

# Non-invasive prenatal diagnosis by plasma nucleic acid analysis

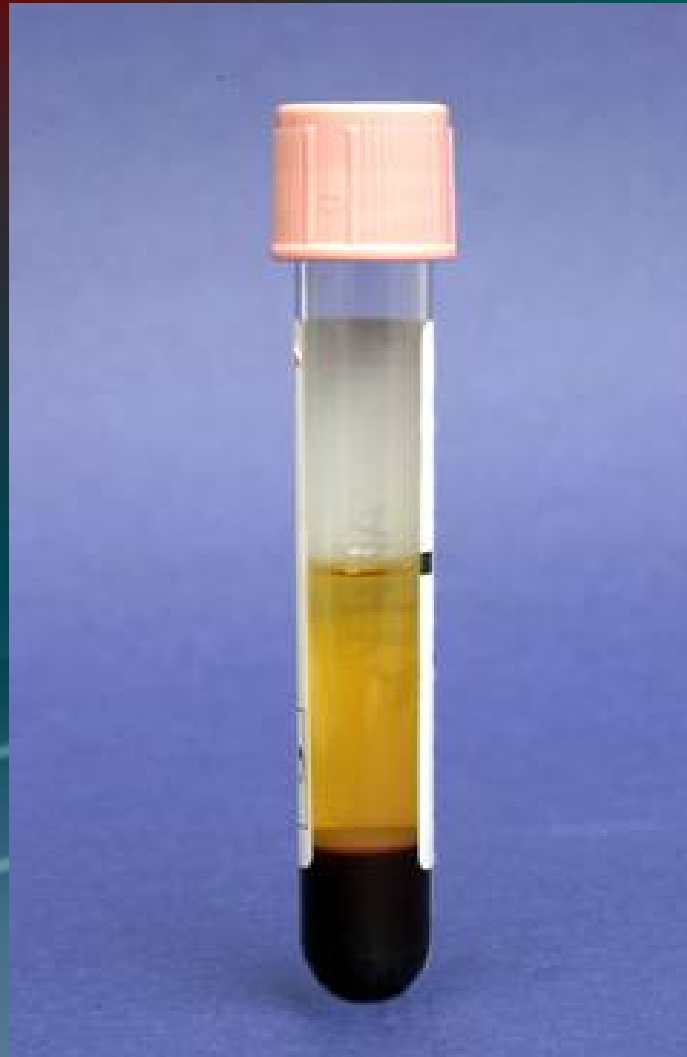
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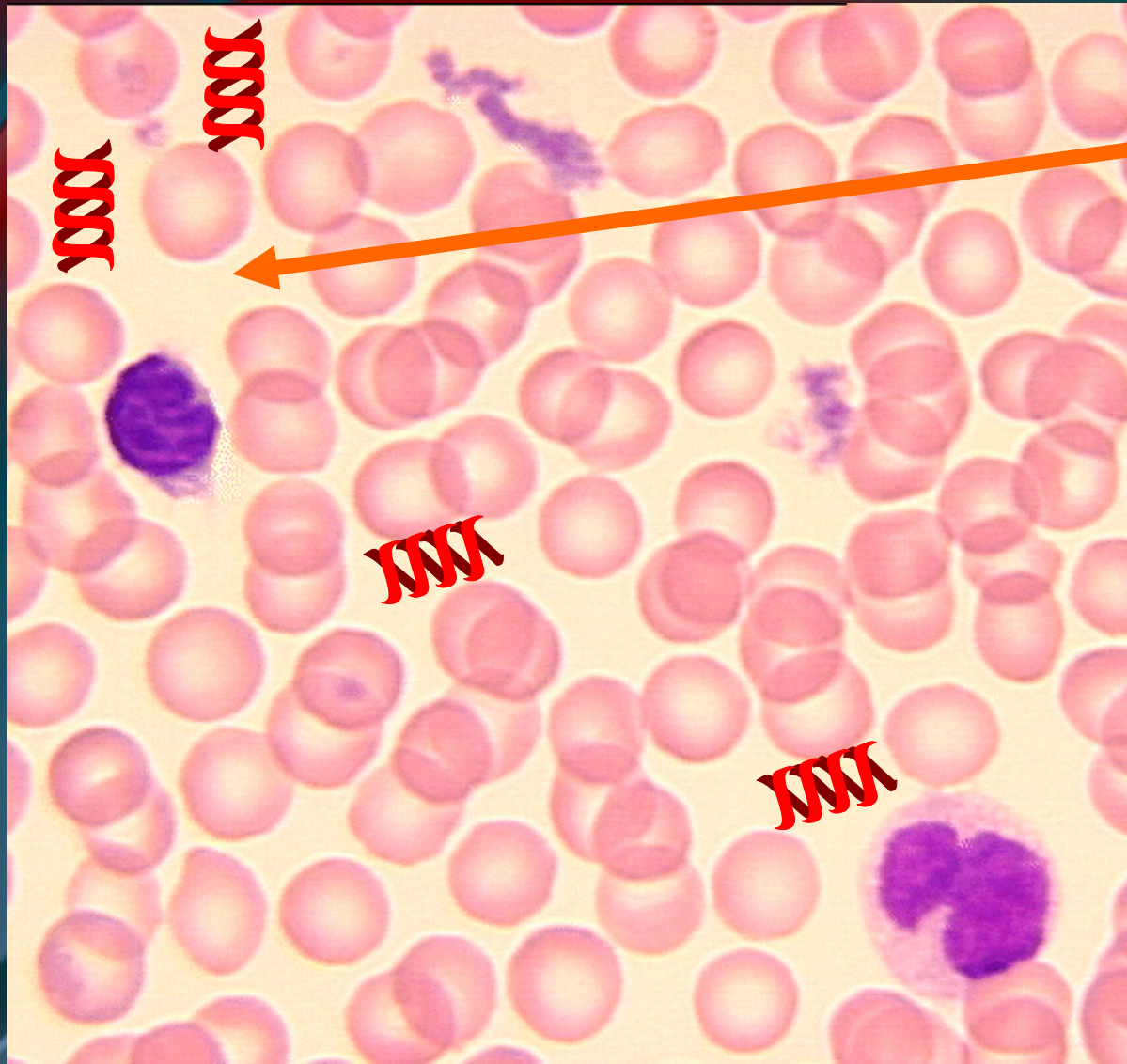
*Y.M. Dennis Lo*

*Li Ka Shing Institute of Health Sciences*

*The Chinese University of Hong Kong*

# Non-invasive prenatal diagnosis?





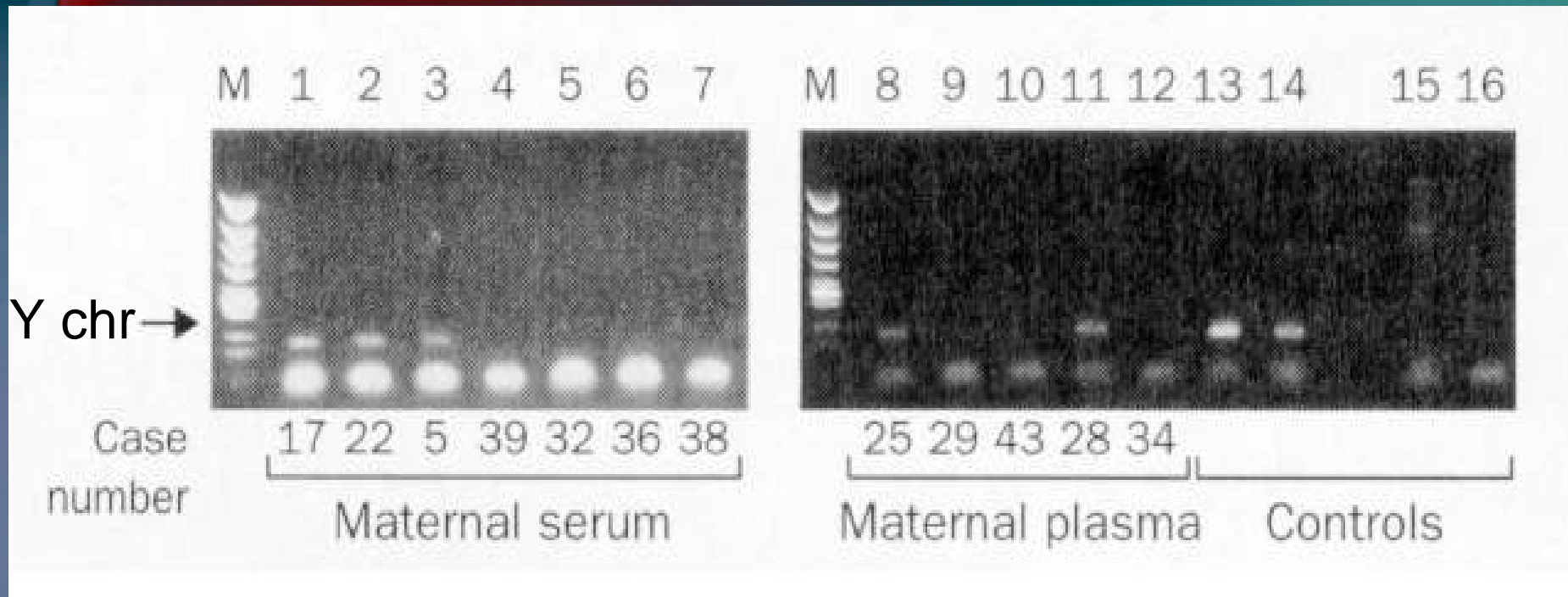
Plasma

# Placenta as a Pseudomalignant Tissue

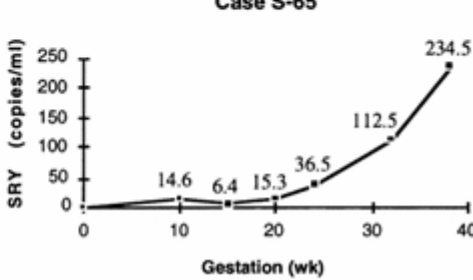
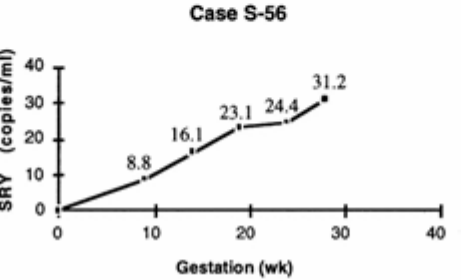
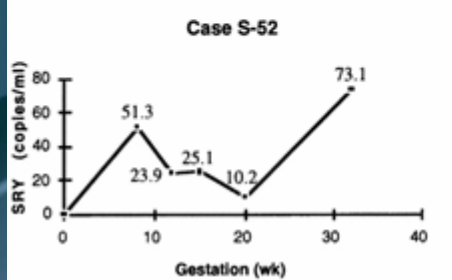
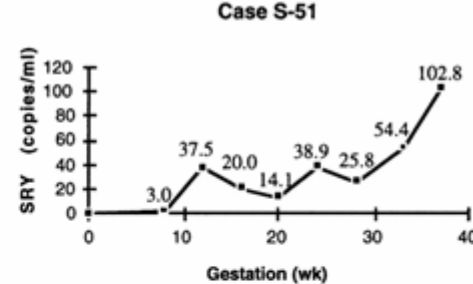
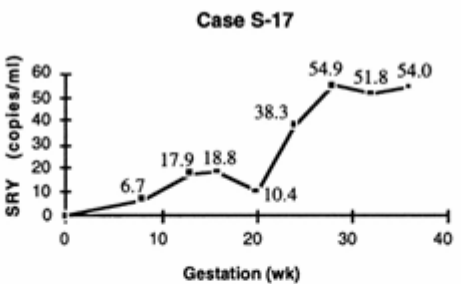
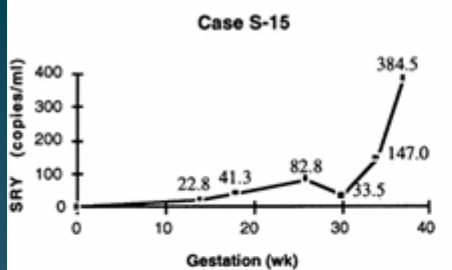
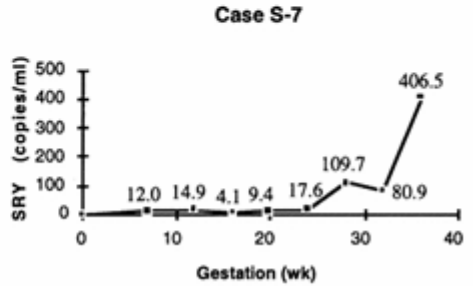
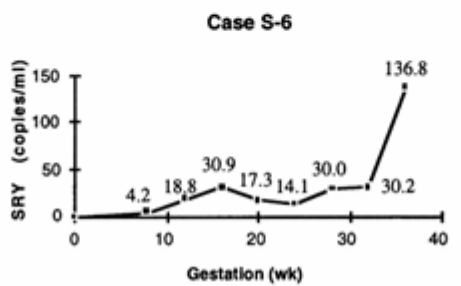
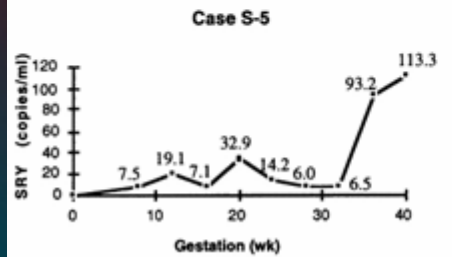
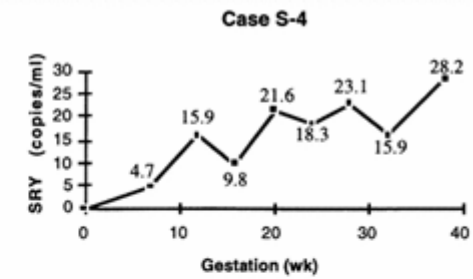
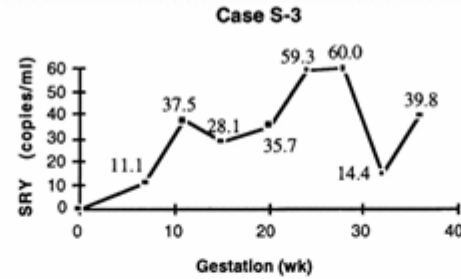
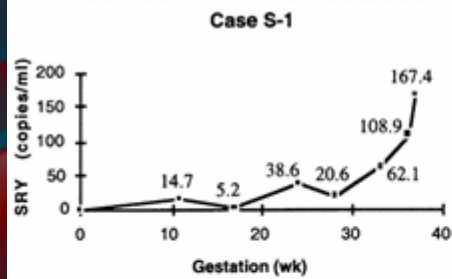
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- high mitotic rate
- invasive
- expression of certain proto-oncogenes
- apparent immune privilege

?presence of fetal DNA in maternal plasma



Lo et al. *Lancet* 1997; 350:485



# Fetomaternal Ratio in Plasma and Cellular Fractions

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<b>Gestation</b>	<b>Fetal DNA in Maternal Plasma</b>	<b>Fetal Cells in Maternal Blood</b>	<b>Fold of Enrichment</b>
<b>11-17 wk</b>	<b>3.4%</b>	<b>0.001%</b>	<b>3400</b>
<b>37-43 wk</b>	<b>6.2%</b>	<b>0.01%</b>	<b>620</b>

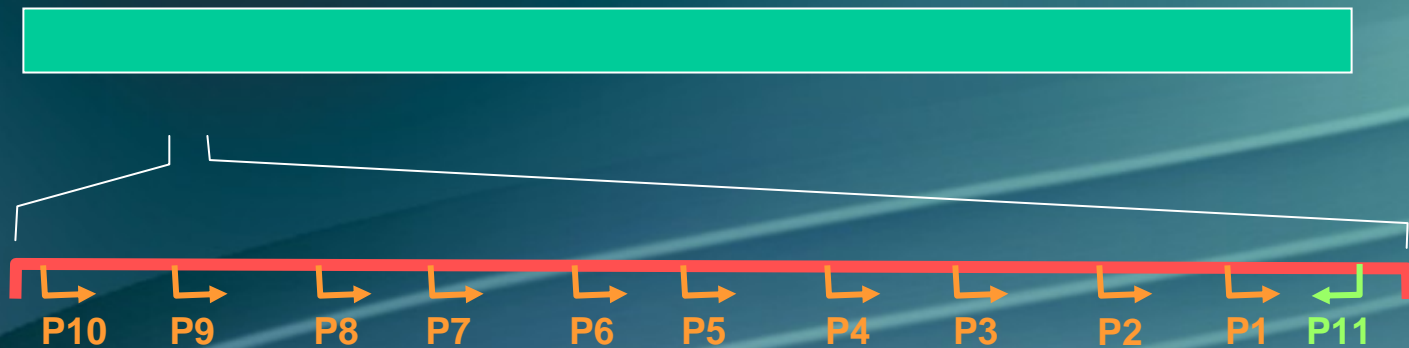
How long are the circulating  
DNA fragments?

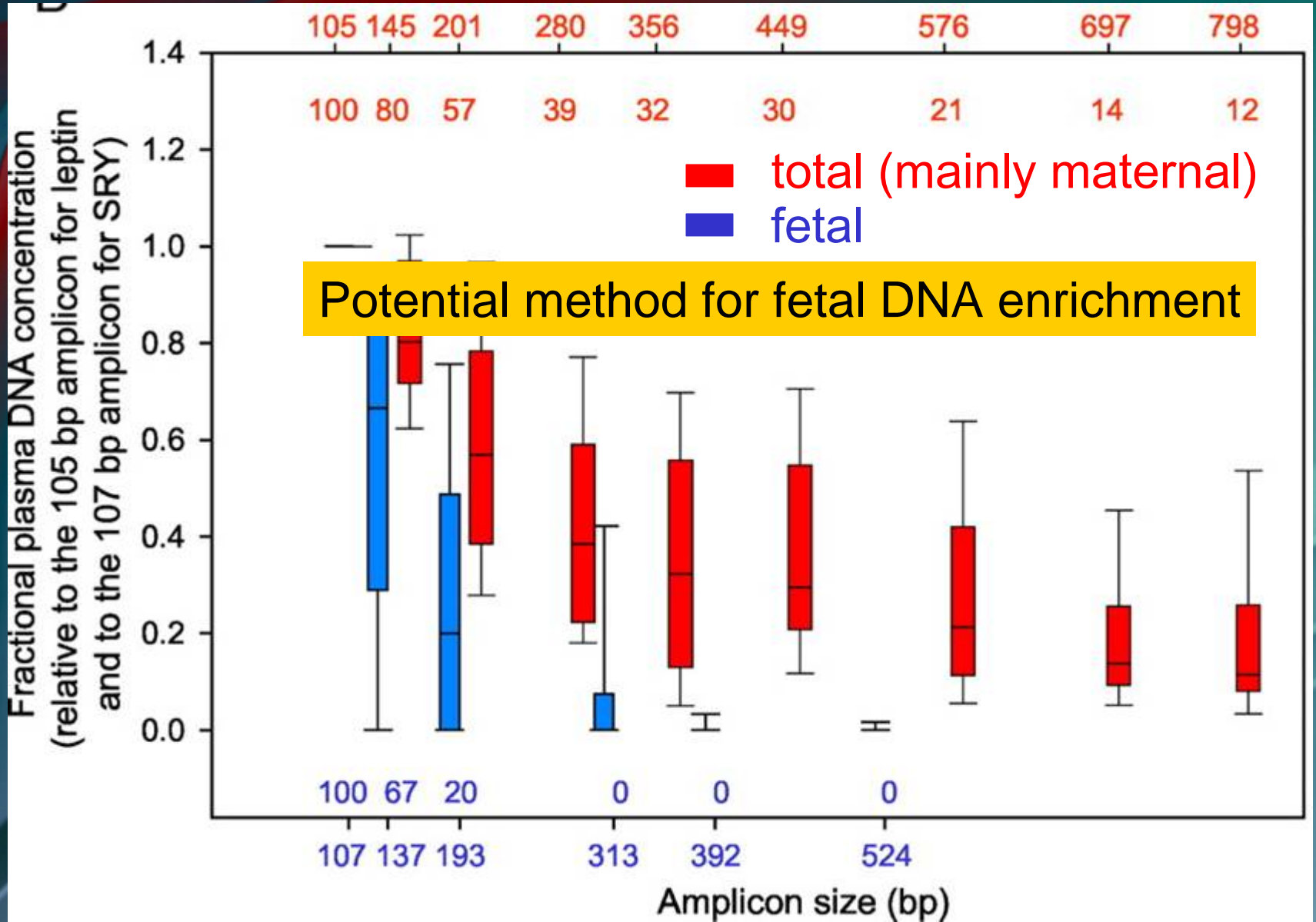


# Size of DNA fragments

SRY: Fetal

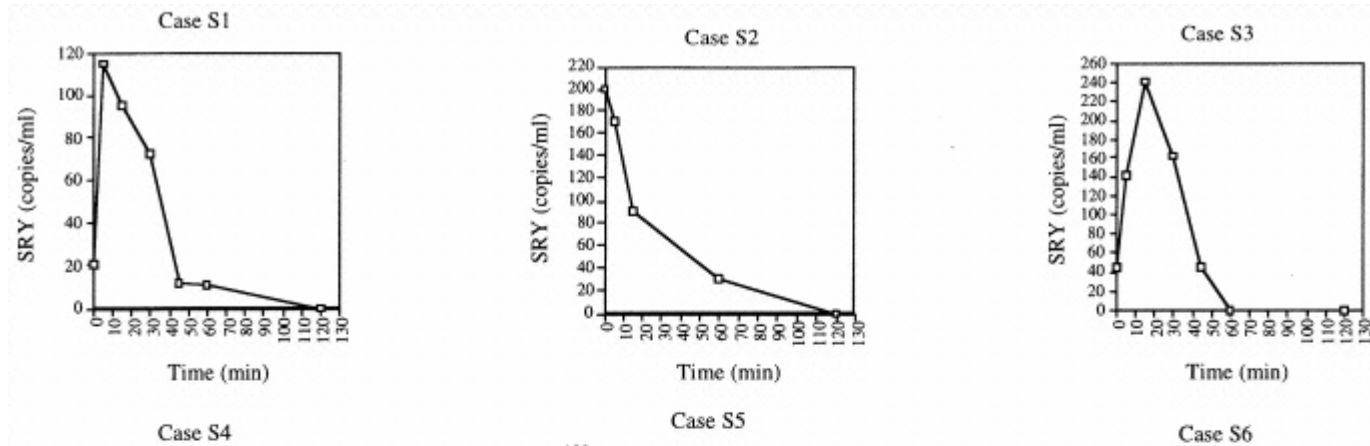
Leptin: Total (predominantly maternal)



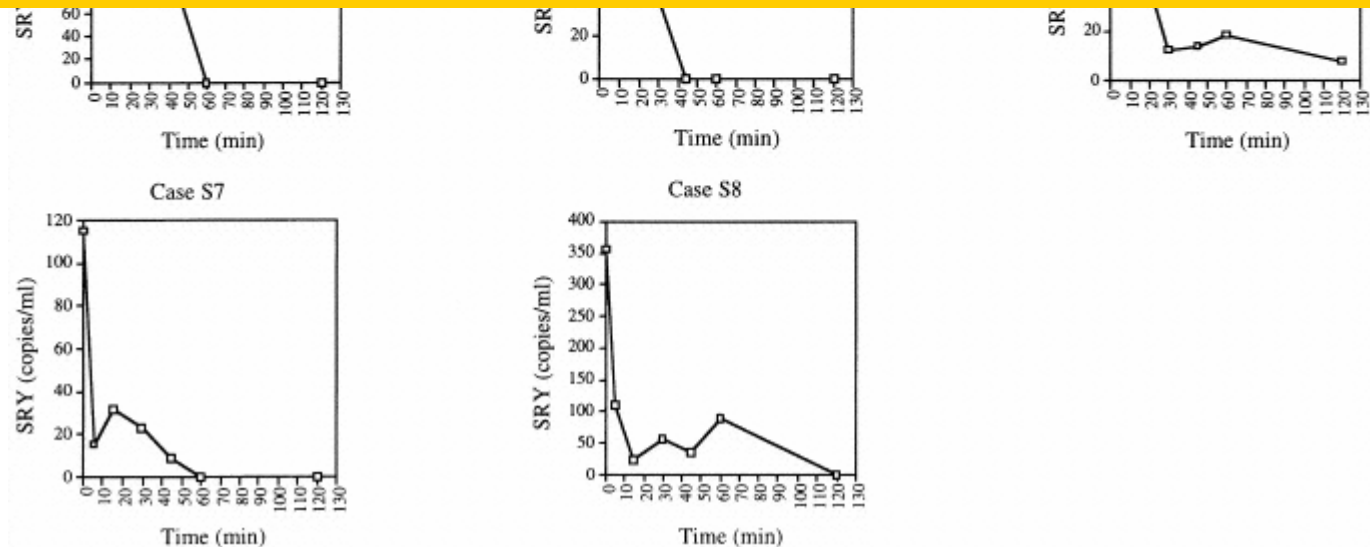


What happens after delivery?

# Rapid Fetal DNA Clearance



No persistence after delivery



# Prenatal Diagnostic Applications

# Fetal Rhesus D genotyping



# Fetal RhD genotyping

RhD-positive

RhD-negative

*RHD*  
gene



*RHCE*  
gene



# Universal fetal DNA control



# CpG methylation



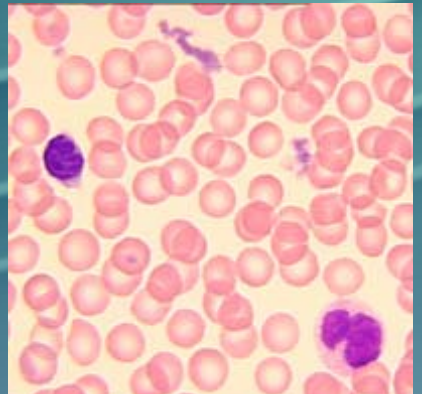
CGCGCGCGC

CGCGCGC



Placenta

Differential methylation

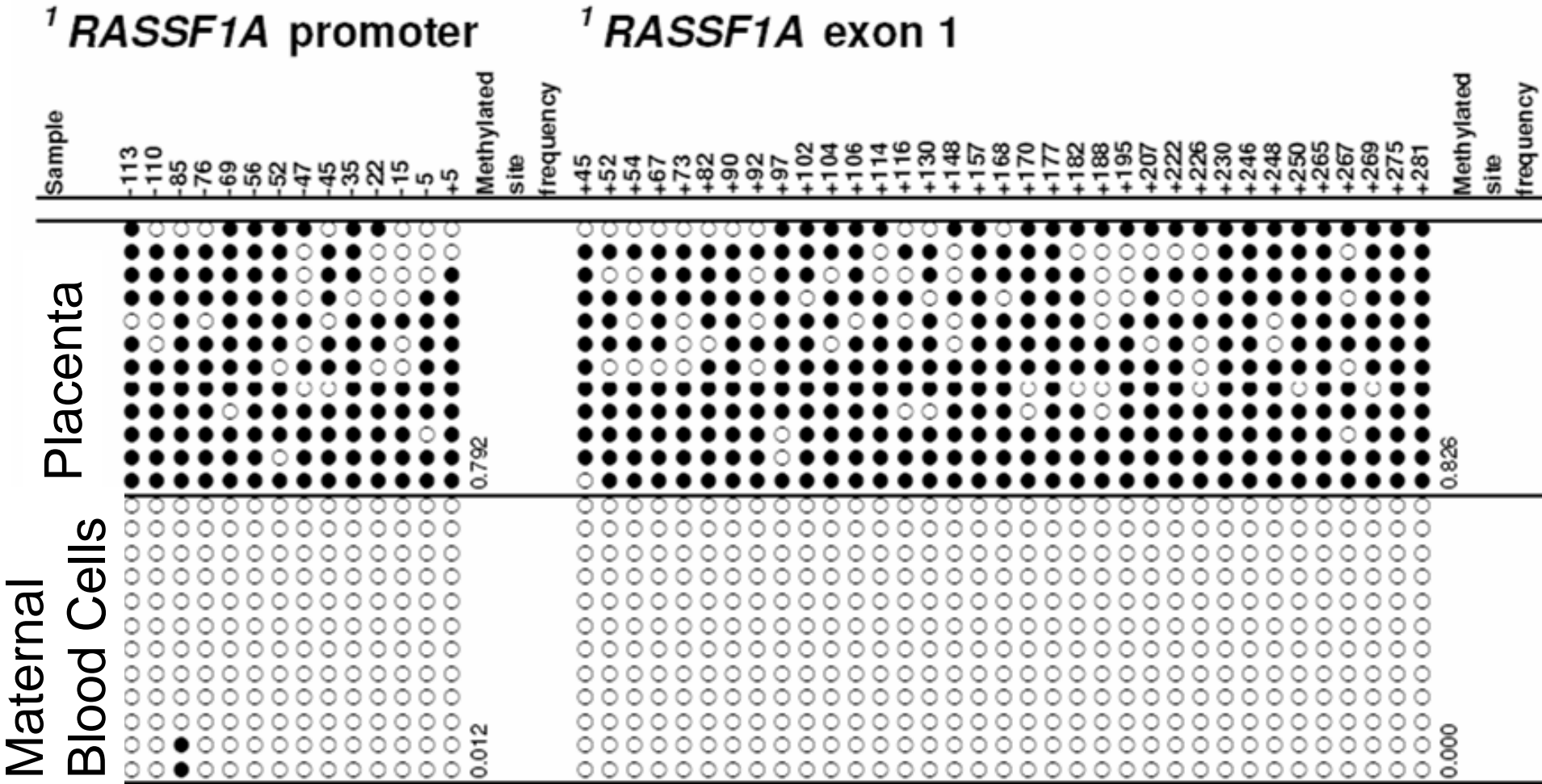


Maternal Blood Cells

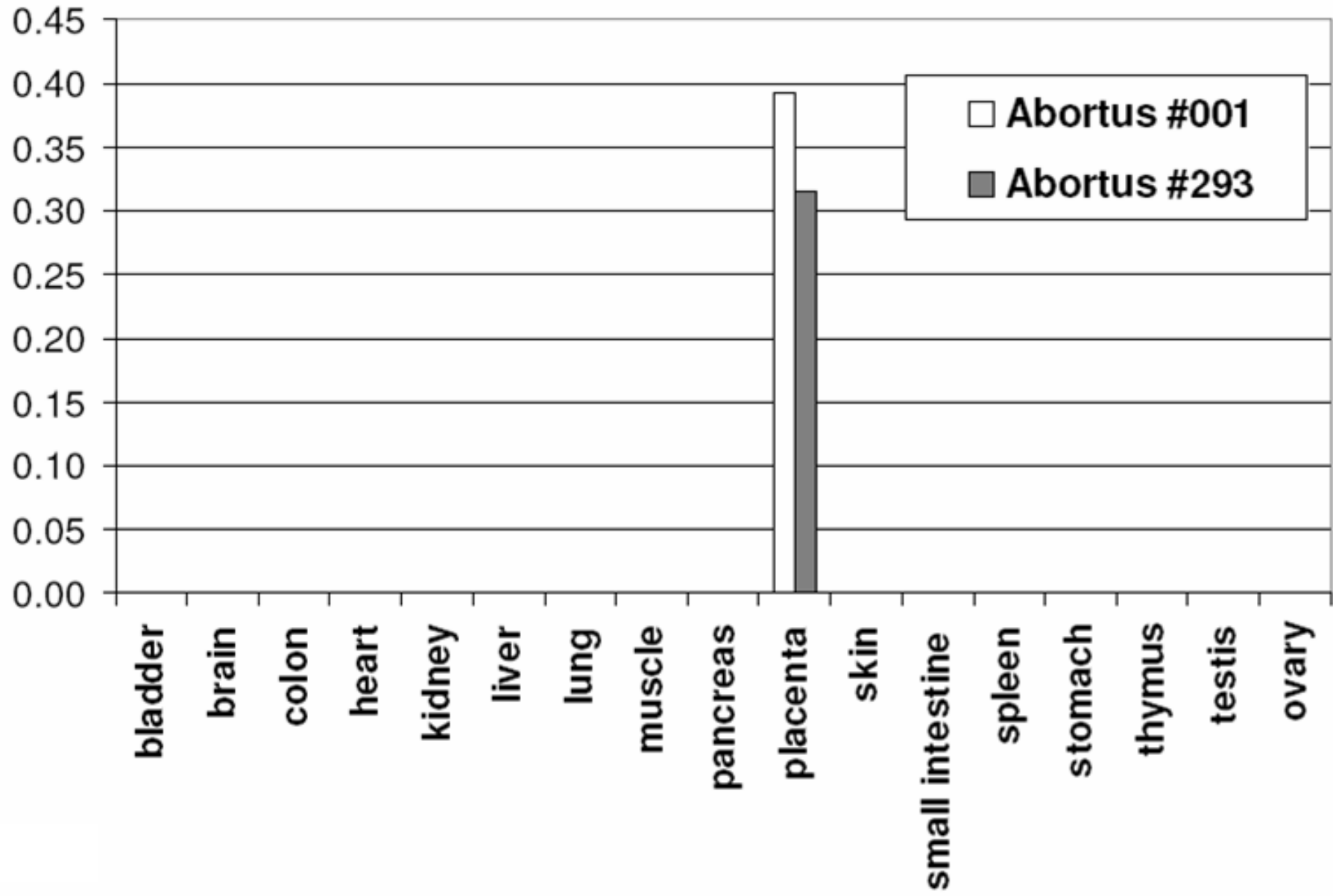
# The Pseudomalignancy Analogy

- Does this extend to the epigenetic level?
- Tumour suppressor genes (TSG): hypermethylated in cancer
- Are TSGs hypermethylated in placenta?

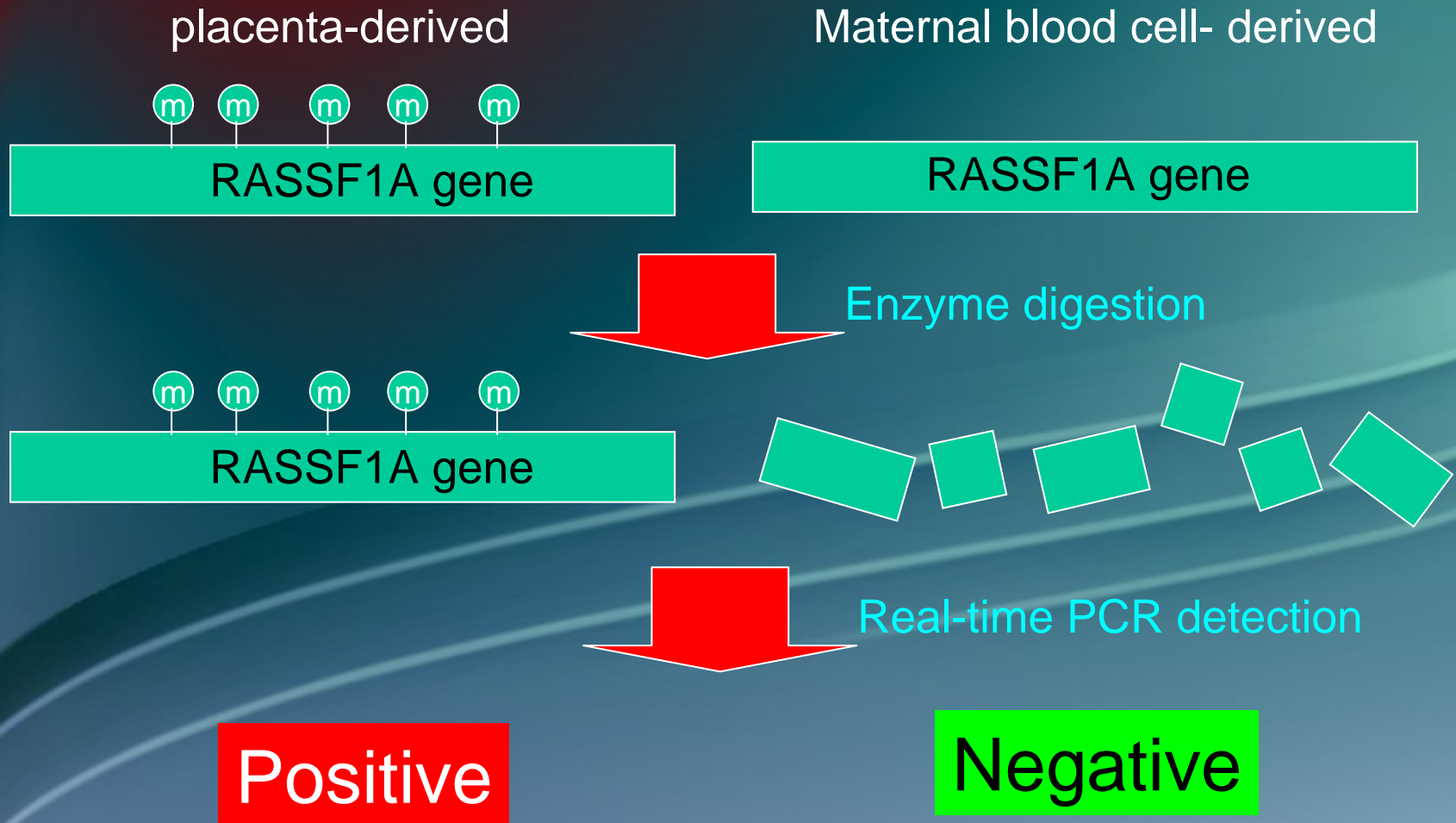
# Bisulfite Sequencing



**Methylation index of *RASSF1A***

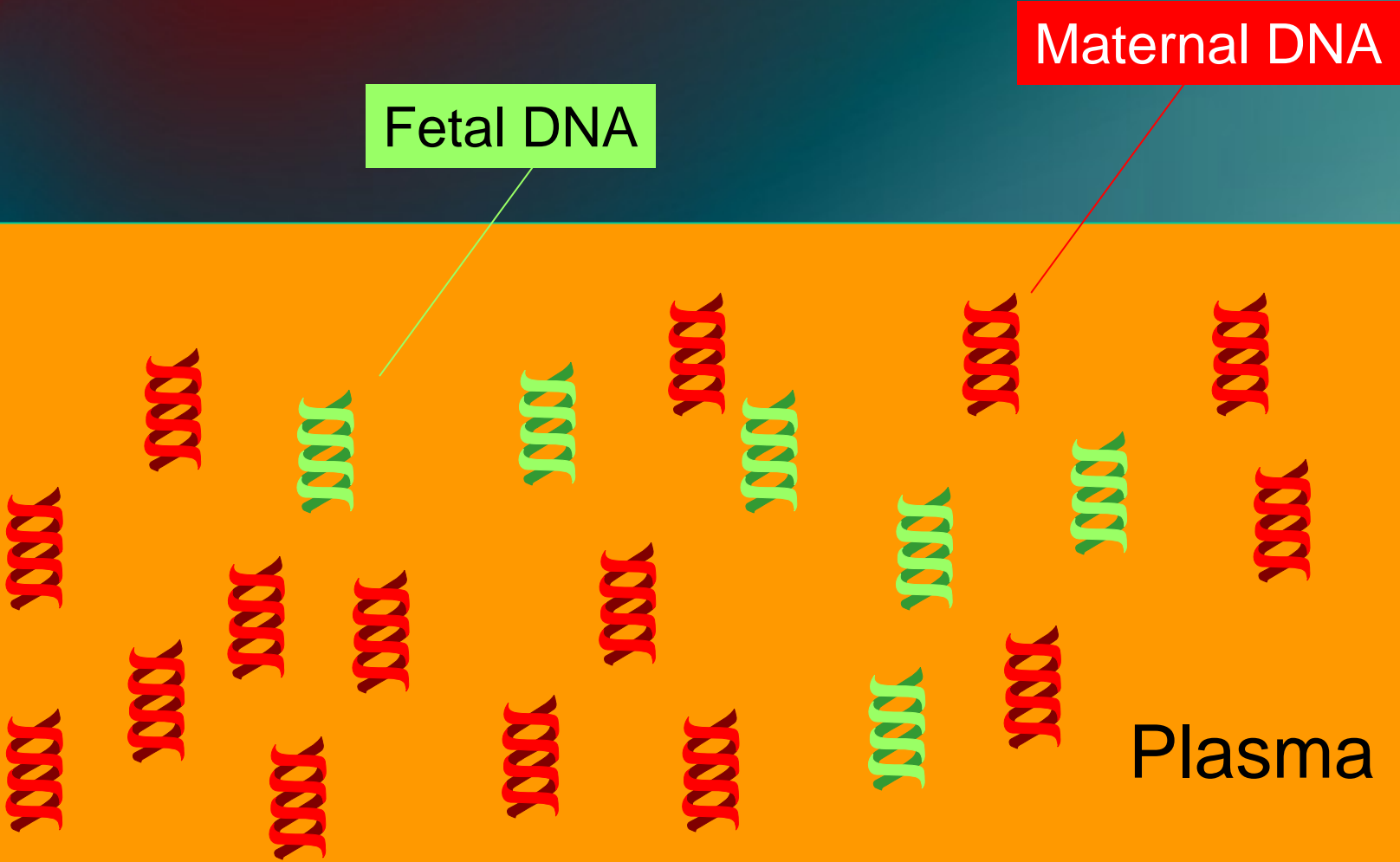


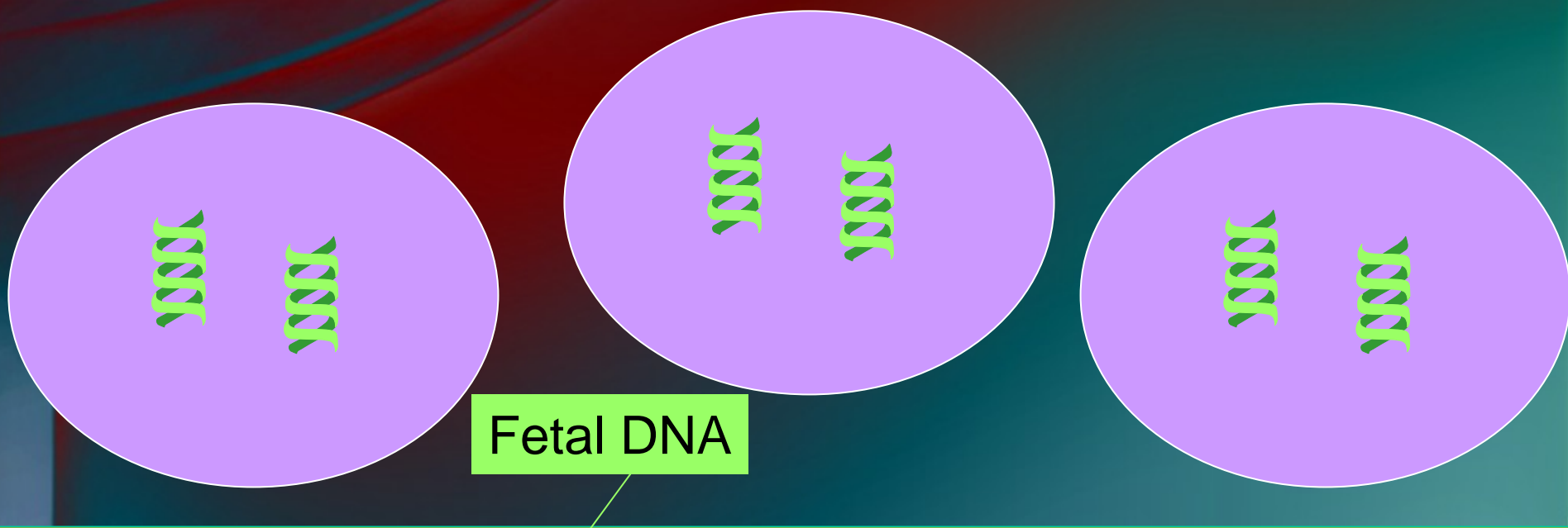
# Methylation-sensitive restriction enzyme analysis



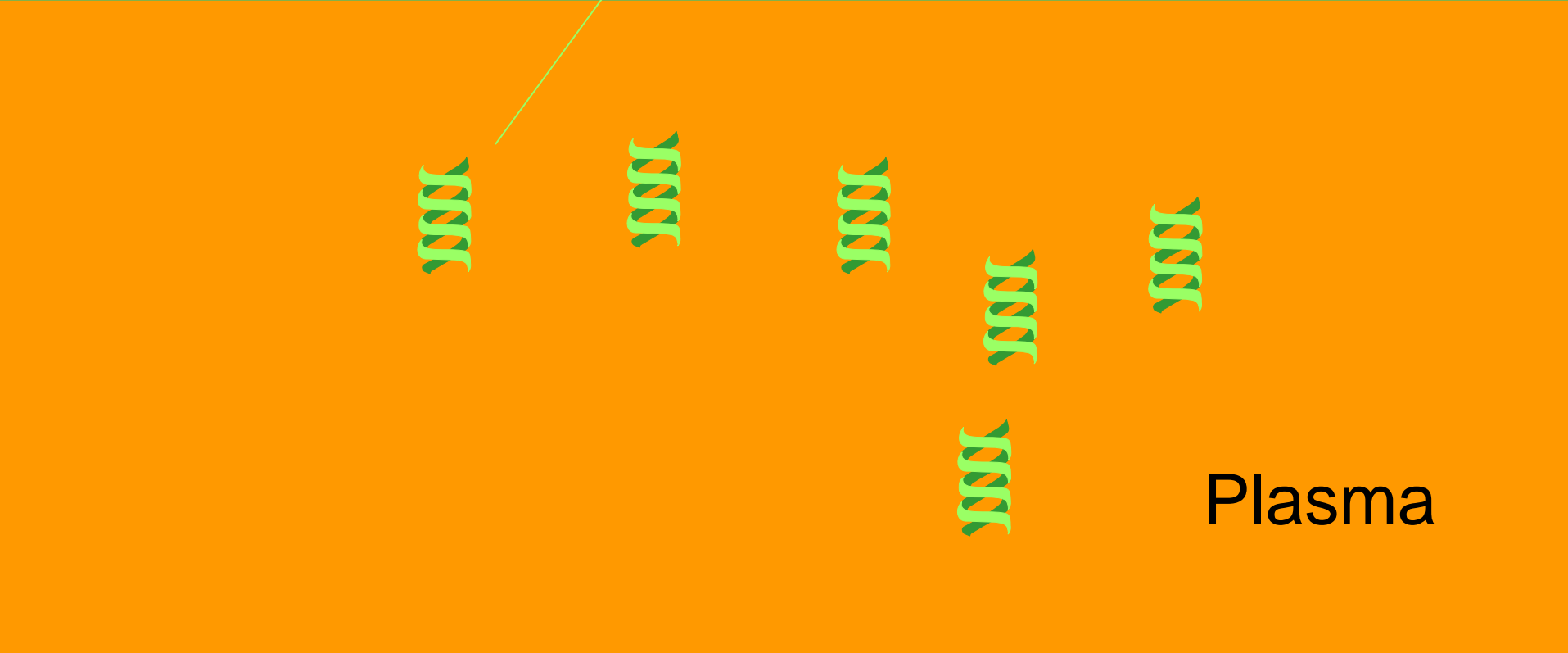
Can this method be used for the detection of Down syndrome?

Total amount of chromosome 21:  
largely reflects maternal chromosome status





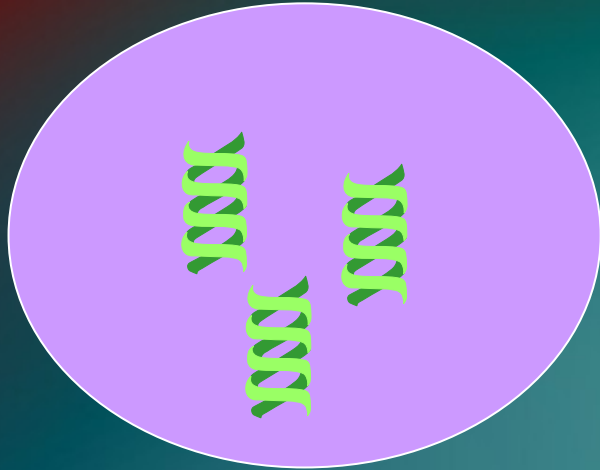
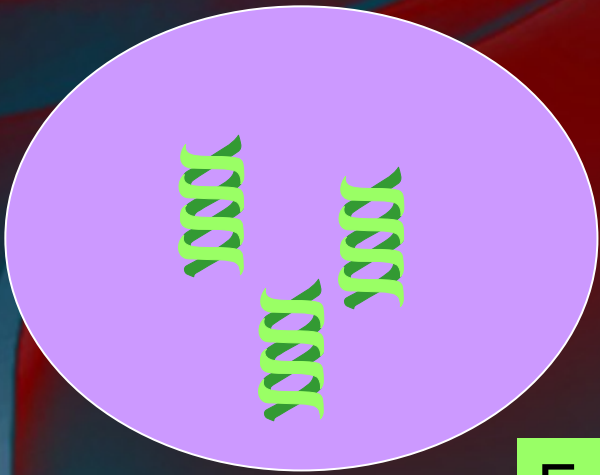
Fetal DNA



Plasma



Fetal DNA



Plasma

# Summary of Difficulties

- Low fractional concentration of fetal DNA
- Need of a method to determine chromosome dosage

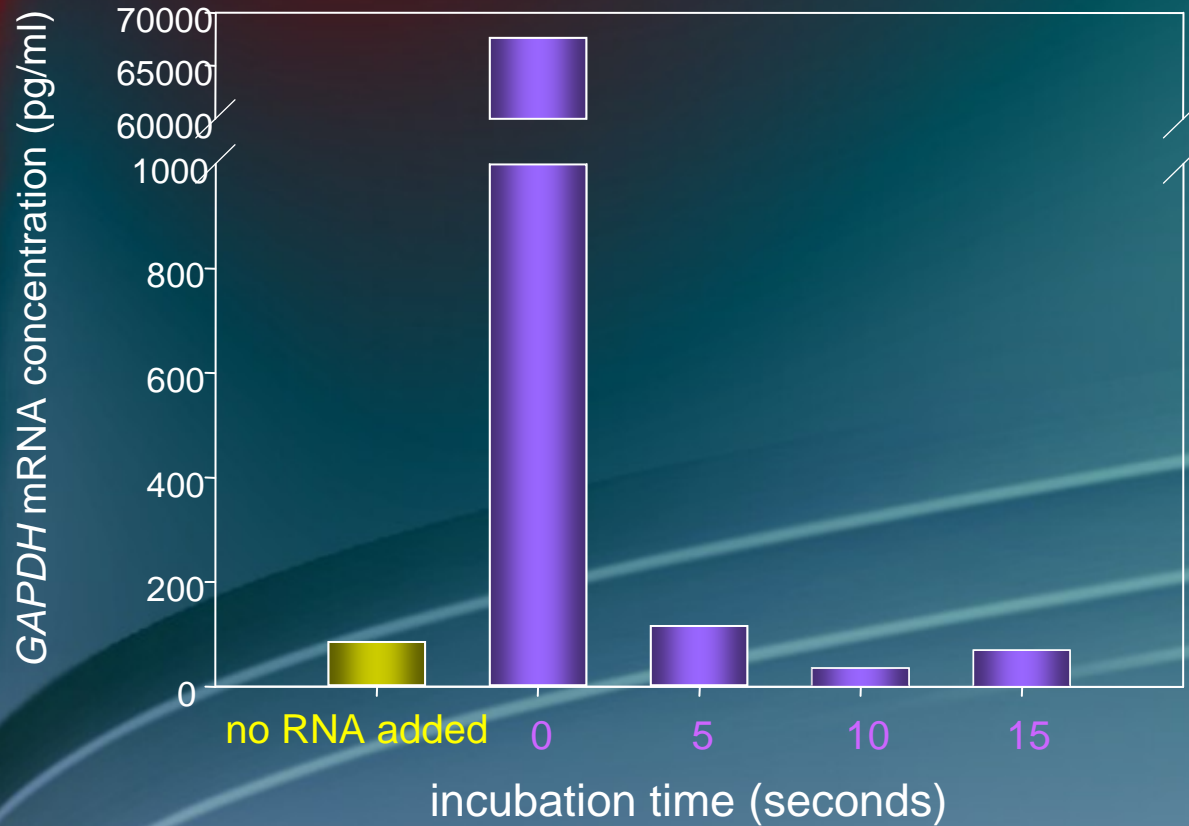
A proposed solution

# Low fractional concentration

- Target a subfraction of plasma nucleic acid that is completely fetal-specific
- RNA markers

# Stability of Plasma RNA

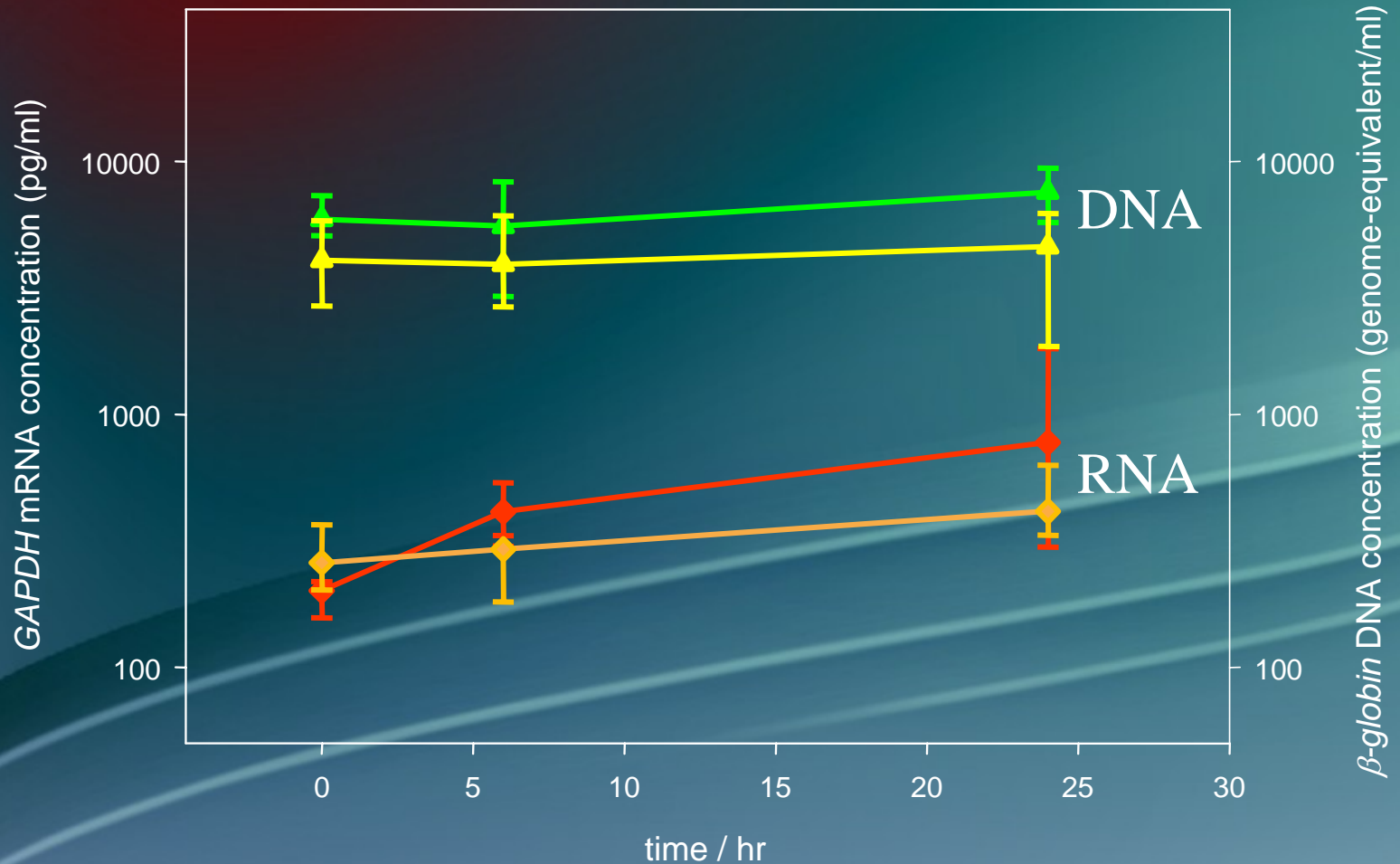
# Rapid degradation of free RNA in plasma



> 99% of free RNA was degraded in 15-s time

# Stability of nucleic acids in plasma

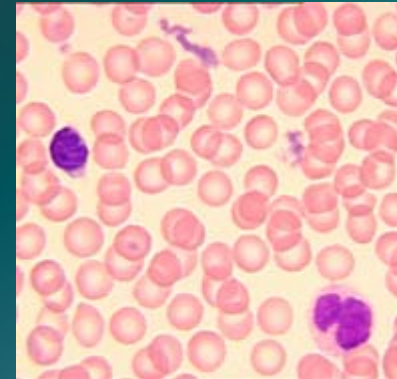
- ◆ RNA, rm temp
- ◆ RNA, 4°C
- ▲ DNA, rm temp
- ▲ DNA, 4°C



Placental tissues

PAIRED

Maternal whole blood



Mine for chromosome 21 transcripts

*PLAC4*

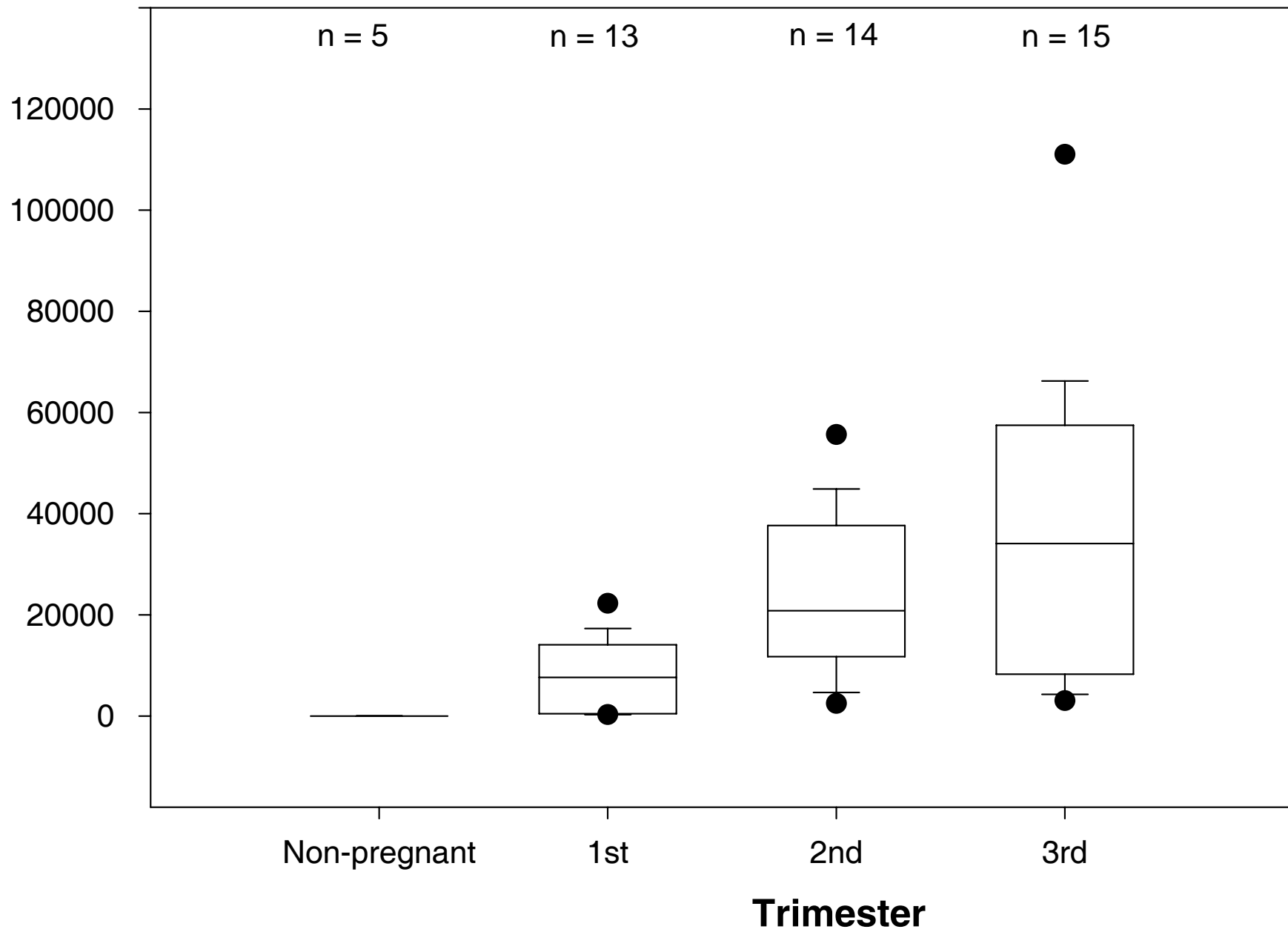
Gene expression analysis

U133A

U133A



**Plasma *PLAC4* mRNA concentration (copies/ml)**



Plasma *PLAC4* mRNA concentration (copies/ml)



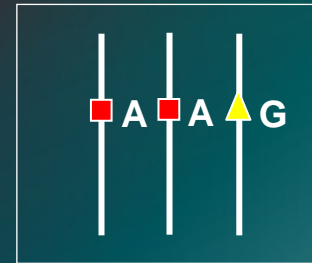
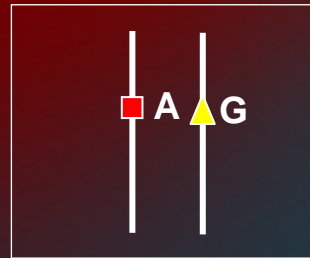
How to derive chromosome dosage information?

# The RNA-SNP Approach

normal fetus

trisomy 21 fetus

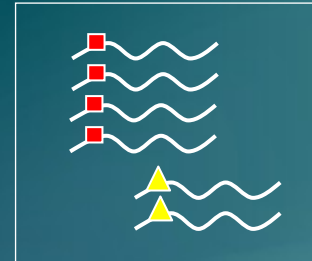
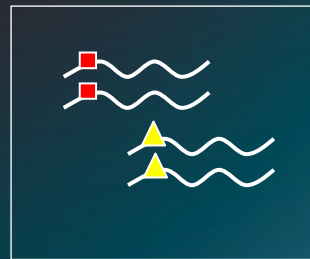
SNP on *PLAC4*  
(chromosome 21)



transcription



*PLAC4* RNA  
expressed in placenta



deviation in  
RNA-SNP  
allelic ratio



release into  
maternal circulation



circulating *PLAC4* RNA  
in maternal plasma



deviation in  
RNA-SNP  
allelic ratio

# RNA-SNP allelic ratio

RNA → cDNA



PCR amplification



Base extension

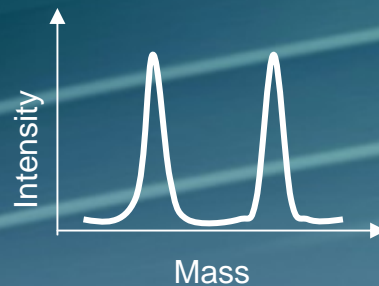


Mass detection

normal fetus



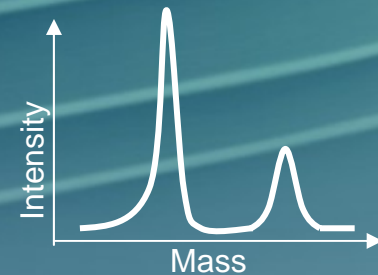
allele-A allele-G



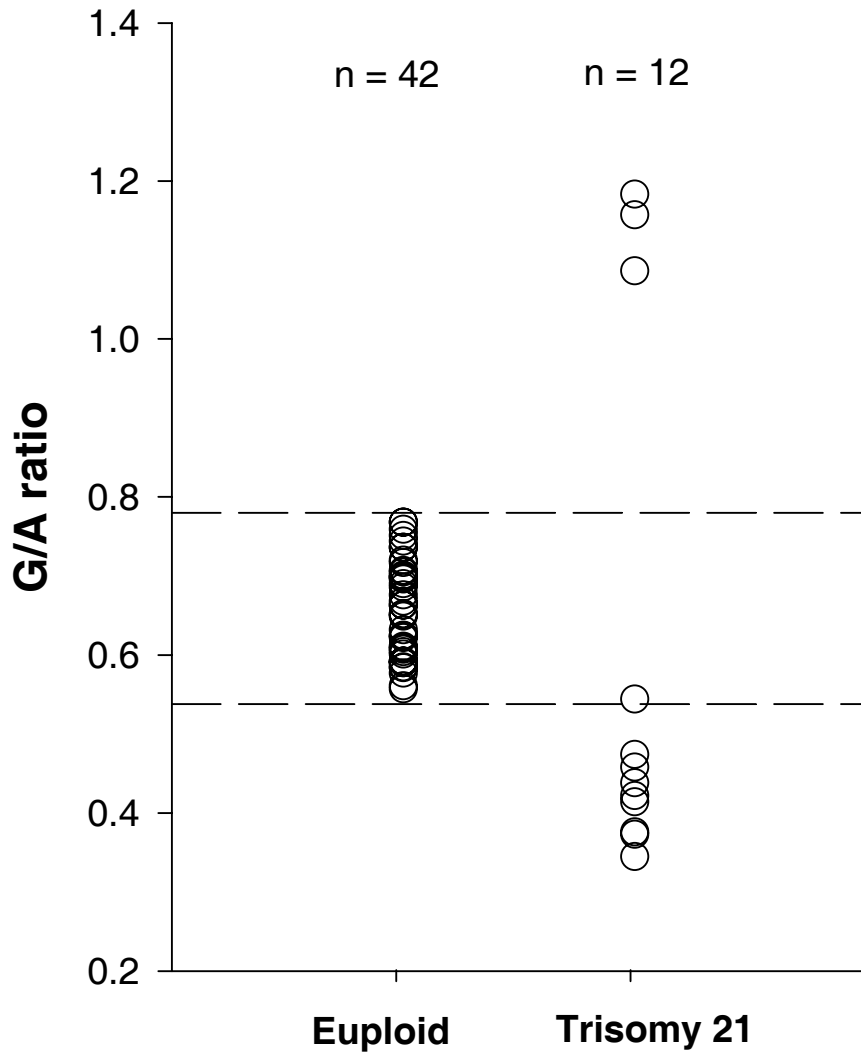
trisomy 21 fetus



allele-A allele-G



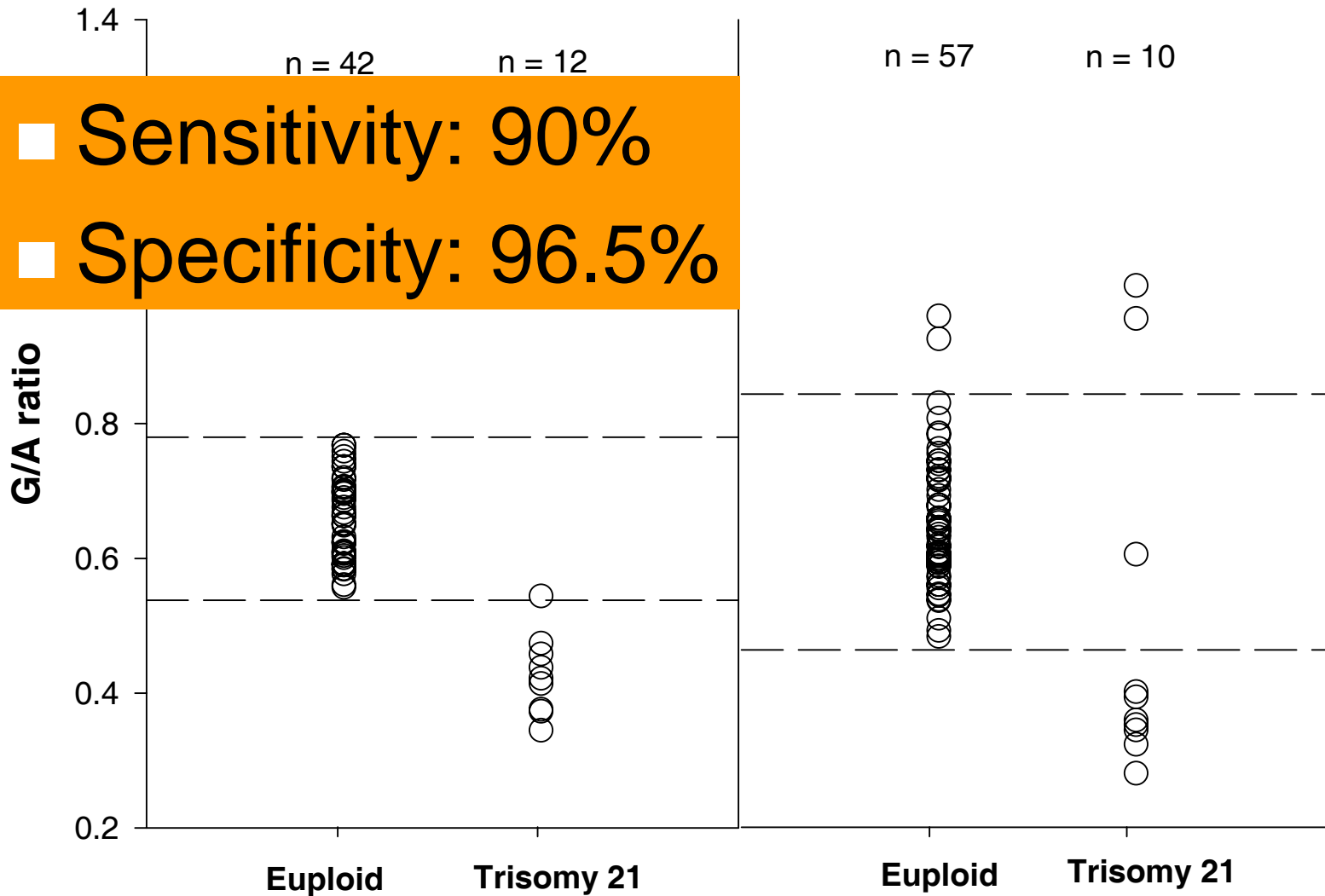
## Placenta



## Placenta

## Maternal plasma

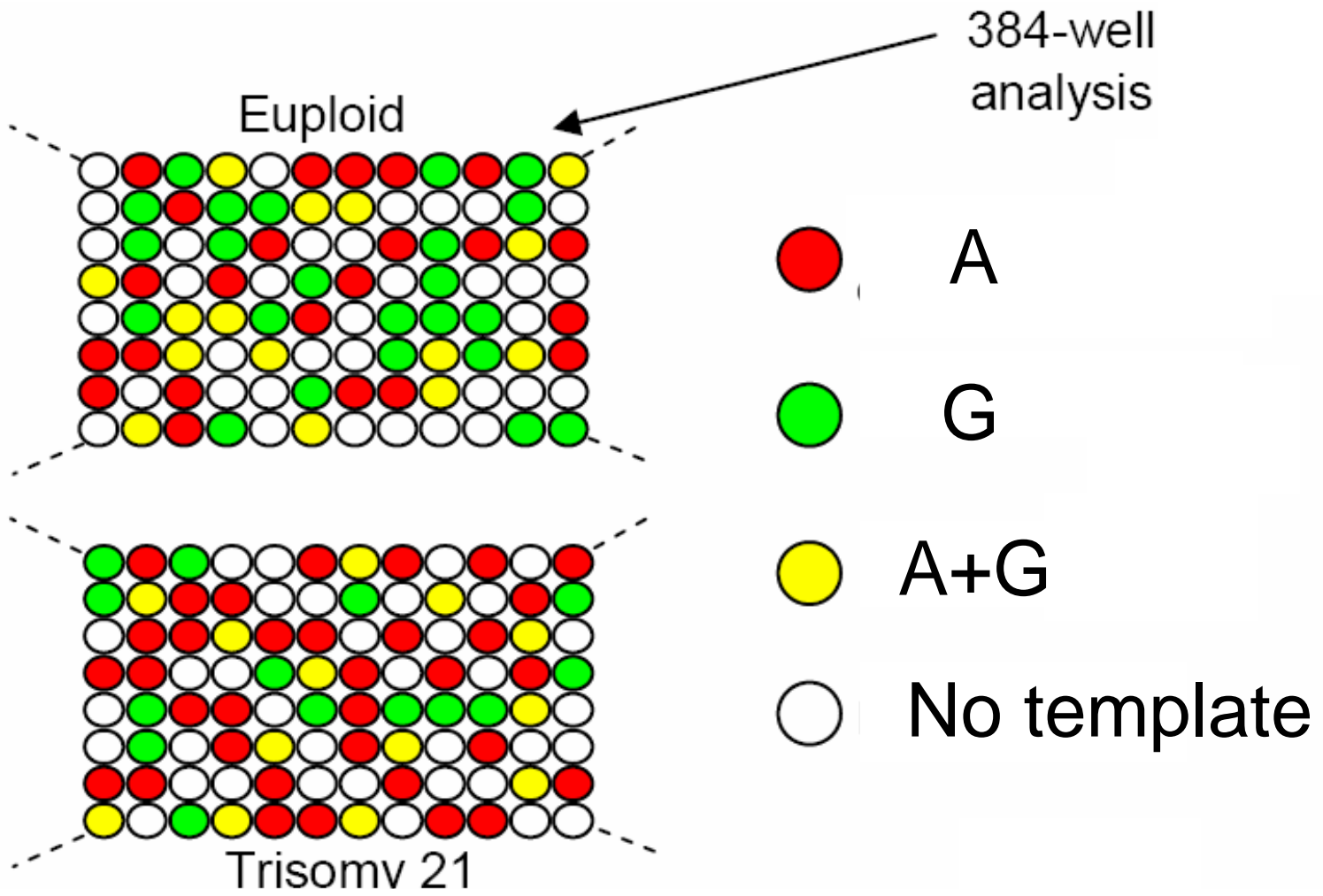
- Sensitivity: 90%
- Specificity: 96.5%





# Digital PCR

Vogelstein and Kinzler  
PNAS 1999



# Conclusions

- Fetal DNA/RNA molecules are present in maternal plasma
- Sex-linked disorders, RhD status
- Fetal epigenetic markers
- Aneuploidy detection: RNA-SNP, high accuracy, heterozygosity requirement
- Digital PCR: high precision, recent automation



# Acknowledgements

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