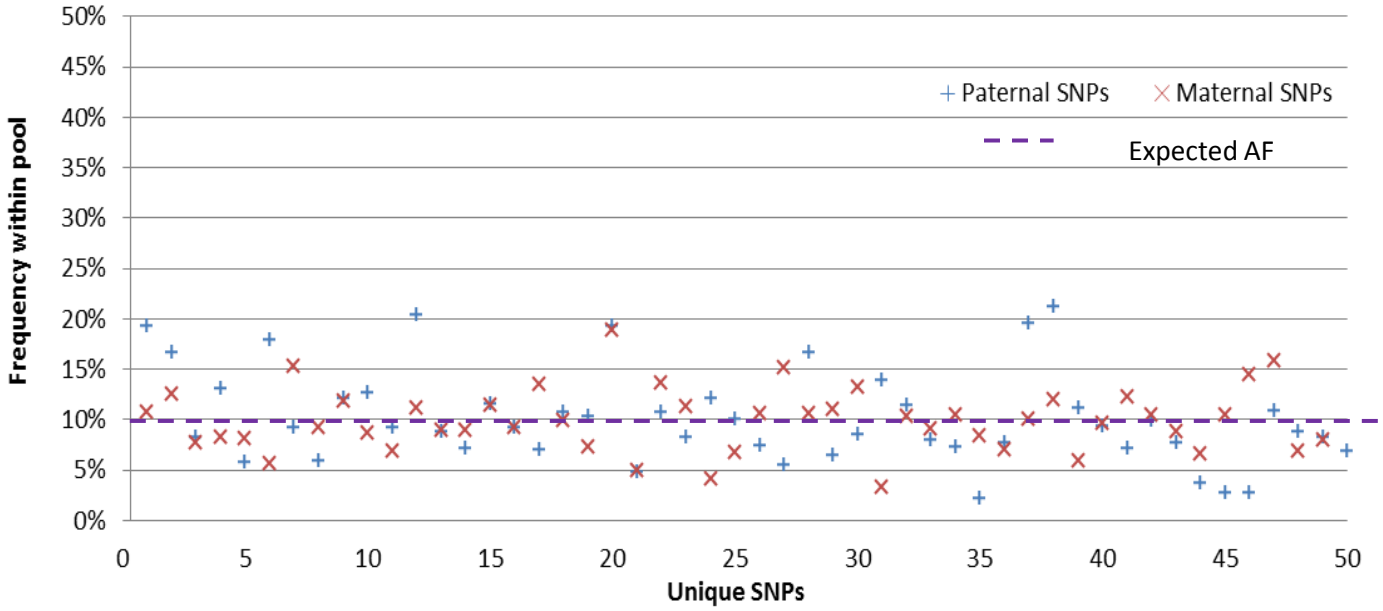
Manchester University NHS Foundation Trust

Heterozygous candidate SNPs:

- Missense
- Quality >20
- GnomAD >0% & <0.02%
- Read depth >50

varSEQ

Proband	Mean frequency		Range		Pool
	Maternal pool	Paternal pool	Maternal pool	Paternal pool	
1.	10.26	9.25	3.76-25.00	2.63-23.33	1
2.	9.58	8.64	1.48-20.42	1.01-15.25	
3.	9.45	9.27	4.92-19.34	4.25-25.83	
4.	9.31	8.94	4.18-14.47	3.13-14.37	
5.	10.25	9.36	5.8-26	4.12-24.06	
6.	9.09	9.00	4.23-17.45	3.76-17.61	2
7.	9.55	9.47	3.88-16.45	3.75-24.93	
8.	9.46	9.65	1.88-22.67	3.57-25.22	
9.	10.65	9.92	3.30-26.43	2.18 - 20.48	
10.	8.71	9.50	5.39-12.94	2.5-17.24	

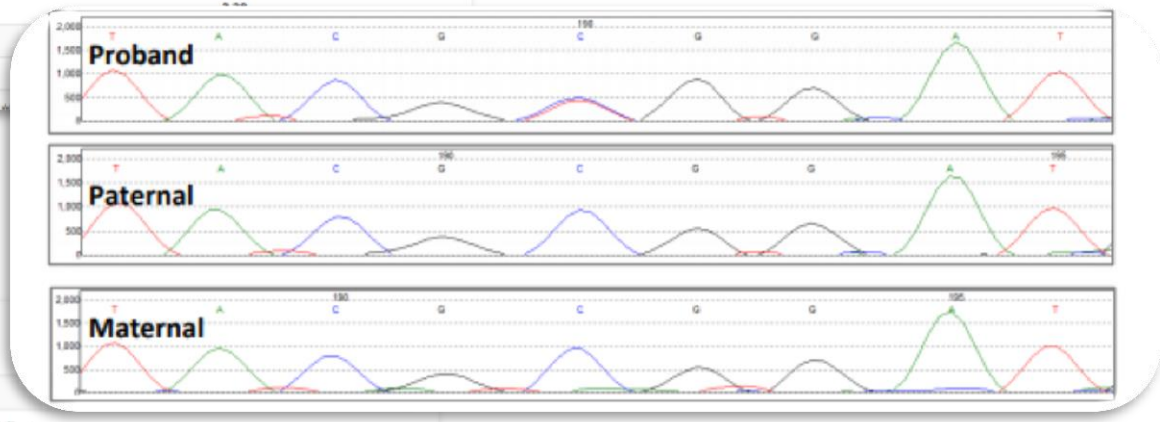


Findings and conclusions

Manchester University
NHS Foundation Trust

KCNQ2 NM_172109 c.794C>T

VEP consequence	Missense variant	Inheritance	De novo fp DNG_ANALYSED
Genotype	Heterozygous	PolyPhen prediction	benign 0.192
HGVSc	NM_172109.1:c.794C>T	SIFT prediction	deleterious 0
HGVSp	NP_742107.1:p.Ala265Val	GERP	
Amino acid	A/V	Reads split	
MAX AF	0	Depth	



HOXA2 NM_006735 c.679G>T

VEP consequence	Stop gained	Inheritance	
Genotype	Heterozygous	PolyPhen prediction	
HGVSc	NM_006735.3:c.679G>T	SIFT prediction	
HGVSp	NP_006726.1:p.Glu227Ter	GERP	5.45
Amino acid	E/*	Reads split	152/138
MAX AF	0	Depth	290
		Other patients with this variant in	0 out of 0 other patients in

1. ***De novo* findings** in 2/10 of this cohort
2. **Additional inheritance information** identified candidate variants (X-linked & AR inheritance)
3. **Time saving** on parental cascades across all modes of inheritance.
4. **Detected** rare SNPs with a mean frequency of 8.64-10.65% in parental pools.
5. **Cost analysis** of parental Pool-Seq (5 parents pooled) - 47% saving per trio analysis

Acknowledgements: S. Ramsden, W. Newman, H. Schlecht, C. Kyle, R. Coates-Brown, R. Wright , D. Gokhale, L. Dutton, R. Lee, R. Mahood

Assessing whole genome sequencing as a diagnostic test for mitochondrial disease

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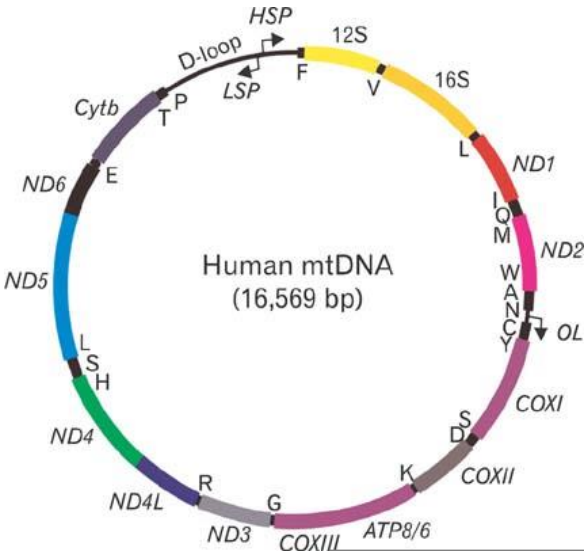
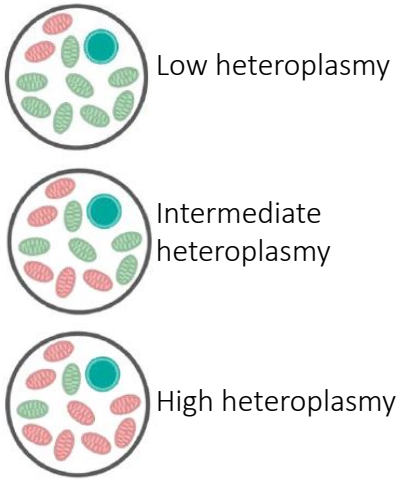
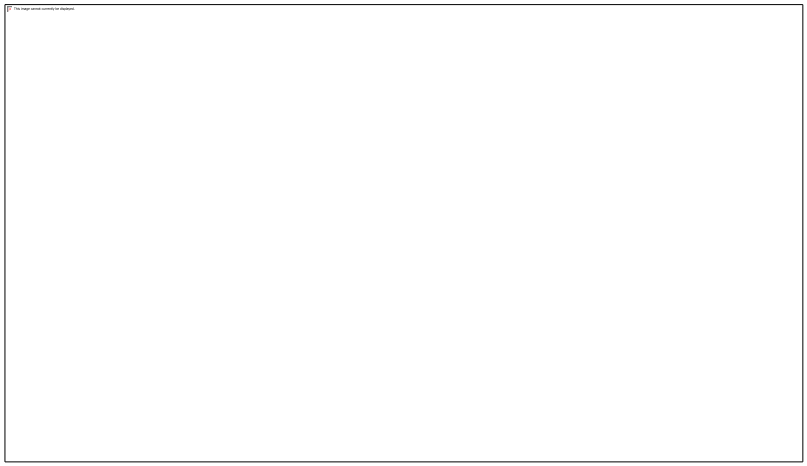
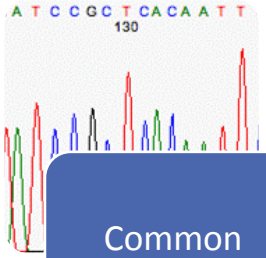
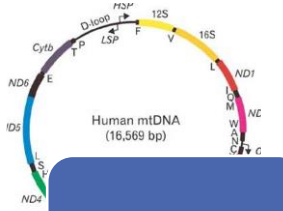




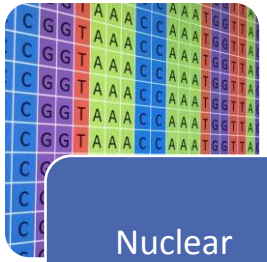




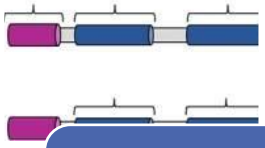
Common mtDNA variants



mtDNA sequencing



Nuclear mitochondrial gene panel



Whole Exome Sequencing

Whole Genome Sequencing

MRC Mitochondrial Biology Unit

MANCHESTER 1824
The University of Manchester

NHS
East Midlands and East of England

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First-line genomic diagnosis of mitochondrial disorders

*F. Lucy Raymond[1,2,3], Rita Horvath[4] and Patrick F. Chinnery[1,5]**

Current approaches for diagnosing mitochondrial disorders involve specialist clinical assessment, biochemical analyses and targeted molecular genetic testing. There is now a strong rationale for undertaking first-line genome-wide sequencing, accelerating the speed of diagnosis and avoiding the need for expensive and invasive investigations.

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support for a variable-size bottleneck

Mingkun Li,[1,2] Rebecca Rothwell,[3] Martijn Vermaat,[4] Ma
Roland Schröder,[1] Jeroen F.J. Laros,[4] Mannis van Oven,[5]
Jasper A.
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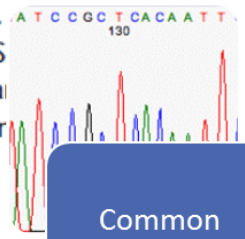
Willem H. Ouwehand§, John R. Bradley§, F. Lucy Raymond§, Mark Caulfield*, Ernest Turro¶, Patrick F. Chinnery¶

ORIGINAL



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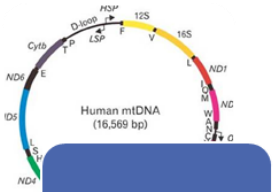
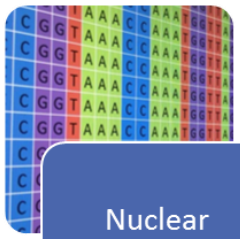
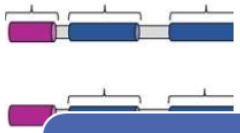


mtDNA sequencing



Nuclear mitochondrial gene panel



Whole Exome Sequencing

Whole Genome Sequencing

Mégy[1],
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MRC Mitochondrial Biology Unit

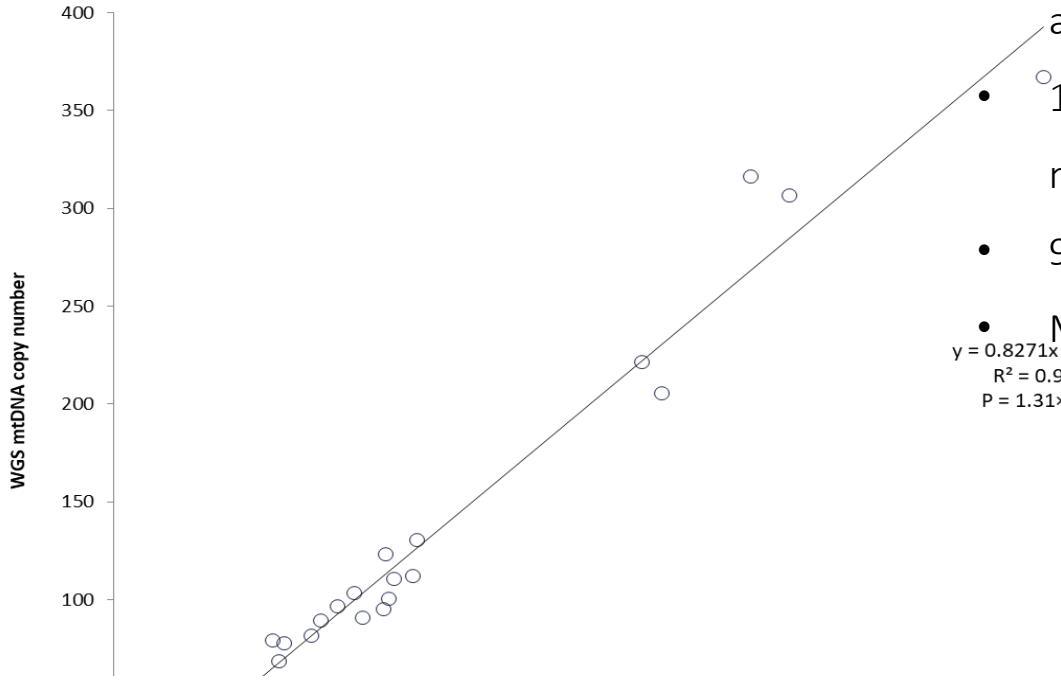
MANCHESTER 1824
The University of Manchester

NHS East Midlands and East of England
NHS Genomic Laboratory Hub

WGS data from 23 individuals- NIHR Bioresource

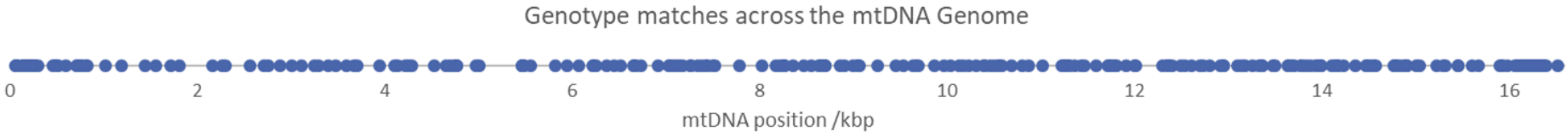
Mitochondrial DNA Copy Number

- WGS estimation compared to ddPCR
- No significant difference between datasets



Single Nucleotide Variants

- Long-range PCR followed by NGS
- High heteroplasmic and homoplasmic SNPs across the mtDNA genome
- 100% concordance between the two methods (269 unique SNPs)
- 98.9% sensitivity (95%CI)
- More work needed:
  - other variant types
  - limit of detection with respect to low level heteroplasmies



Whole genome sequencing in a series of primary ciliary dyskinesia cases identifies molecular diagnoses in *DNAH5*

Holly Black

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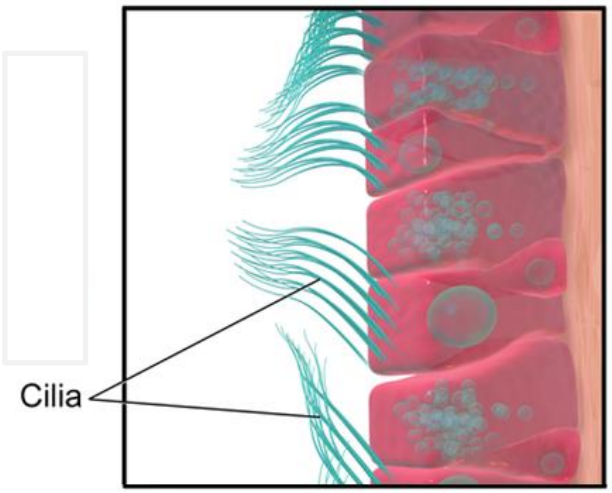
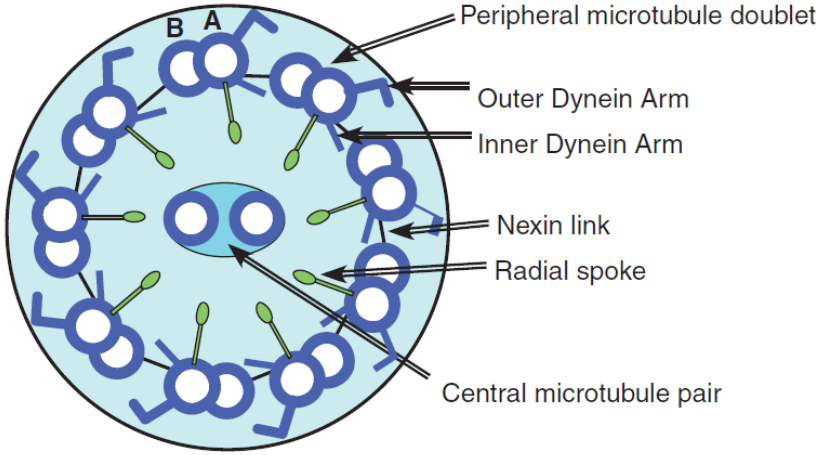
Primary Ciliary Dyskinesia (PCD)

- Disorder of motile cilia
- ~40 genes associated with PCD
- WGS in 8 families (SGP)
  - Variants filtered for PanelApp PCD genes (32 “green” genes)



Blausen.com (2014) WikiJournal of Medicine



Leigh, 2012, Kendig and Chernick’s Disorders of the Respiratory Tract in Children

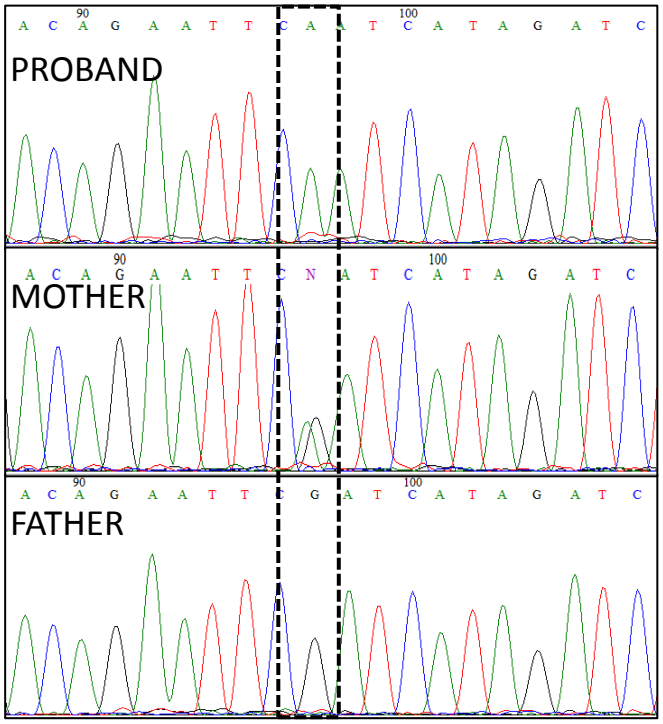
WGS in PCD

- Genetic diagnosis in 6 of 8 families
  - *DNAH5* (3), *DNAH11* (2), *DNAAF4* (1)

- 3 have biallelic variants in *DNAH5*
  - Case 1:
    - Compound heterozygote
  - Case 2:
    - Singleton, phase unknown- investigating using ddPCR
    - Static cilia
  - Case 3:
    - Compound heterozygote
    - Outer dynein arm defect

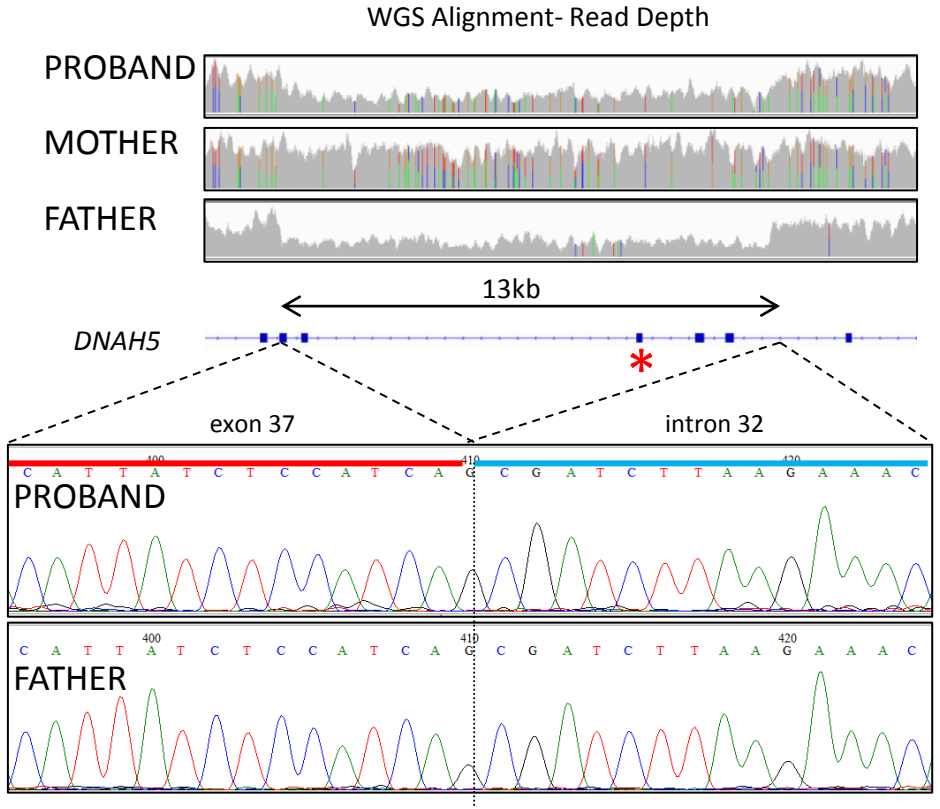
Case 1 – *DNAH5* compound het

c.5281C>T, p.(Arg1761Ter)



Non-paternity excluded

Deletion intron 32 to exon 37



- WGS confirms paternity and enables structural variant identification

The 100,000 Genomes Project; successful cases and cautionary tales – the Wessex experience

Amy Webb

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Variants identified by the 100KGP where previous testing was negative

- **Whole Genome Sequencing offers superior coverage even in comparison to Whole Exome Sequencing**
- **The original test didn't include the gene in which the causal variant was found, or didn't cover the whole gene.**
- **The final diagnosis was never considered and previous tests carried out were not capable of finding the answer**

Example 1

Patient: Dev delay, hypotonia, elbow contracture + motor delay.

Previously suspected: DiGeorge or Freeman-Sheldon Syndrome.

Testing: Array-CGH, Clinical Exome (virtual panels), 22q11.2FISH, karyotype.

100KGP result: UPD14.

Example 2

Patient: Female infant with brain abnormalities, developmental regression and seizures. Died age 5 weeks.

Previously testing: Extensive mitochondrial disorder testing, array-CGH and karyotyping on skin and blood.

100KGP result: Homozygous 45.5kb deletion involving *SLC19A3.* This is the first causal 100KGP CNV confirmed in our GMC

Intronic variants outside of the splice regions

Variants missed due to poor read depth or QC

Causal variants missed as they weren't on the panel/s applied

All variants were identifiable in the raw data but not called

SNVs filtered out if they are the only SNV in a recessive gene

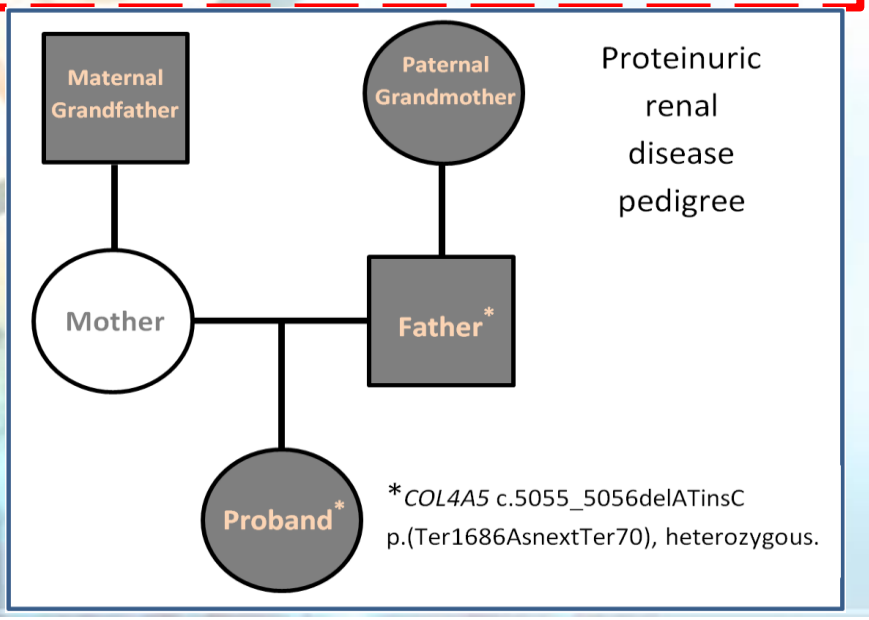
CNVs will not be called if they are below GEL's 10kb cut off

Affected individuals on both sides of the family can cause a dominant variant to be filtered out

Example: Compound heterozygous *DNAAF4* variants:
1 SNV and 1 CNV

Both the nonsense mutation and the 3.5kb CNV were filtered out.

WRGL PCD gene panel found nonsense mutation and further investigation identified the CNV



The 100,000 Genomes Project; successful cases and cautionary tales

IN SUMMARY…

Many cases solved

Many cases could probably have been solved by conventional testing without whole genome sequencing had the patient been well phenotyped and/or seen by a clinical geneticist

We must learn from missed variants to develop better filtering strategies

WGS is not the end!!!

WGS does not always = WG analysis

Targeted microarray design increases detection of clinically-relevant variants across multiple NHS genomics centres

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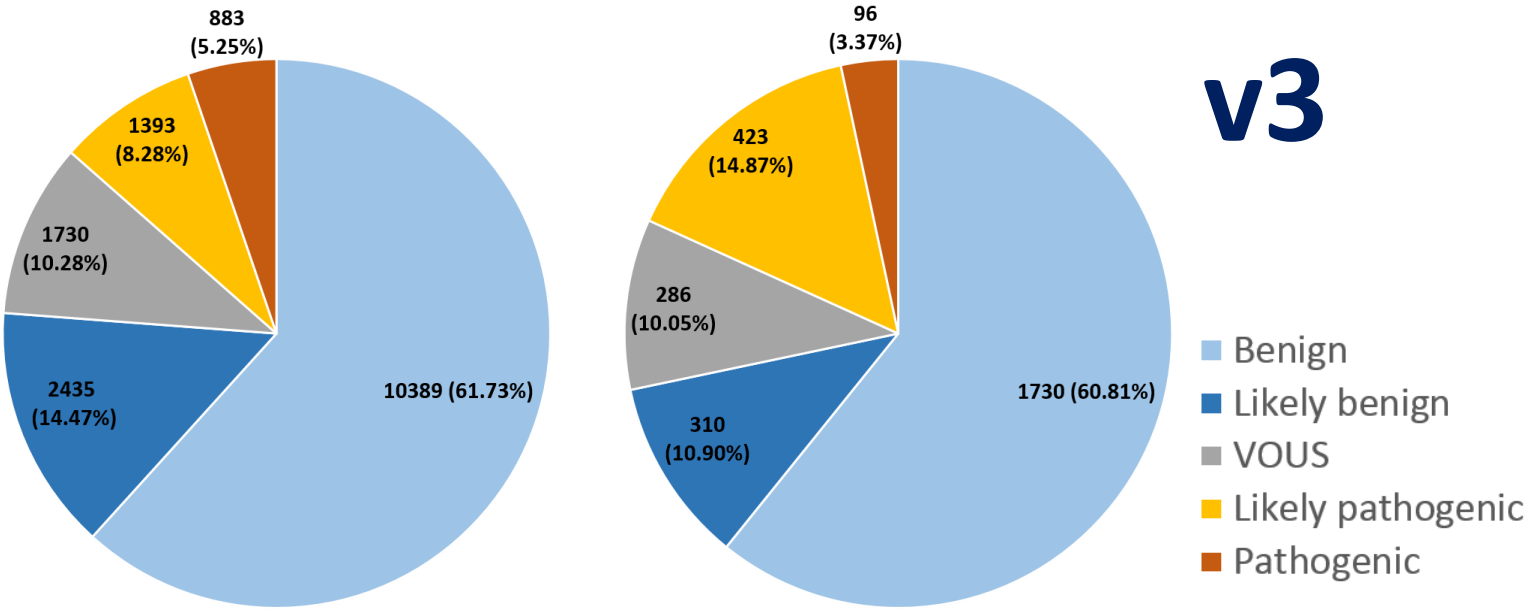
Exon-focussed targeted oligo-array design provides an increased diagnostic yield: Data analysis of 27,756 patients from a consortium of UK diagnostic genomic centres

Traditional design
CytoSure ISCA v2 oligo-array

Targeted design
CytoSure Constitutional v3 oligo-array



4.7%
increase for v3

	Total number of samples	Samples with likely pathogenic or pathogenic CNV	% samples with likely pathogenic or pathogenic CNV	Number of samples reported*	Reporting rate
CytoSure v2	16830	2276	**13.5%**	4006	**23.80%**
CytoSure v3	2845	519	**18.24%**	805	**28.29%**

4.5%
increase for v3
(p-value = 2.85e-07)

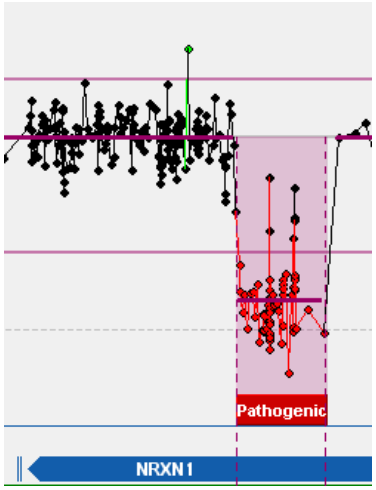
Table 2: Reporting rate of CytoSure v3 and v2 oligo-arrays.
* Number of samples classified as either VOUS, likely pathogenic or pathogenic.

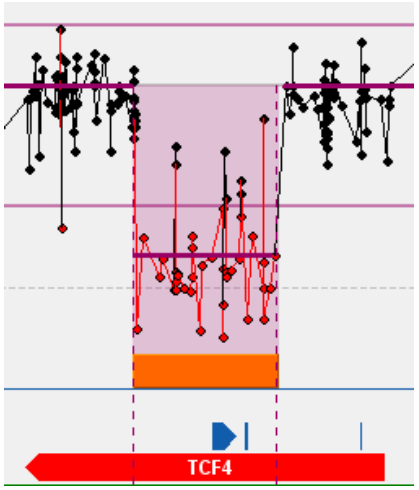
The v3 exon-focused array design shows a significant increase in intragenic CNVs per sample when compared to the v2 array:

v3: 0.73 CNVs per sample vs v2: 0.5 CNVs per sample (P < 2.2e-16)

64 cases

with pathogenic or likely pathogenic intragenic CNVs identified in the following genes that would have been **missed** by the v2 array:





Summary

- Our results show that the v3 exon-focused array design provides a powerful tool for detection of small pathogenic intragenic deletions, providing a clinical answer for a proportion of patients that would have remained undiagnosed.
- At this time, targeted arrays offer a robust method of copy number analysis to supplement NGS based tests.

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Jade Heath, Deborah Barrell

Oxford Gene Technology, UK:
Frank Smith,
Jolyon Holdstock,
Douglas Hurd

Contributing NHS Laboratories:
All Wales Medical Genomics Service, NHS Wales
Bristol Genetics Laboratory – North Bristol NHS Trust
Wessex Regional Genetics Laboratory – Salisbury NHS Foundation Trust
West Midlands Regional Genetics Laboratory - Birmingham Women's NHS Foundation Trust

Detection of fetal microdeletions by non-invasive prenatal testing

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HSST Innovation Project

North West
NHS Genomic Laboratory Hub

- Detection of fetal microdeletion Syndromes by non-invasive prenatal testing (NIPT)
- Sequence to determine what deletions and duplications can be detected at differing fetal fractions and read depths
- Statistical analysis of the results to determine the likelihood that a sample is affected with a microdeletion
- Determining level of abnormality in relation to fetal fraction
- Potential to reduce the number of false positive results

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Method

North West
NHS Genomic Laboratory Hub

- Sourced abnormal cell lines from commercial suppliers for the common microdeletion syndromes
- Microarray – cell lines and ‘normal’ background
- Combine these cell lines with a ‘normal’ background to different artificial fetal fractions
  - 5, 10 and 15% ff’s
  - 1p36, Wolf-Hirschhorn Syndrome, Cri du Chat Syndrome, Prader-Willi/Angelman Syndrome, Di George Syndrome
- Fragment
- Size selection
- Library Prep
- Sequence
  - Using Ion Torrent
- Analyse
  - Using R

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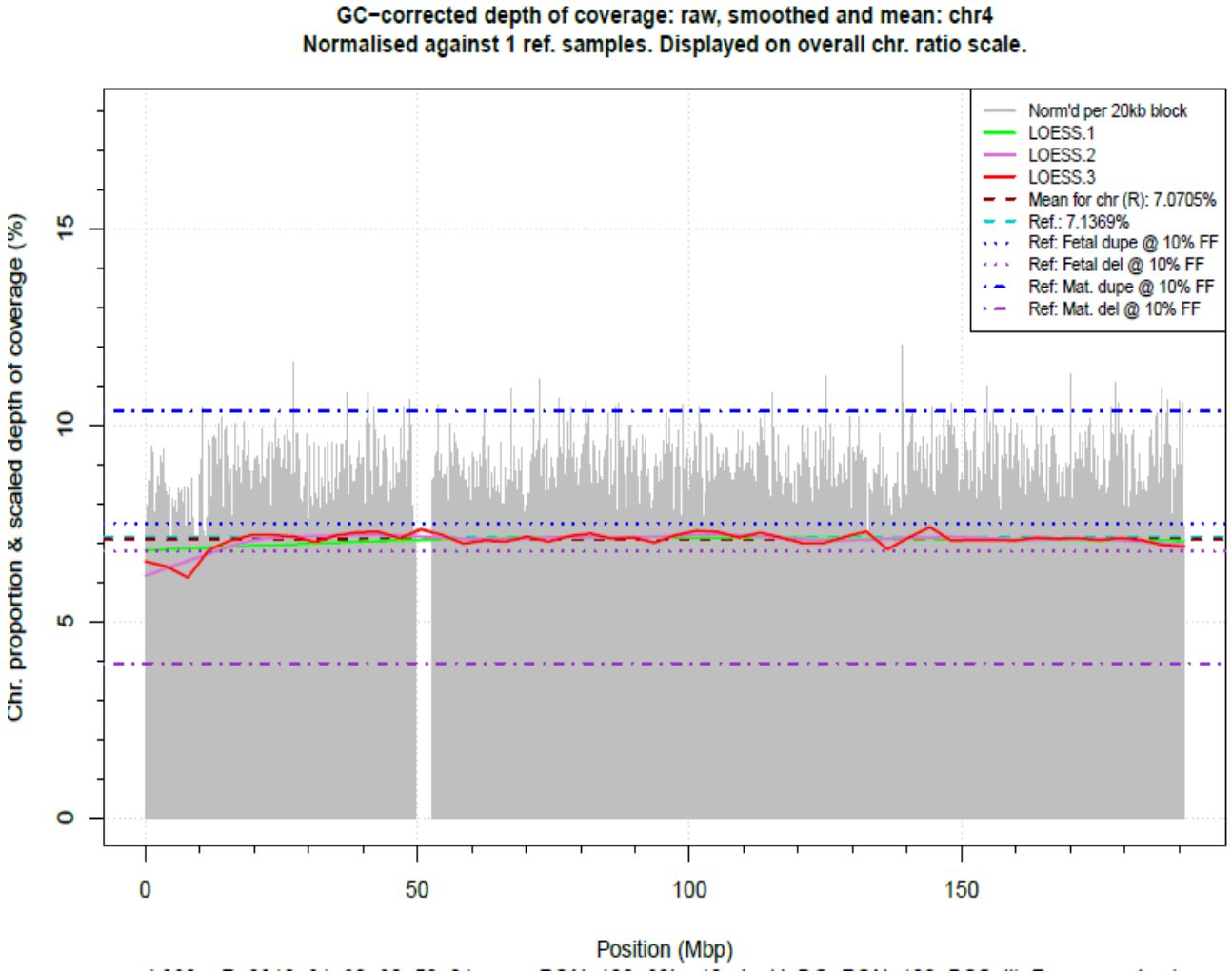
Wolf-Hirschhorn Syndrome – 11.0-11.1Mb deletion from 4p15.33 to 4p16.3 – Interstitial

North West
NHS Genomic Laboratory Hub

Region 2	Start Cyto	End Cyto	Assessment	Type	Copy #	Chromosome	Start	End	Size (bp)	Man Change	StdDev	Included	Excluded	%Included
min	4p16.3	4p15.33	Pathogenic?	LOSS	-0.81	4	428,761	11,431,717	11,002,957	no	0.14	246	0	100.00
max	4p16.3	4p15.33	Pathogenic?	LOSS	-0.81	4	374,586	11,507,196	11,132,611	no	0.14	246	0	100.00



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A new diagnostic approach to calling CNV's from low read-depth genomic sequencing data

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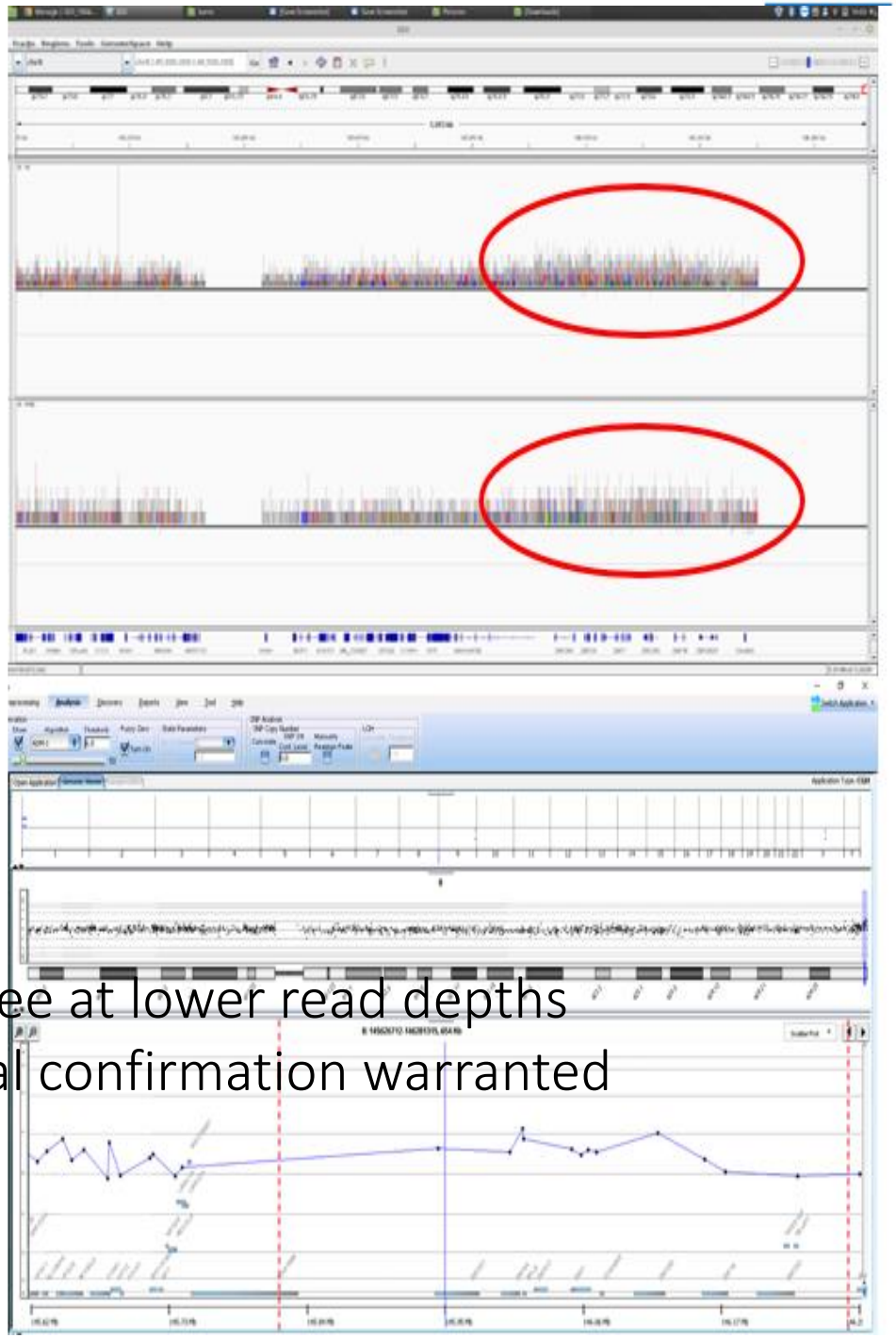
Project Aims

Primary Goal – To assess if low read depth Genome Sequencing (GS) is a clinically feasible, cost effective alternative to Chromosomal Microarray Analysis (CMA) for CNV detection

- Read depth?
- Resolution?
- Cost per sample?
- CNV-calling algorithms? WISECONDORX, Canvas, CNVSeq

Secondary Goal - Can GS provide a better quality result for tissue samples with low-quality DNA?

- Products of Conception (POCs), Fetal Tissues (FT) and Stillbirths
- 30% of 1,144 miscarriage tissues achieved a sub-optimal resolution result in 2017



- Difficult to see at lower read depths
- Experimental confirmation warranted

Conclusions

- GS comparable to CMA

- WISECONDORX optimal of all algorithms tested
- Bin size 50,000 bp
- Carefully curated reference

- Shallow WGS detected all CNVs 300 kb and greater in size (resolution may improve with further development)
- 500 kb CNV detected at 0.04X

- Seems to work equally well for poor quality DNA

- Sample preparation demonstrated to be feasible

- Reagent cost per sample as low as £75