

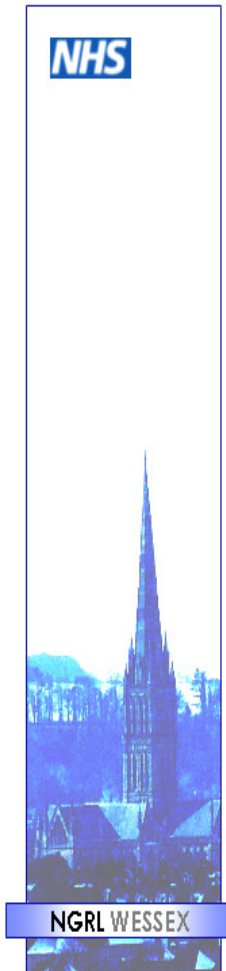


Development of RASSF1A assay: universal fetal DNA marker

Helen White

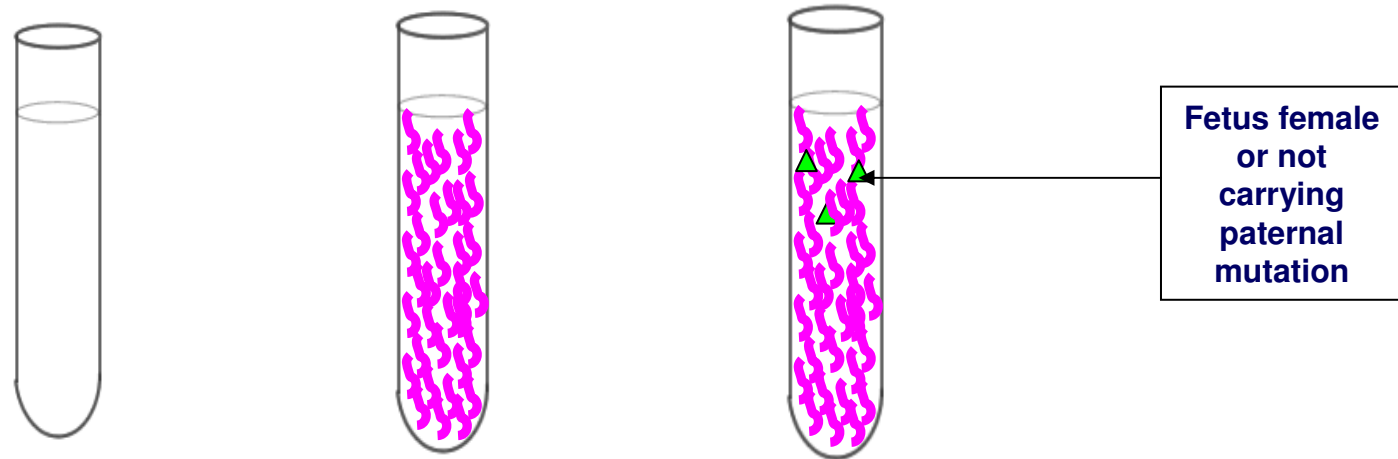
Senior Scientist

National Genetics Reference Lab (Wessex)



Fetal specific markers

- To confirm presence of cf fetal DNA if testing result is negative



- **Several candidates**

Control Genes (limited) – will determine that cf DNA extracted but not distinguish fetal and maternal

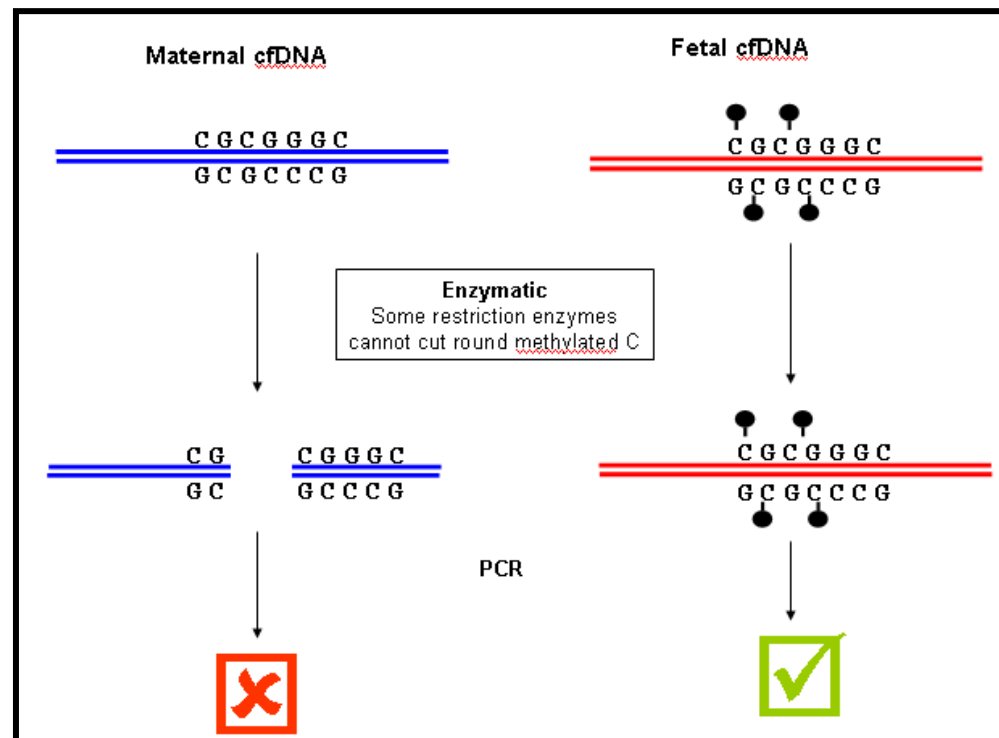
Paternal single nucleotide polymorphisms

Epigenetic markers

No one clear approach at present – many candidate markers under development

Hypermethylated *RASSF1A* in Maternal Plasma: A Universal Fetal DNA Marker that Improves the Reliability of Noninvasive Prenatal Diagnosis

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Protocol Modifications



NHS

Chan *et al.* protocol (2006)

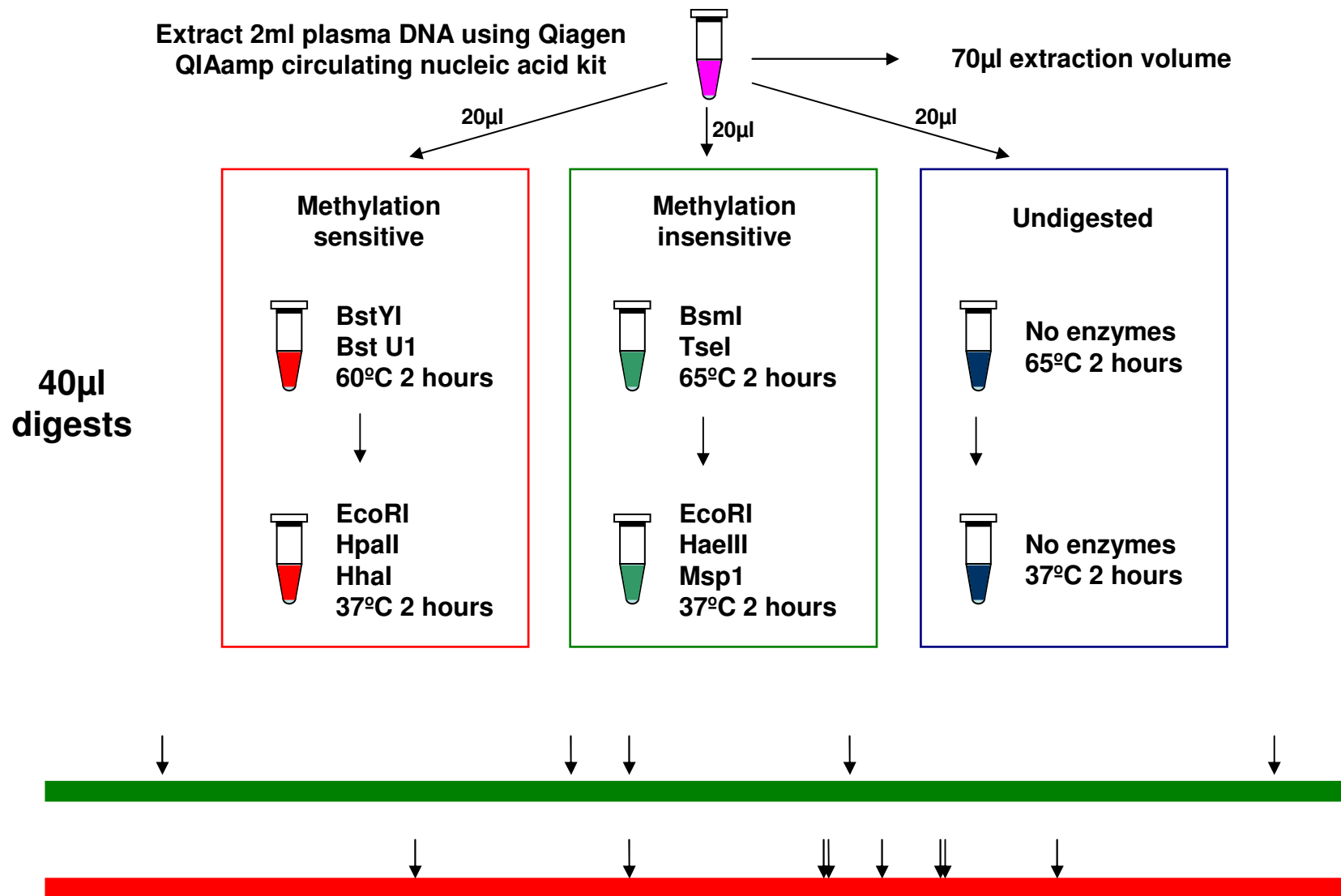
- Single enzyme digest (BstUI 60°C 16 hours)
- Probe based assay
- Beta actin promoter as digest control

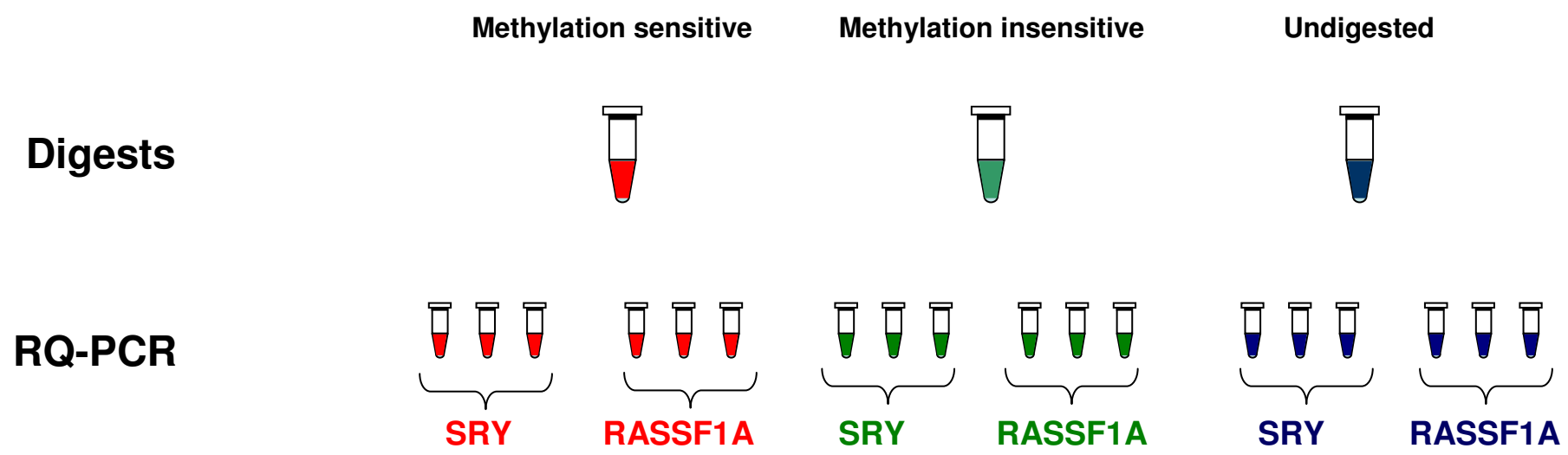
Modified protocol

- Multiple enzyme digest (total digest time 4 hours)
- SYBR green assays for SRY and RASSF1A
- Melt to check amplicon specificity
- Methylation insensitive digest control on RASSF1A
- (Beta actin promoter as digest control)

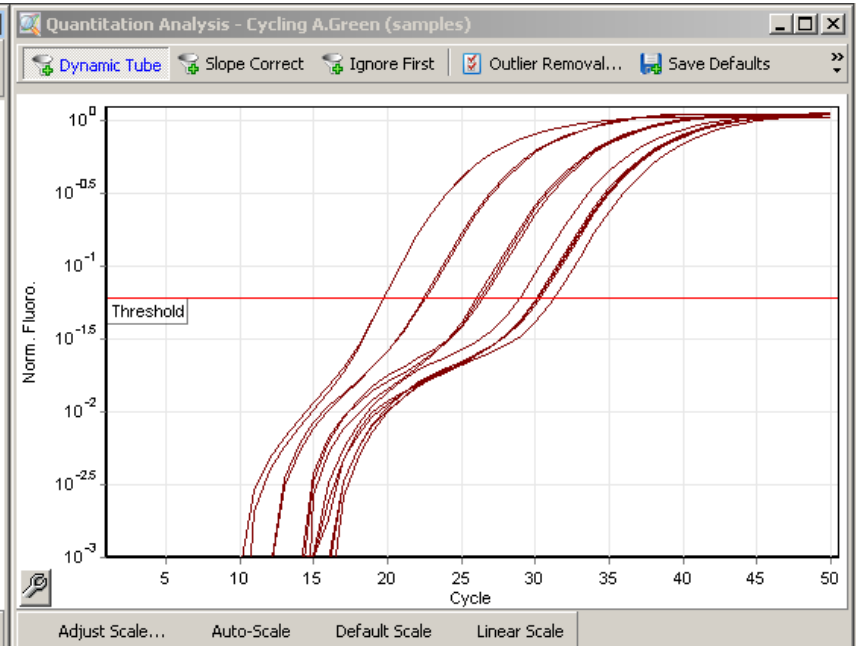
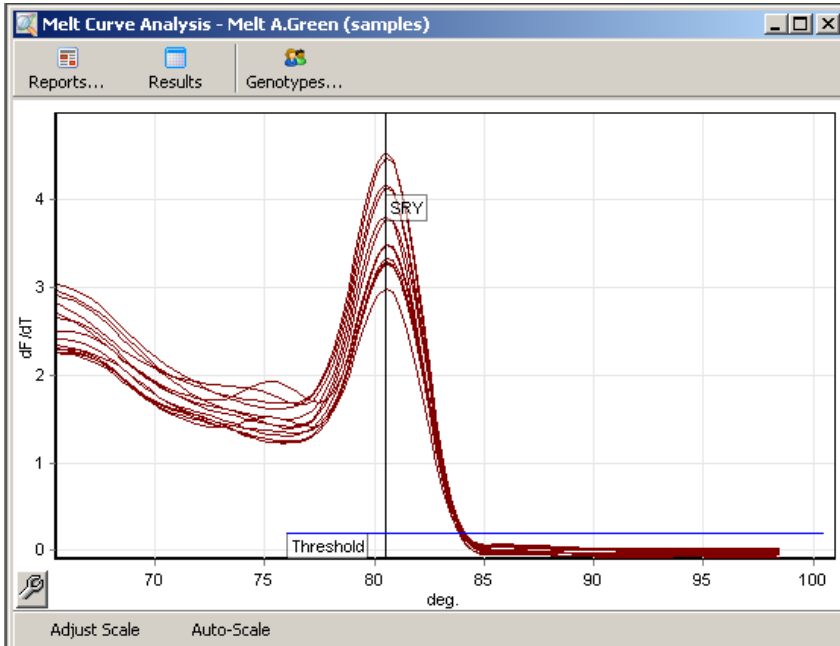


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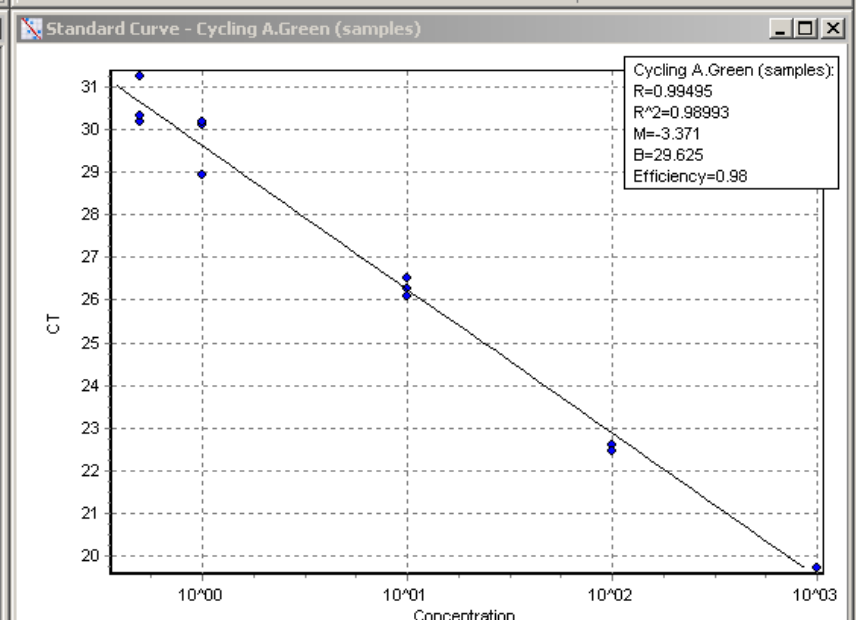


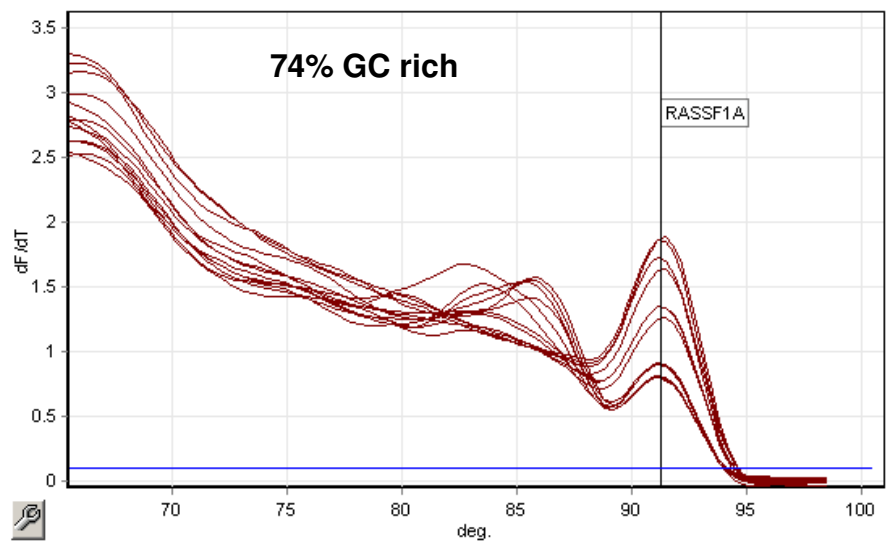
Male fetus	✓	✓	✓	✗	✓	✓
Female fetus	✗	✓	✗	✗	✗	✓
No fetal DNA	✗	✗	✗	✗	✗	✓
No cell free DNA	✗	✗	✗	✗	✗	✗



Melt Curve Results - Melt A.Green (samples)

No.	Name	Genotype	Peak 1
1	6.6ng		80.5 (SRY)
2	6.6ng		80.5 (SRY)
3	660pg		80.5 (SRY)
4	660pg		80.5 (SRY)
5	66pg		80.7 (SRY)
6	66pg		80.7 (SRY)
7	66pg		80.5 (SRY)
8	6.6pg		80.5 (SRY)
9	6.6pg		80.5 (SRY)
10	6.6pg		80.5 (SRY)
11	3.3pg		80.5 (SRY)
12	3.3pg		80.5 (SRY)
13	3.3pg		80.5 (SRY)

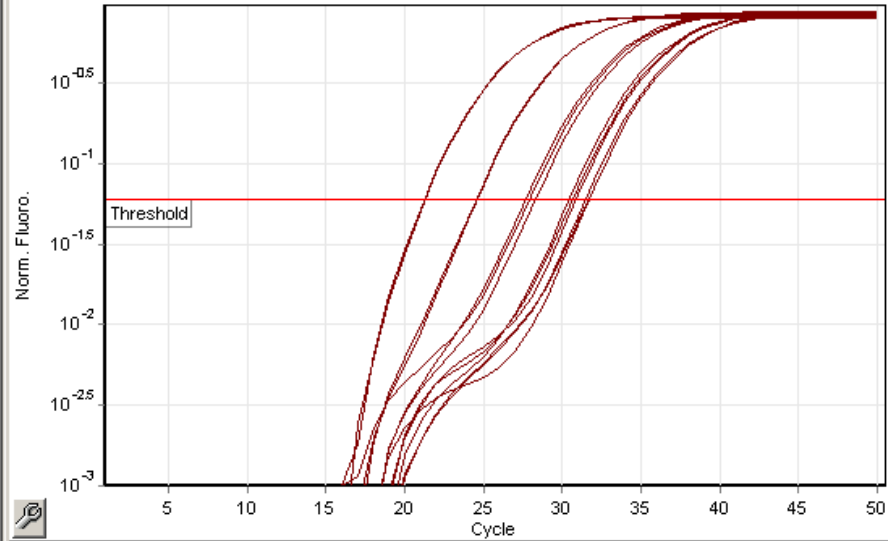




Adjust Scale Auto-Scale

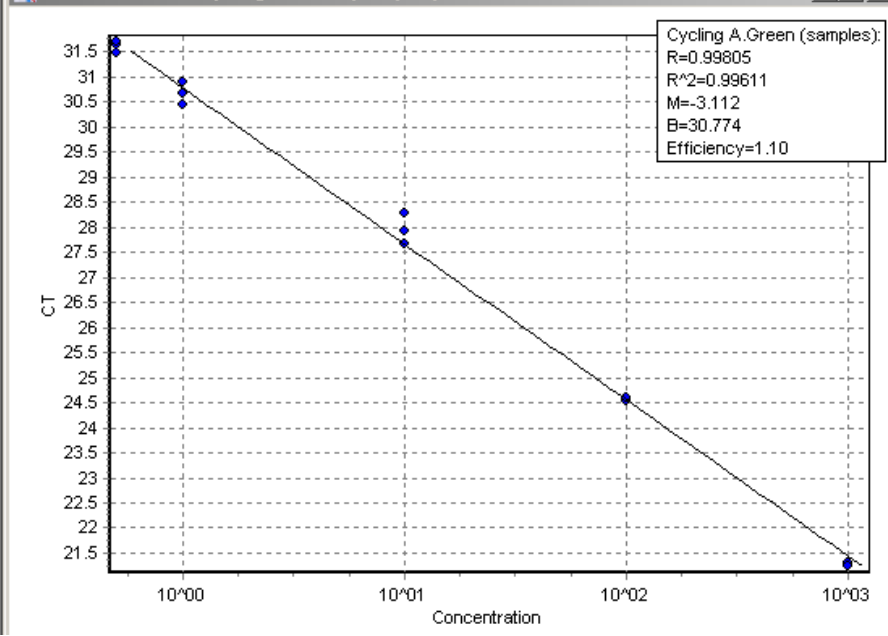
Melt Curve Results - Melt A.Green (samples)

No.	Name	Genotype	Peak 1	Peak 2	Peak 3
1	6.6ng	RASSF1A	91.5 (RASSF1A)		
2	6.6ng	RASSF1A	66.0	91.3 (RASSF1A)	
3	660pg	RASSF1A	66.0	91.2 (RASSF1A)	
4	660pg	RASSF1A	66.0	91.3 (RASSF1A)	
5	66pg	RASSF1A	83.2	91.3 (RASSF1A)	
6	66pg	RASSF1A	82.8	91.3 (RASSF1A)	
7	66pg	RASSF1A	82.5	91.3 (RASSF1A)	
8	6.6pg	RASSF1A	79.8	85.7	91.2 (RASSF1A)
9	6.6pg	RASSF1A	65.7	82.7	91.2 (RASSF1A)
10	6.6pg	RASSF1A	85.5	91.2 (RASSF1A)	
11	3.3pg	RASSF1A	85.7	91.2 (RASSF1A)	
12	3.3pg	RASSF1A	66.2	85.3	91.2 (RASSF1A)
13	3.3pg	RASSF1A	75.0	83.5	91.2 (RASSF1A)



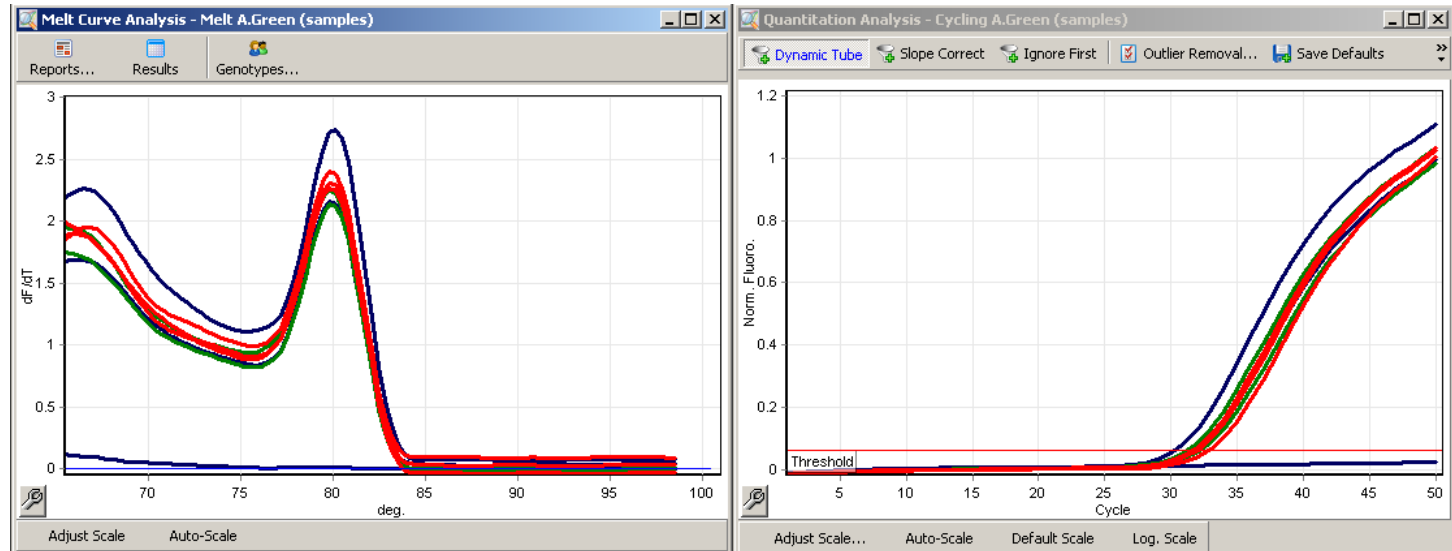
Adjust Scale... Auto-Scale Default Scale Linear Scale

Standard Curve - Cycling A.Green (samples)

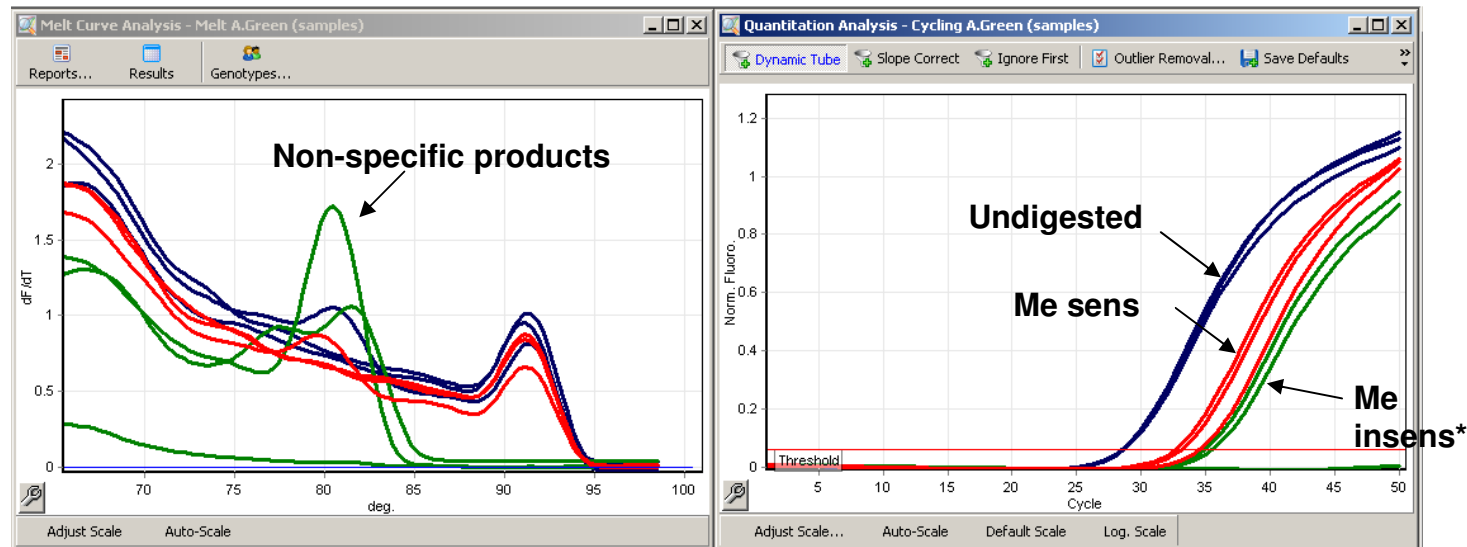


Male fetus: SRY +ve and RASSF1A +ve

SRY: 8/9 +ve

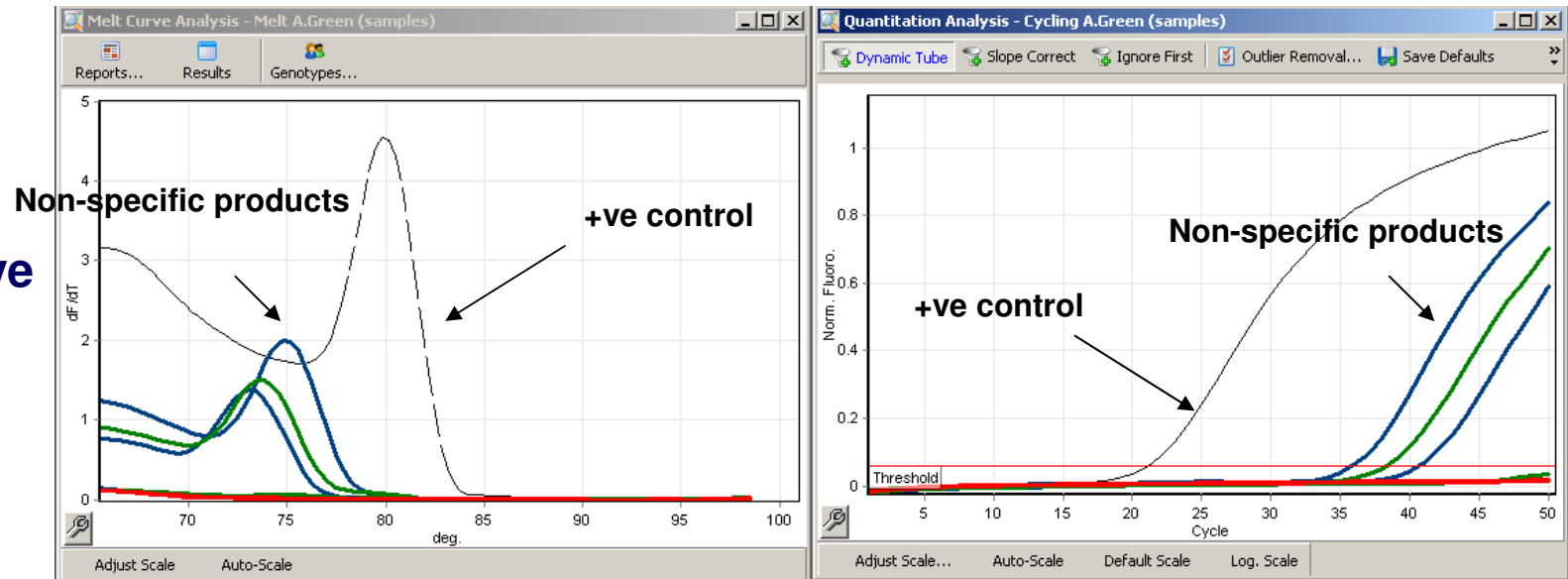


RASSF1A:

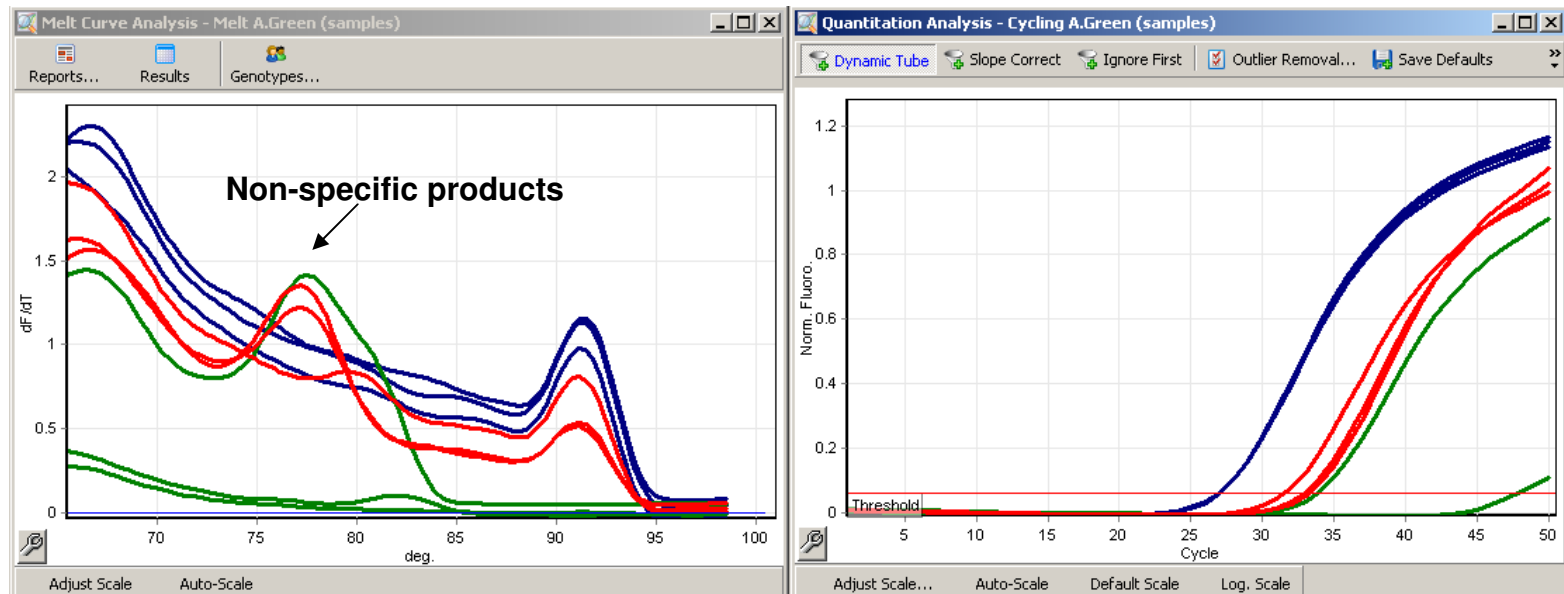


Female fetus: SRY -ve and RASSF1A +ve

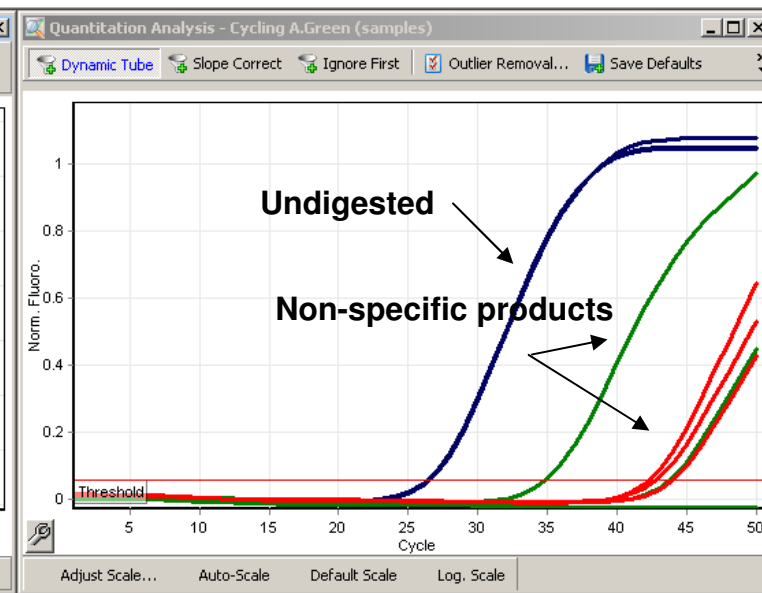
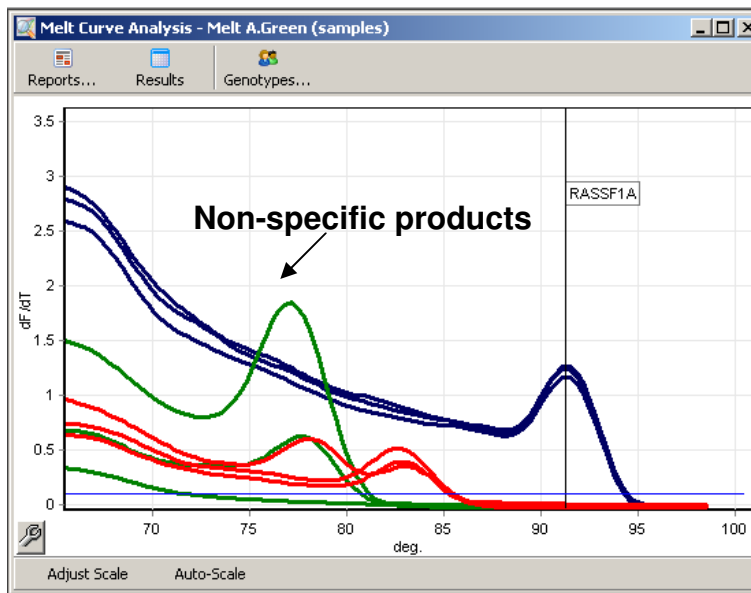
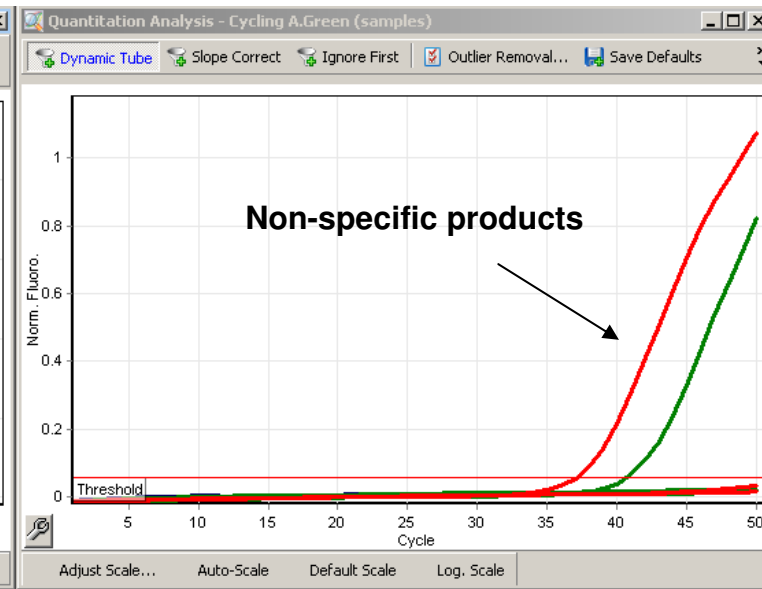
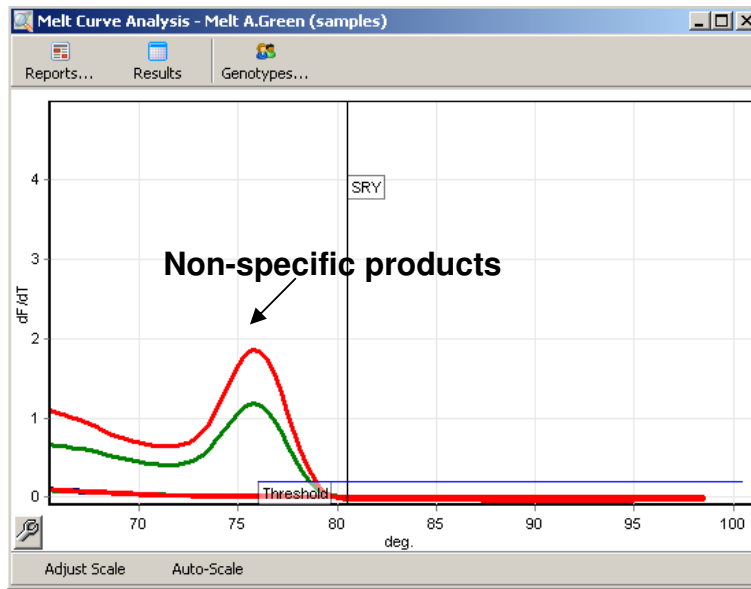
SRY: 0/9 +ve



RASSF1A



No fetal DNA: SRY -ve and RASSF1A -ve



Future work

- NGRL (W) / RAPID validation ongoing
- Retrospective study of 66 samples - analysis near completion

Undigested Samples (total cell free DNA): 100% had 3 replicates +ve

Methylation sensitive (fetal DNA only)

5 (7.5%)	3 replicates negative (no fetal DNA)
61 (92%)	1 or more replicates +ve
54 (83%)	2 or more replicates +ve
48 (72%)	3 replicates +ve

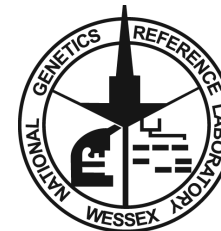
Methylation insensitive (all DNA should digest)

61 (92%)	3 replicates negative
5 (7.5%)	1 replicate +ve

- Prospective study of 100 samples underway - using larger volume of plasma
- Assessment of RASSF1A and beta actin digests - what combination is most useful / appropriate control
- Best practice?



Acknowledgements



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Prof Lyn Chitty

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