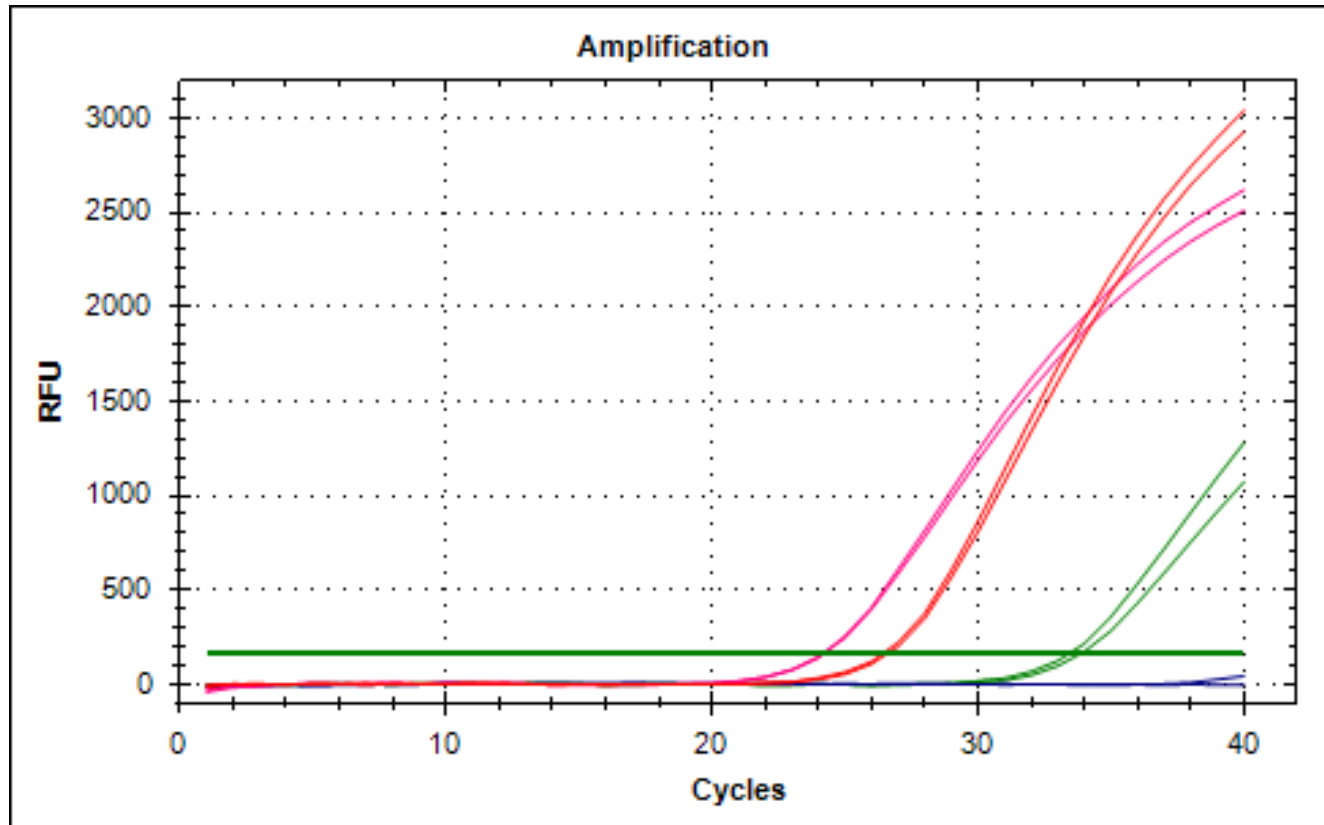


Non-invasive Prenatal Diagnosis



Peerapon Wong MD, Faculty of Medicine
Naresuan University

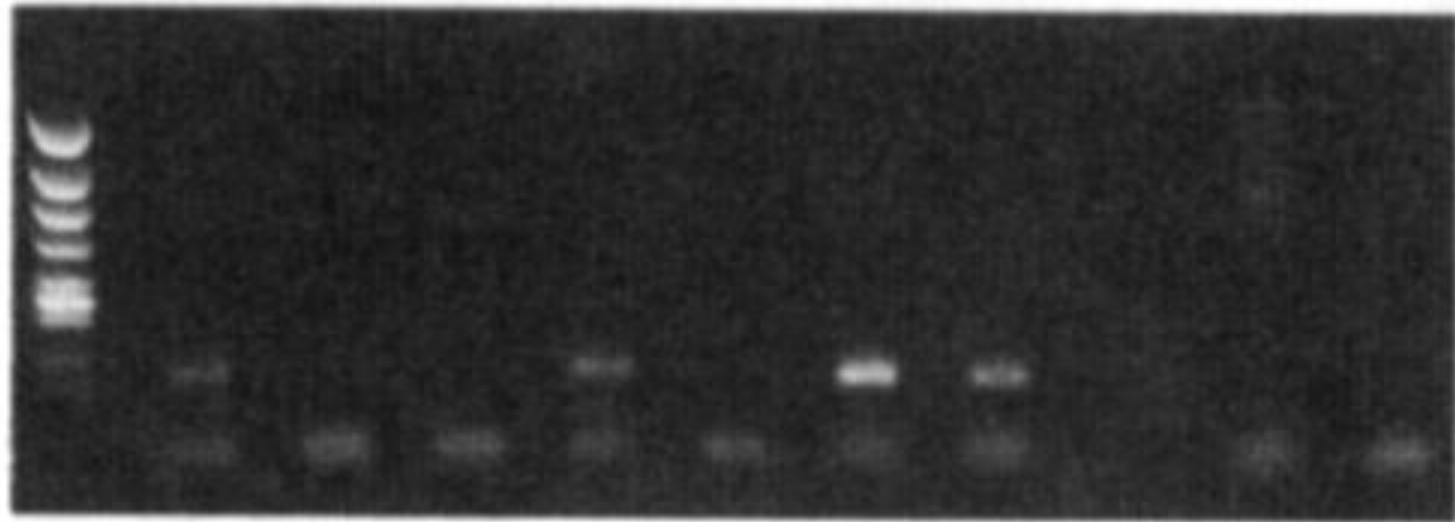
Fetus-derived Y-sequence from maternal blood

Plasma	24 (80%) of 30
Serum	21 (70%) of 30
Nucleated cell	5 (17%) of 30

Lo YMD, et al. Lancet 1997;350(9076):485-87.

Amplification of fetal Y-chromosomal sequences from maternal plasma

M 8 9 10 11 12 13 14 15 16



25 29 43 28 34

Maternal plasma

Controls

Lo YMD, et al. Lancet 1997;350(9076):485-87.

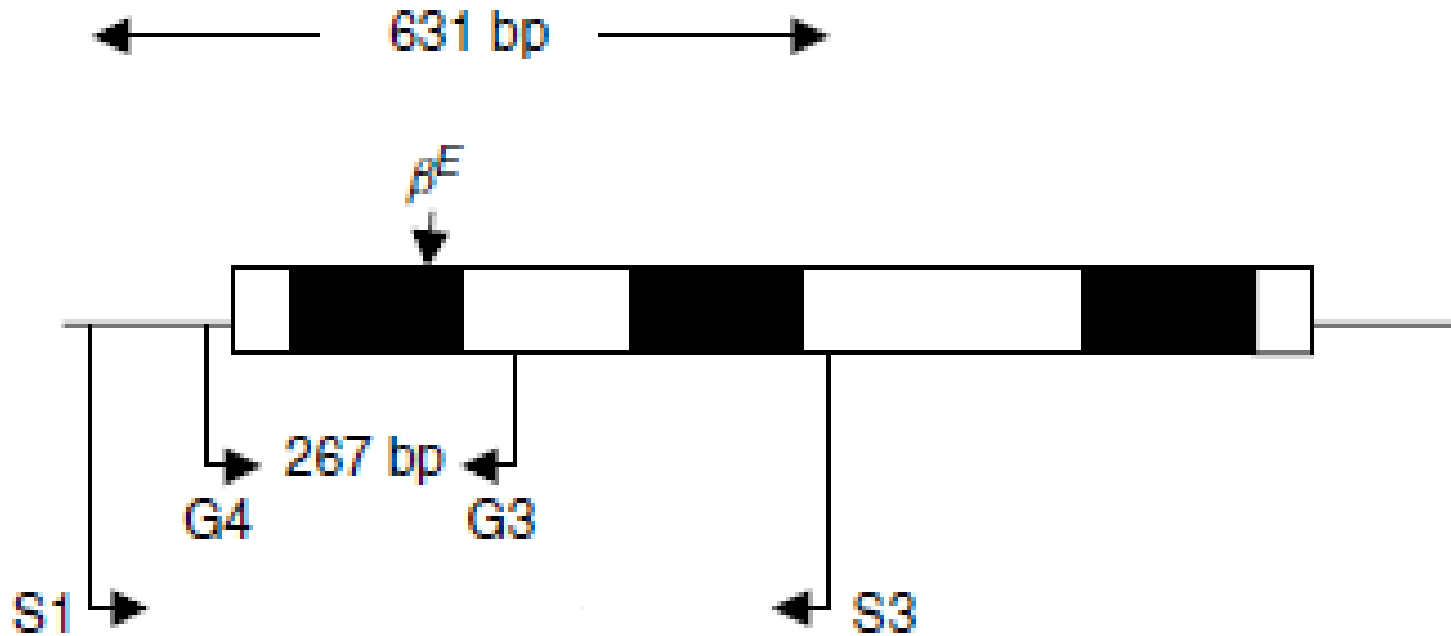
Quantitation of fetal DNA in maternal plasma and serum

SRY CONCENTRATION (copies/ml)

	EARLY PREGNANCY		LATE PREGNANCY	
	Plasma	Serum	Plasma	Serum
Range	3.3–69.4	4.0–58.1	76.9–769	33.8–900
Mean	3.4%	28.7	6.2%	342.1
Median	20.6	19.5	244.0	286.0

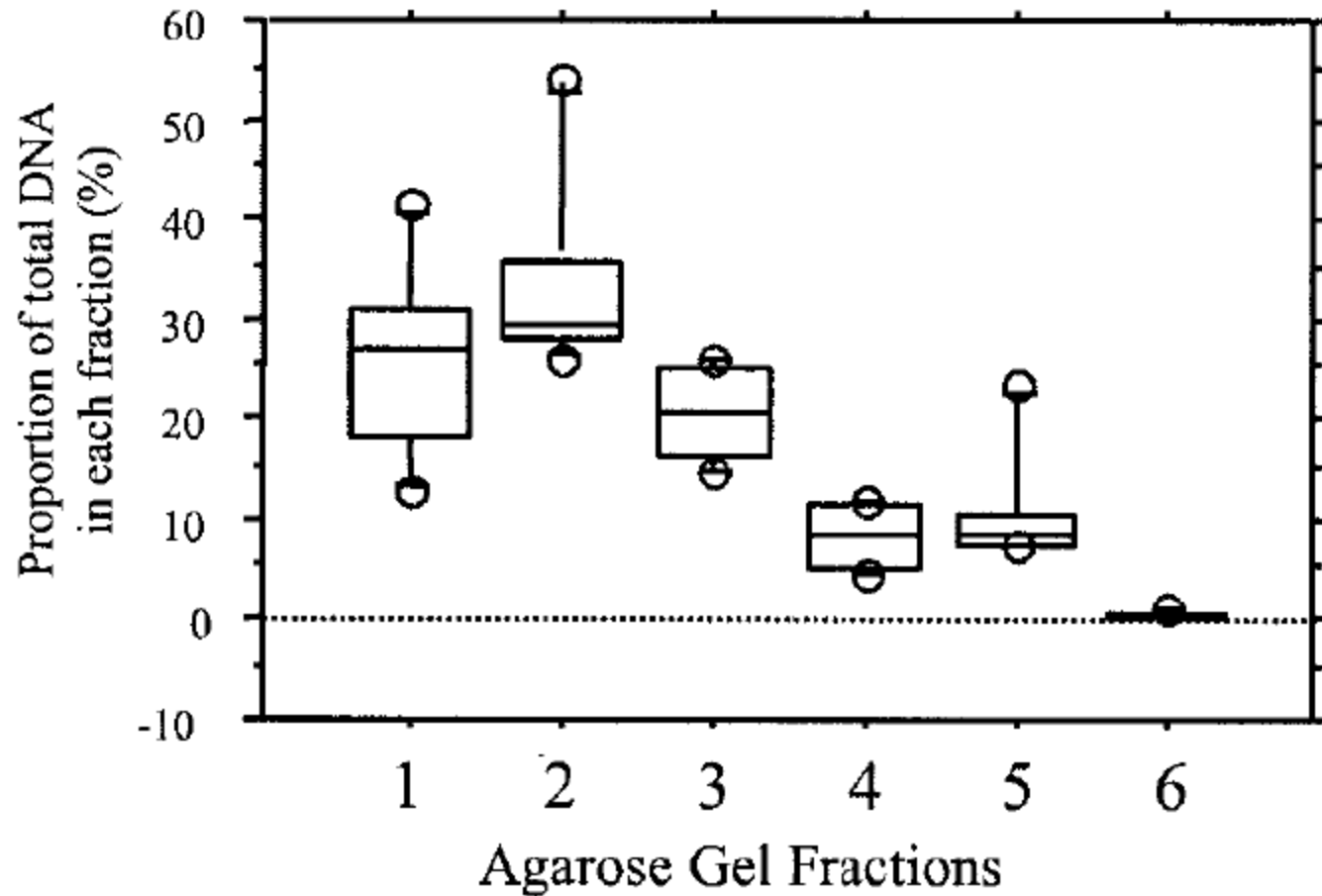
Lo YMD, et al. Am J Hum Genet 1998;62(4):768-75.

Amplification of β^E segment by nested PCR from maternal plasma



Fucharoen G, et al. *Prenat Diagn* 2003;23:393-6.

Size distribution of total and fetal circulatory DNA in third-trimester maternal plasma samples



Size distribution of total and fetal circulatory DNA in third-trimester maternal plasma samples

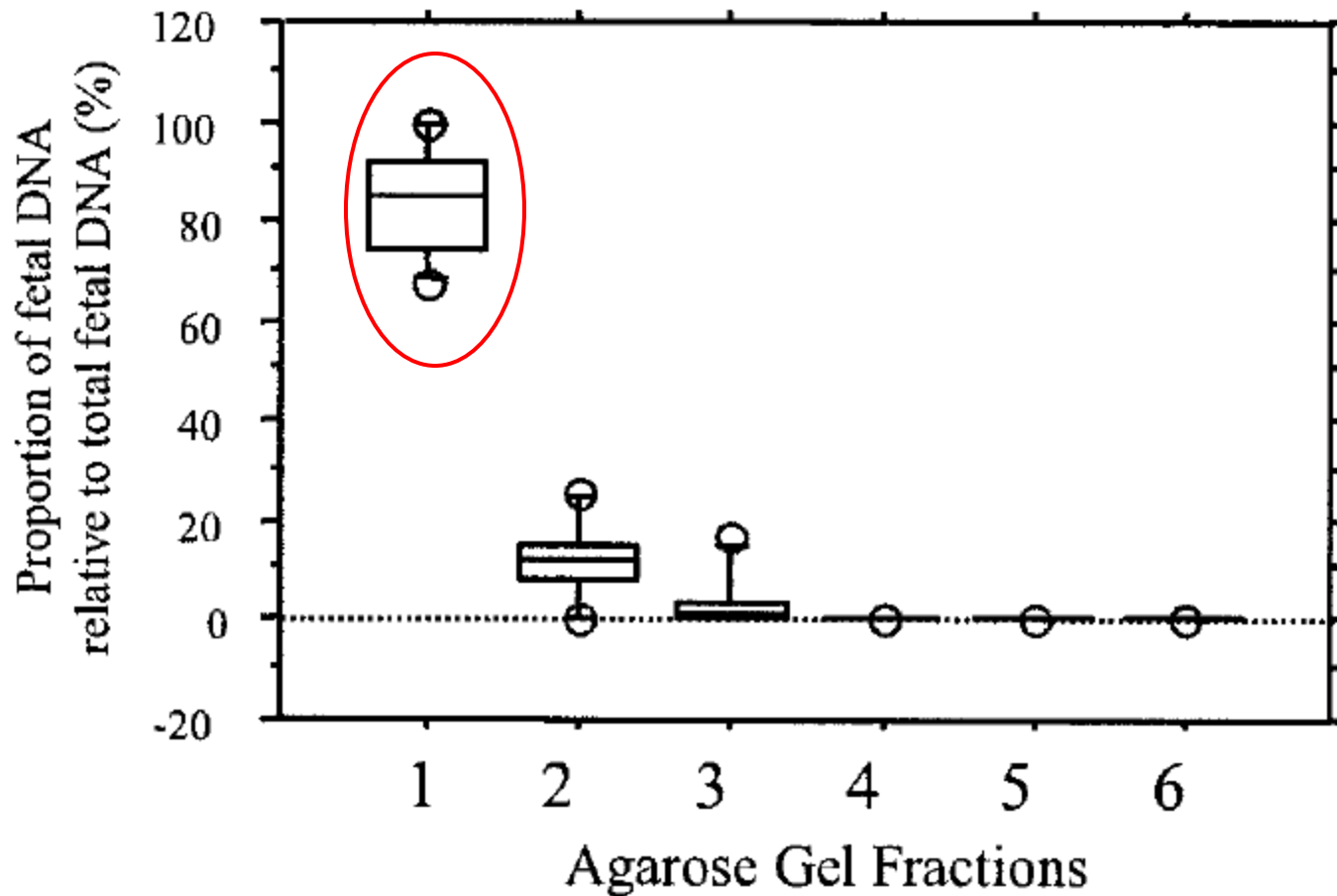


Table 1. Size distribution of total and fetal circulatory DNA in third-trimester maternal plasma samples.^a

Size of DNA fraction, kb	Median (range)		
	Size distribution of total DNA, ^b %	Size distribution of fetal DNA, ^c %	Proportion of fetal DNA per fraction, ^d %
<0.3	22.4 (15.7–26.7)	70.0 (51.0–82.3)	68.7 (22.2–87.1)
0.3–0.5	28.4 (15.7–35.2)	24.3 (13.8–31.6)	15.4 (6.4–31.4)
0.5–1.0	23.0 (15.0–26.8)	3.8 (0.0–17.4)	2.6 (0.0–7.8)
1.0–1.5	7.5 (2.2–11.4)	0.0 (0.0–8.7)	0.0
1.5–23	21.1 (10.3–35.7)	0.0	0.0

Size distribution of total and fetal circulatory DNA in maternal plasma samples obtained early in pregnancy

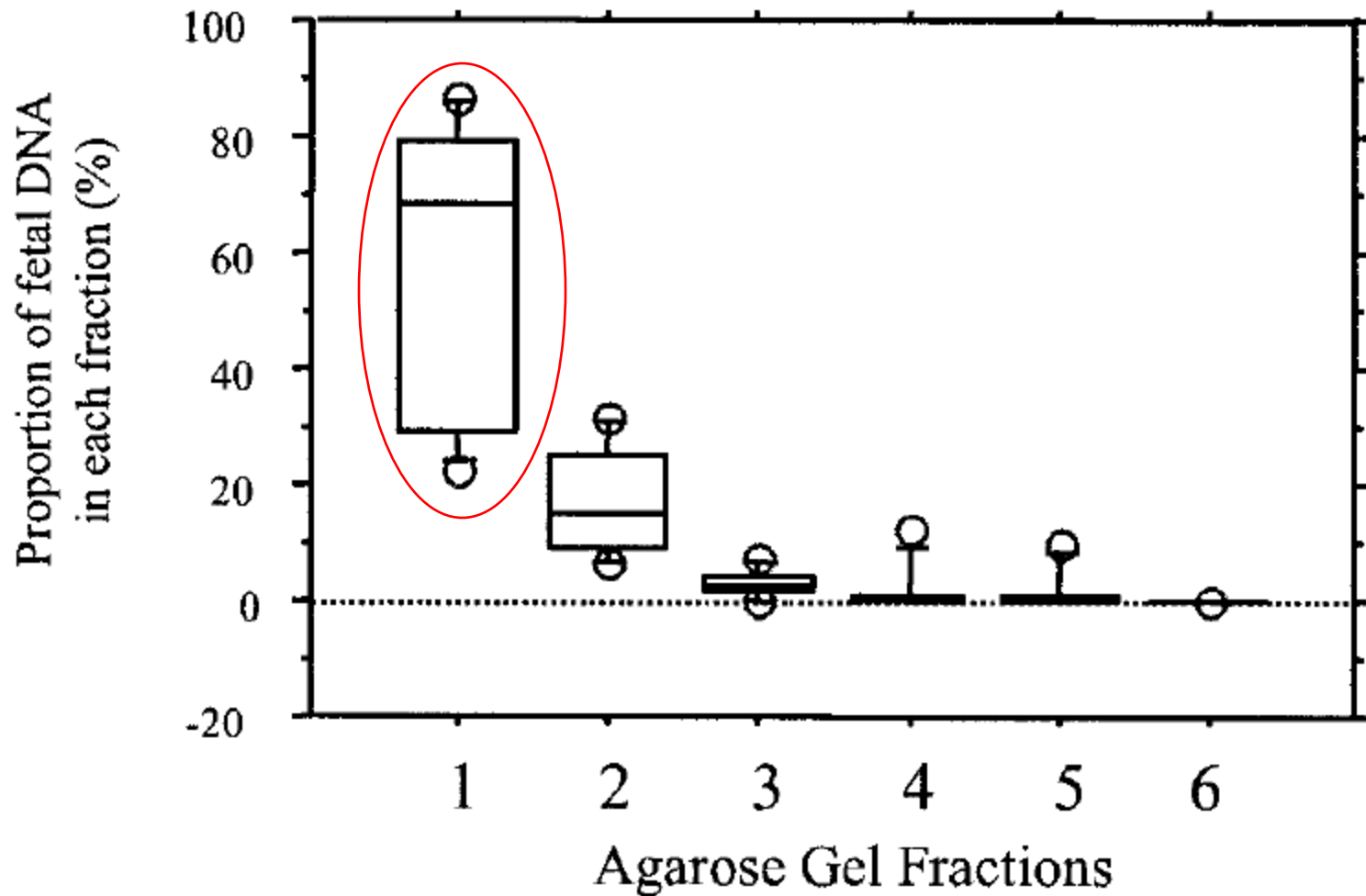
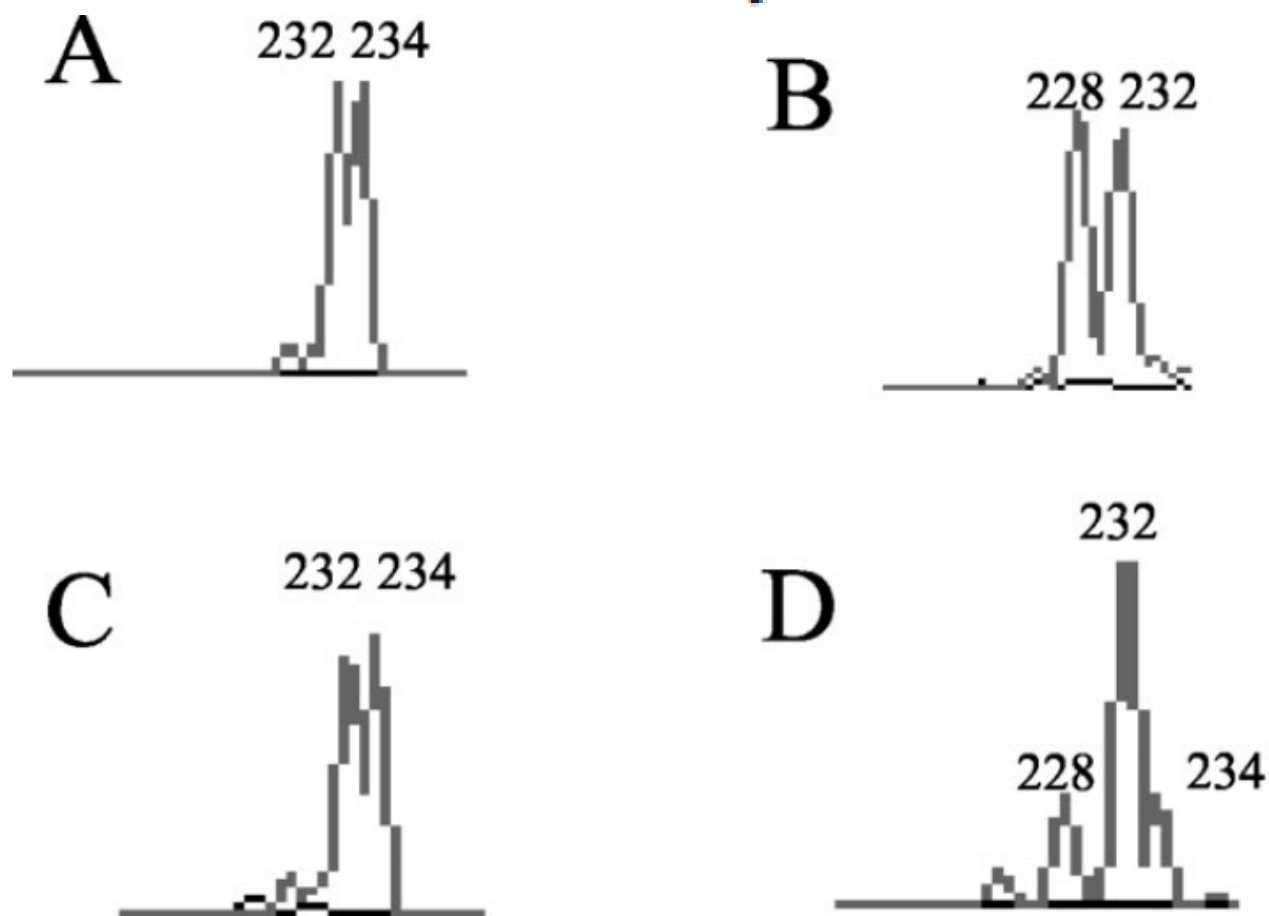


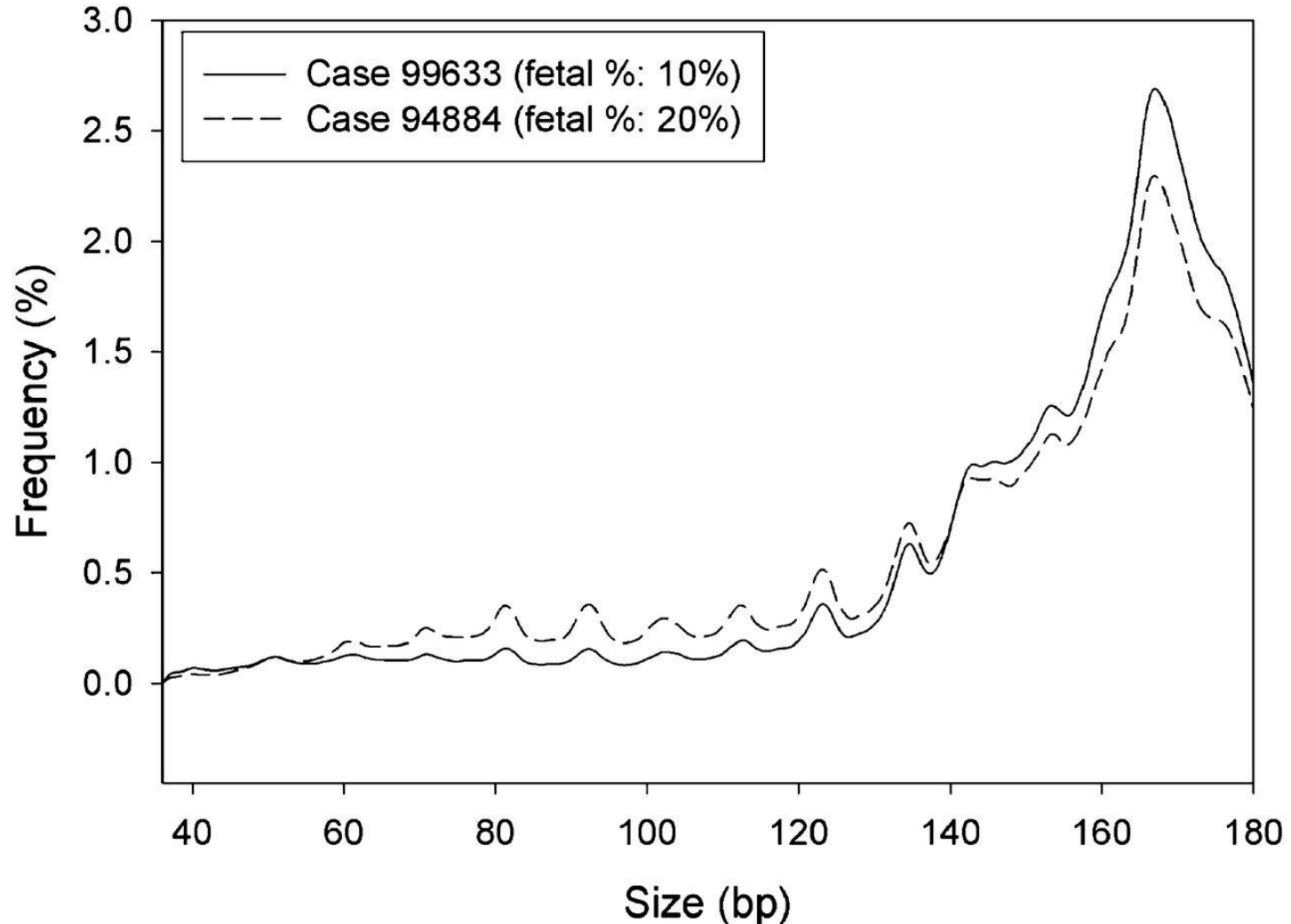
Table 2. Size distribution of total and fetal circulatory DNA in maternal plasma samples obtained early in pregnancy.^a

Size of DNA fraction, kb	Median (range)		
	Size distribution of total DNA, %	Size distribution of fetal DNA, %	Proportion of fetal DNA per fraction, %
<0.3	26.9 (12.7–41.3)	85.5 (67.8–100.0)	28.4 (11.6–56.6)
0.3–0.5	29.1 (26.1–54.4)	11.7 (0.0–15.5)	4.0 (0.0–13.5)
0.5–1.0	28.5 (14.5–25.9)	1.2 (0.0–16.8)	0.4 (0.0–5.2)
1.0–1.5	8.2 (4.5–12.1)	0.0	0.0
1.5–23	8.6 (7.6–23.7)	0.0	0.0

Fig. 5. Detection of both paternally and maternally inherited highly polymorphic microsatellite markers in size-fractionated circulatory DNA.



Size distributions of DNA fragment in two maternal plasma samples with different fetal DNA fractions



Yu SCY, et al. PNAS 2014;111:8583-8588

PNAS

Prenatal diagnosis of homozygous alpha-thalassemia-1 by cell-free fetal DNA in maternal plasma

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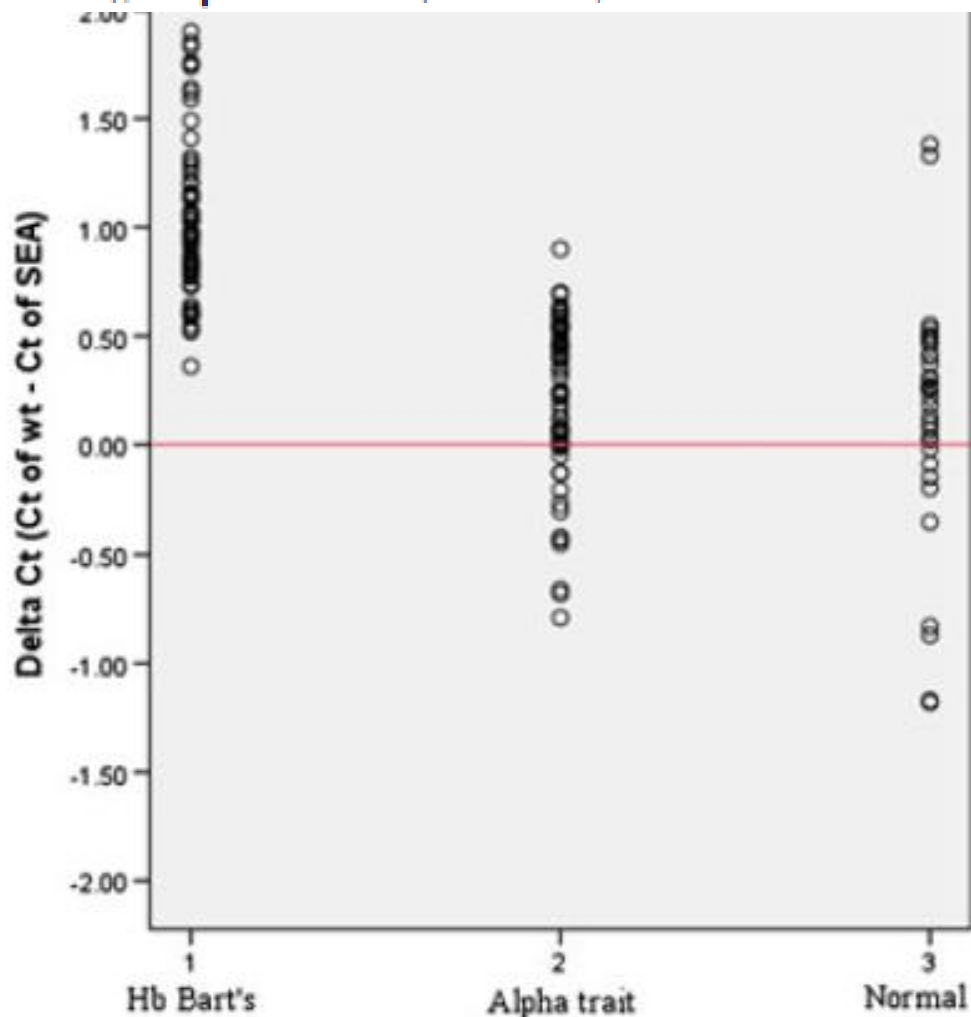
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Prenatal Diagnosis 2011, 32, 45–49

Figure 1 ΔCt (Ct of wt allele – Ct of SEA allele) of maternal plasma DNA analysis from pregnant women who carried Hb Bart's hydrops (N = 62), alpha-trait (N = 62) and normal fetuses (N = 34)



ARMS-PCR Codon 41/42

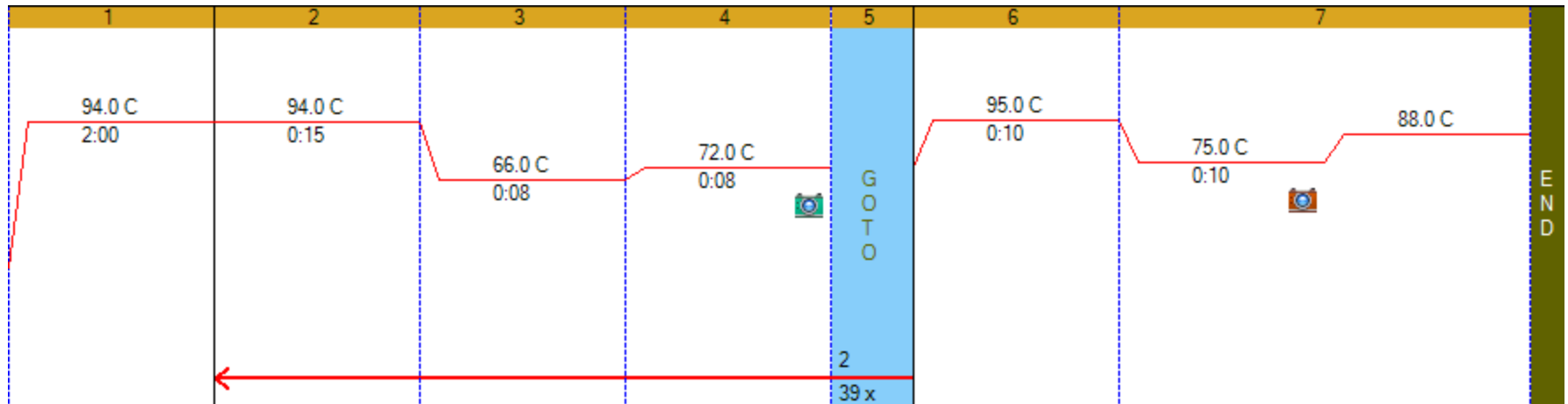
PCR Mixture

Master mix	Final conc.	1X
10X PCR	1X	2.5
50mM MgCl ₂	1.5mM	0.75
10 mM dNTPs	0.2mM	0.5
5μM Primer F	0.2μM	1
5μM Primer R	0.2μM	1
50μM Syto9	2μM	1
Platinum Taq	1U/μl	0.2
dH ₂ O	-	13.05
DNA	-	5
Total	-	25

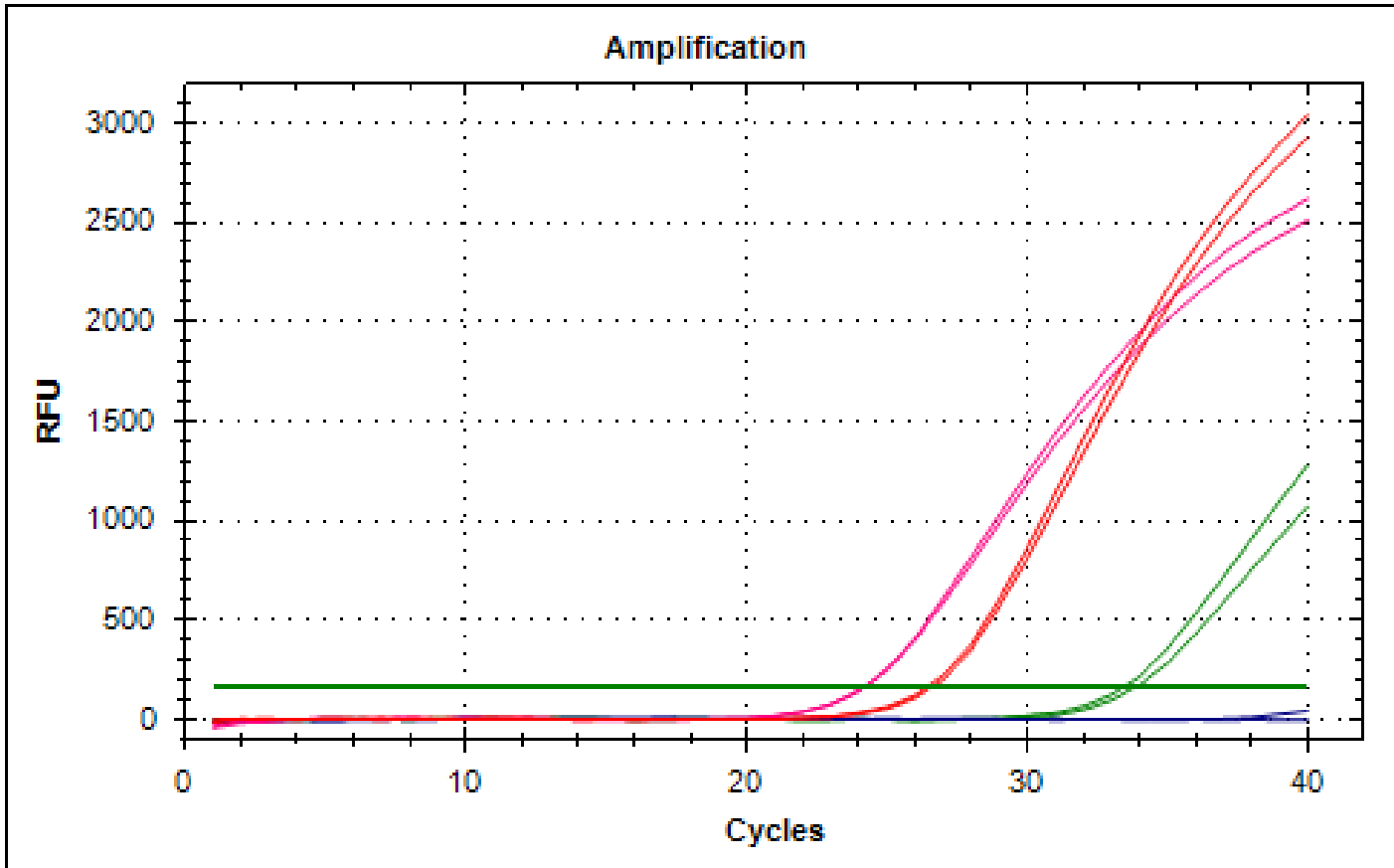
PCR Primers

Primer	Sequence
Forward_CD41/42	5'- TTT TCC CAC CCT TAG GCT GCT -3'
Reverse_CD41/42	5'- GAG TGG ACA GAT CCC CAA AGG ACT CAA CCT-3'

PCR Condition



ARMS-PCR Codon 41/42



Maternal plasma (P1/60)



Maternal blood (Blood-41/42)



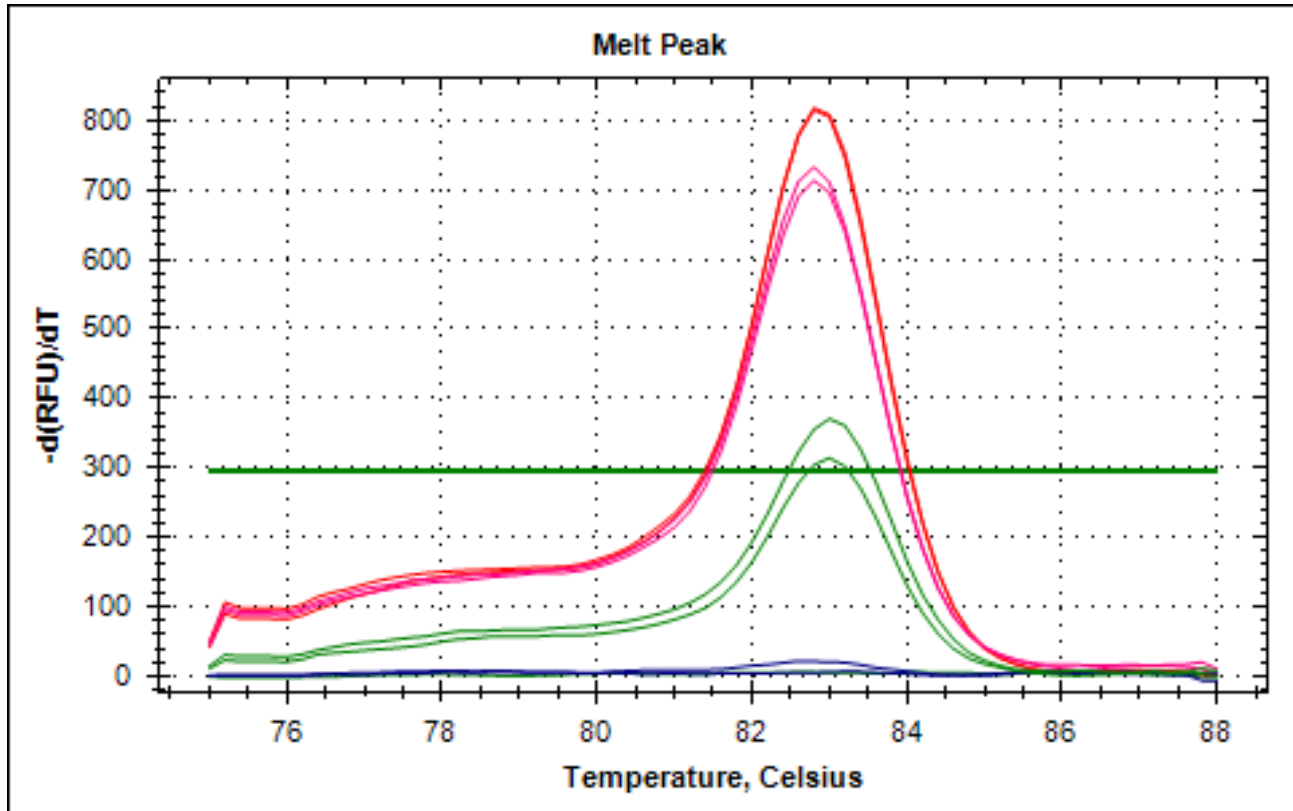
Paternal plasma (P1/60-F)



Amniotic fluid (AF105-41/42)



ARMS-PCR Codon 41/42



Sample	Melt Temp
P1/60	83.00
P1/60	83.00
P1/60-F	None
P1/60-F	None
Blood-41/42	82.80
Blood-41/42	82.80
AF105-41/42	82.80
AF105-41/42	82.80

Maternal plasma (P1/60)



Maternal blood (Blood-41/42)



Paternal plasma (P1/60-F)



Amniotic fluid (AF105-41/42)



Thank you for your attention

