Figure S2a: Amplification plots showing the difference in efficiency of three methylation sensitive RASSF1A restriction enzyme digest protocols using cell free DNA extracted from the plasma of a non-pregnant female. The samples should digest completely and therefore no RASSF1A should be detected as there is no hypermethylated cell free fetal DNA present in the sample. Protocol D was the only one shown to give complete digestion.

A. Undigested samples (Ct 23.8)

B. Sample digested at 60°C with BstUI only (Ct 36.8) (e.g. ref 12).

C. Sample digested at 37°C with HpaII, HhaI and EcoRI (Ct 40.3).

D. Sample (purple line) digested at 60°C with BstUI and BstYI followed by digestion at 37°C with HpaII, HhaI and EcoRI. No amplification (purple line)

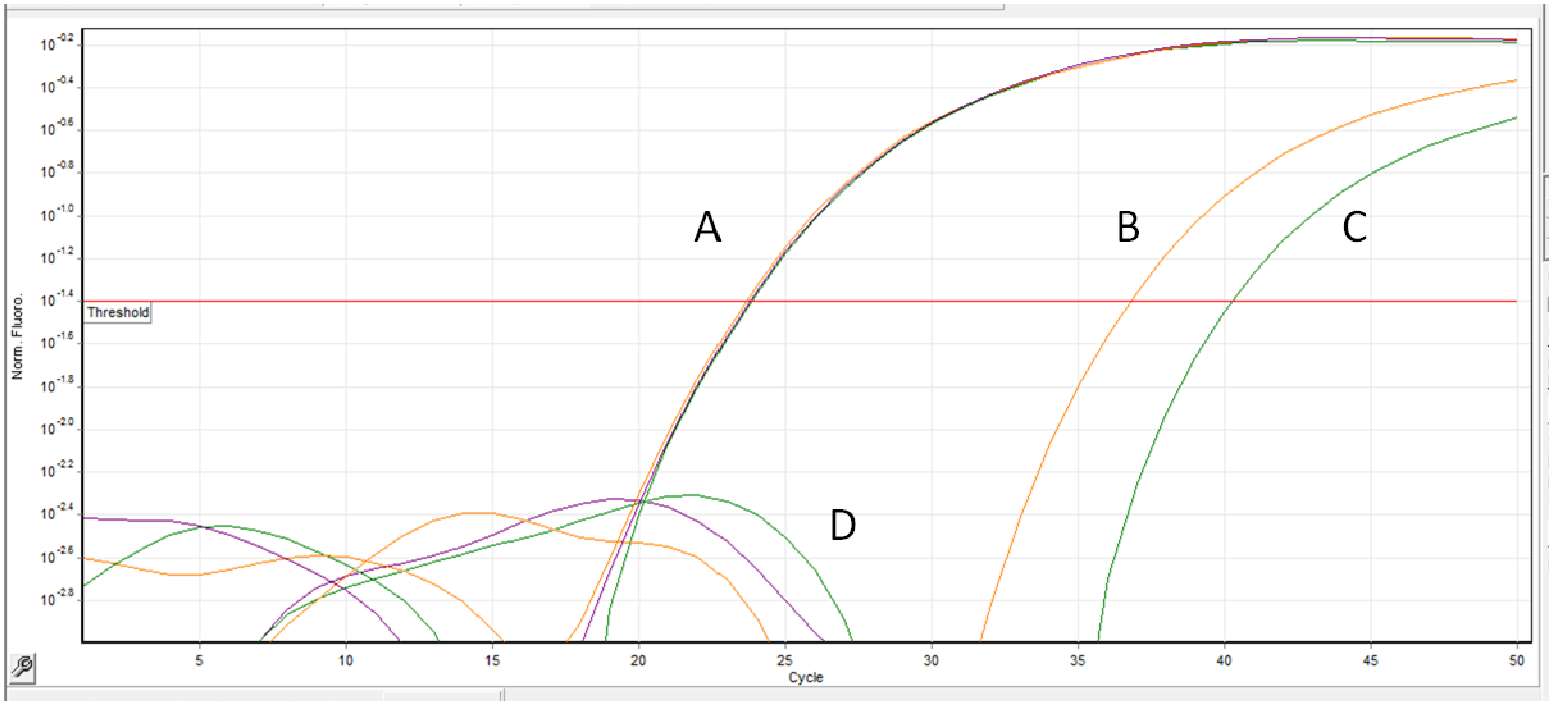

Figure S2b: Amplification plots showing an example of the difference in efficiency of two methylation sensitive RASSF1A restriction enzyme digest protocols using cell free DNA extracted from the same plasma sample from a pregnant female.

A. Undigested sample (Ct 29.1)

B. Plasma sample (red line) digested at 60°C with BstUI only (Ct 34.5)

C. Plasma sample (purple line) digested at 60°C with BstUI and BstYI followed by digestion at 37°C with HpaII, HhaI and EcoRI (Ct 35.6)

The difference in Ct values (1.1) between protocols B and C suggest that the digestion of unmethylated RASSF1A has been less efficient with protocol B than with protocol C. The amount of detectable RASSF1A is higher using protocol B than C an interpretation of that result is that protocol B leaves some of the unmethylated RASSF1A undigested.