Non-Invasive Prenatal Diagnosis for Sickle Cell Disease by Droplet Digital PCR and Next Generation Sequencing

Joe Shaw[1], Sophie Sheppard[1], Natalie Chandler[1], Helena Ahlfors[1], Chris Sale[2], Michael Parks[2], Lee Silcock[2], Lyn S Chitty[1]

[1] London North Genomic Laboratory Hub; [2] Nonacus Ltd

Joseph.Shaw@gosh.nhs.uk

NHS
Great Ormond Street Hospital for Children
NHS Foundation Trust

NHS
London North
NHS Genomic Laboratory Hub

Sickle Cell Disease

Most common single-gene invasive prenatal test in the UK

62% of cases ***HBB* c.20 A>T p.(Glu7Val)**

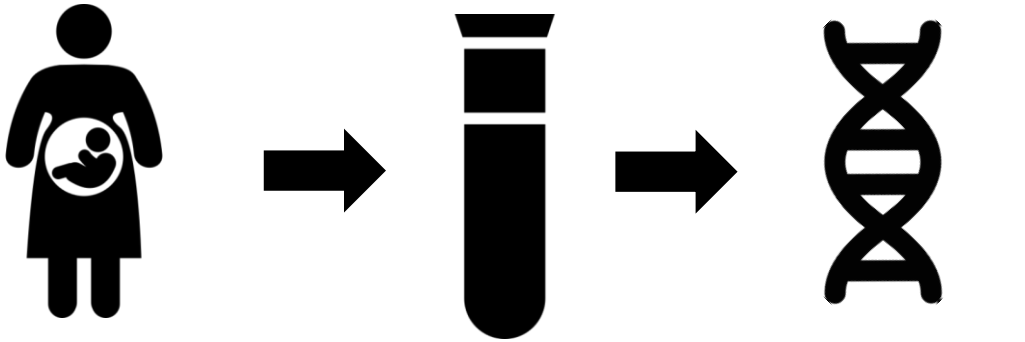
Challenges to NIPD with cfDNA

High background of maternal mutation

Mother and father – same mutation

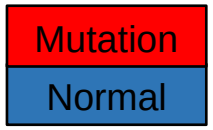
Paternal sample not received in ~40% of cases

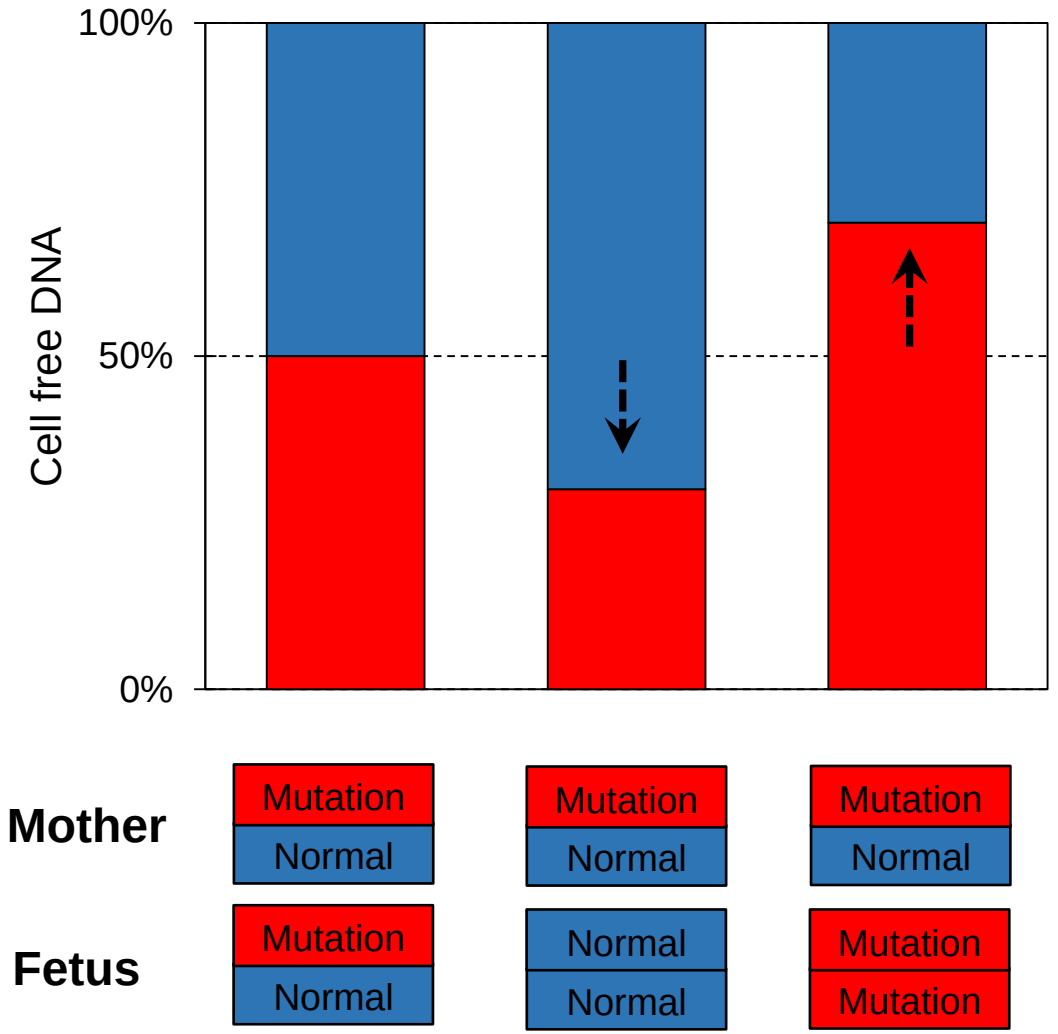
Solution: relative mutation dosage using a maternal sample



Carrier mother

cell free DNA





Study Structure

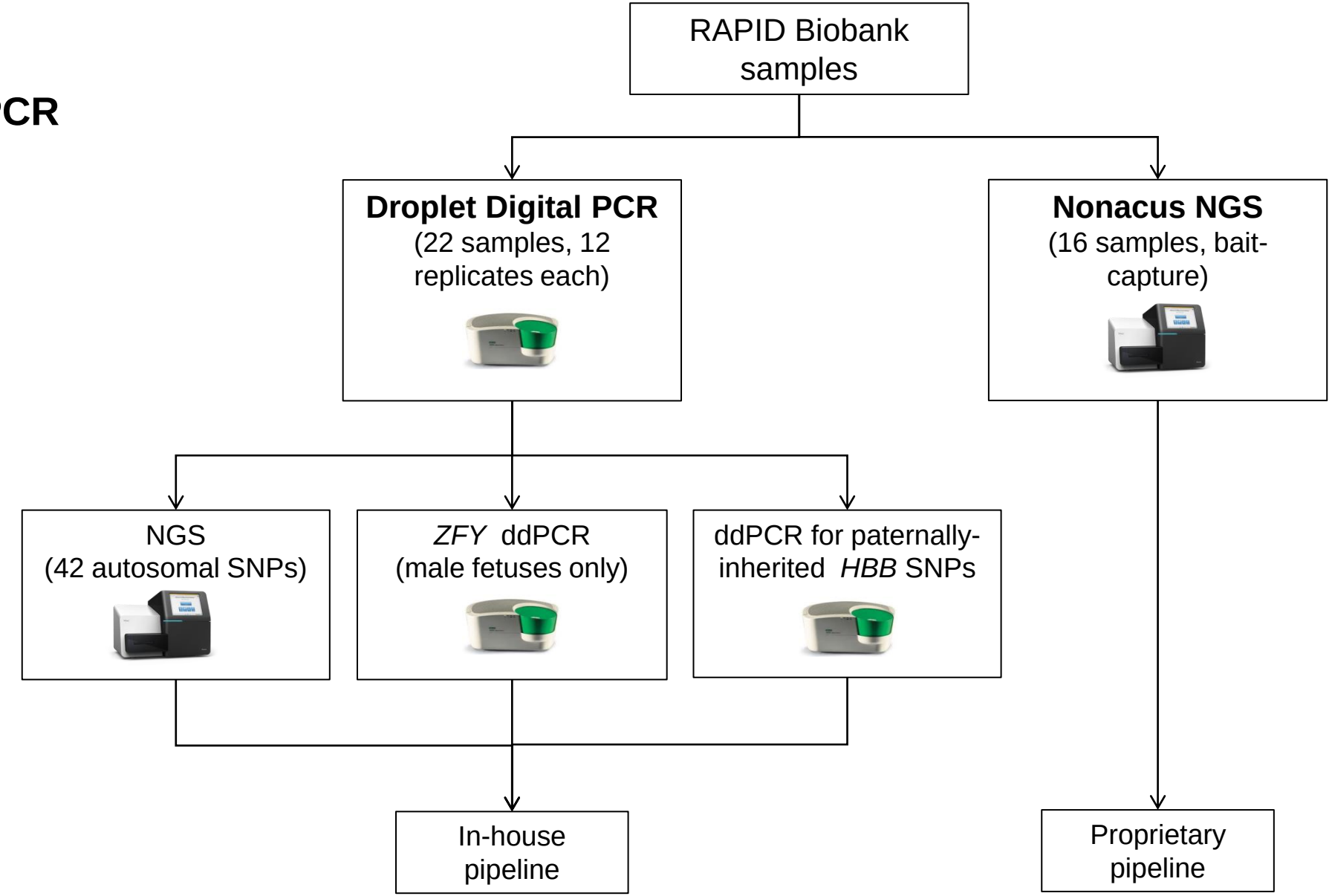
NGS and droplet digital PCR

1. **Determine abundance of normal and mutant alleles**

2. **Determine the fetal fraction** (measure a marker that is present in fetus but not mother)

3. Relative mutation dosage (SPRT)*

*Sequential probability ratio test



Results: Nonacus NGS

Sample ID	Median FF(%)	Fetus	Predicted Fetal Genotype: Standard analysis	Predicted Fetal Genotype: Reads less than 155bp
16186	10.4	SS	SS	SS
14751	14.7	SS	SS	SS
16794	8.6	SS	SS	Inconclusive
14248	4.2	SS	SS	Inconclusive
14004	11.7	AS	AS	Inconclusive
12368	7.1	SS	Inconclusive	AA
13809	15.1	AS	Inconclusive	SS
14000	9.4	AA	Inconclusive	AA
14182	8.9	AS	Inconclusive	AS
18135	16	SS	Inconclusive	SS
16023	5.9	AA	Inconclusive	Inconclusive
14281	6.9	AA	Inconclusive	Inconclusive
18893	10.3	AS	Inconclusive	Inconclusive
18579	14.6	AS	Inconclusive	Inconclusive
17729	8.1	AA	Inconclusive	Inconclusive
14650	12	AA	Inconclusive	Inconclusive
			5 correct **11 inconclusive**	**5 correct** **2 incorrect** **9 inconclusive**

Key
AA: homozygous wild type
AS: heterozygous
SS: homozygous mutant

Overall	
Correct:	8
Incorrect:	2
Inconclusive:	6

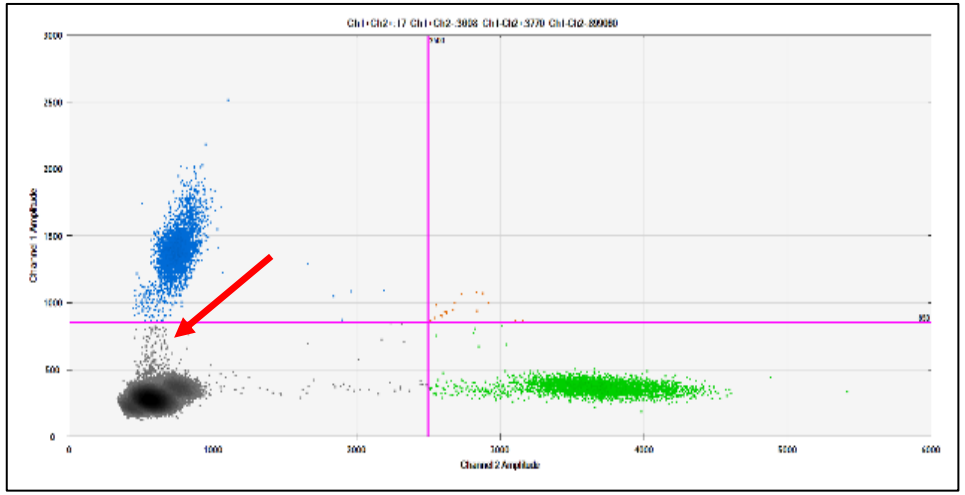
Results: droplet digital PCR

		Fetal Load NGS		*ZFY* ddPCR	
Lab ID	Fetus	Median FF (%)	Prediction	Median FF (%)	Prediction
14964	AS	9.1	AS	6.8	AS
14596	SS	3.7	SS	7.0	SS
16471	AA	7.9	AA	10.6	AA
18135	SS	10.5	SS	10.8	SS
14713	AS	2.3	Inconclusive	7.4	AS
16731	SS	9.5	Inconclusive	8.8	SS
16808	AA	0.8	Inconclusive	12.4	AA
14136	AA	4.3	Inconclusive	6.0	Inconclusive
10118	AA	10.5	AA		
17500	SS	5.4	SS		
18183	AS	8.2	AS		
16794	SS	6.2	SS		
17473	AA	9.6	AA		
18111	AS	5.9	AA		
19349	AS	2.5	AA		
16186	SS	5.4	Inconclusive		
17555	AA	0.2	Inconclusive		
19298	AS	3.0	Inconclusive		
19350	AS	4.9	Inconclusive		
19292	AS	4.7	Inconclusive		
18447	SS	0.6	Inconclusive		
18459	AS	3.6	Inconclusive		

Overall

Correct:	12
Incorrect:	2
Inconclusive:	8

- Limited correlation between NGS and ZFY ddPCR results
- Paternally inherited SNPs: assays designed and proof-of-principle demonstrated for 4 cases
- 2 carrier fetuses incorrectly predicted to be homozygous normal



Problem: systematic under-representation of the mutant allele

Solution: further PCR optimisation

Comparison: ddPCR and NGS

	ddPCR	Nonacus NGS
Correct predictions	12/22	8/16
Incorrect predictions	2/22	2/16
Inconclusive predictions	8/22	6/16
Lab work	1 morning	2 days
Throughput	Flexible	6 samples per hybridisation
Estimated cost per sample	£149*	£342*
Fetal fraction	Extra assay required	Within assay
Mutation Spectrum	*HBB* c.20A>T	Any *HBB* sequence variant

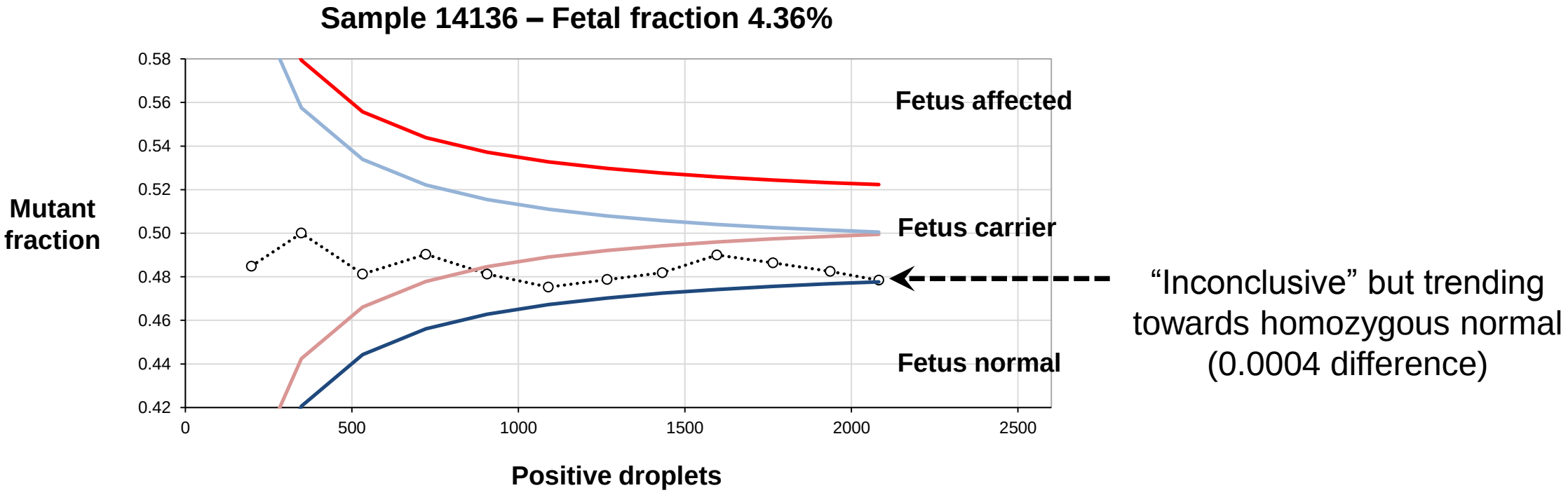
*Depends on how many samples included in a run

Limitations

No reflex testing of inconclusive samples

ddPCR assay not fully optimised

Combining NGS and ddPCR – potential for PCR bias



Conclusions

NIPD for sickle cell disease with ddPCR and NGS is possible, but assay must be robustly validated

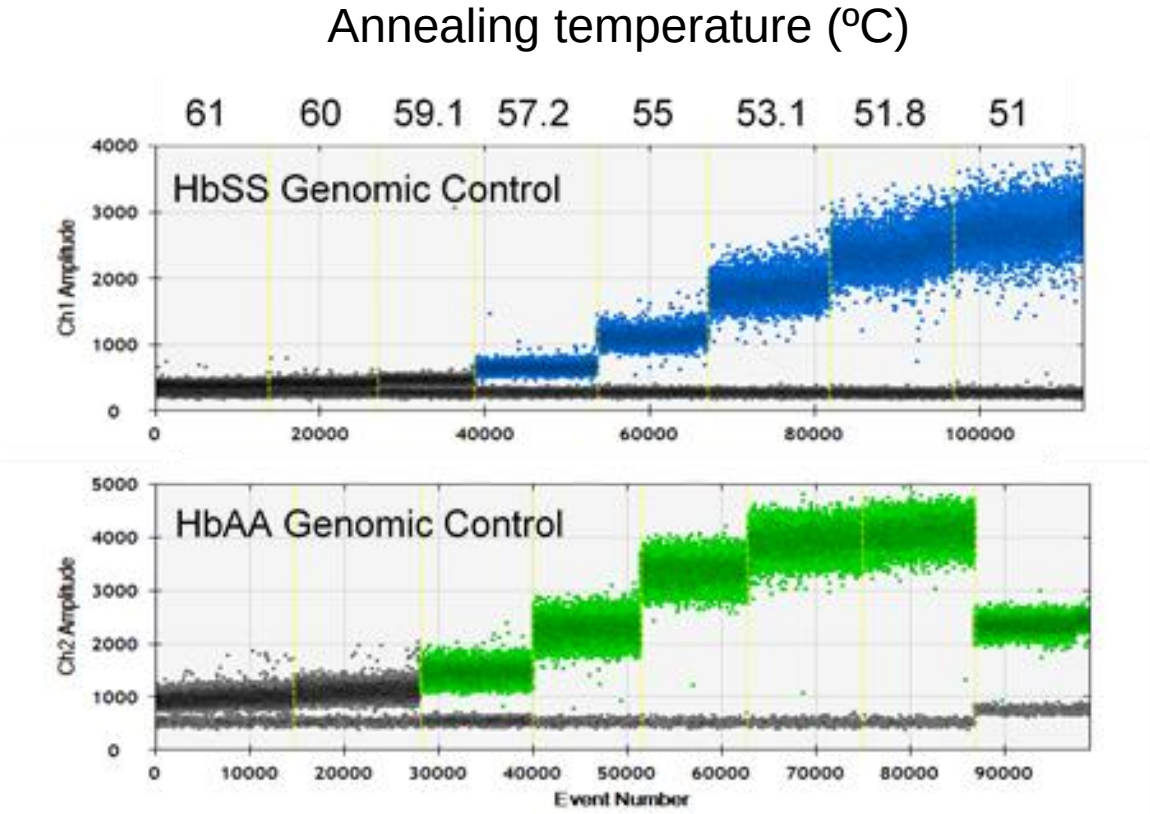
Fetal fraction determination is a major obstacle, and measurements vary between techniques

Future Directions

Fully optimise the sickle cell ddPCR assay

Blinded study – ddPCR using NGS for fetal fraction

Optimised workflow – repeat those with inconclusive results



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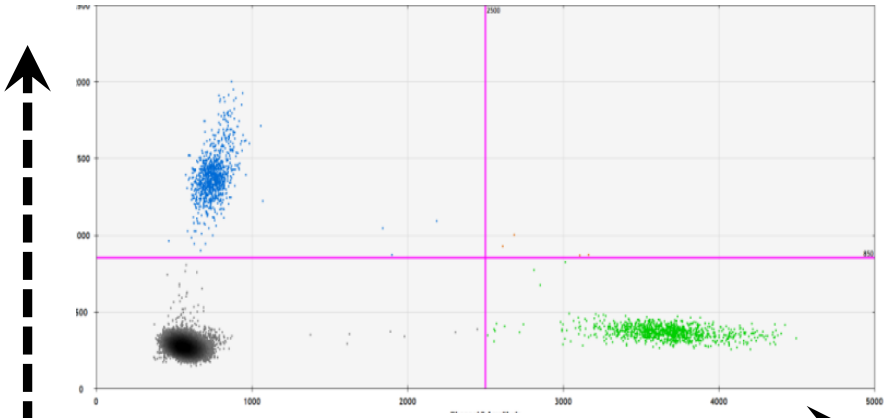
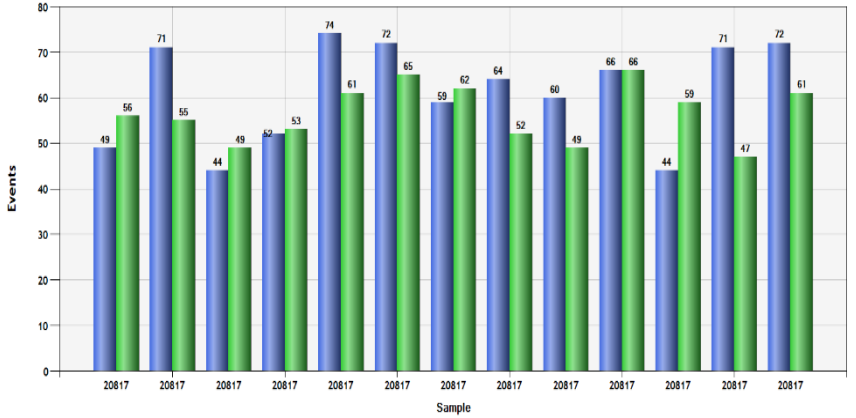
Results: droplet digital PCR

Cell free DNA



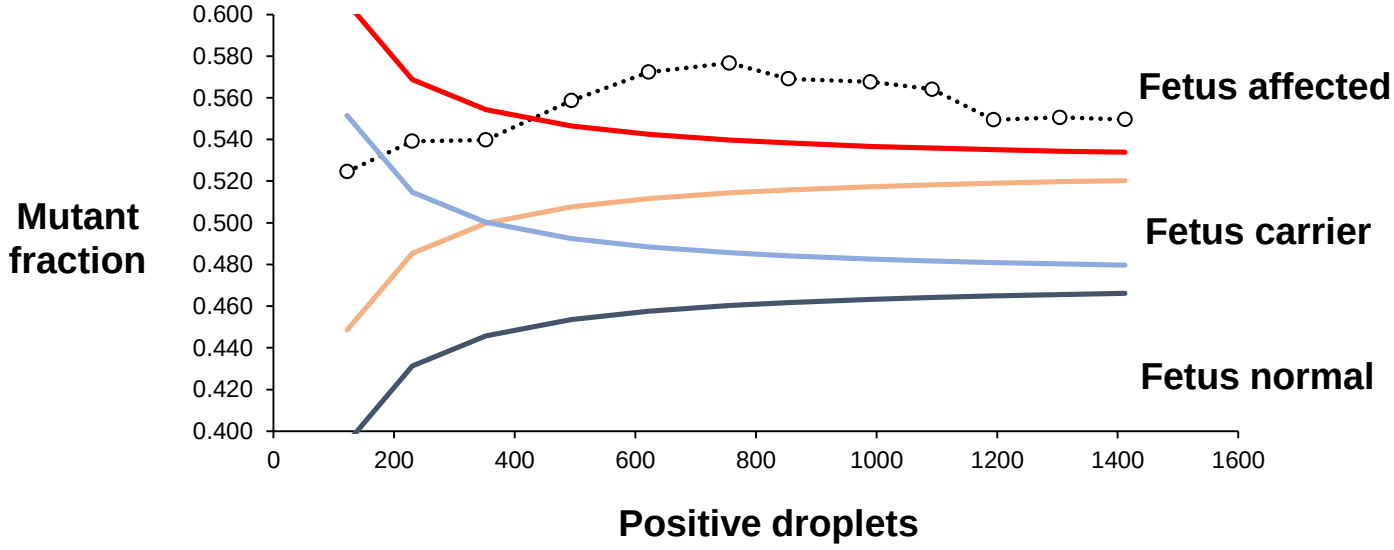
Blue (mutant)

Green (normal)



Results per replicate

+ fetal fraction



Affected fetus
10.8% fetal fraction