Cell-Free DNA: Biology and Applications

November 2019

AJHG

ASHG
American Society of Human Genetics

Today's Presenters

Dennis Lo, MD

Diana Bianchi, MD

Bruce Korf, MD, PhD
Moderator

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香港中文大學
The Chinese University of Hong Kong

香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

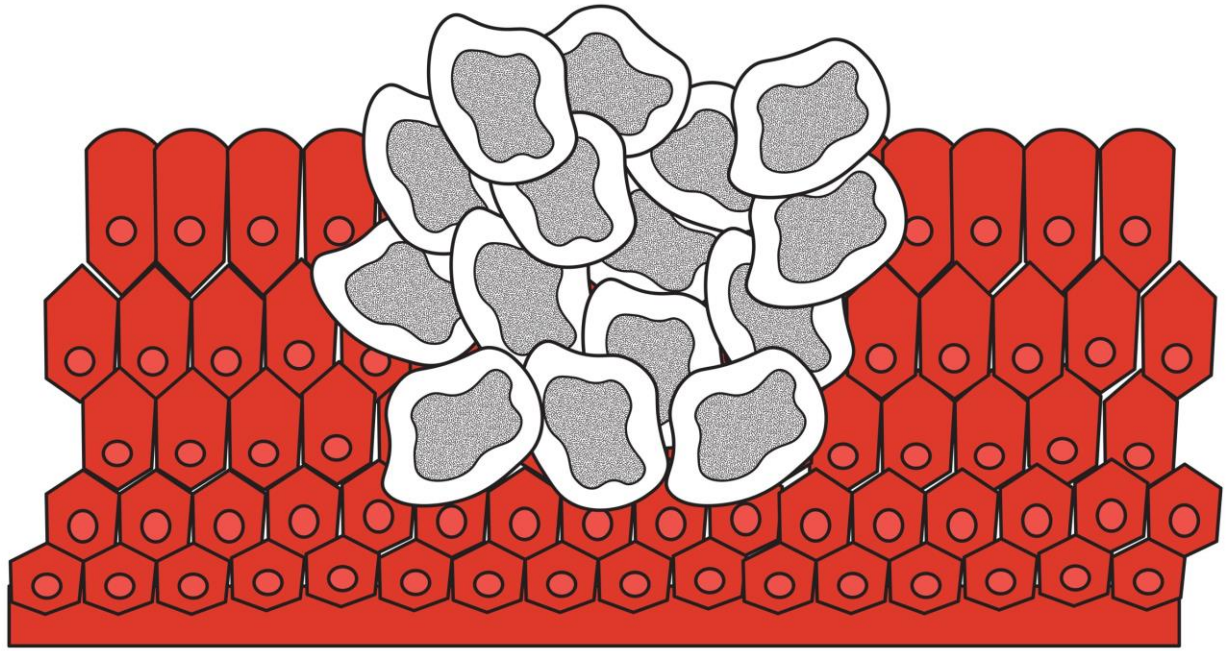
Plasma DNA fragmentomics, topologics and beyond

Dennis Lo
Li Ka Shing Institute of Health Sciences
The Chinese University of Hong Kong

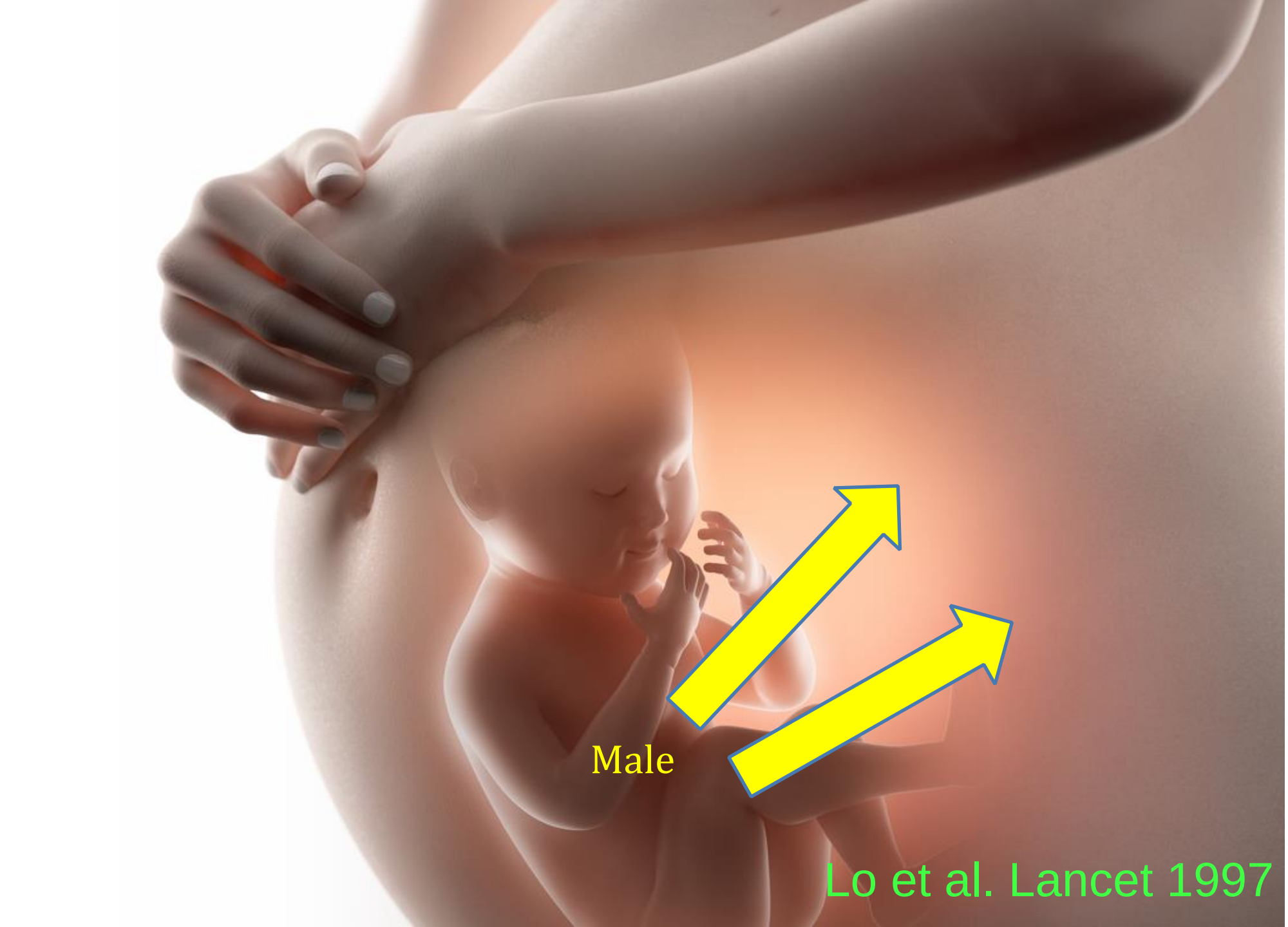
Plasma-DNA based molecular diagnostics

Fetus

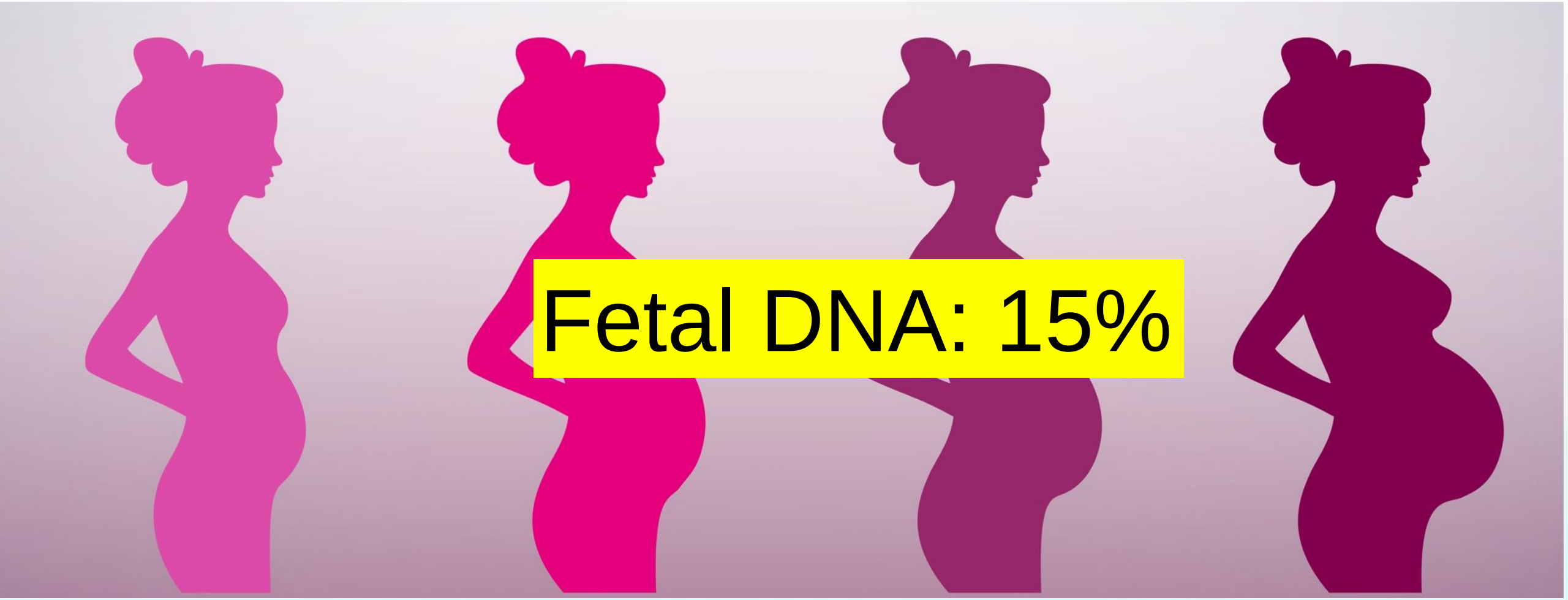
Cancer



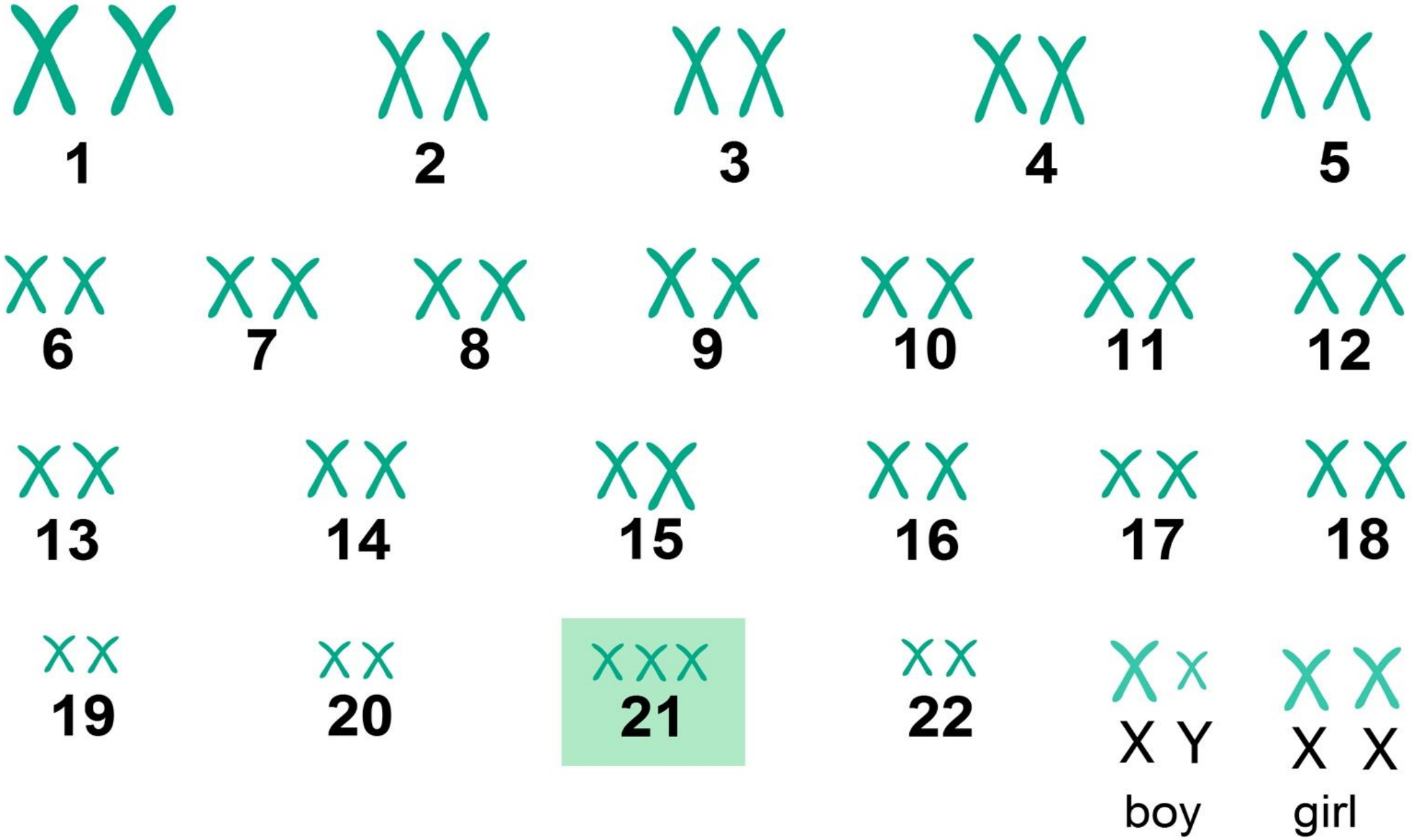

Lo et al. Lancet 1997

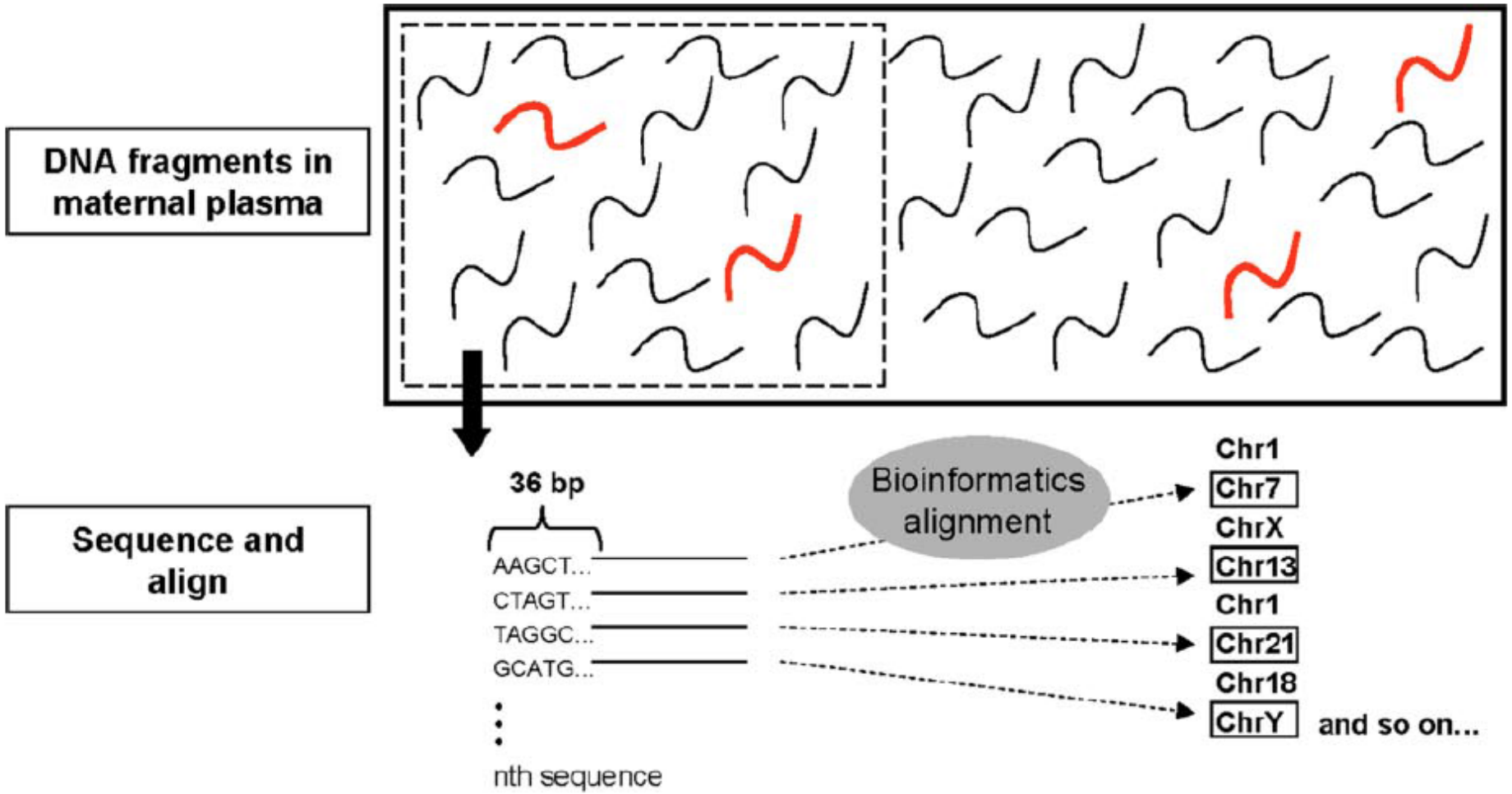


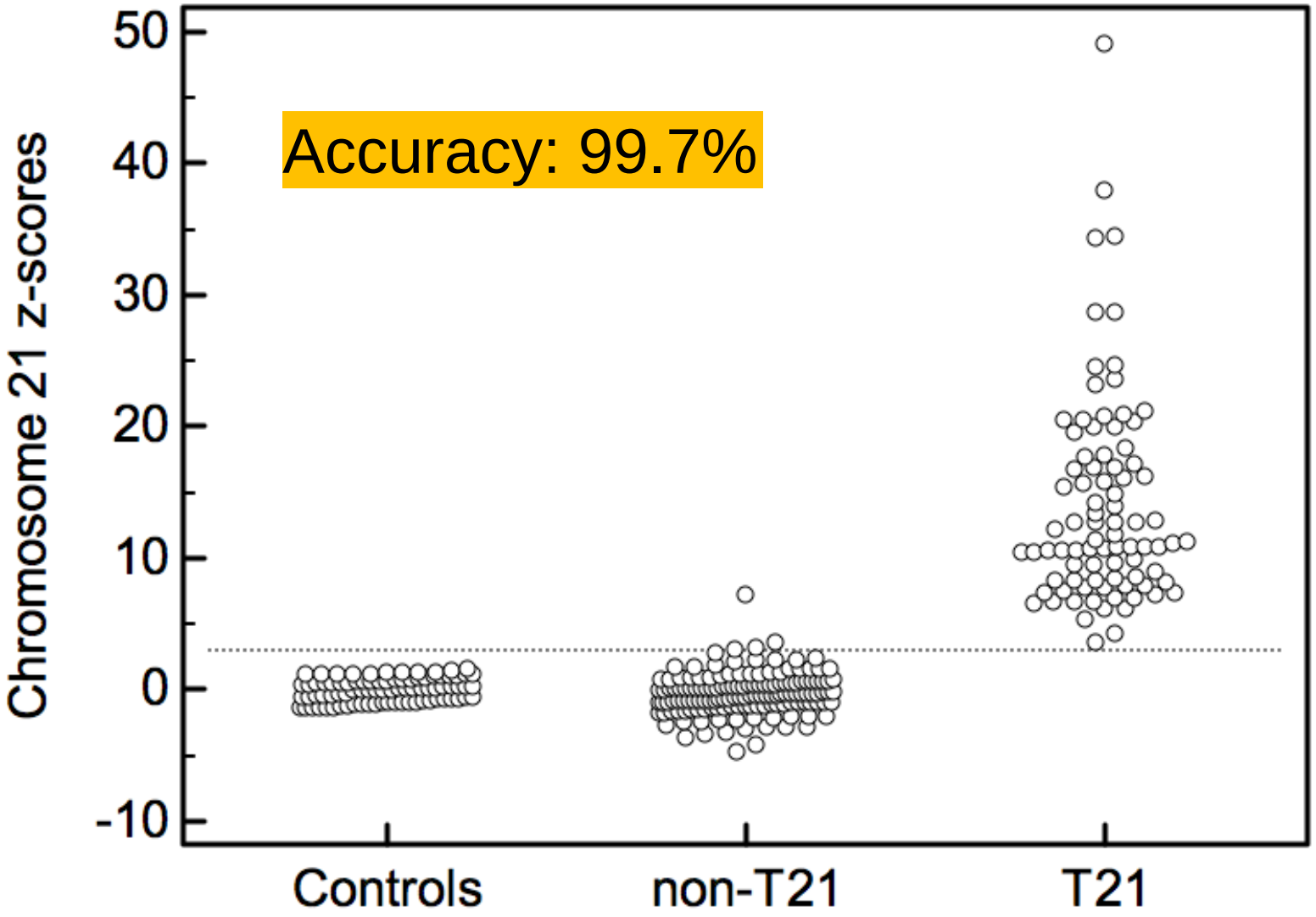
Lo et al. Lancet 1997


Fetal DNA: 15%

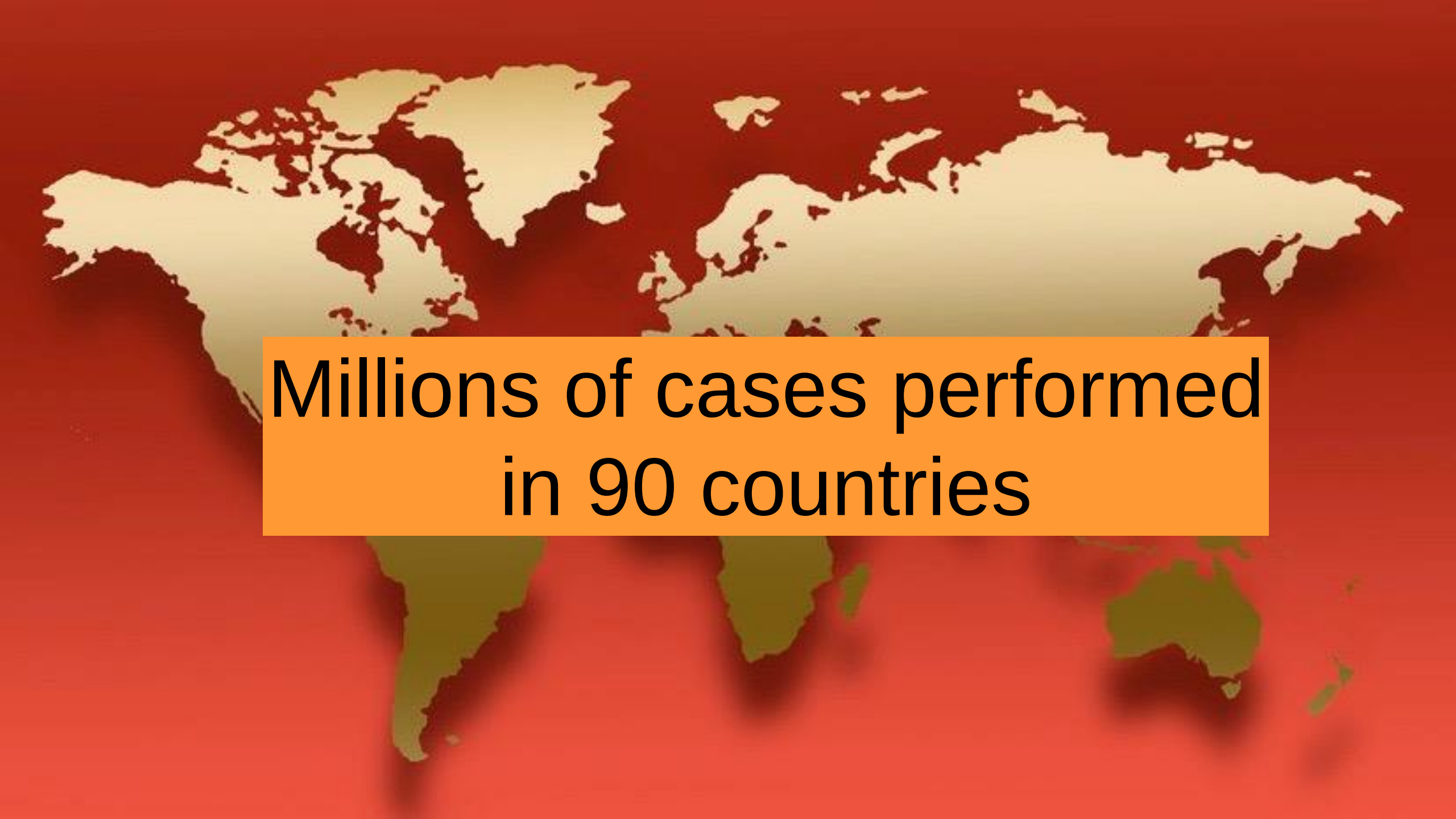
Down Syndrome



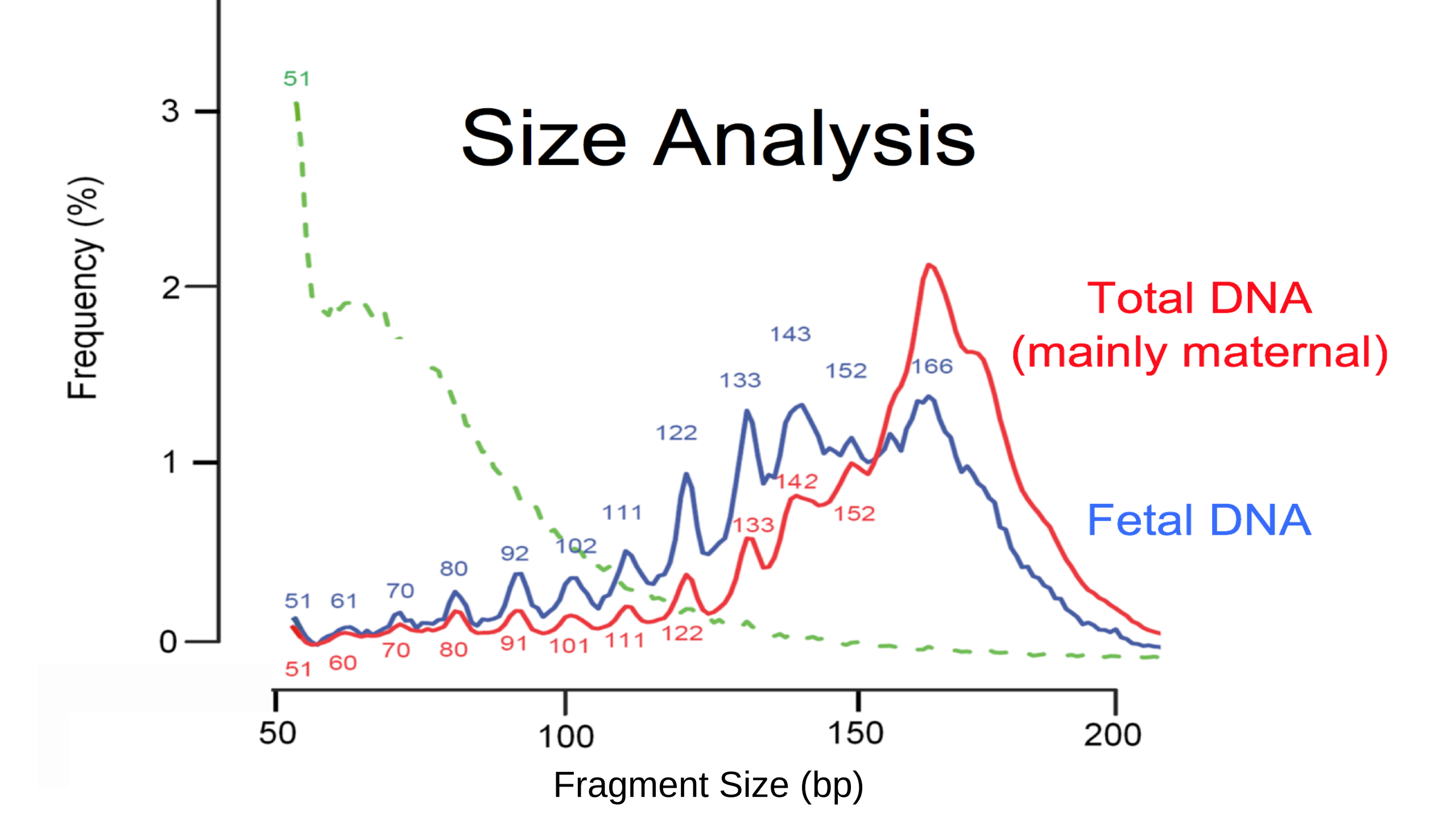

DNA fragments in maternal plasma
Sequence and align
36 bp
AAGCT...
CTAGT...
TAGGC...
GCATG...
nth sequence
Bioinformatics alignment
Chr1
Chr7
ChrX
Chr13
Chr1
Chr21
Chr18
ChrY
and so on...
Sequence counting
chromosome 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y

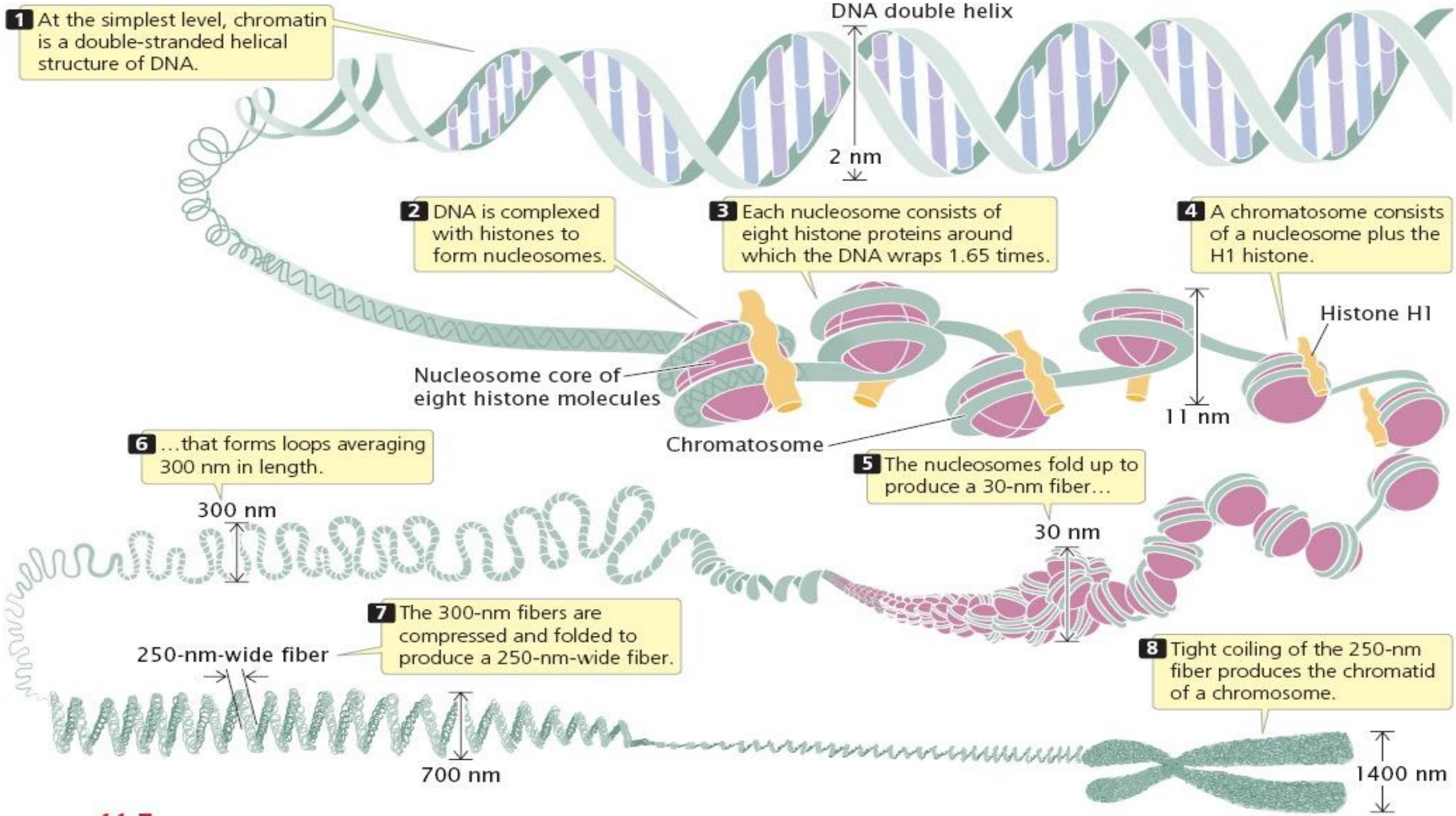


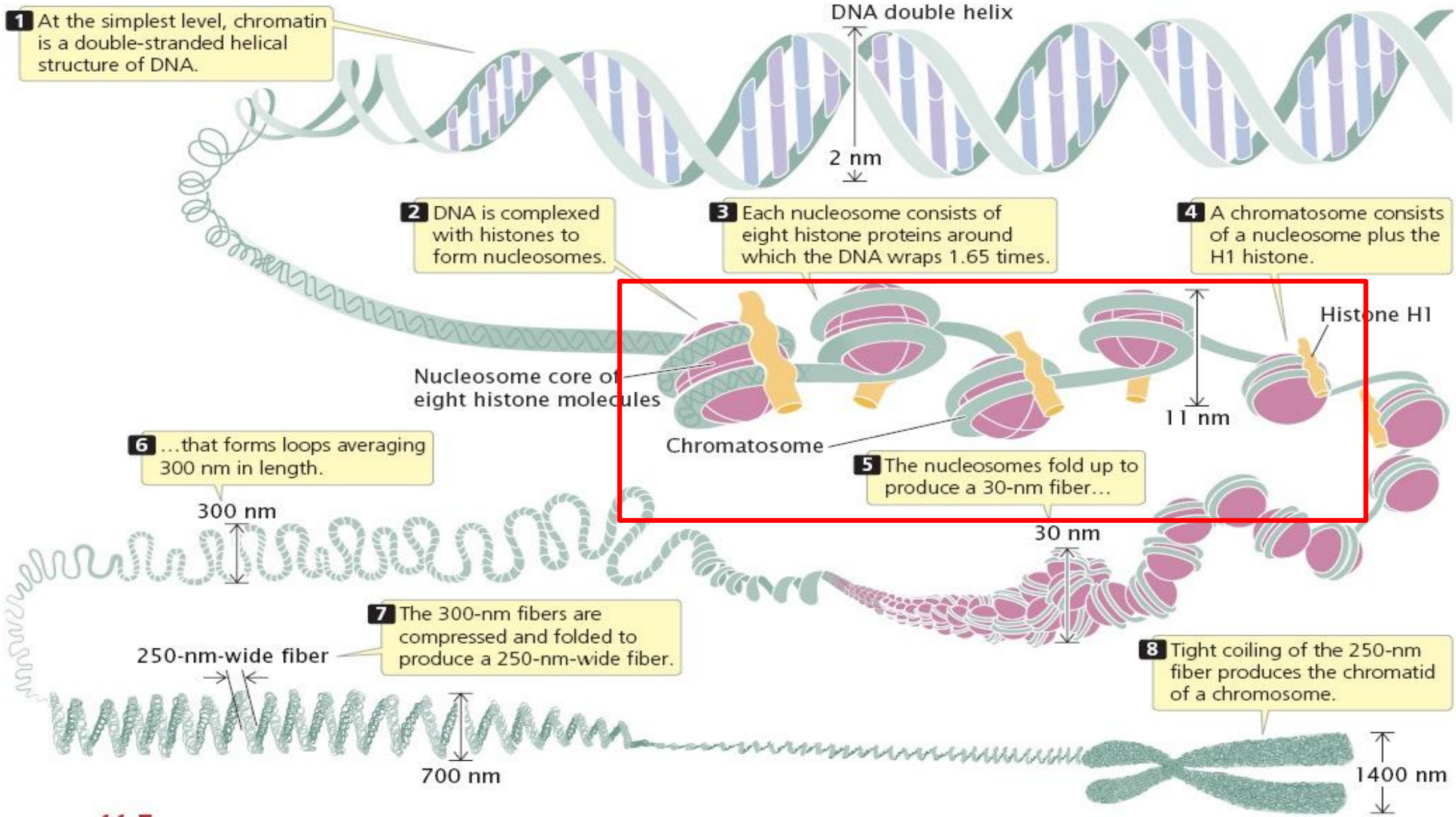
Chiu et al BMJ 2011


Millions of cases performed
in 90 countries

Size of Plasma DNA Molecules


Size Analysis
Frequency (%)
0
1
2
3
50
100
150
200
Fragment Size (bp)
Total DNA
(mainly maternal)
Fetal DNA
51
51
61
70
80
92
102
111
122
133
143
152
166
51
60
70
80
91
101
111
122
133
142
152


1 At the simplest level, chromatin is a double-stranded helical structure of DNA.
DNA double helix
2 nm
2 DNA is complexed with histones to form nucleosomes.
3 Each nucleosome consists of eight histone proteins around which the DNA wraps 1.65 times.
4 A chromatosome consists of a nucleosome plus the H1 histone.
Histone H1
Nucleosome core of eight histone molecules
11 nm
Chromatosome
6 ...that forms loops averaging 300 nm in length.
5 The nucleosomes fold up to produce a 30-nm fiber...
300 nm
30 nm
7 The 300-nm fibers are compressed and folded to produce a 250-nm-wide fiber.
250-nm-wide fiber
8 Tight coiling of the 250-nm fiber produces the chromatid of a chromosome.
700 nm
1400 nm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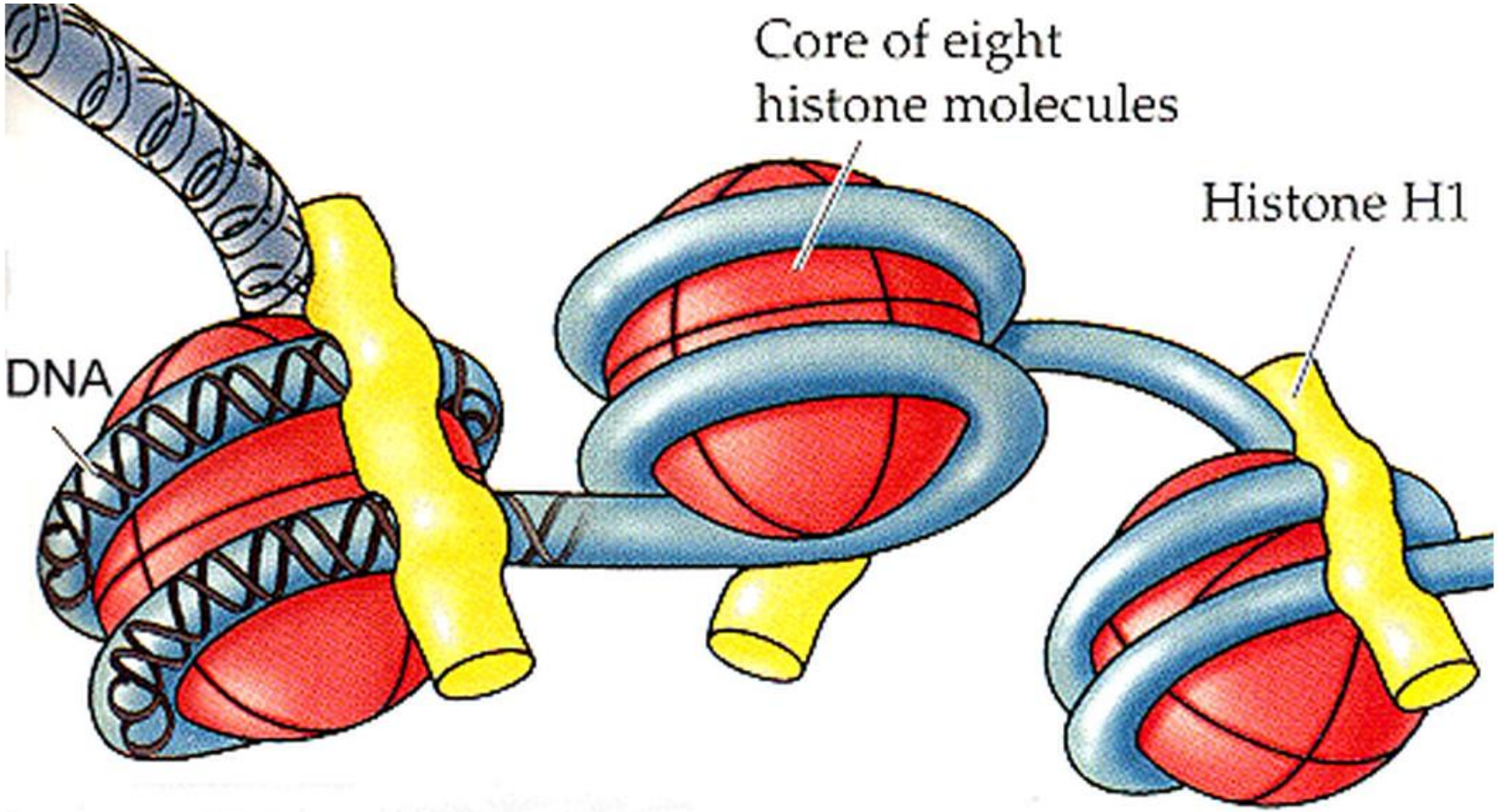
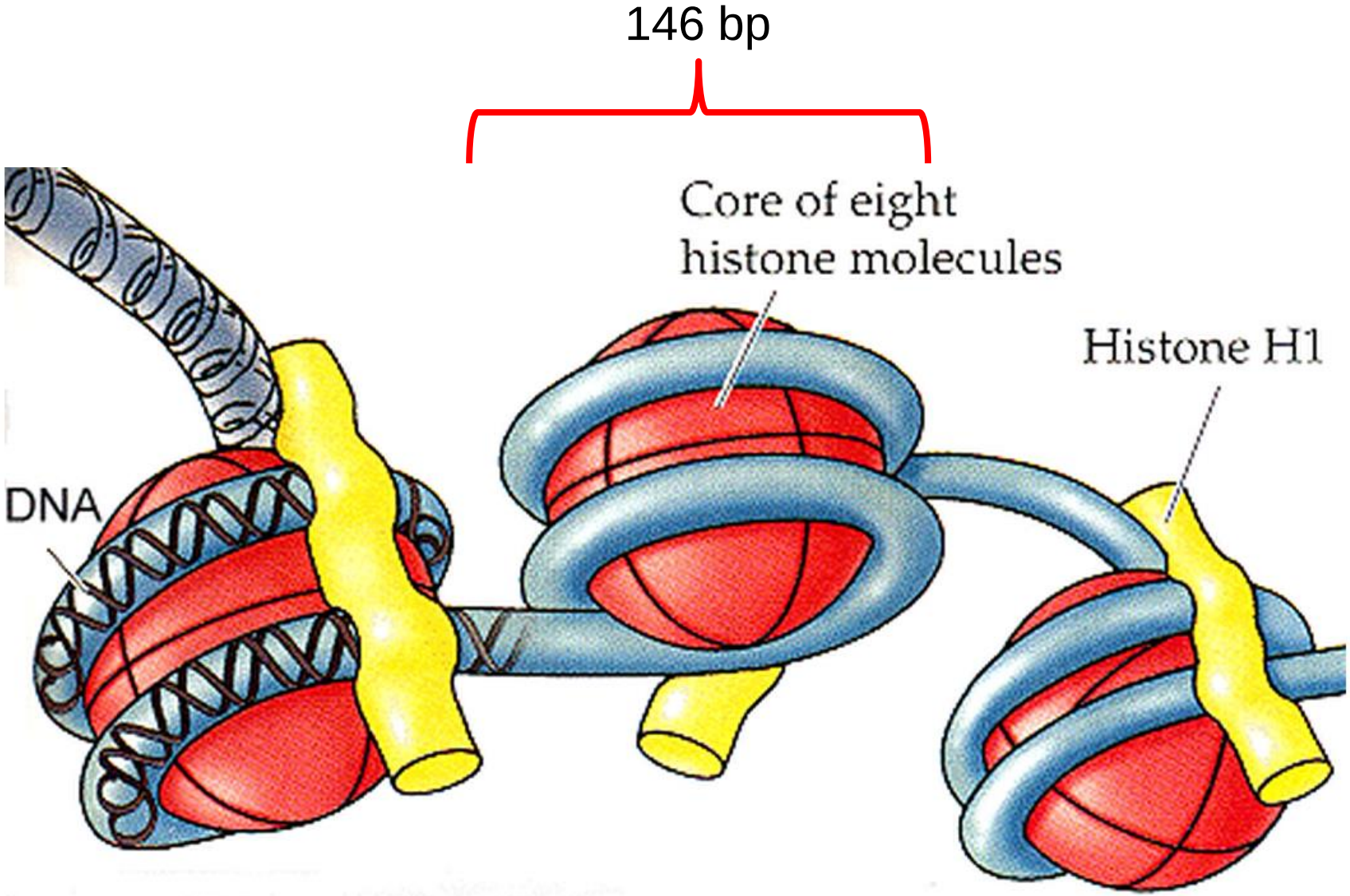
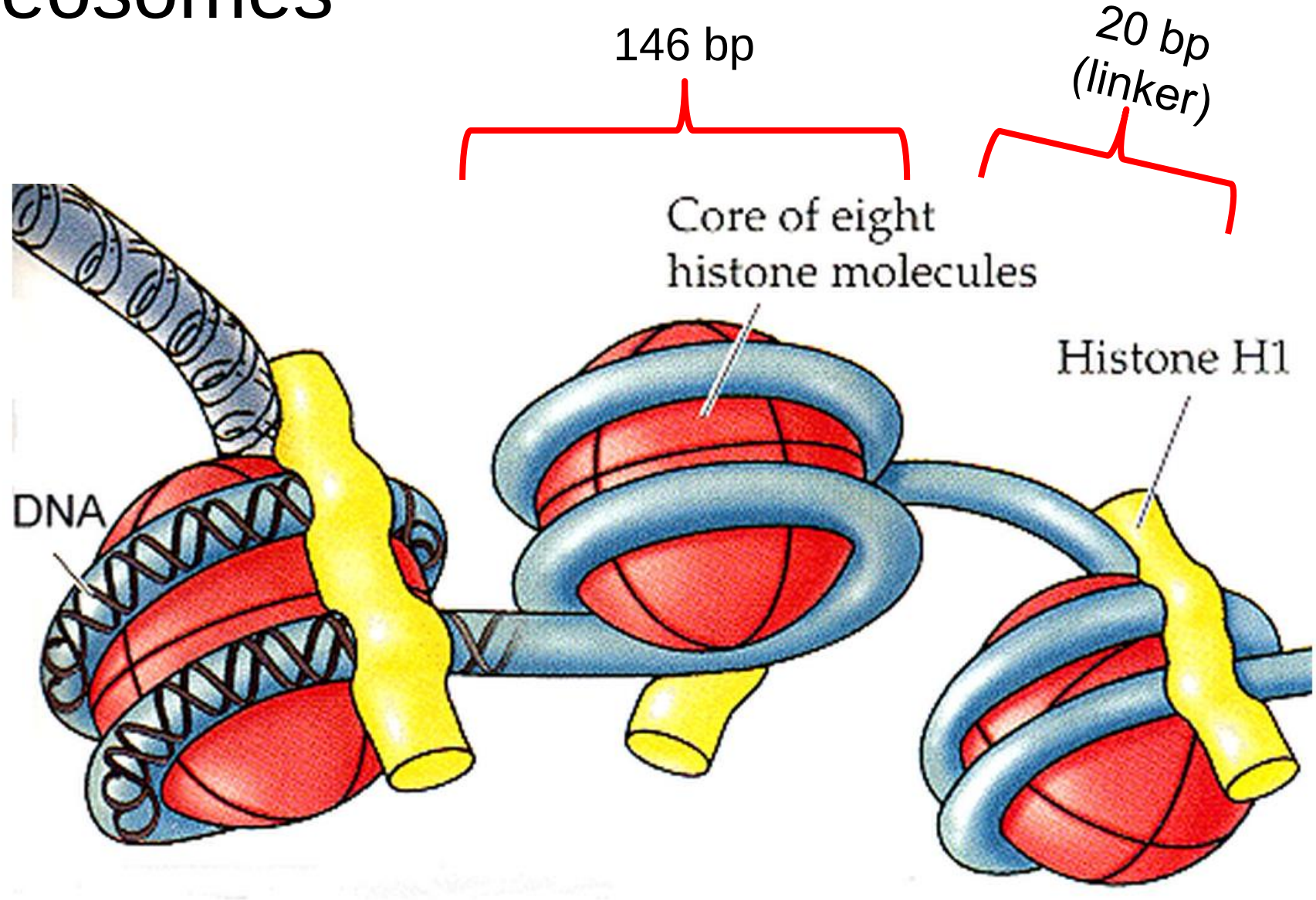
Nucleosomes



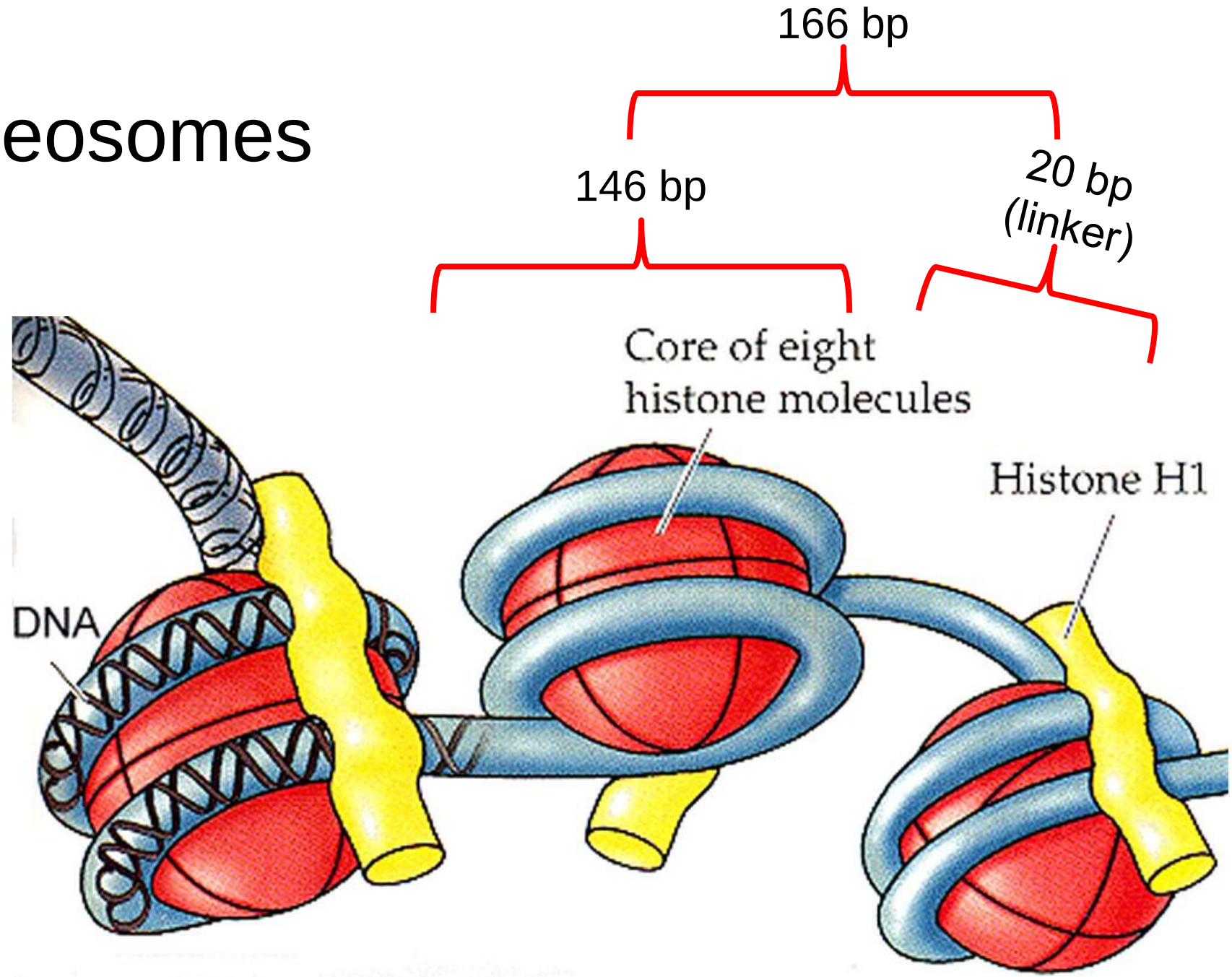
Nucleosomes



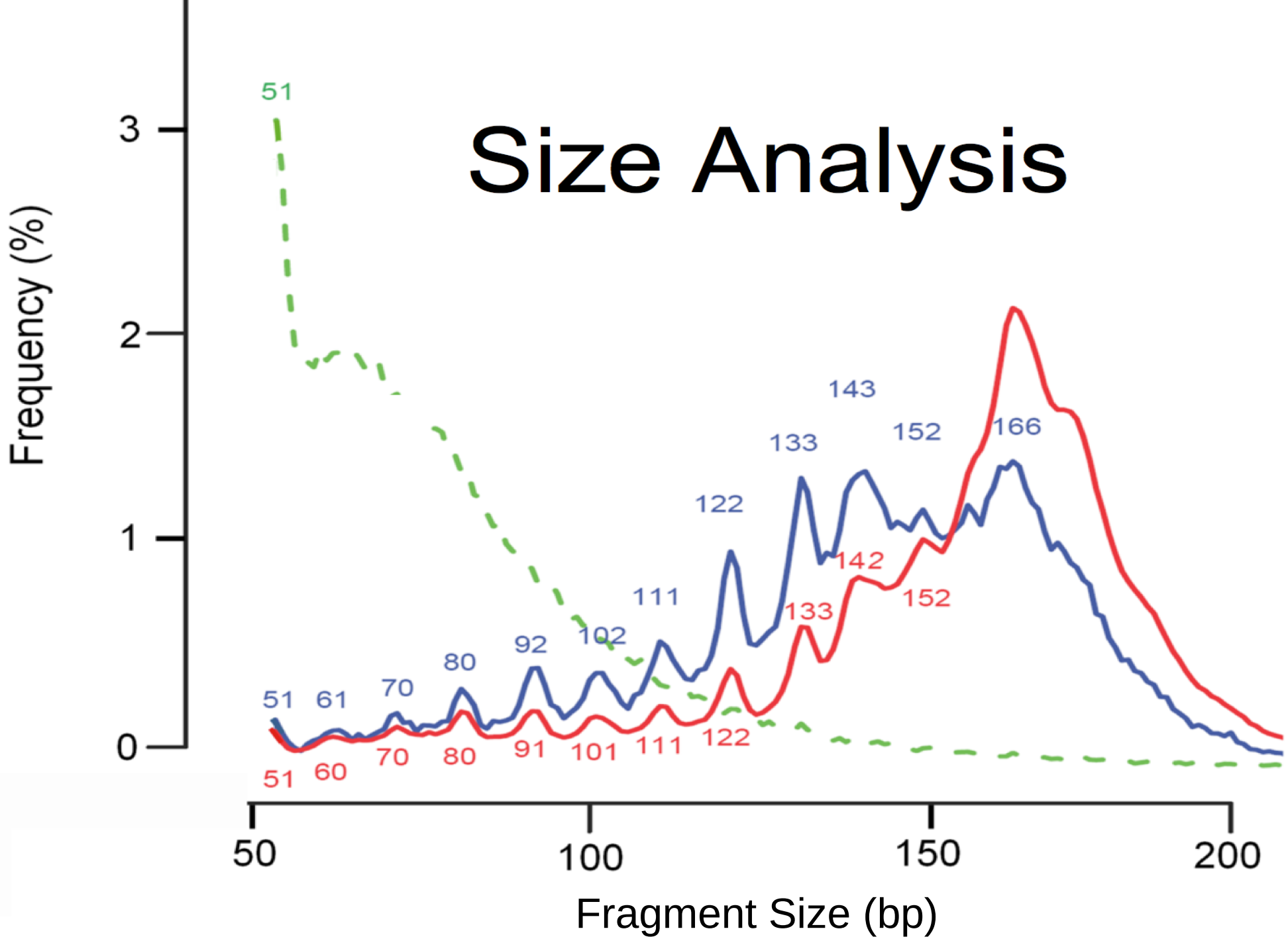
Nucleosomes



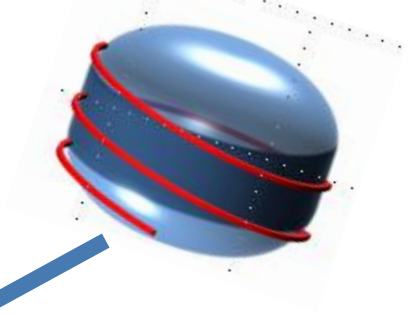
Nucleosomes

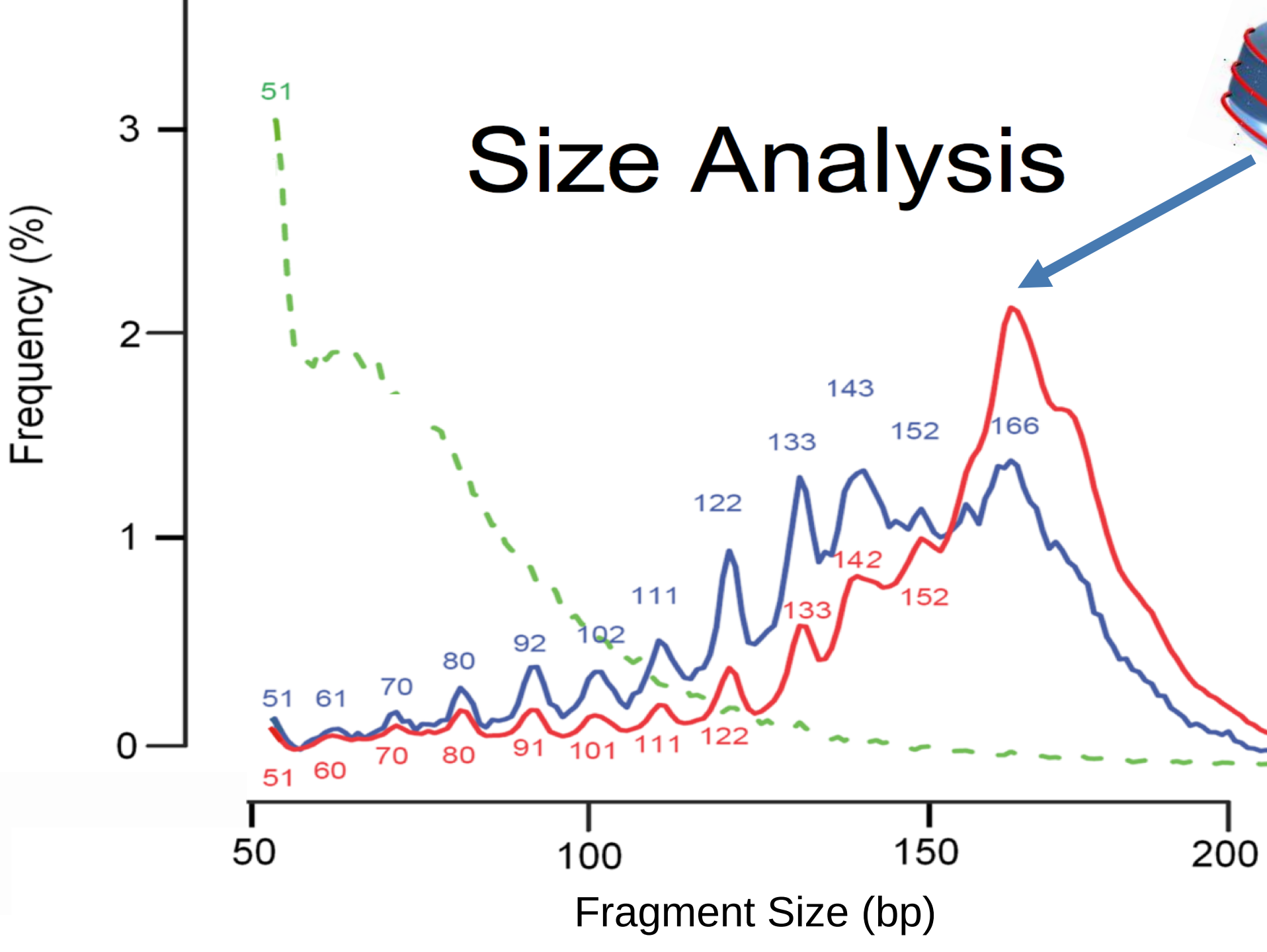


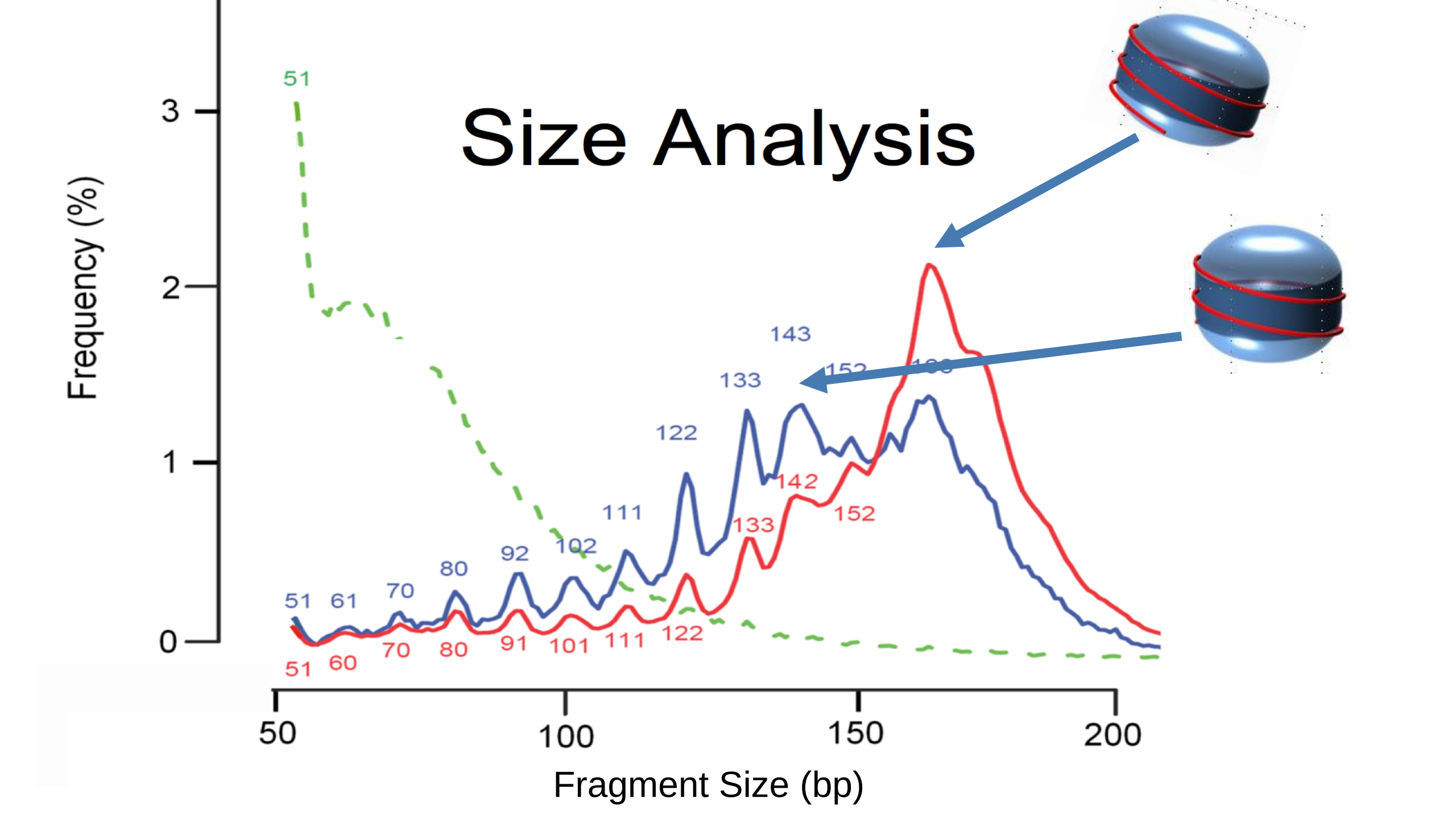
Size Analysis



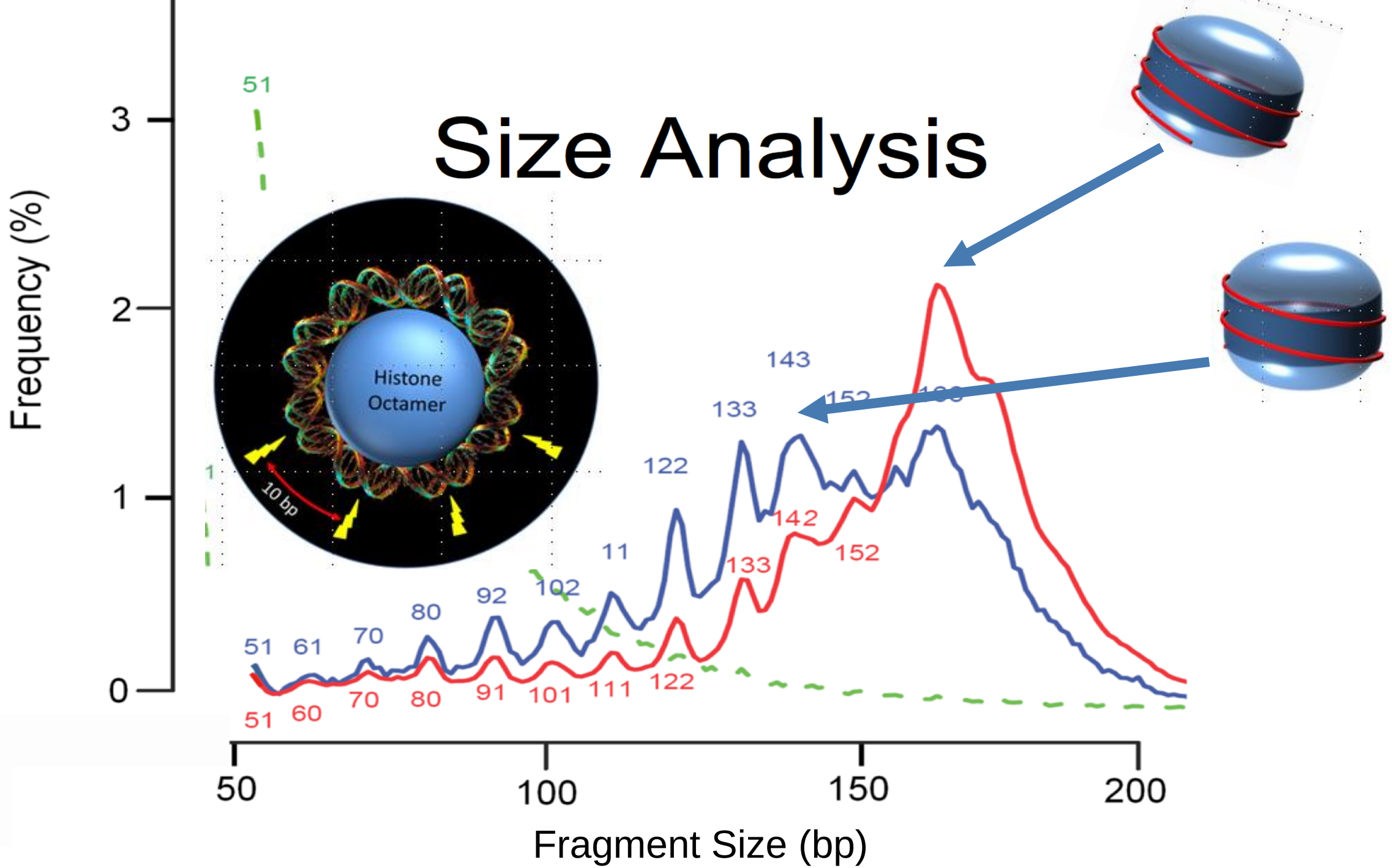
Size Analysis

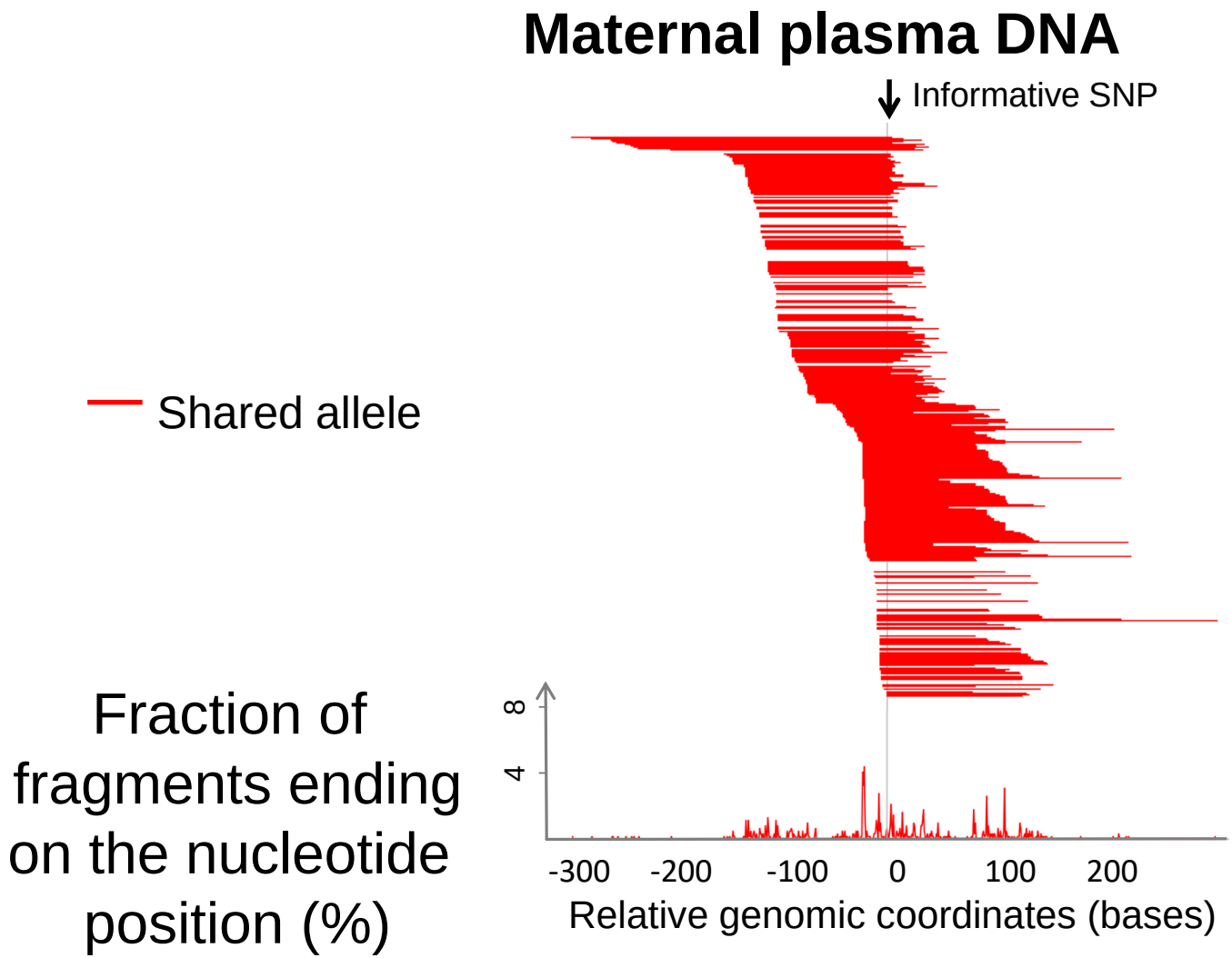




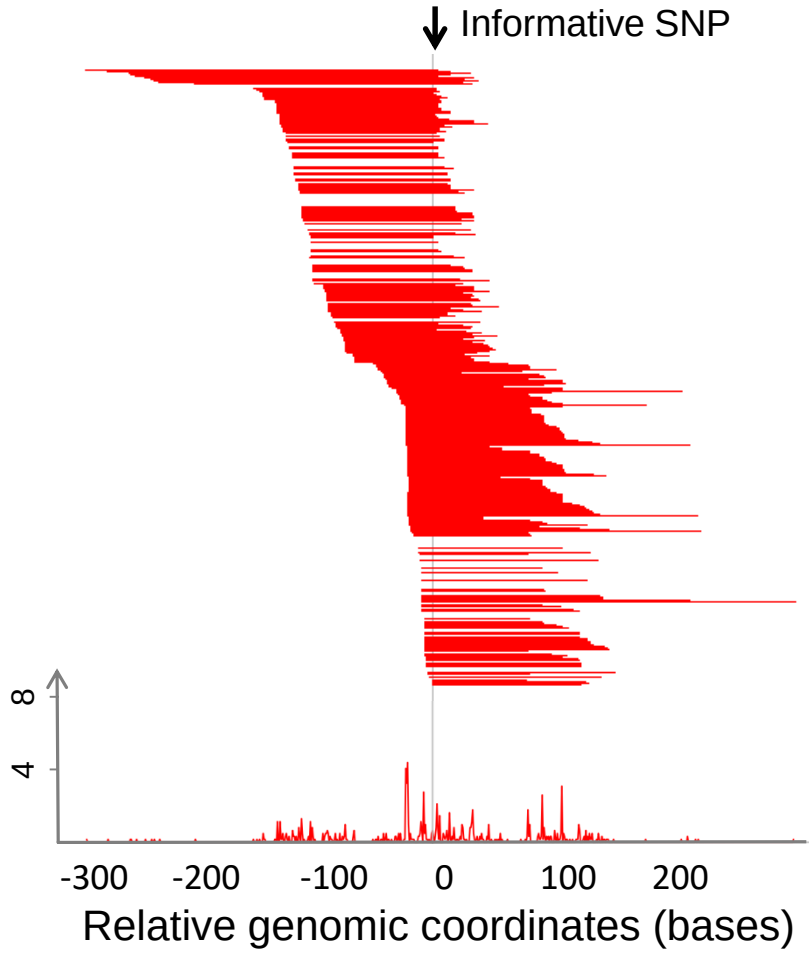

Size Analysis
Frequency (%)
0
1
2
3
50
100
150
200
Fragment Size (bp)
51
51 61 70 80 92 102 111 122 133 143
51 60 70 80 91 101 111 122 133 142 152

Size Analysis

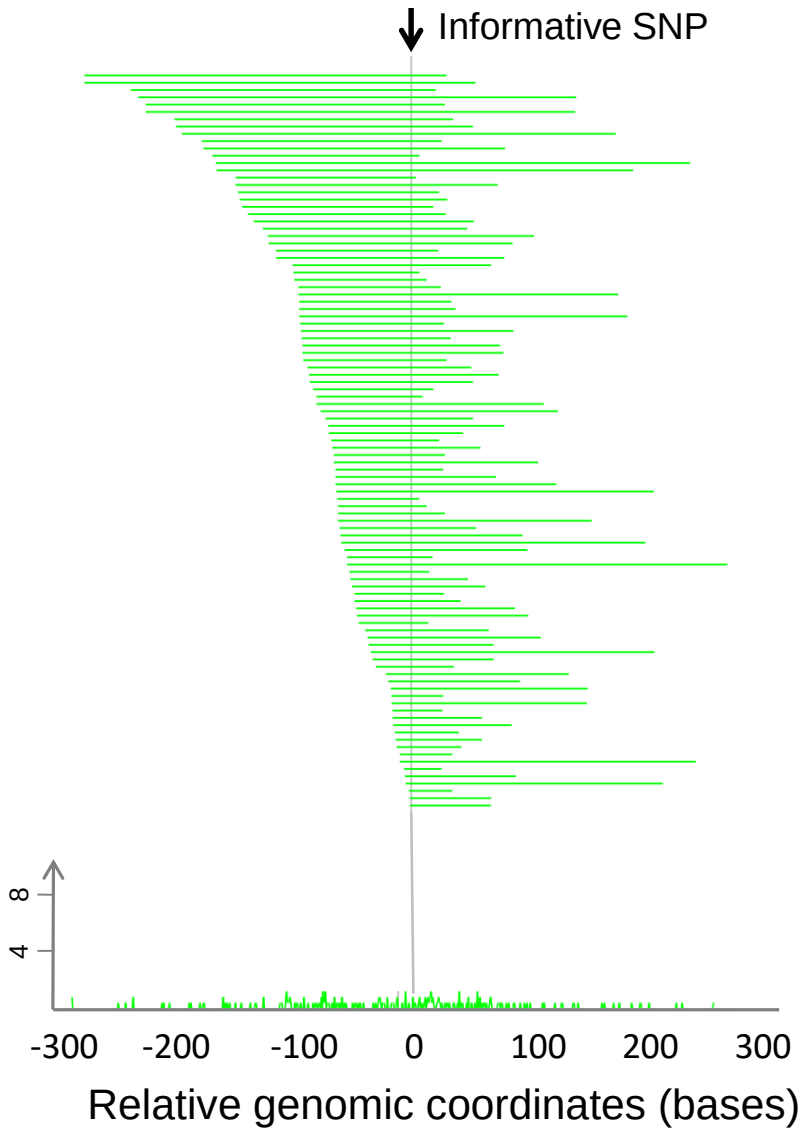



Maternal plasma DNA
Informative SNP
Shared allele
Fraction of fragments ending on the nucleotide position (%)
8
4
-300
-200
-100
0
100
200
Relative genomic coordinates (bases)

Maternal plasma DNA

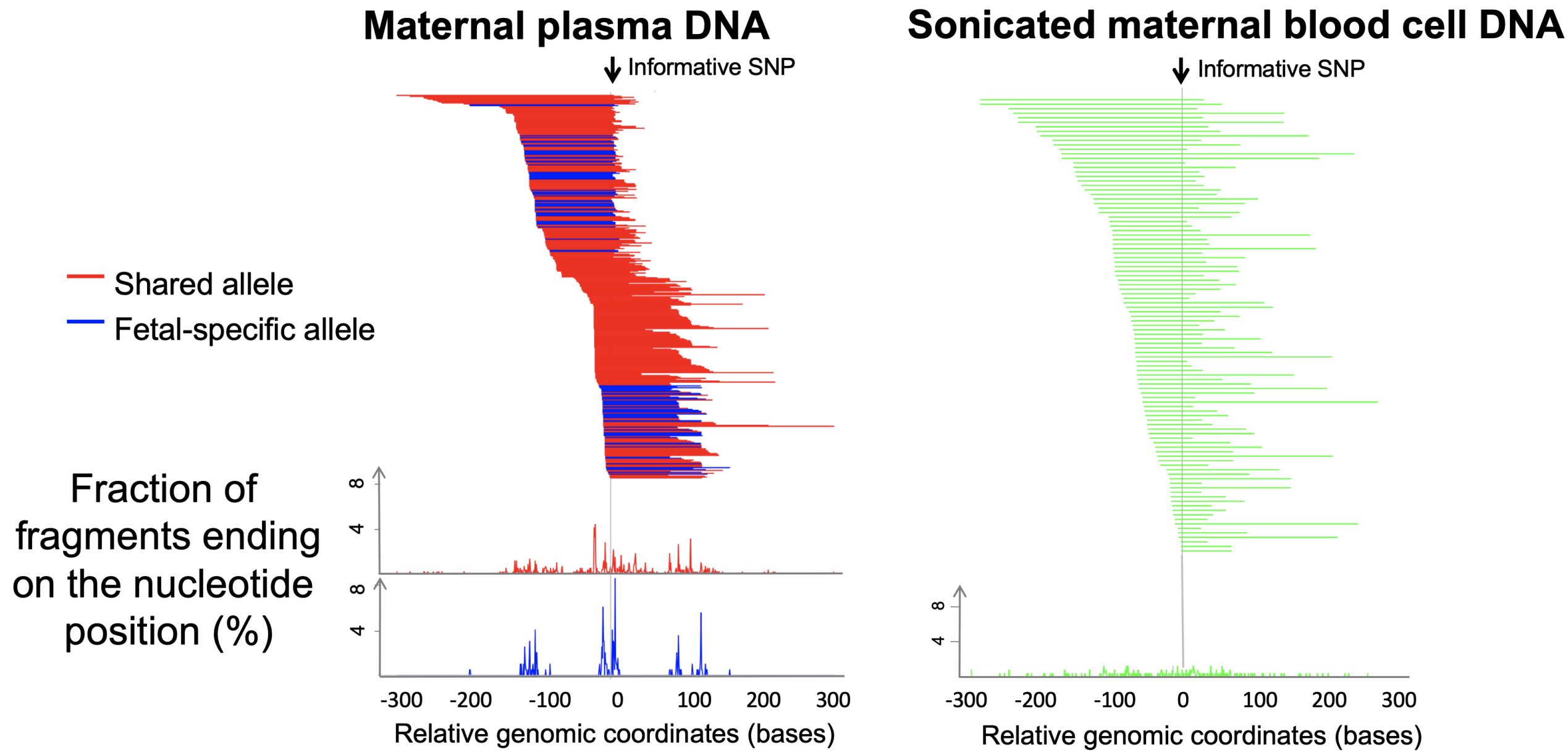


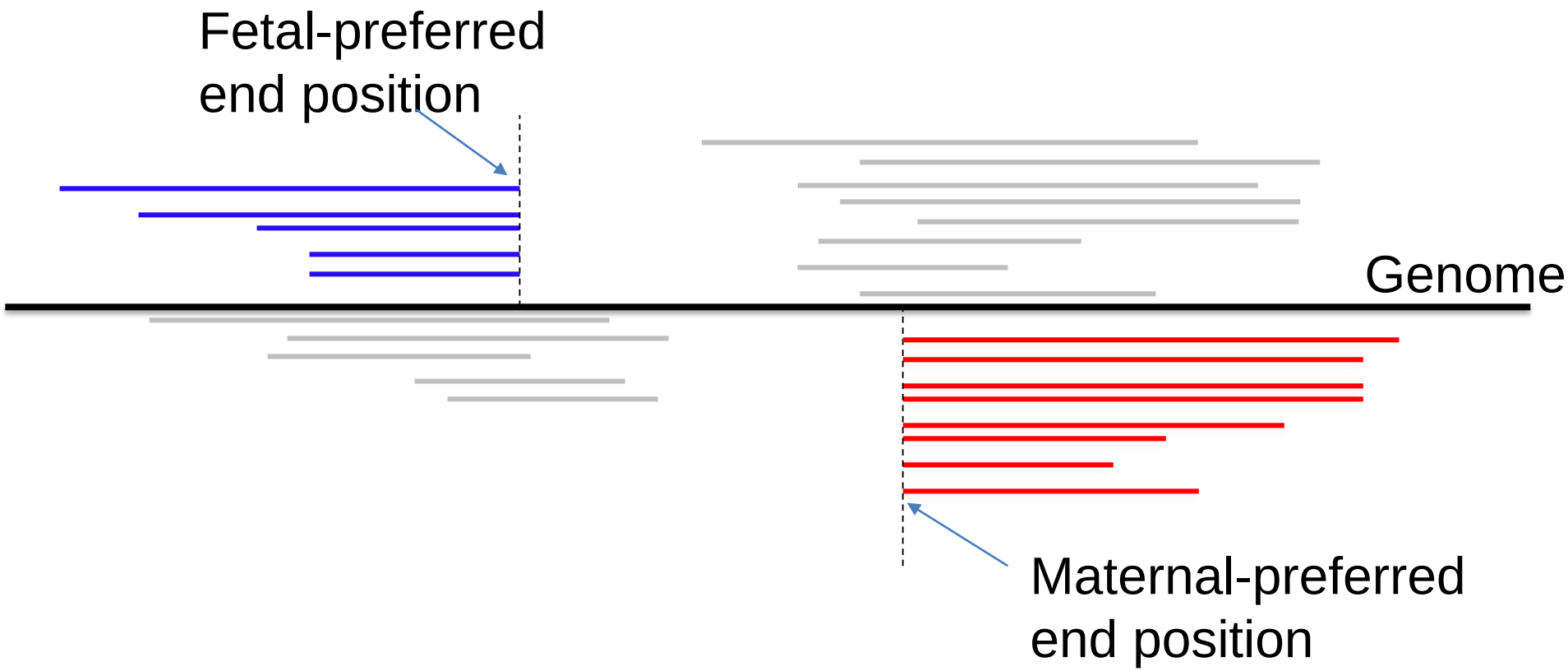
Sonicated maternal blood cell DNA



Shared allele

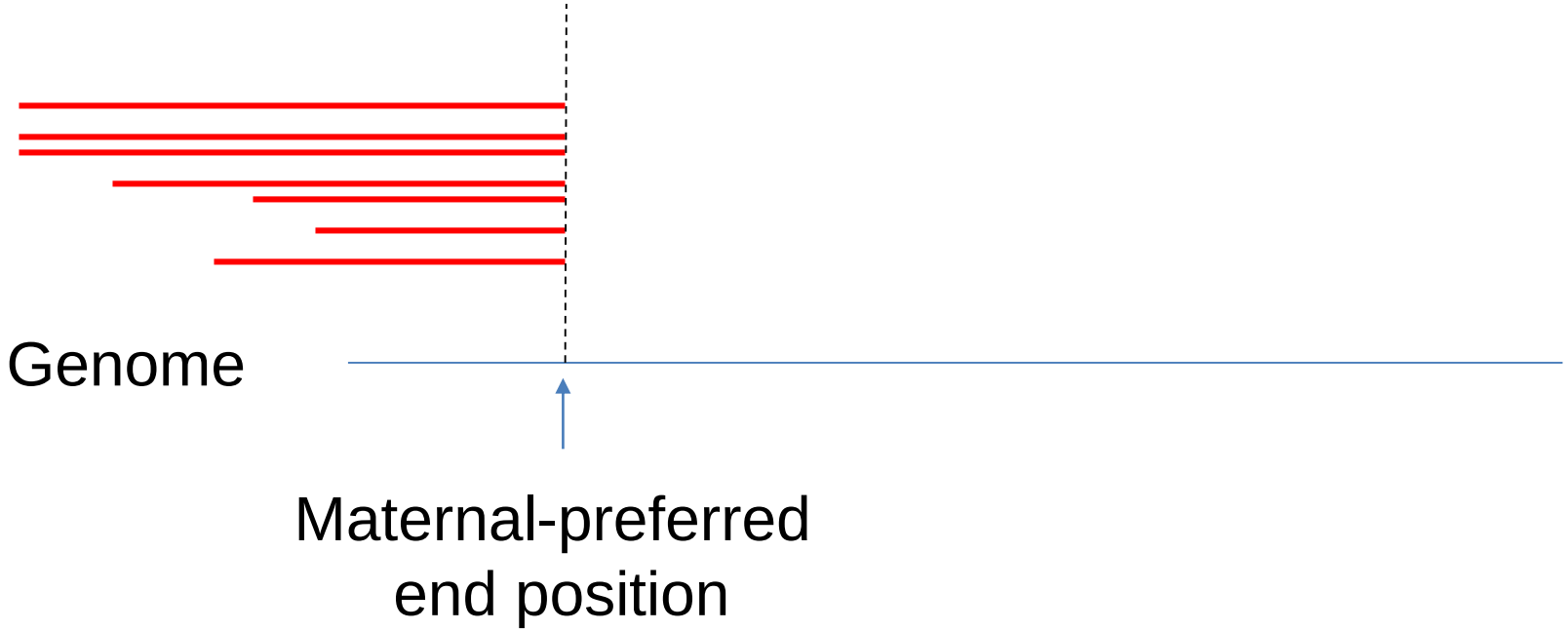
Fraction of fragments ending on the nucleotide position (%)


Maternal plasma DNA
Informative SNP
Shared allele
Fetal-specific allele
Fraction of fragments ending on the nucleotide position (%)
8
4
8
4
-300
-200
-100
0
100
200
300
Relative genomic coordinates (bases)
Sonicated maternal blood cell DNA
Informative SNP
8
4
-300
-200
-100
0
100
200
300
Relative genomic coordinates (bases)

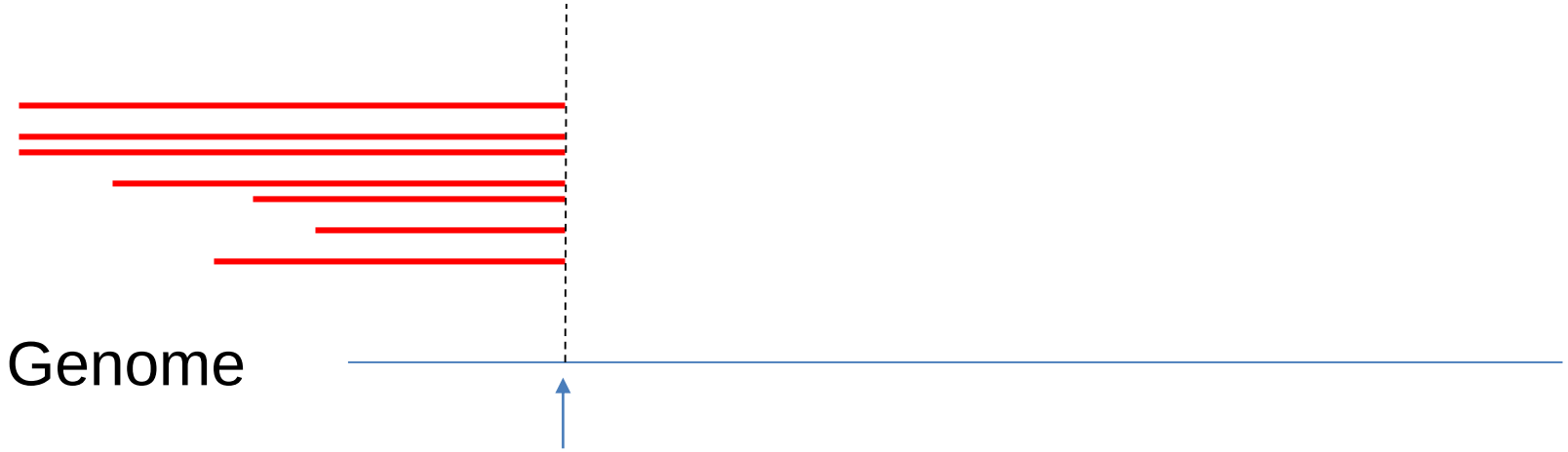

Fetal-preferred
end position
Genome
Maternal-preferred
end position

Hypothesis

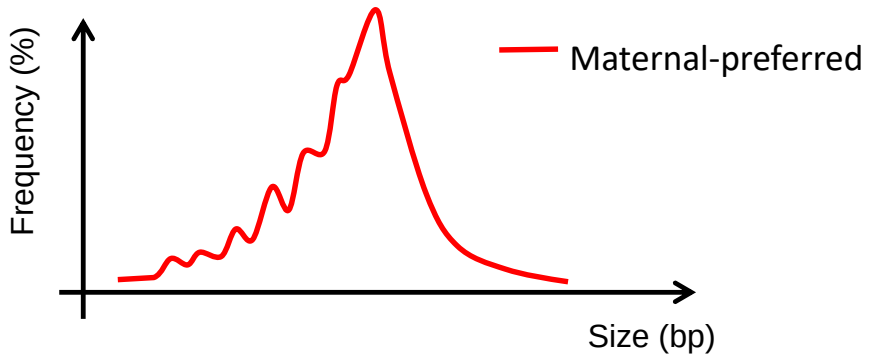
Hypothesis



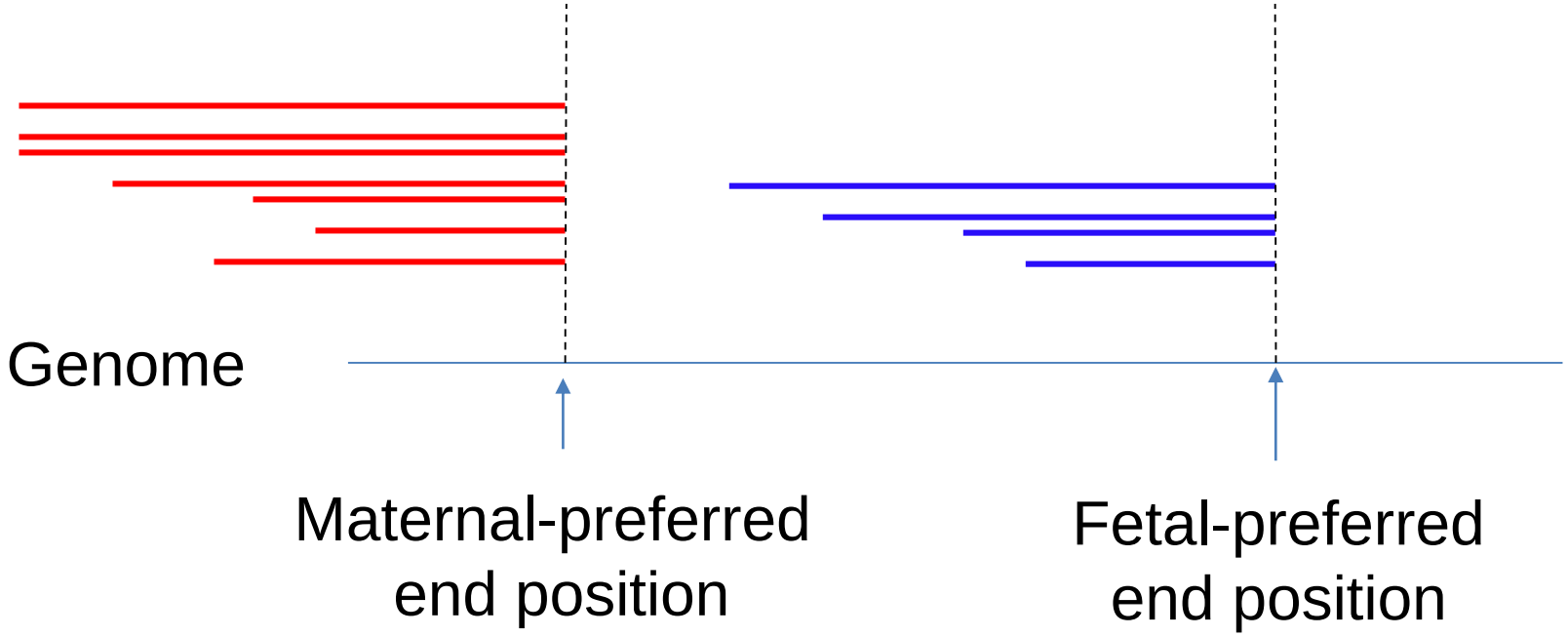
Hypothesis

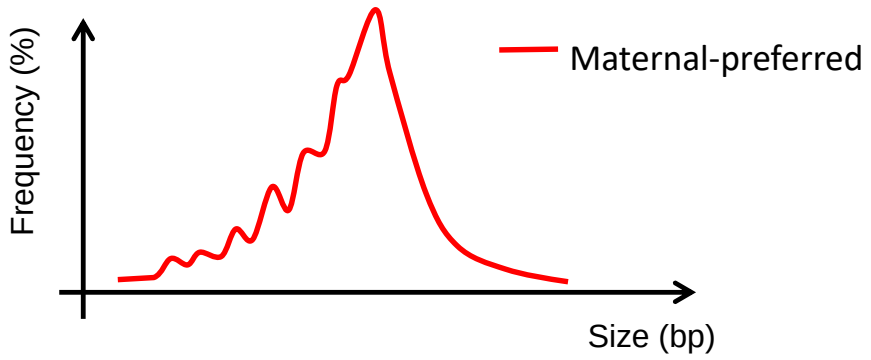


Maternal-preferred
end position

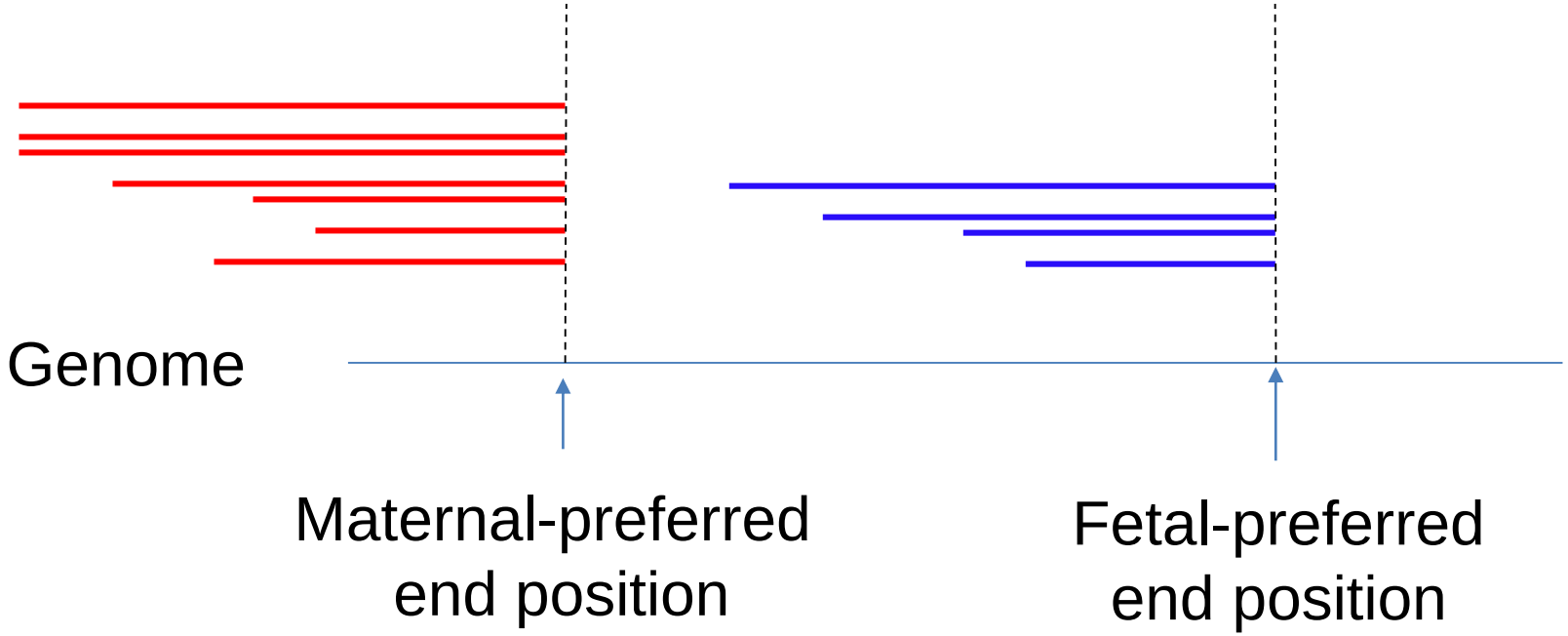


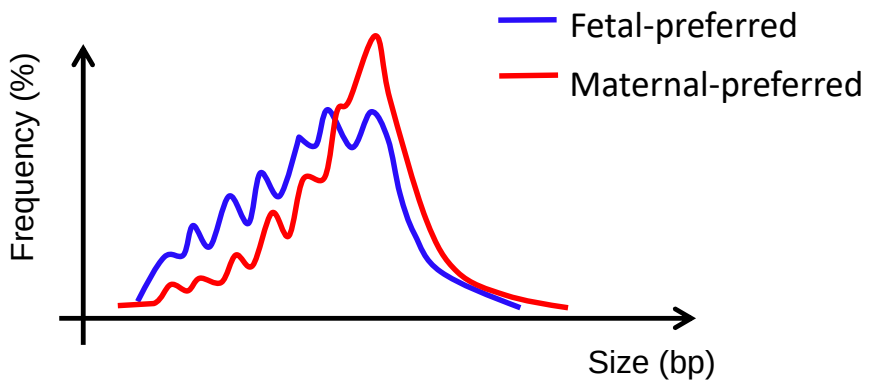
Hypothesis


Genome
Maternal-preferred
end position
Fetal-preferred
end position

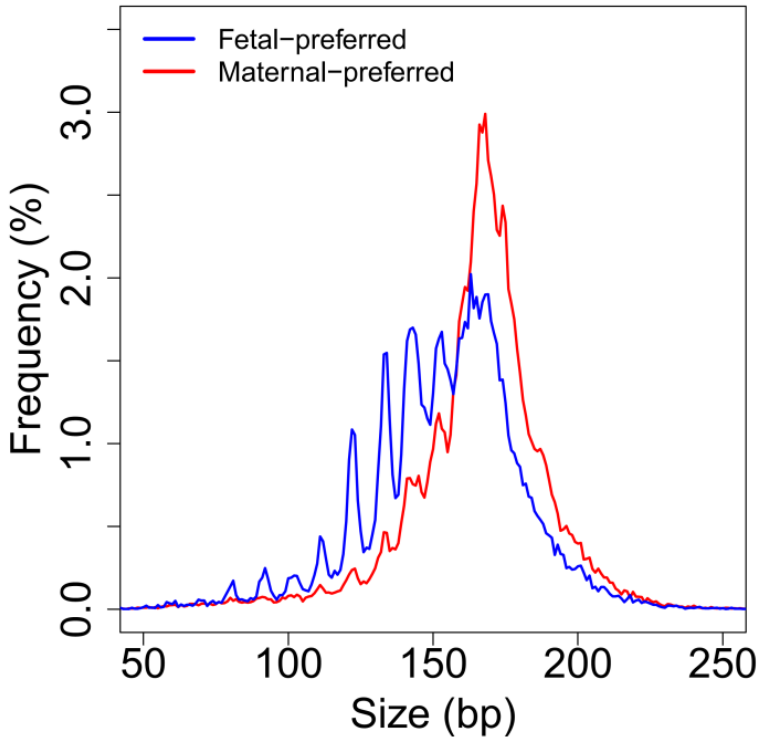

Frequency (%)
Maternal-preferred
Size (bp)

Hypothesis

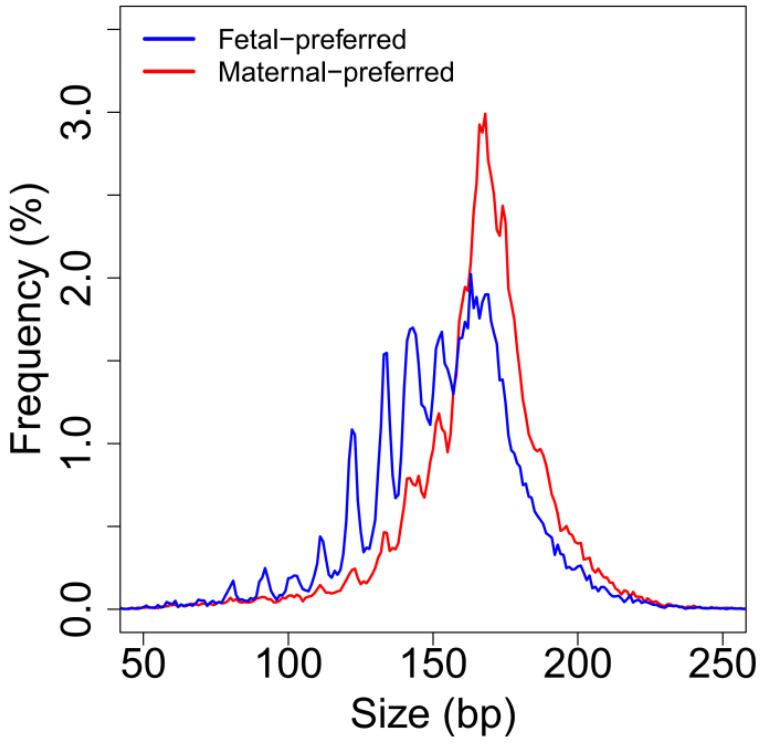

Genome
Maternal-preferred
end position
Fetal-preferred
end position

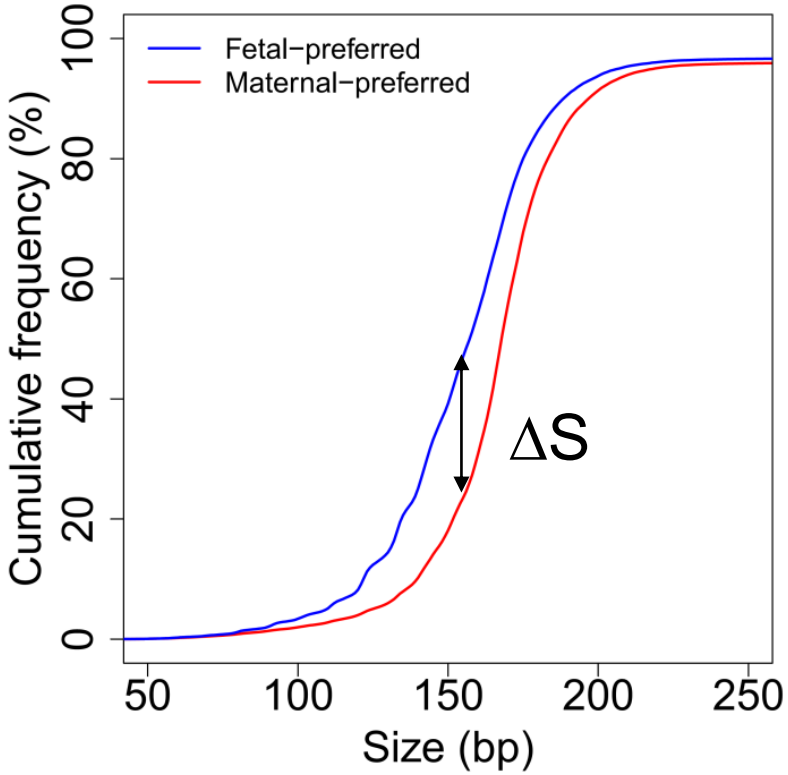

Fetal-preferred
Maternal-preferred
Frequency (%)
Size (bp)

Size distribution of fragments with maternal- and fetal-preferred ends

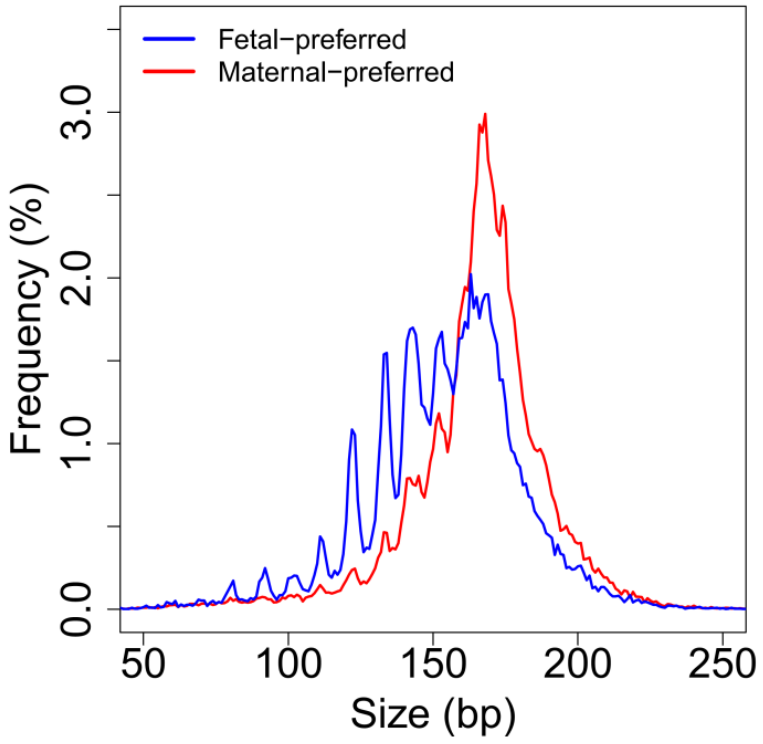


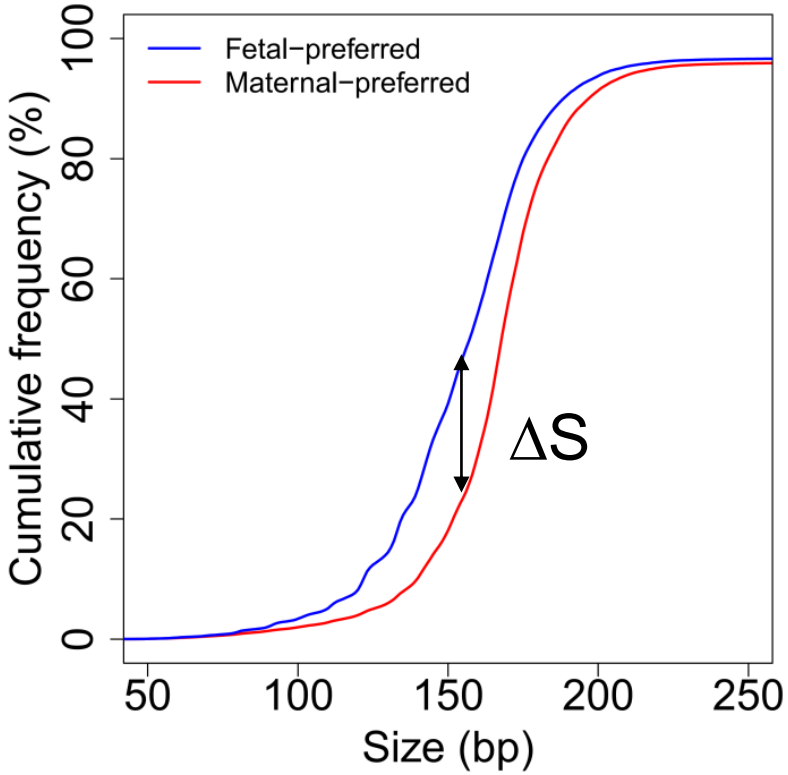
Size distribution of fragments with maternal- and fetal-preferred ends

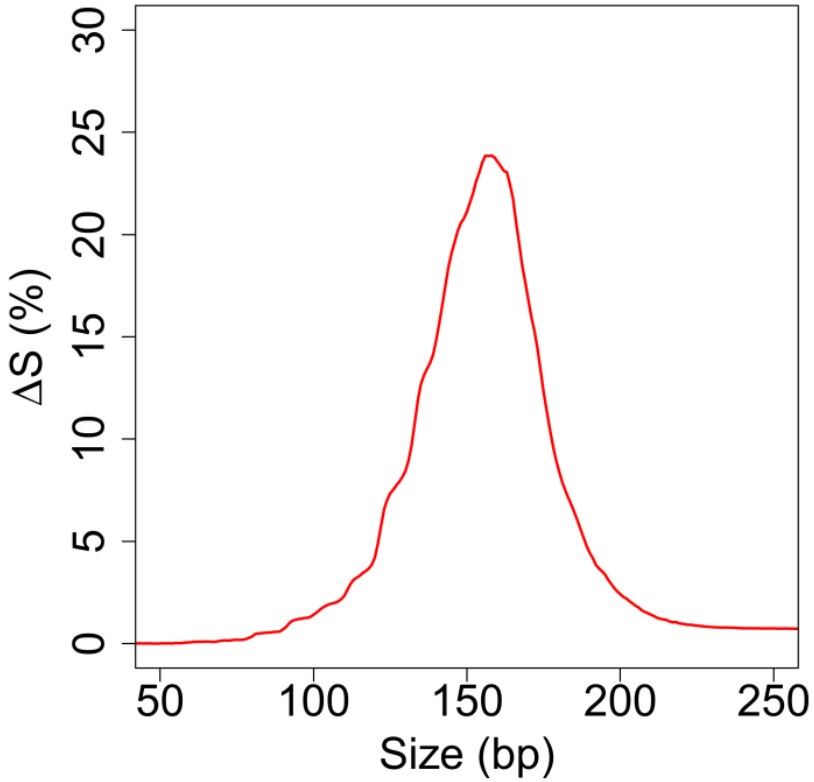


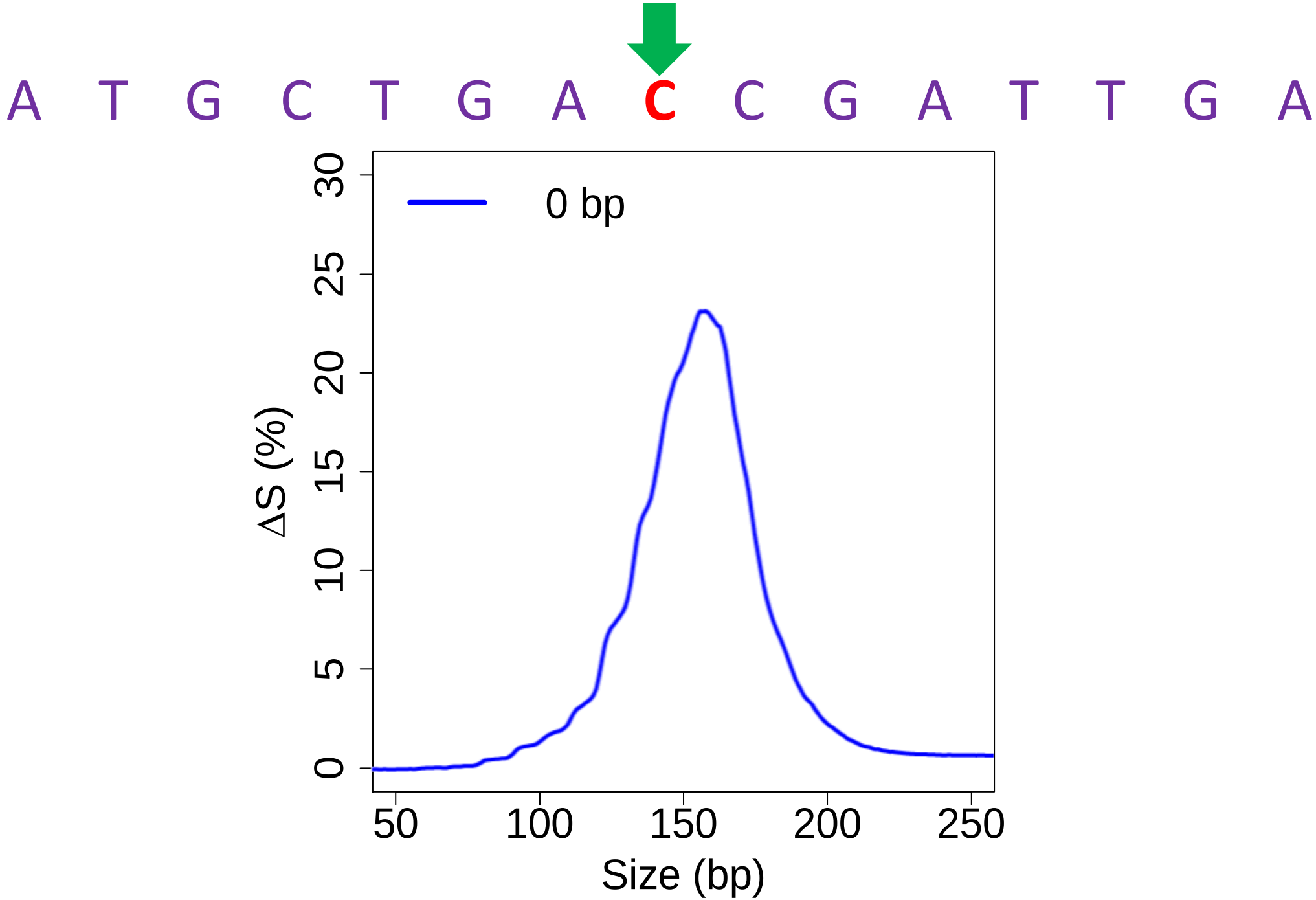


Size distribution of fragments with maternal- and fetal-preferred ends

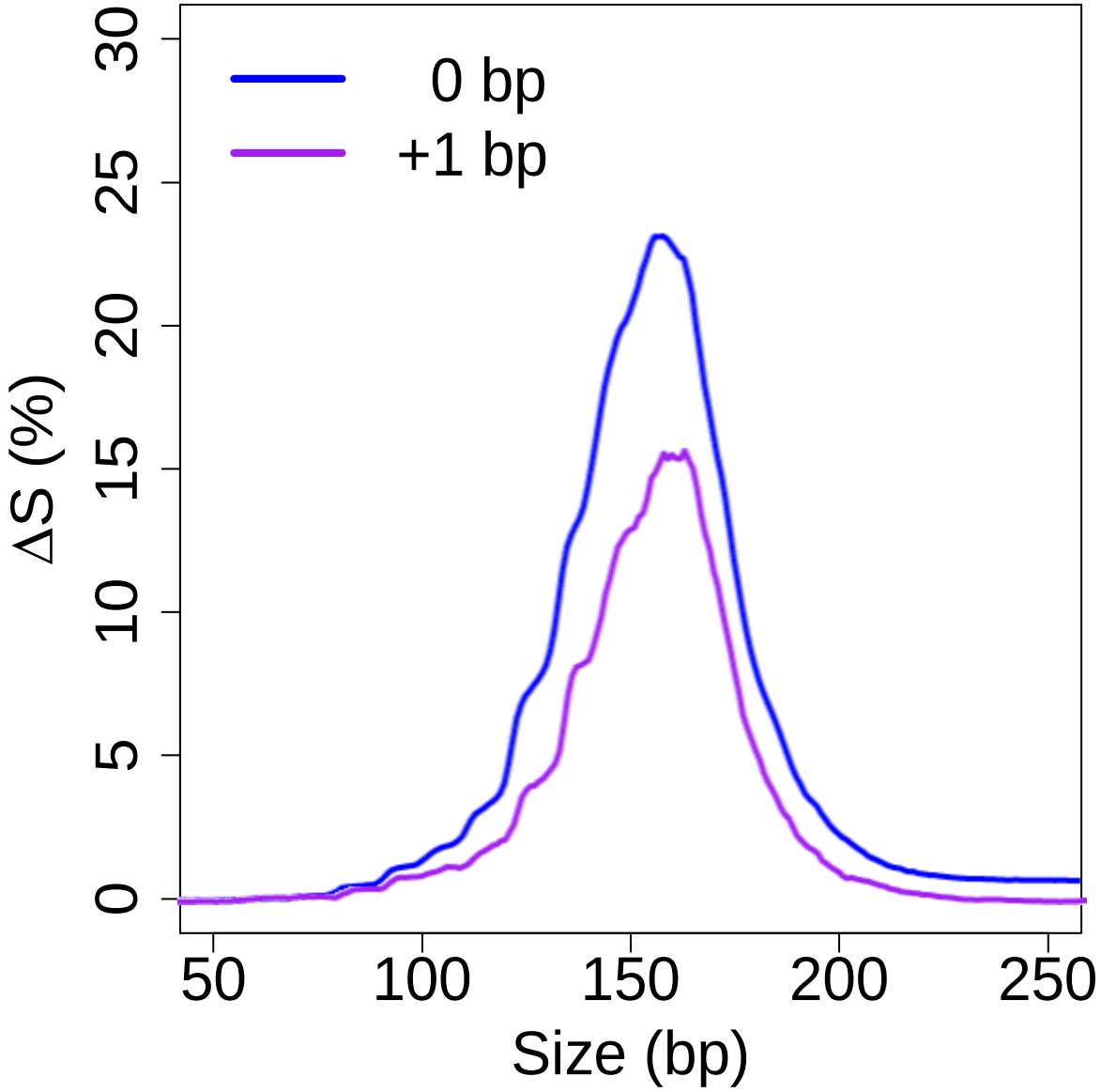




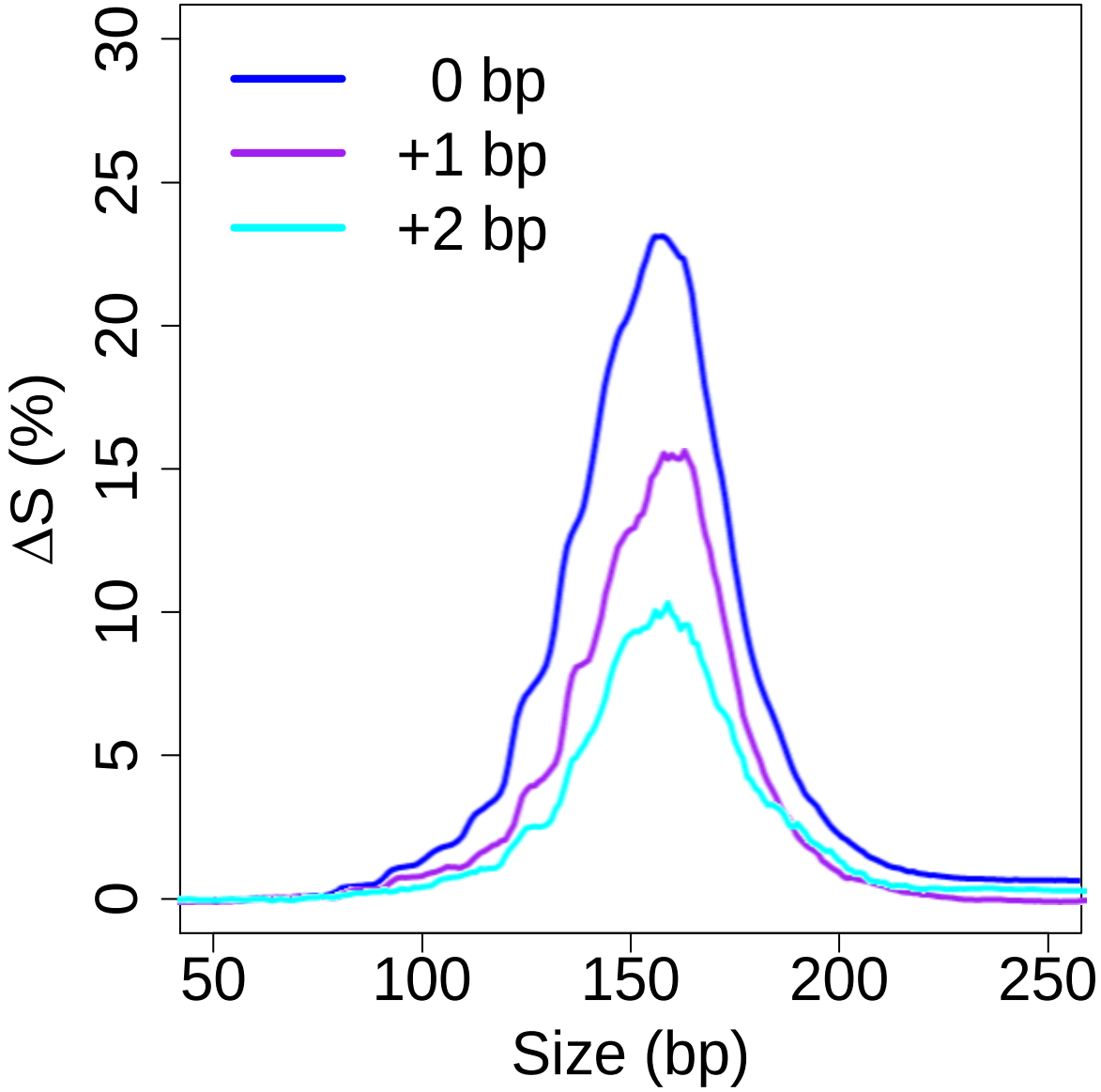



A T G C T G A C C G A T T G A
0 bp
ΔS (%)
0
5
10
15
20
25
30
50
100
150
200
250
Size (bp)

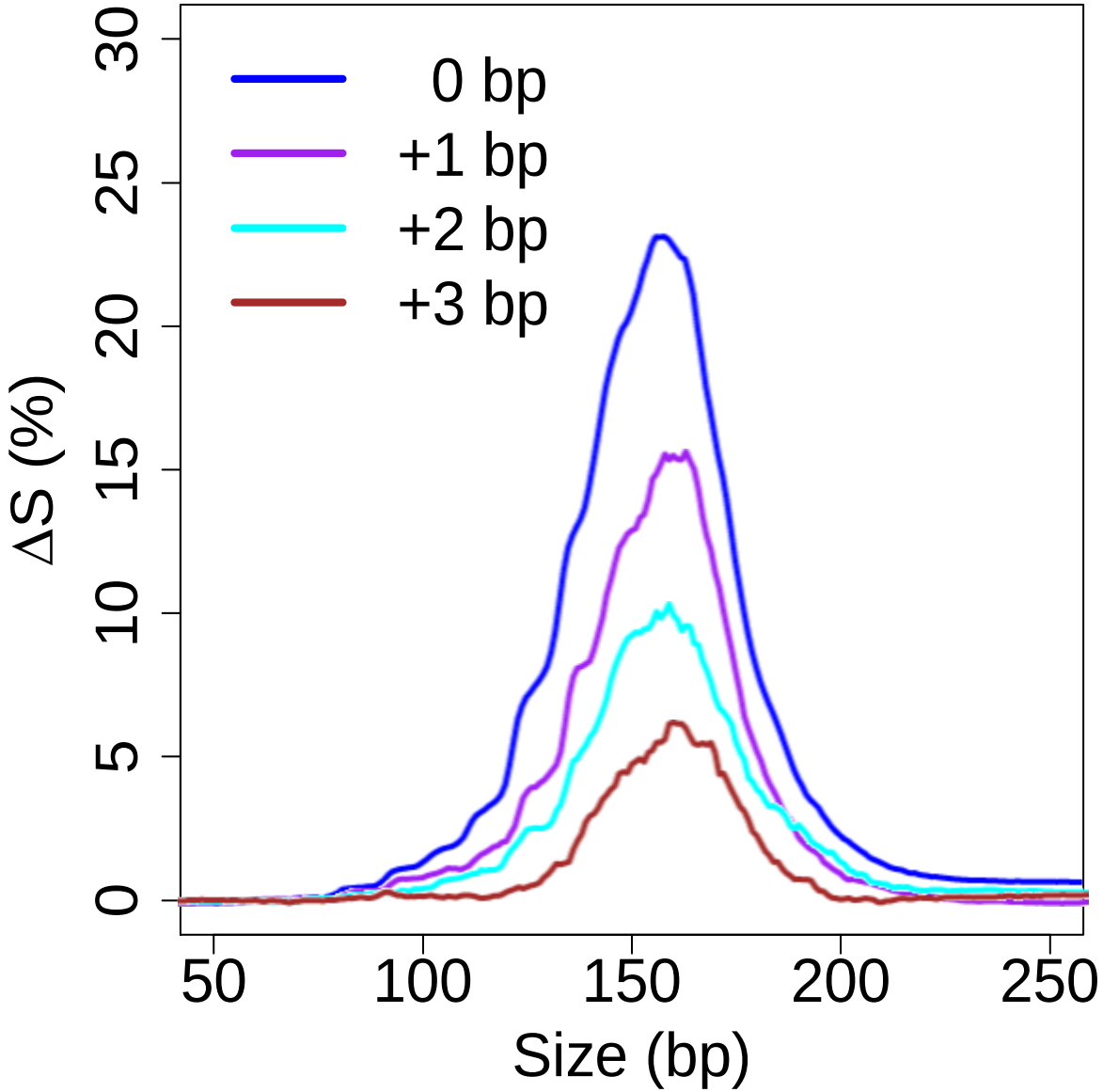
A T G C T G A C C G A T T G A



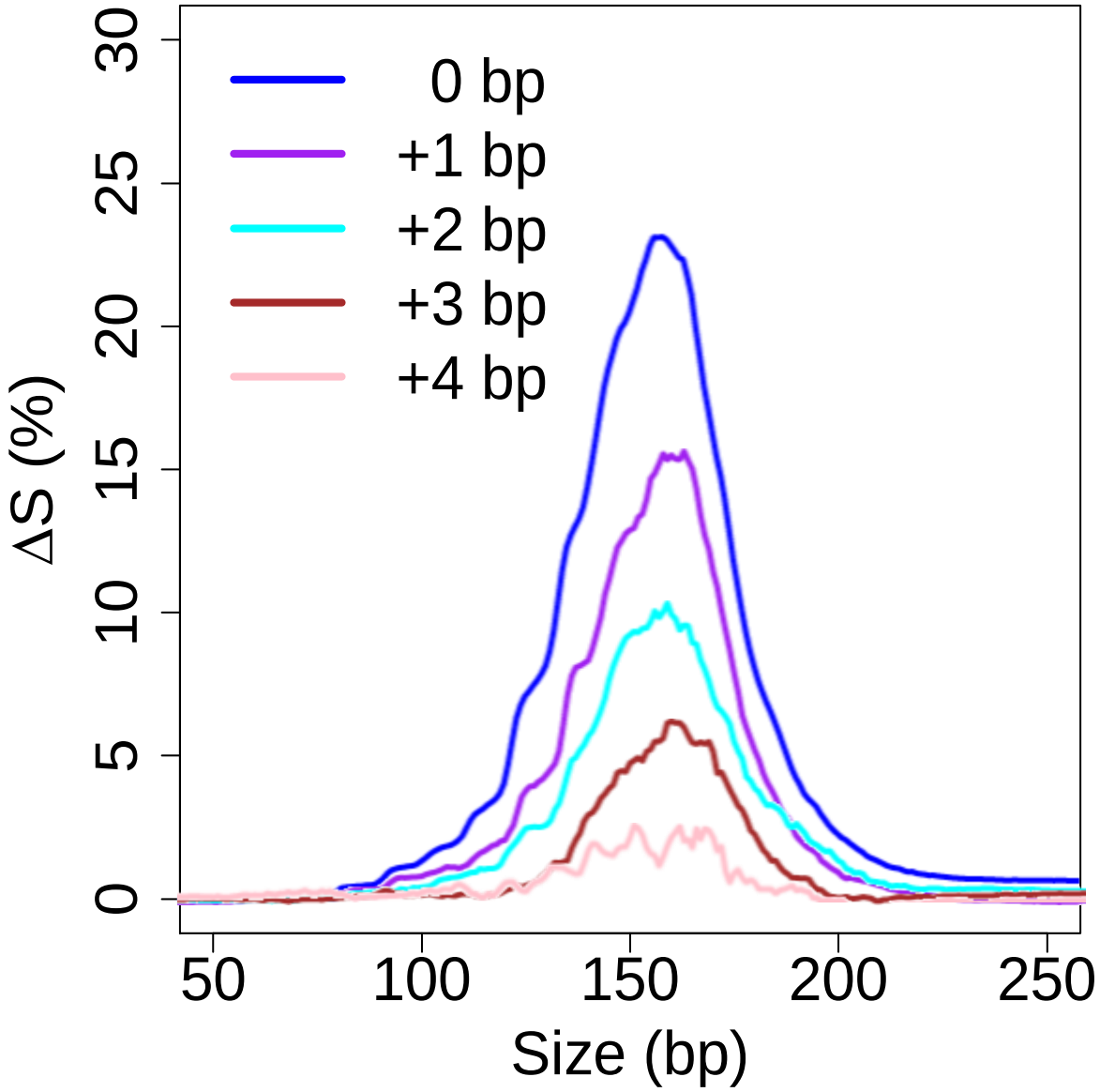
A T G C T G A C C G A T T G A



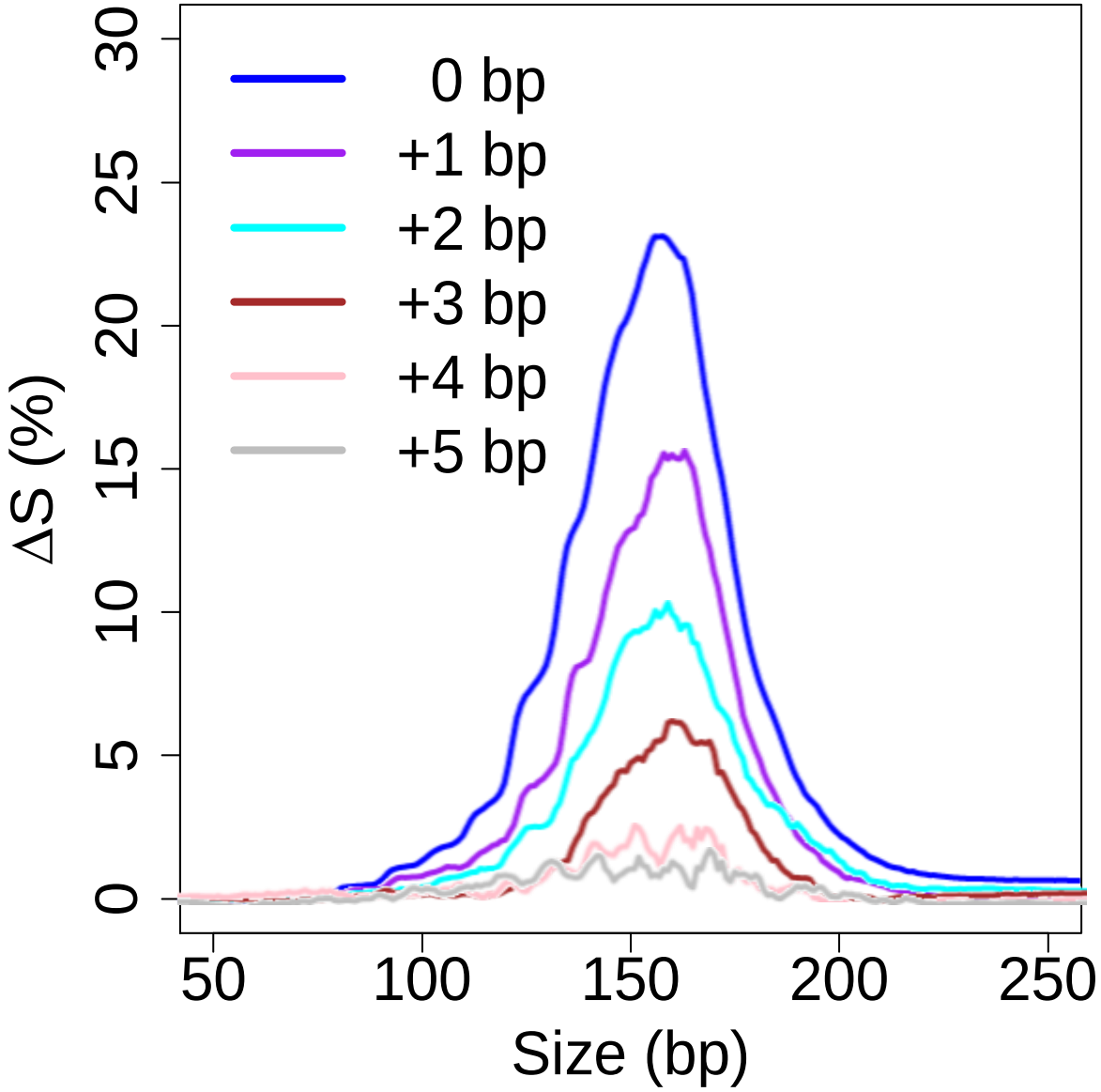
A T G C T G A C C G A T T G A



A T G C T G A C C G A T T G A

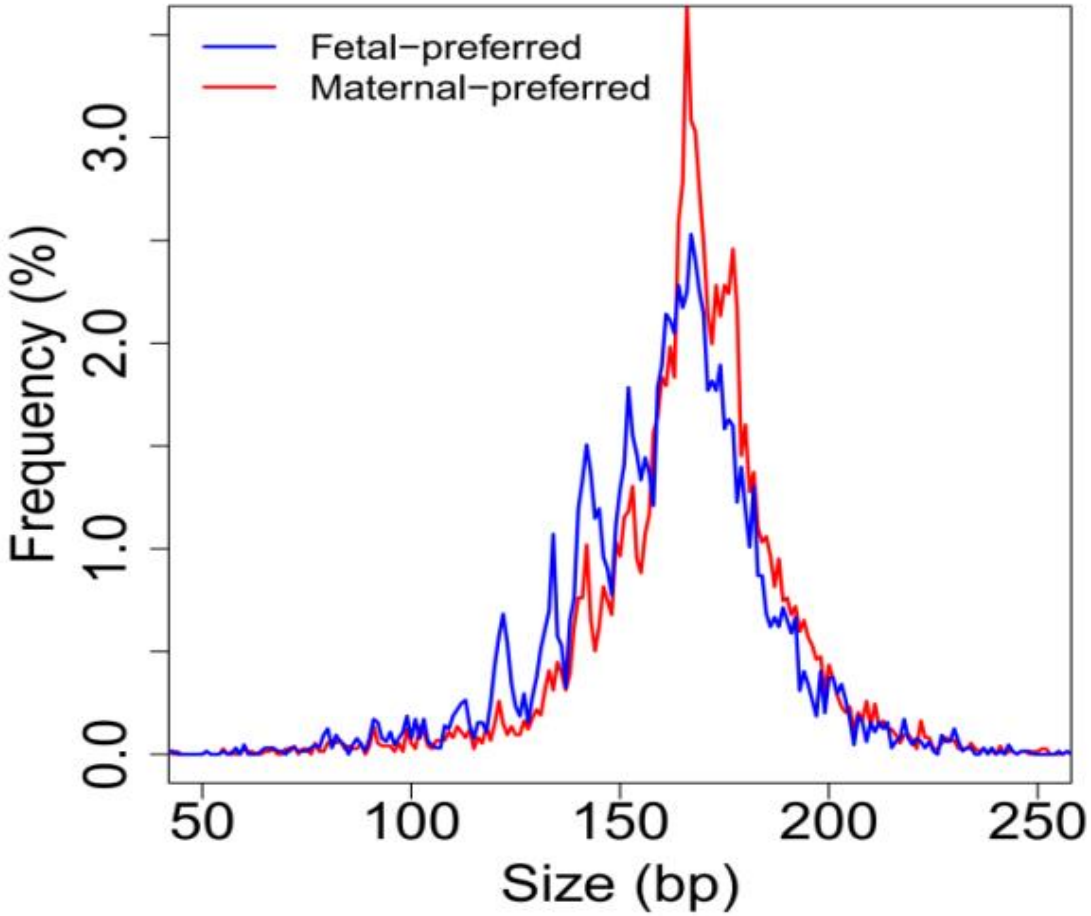


A T G C T G A C C G A T T G A

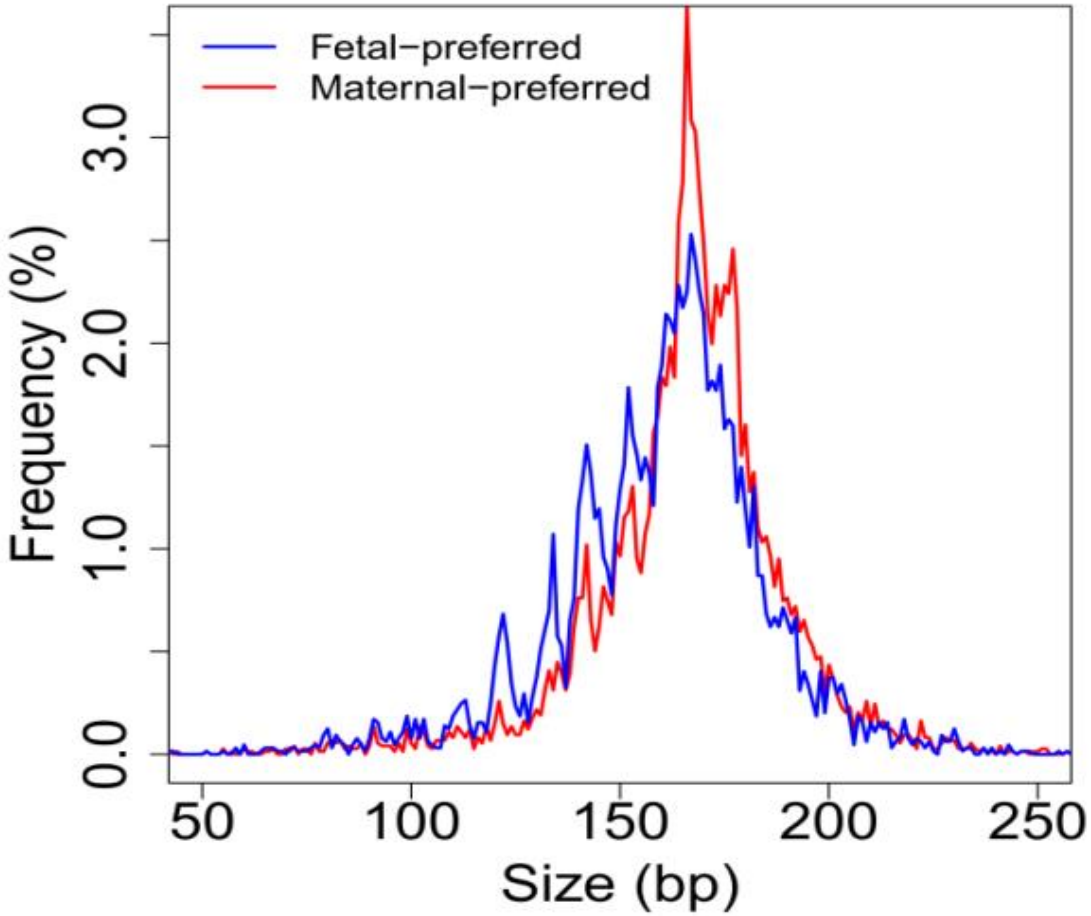


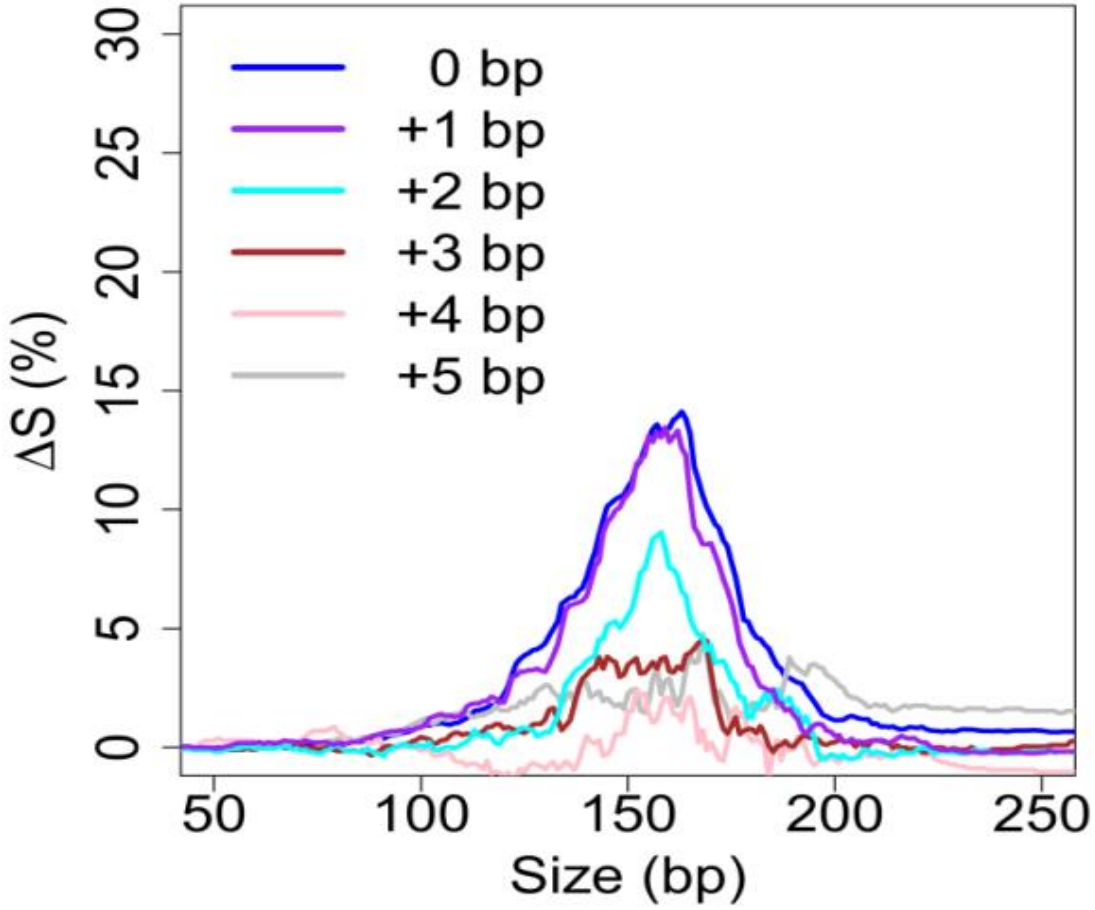

private
public

Pooled sequence reads from 26 first trimester cases

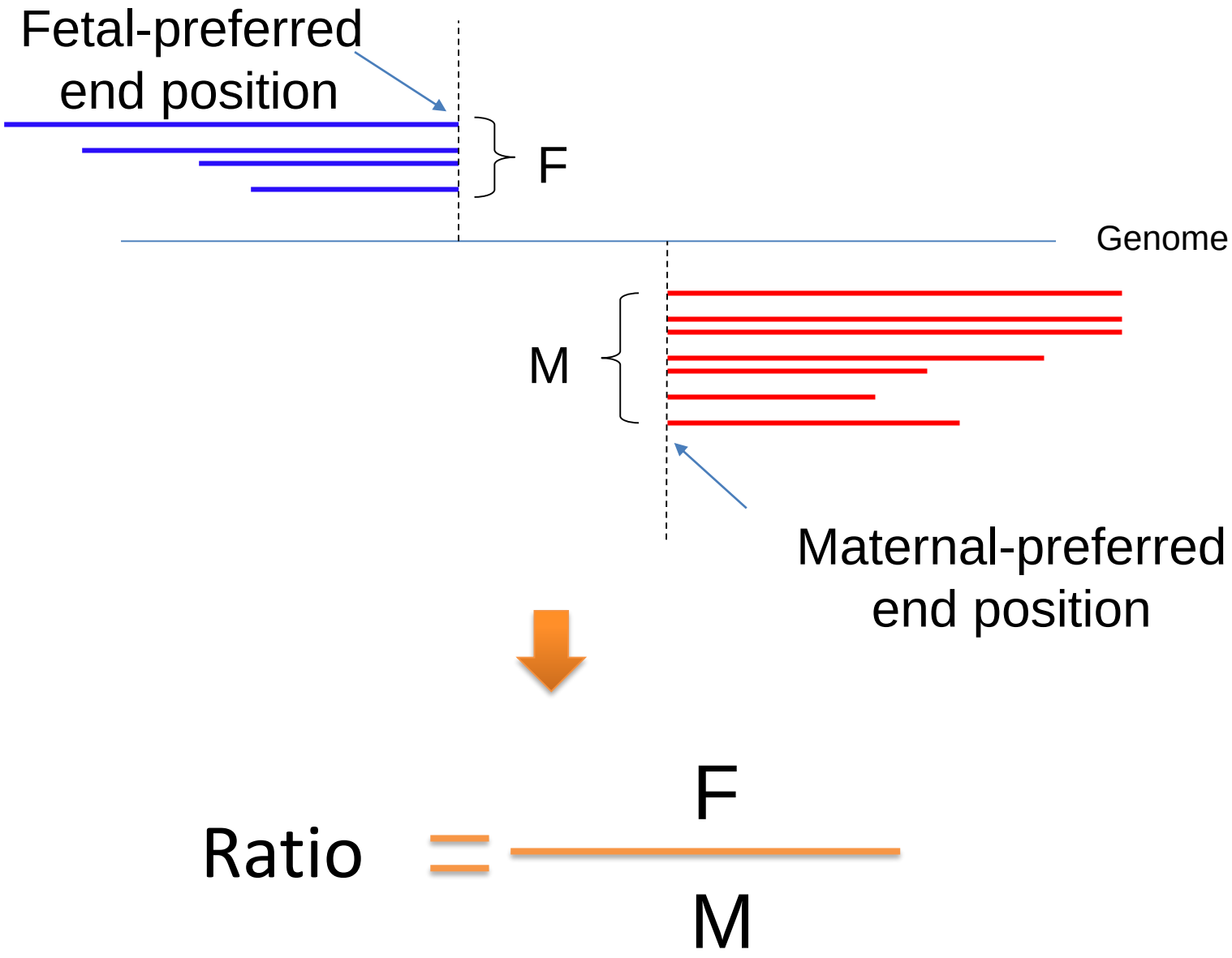


Pooled sequence reads from 26 first trimester cases

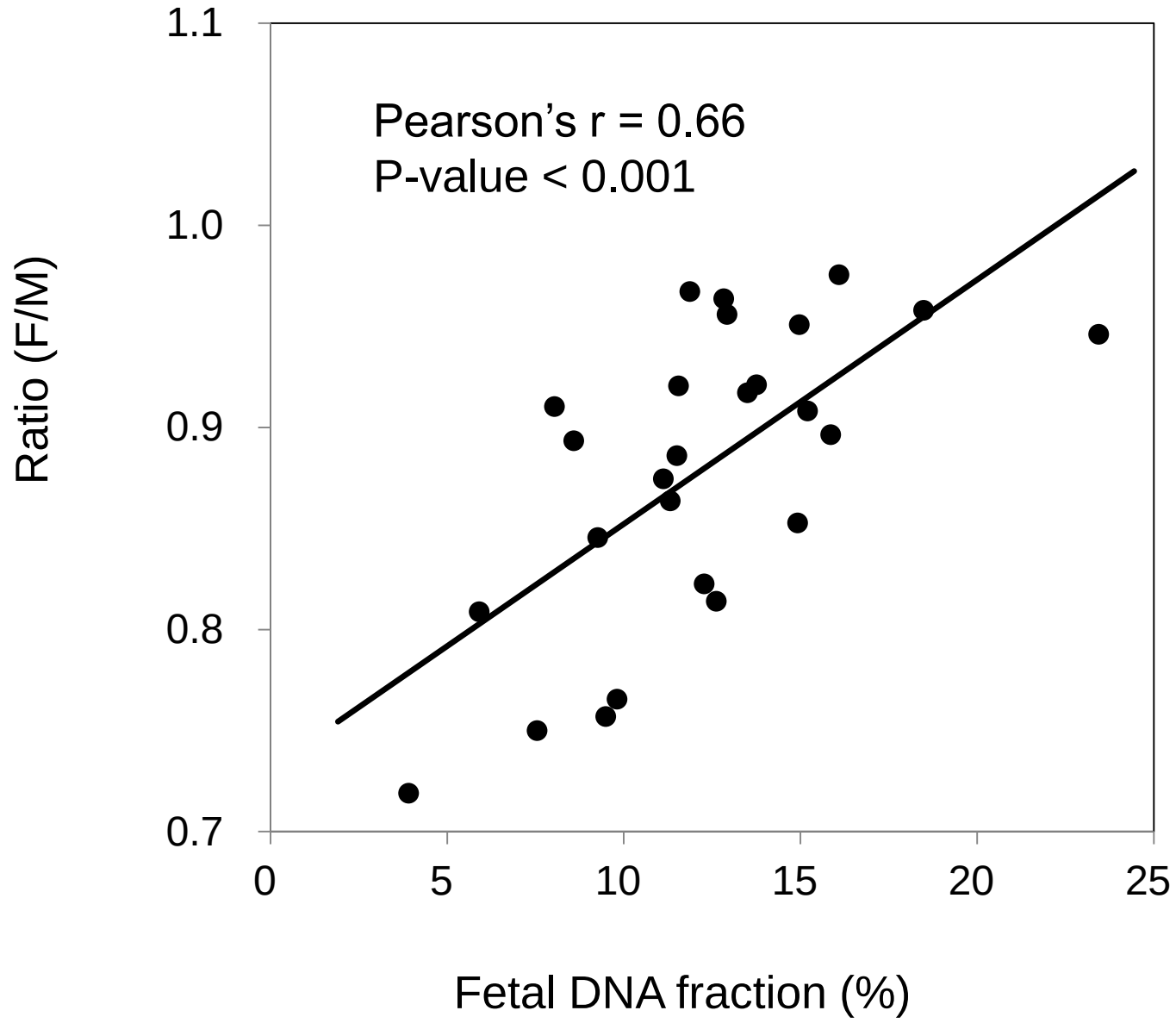




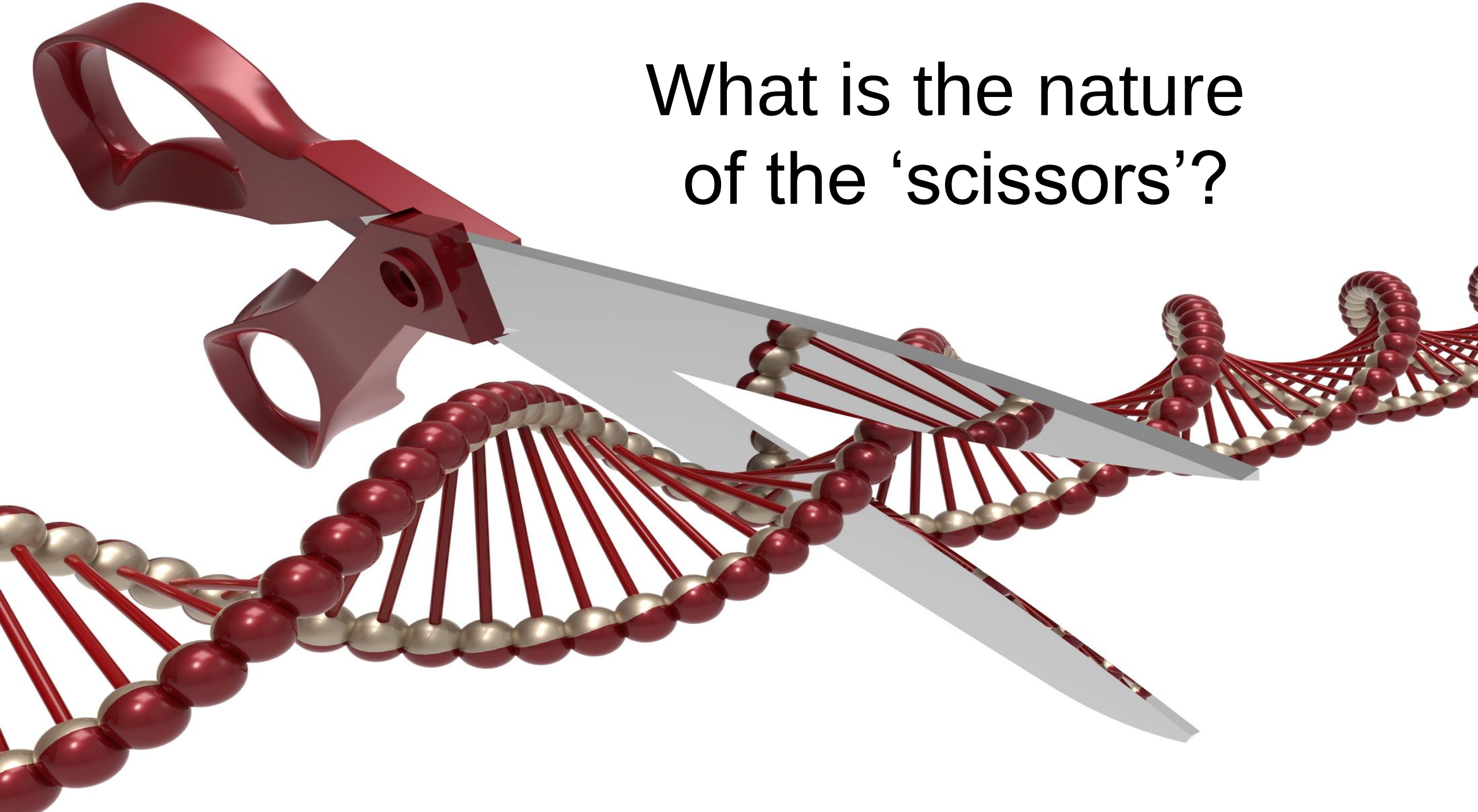
What's the potential utility?

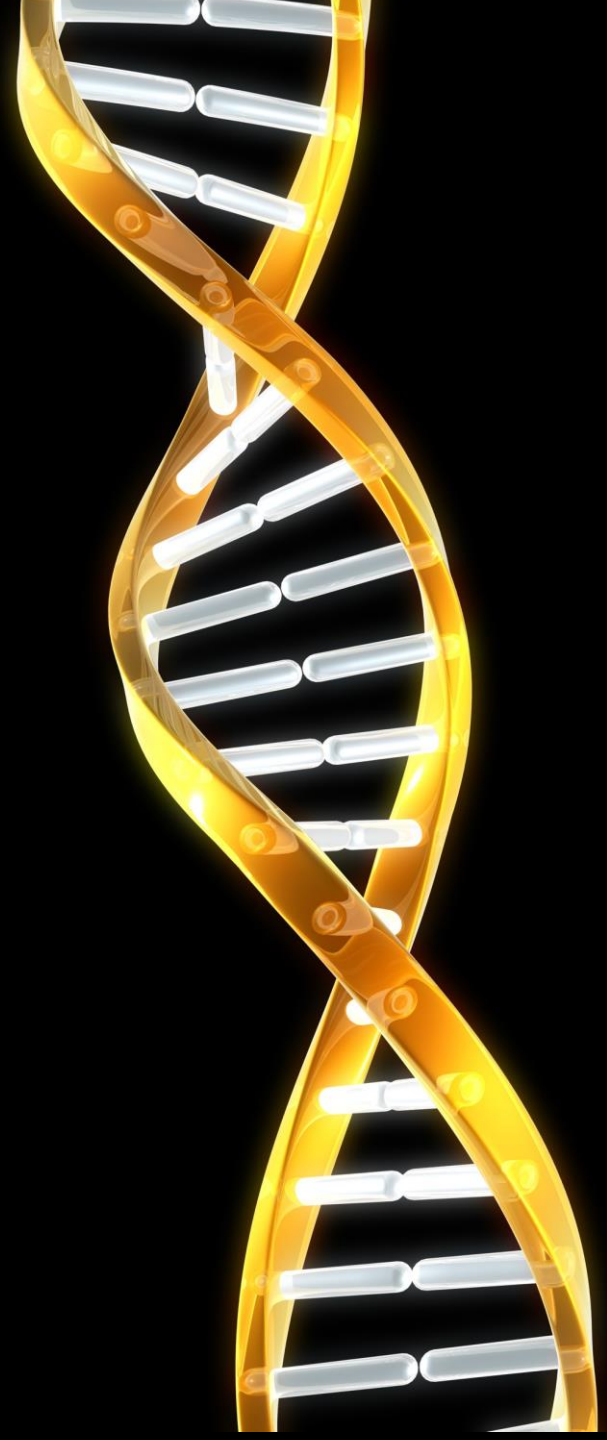

Fetal-preferred
end position
F
Genome
M
Maternal-preferred
end position
Ratio
F
M

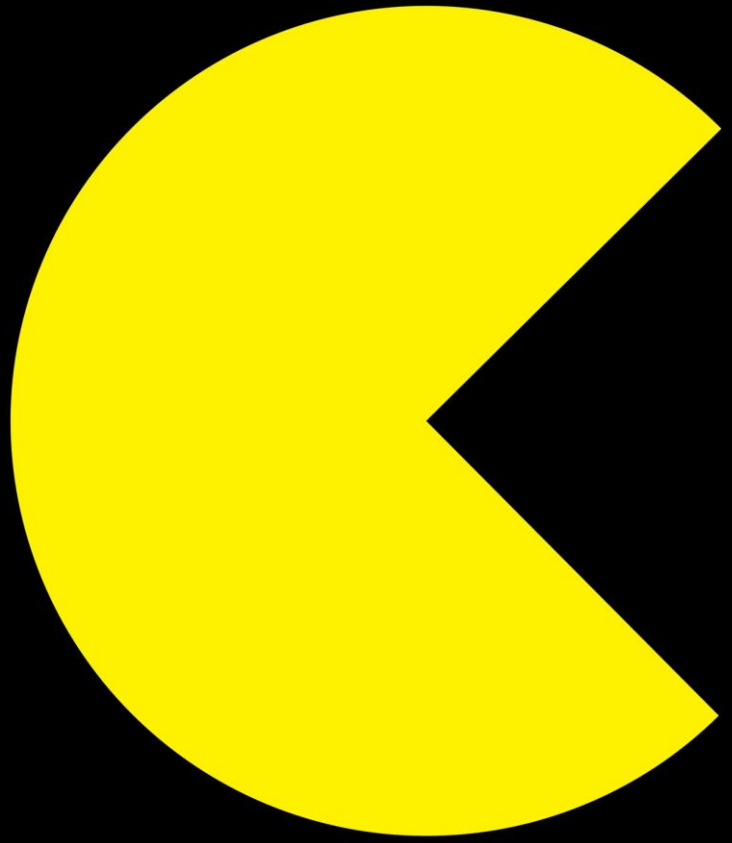
Correlation with fetal DNA fraction



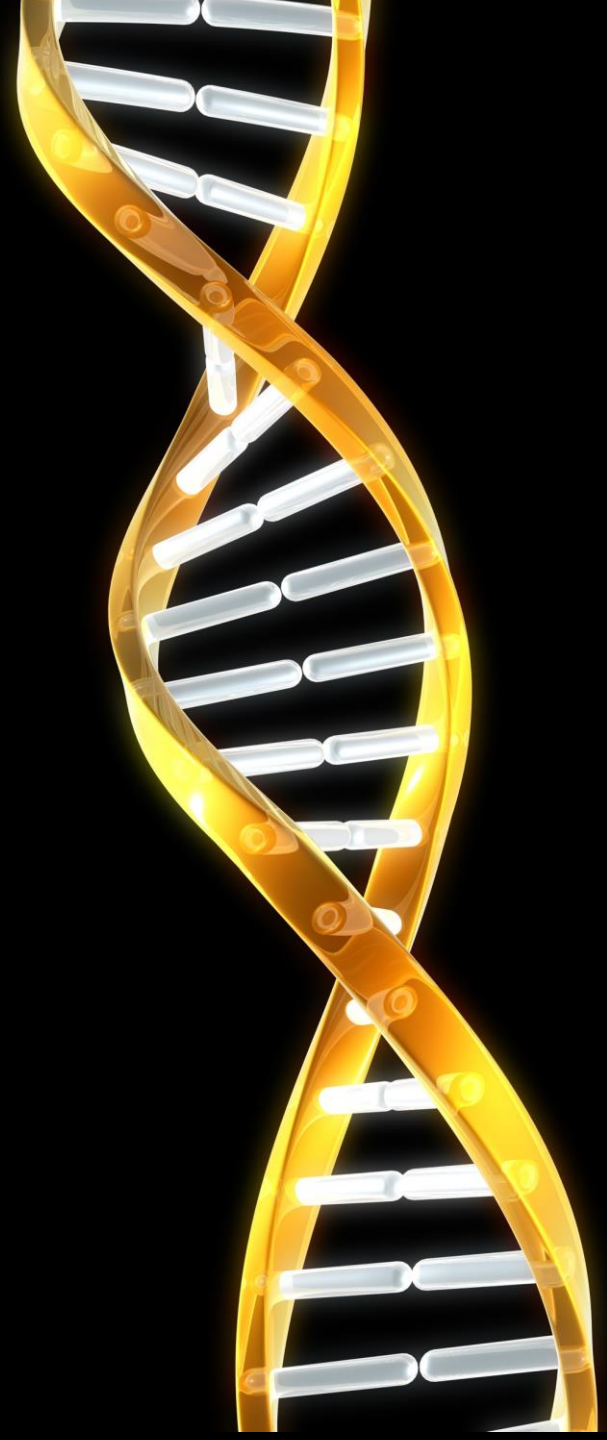
What is the nature of the ‘scissors’?

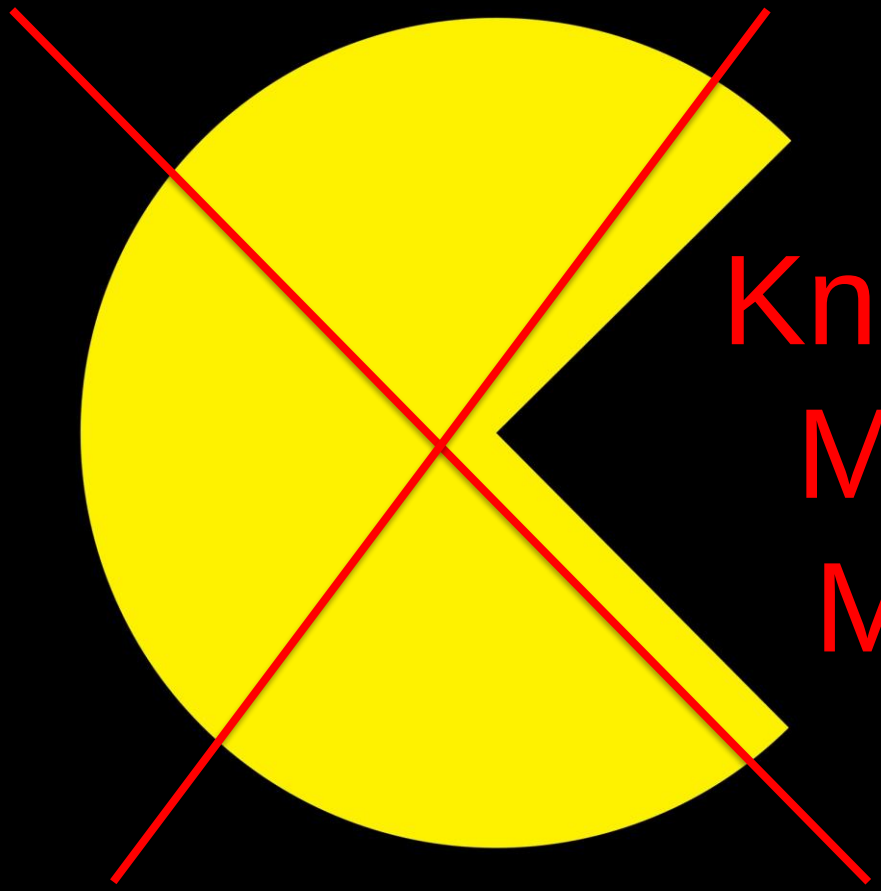




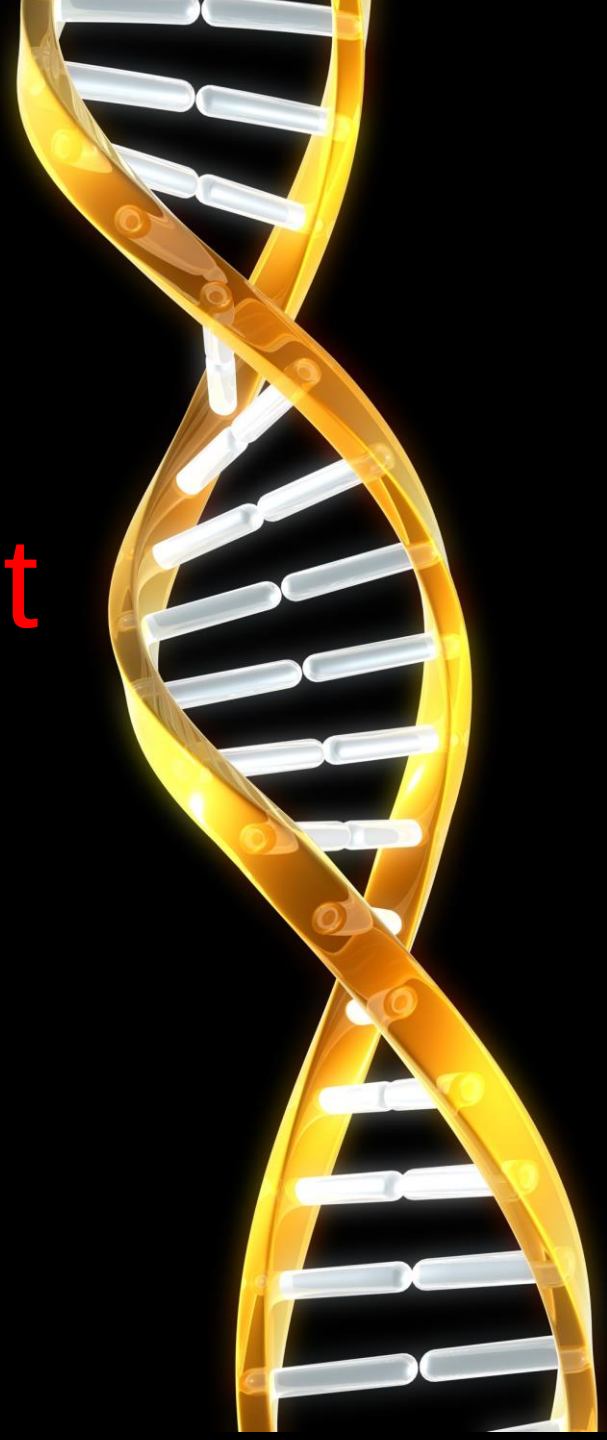


Nuclease



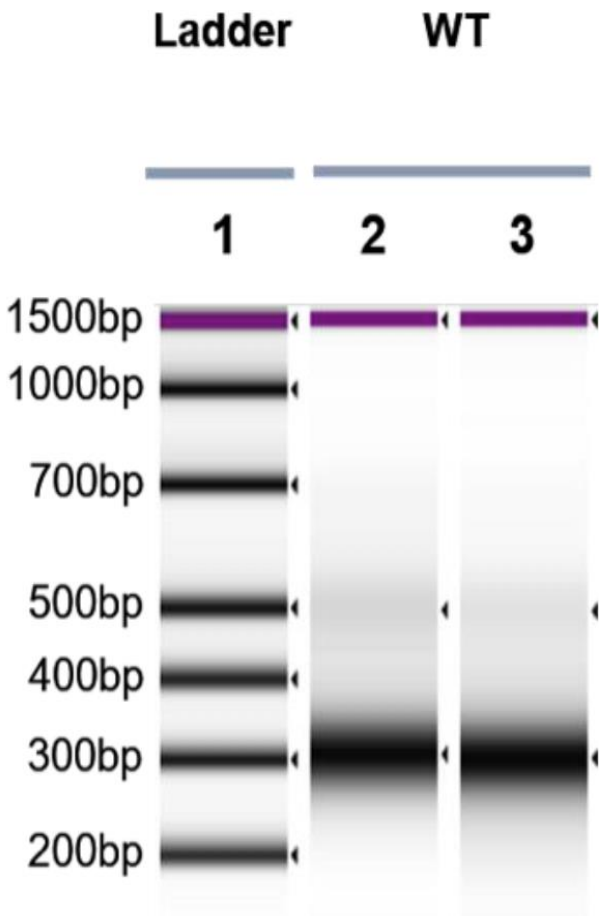


Knockout
Mouse
Model

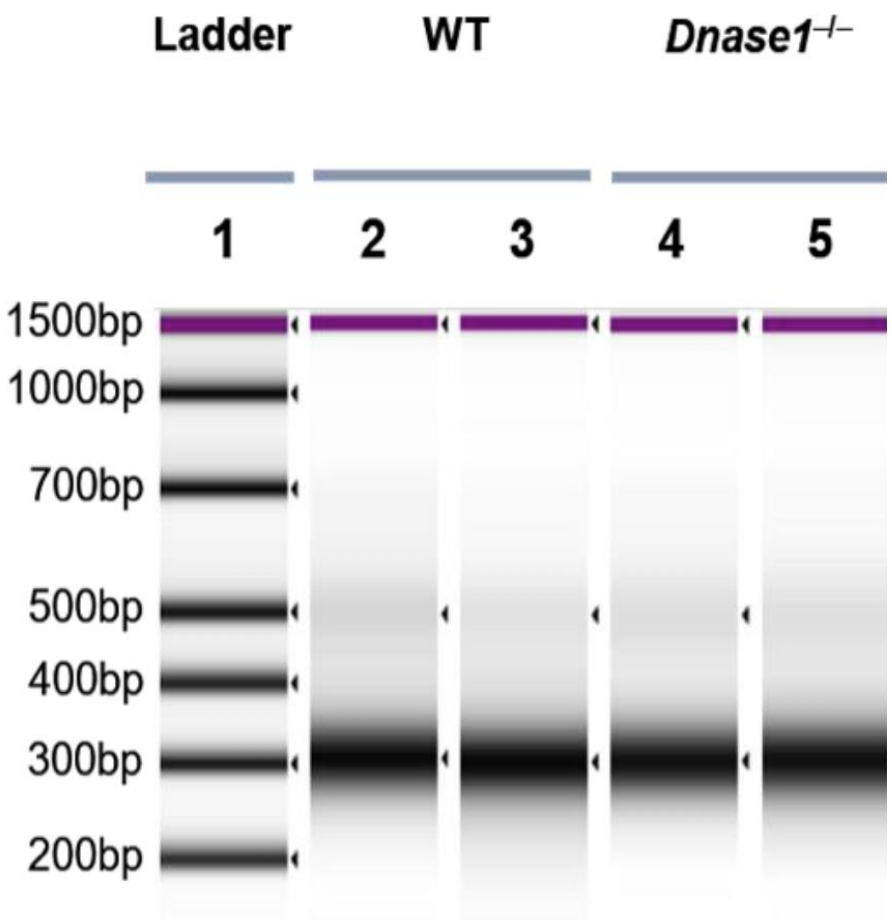


Nuclease

Illumina Sequencing Libraries



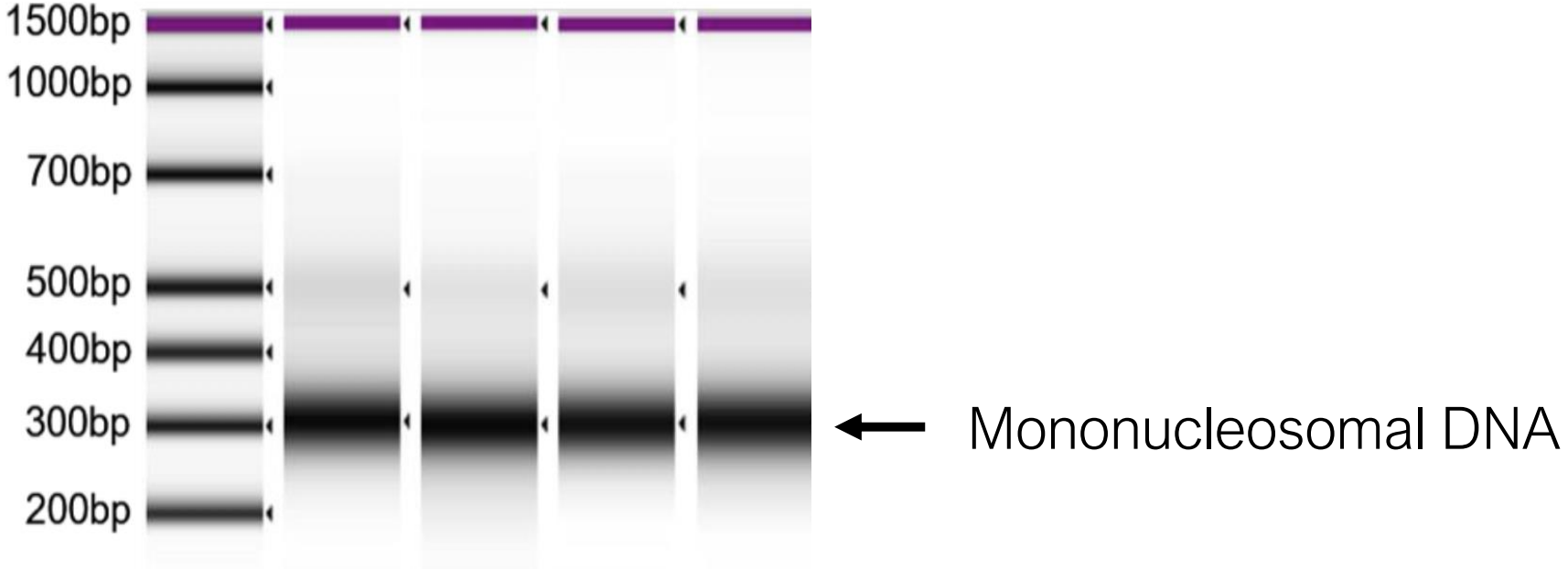
← Mononucleosomal DNA



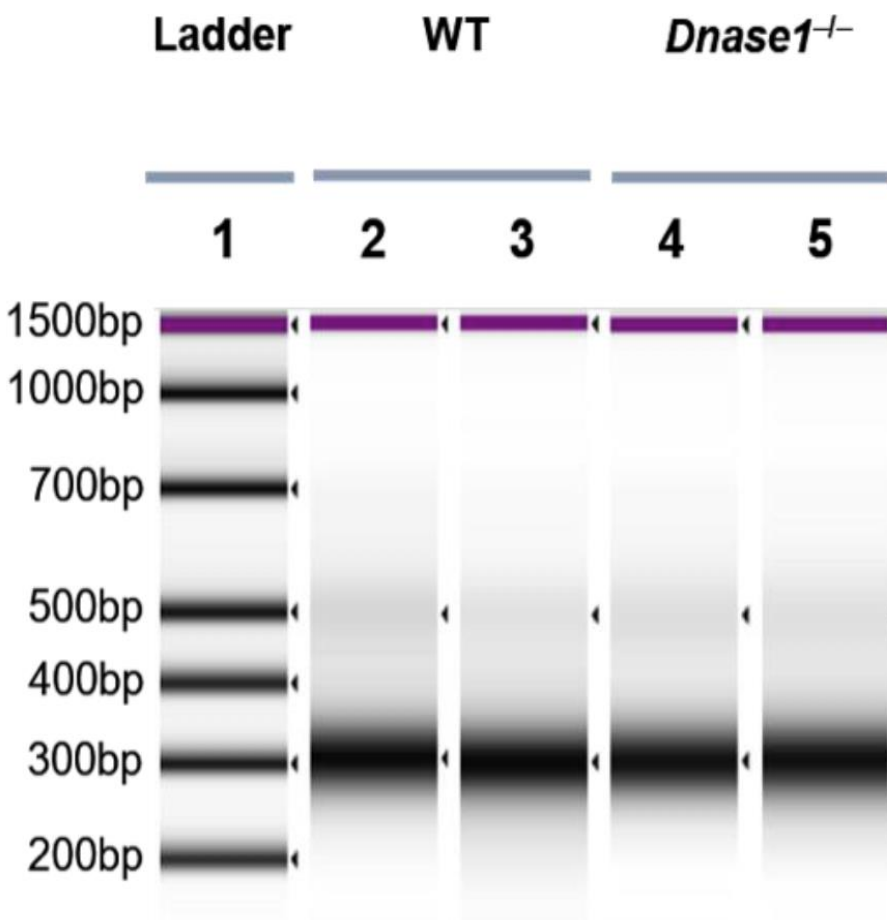
← Mononucleosomal DNA

DNase1 Does Not Appear to Play a Major Role in the Fragmentation of Plasma DNA in a Knockout Mouse Model.

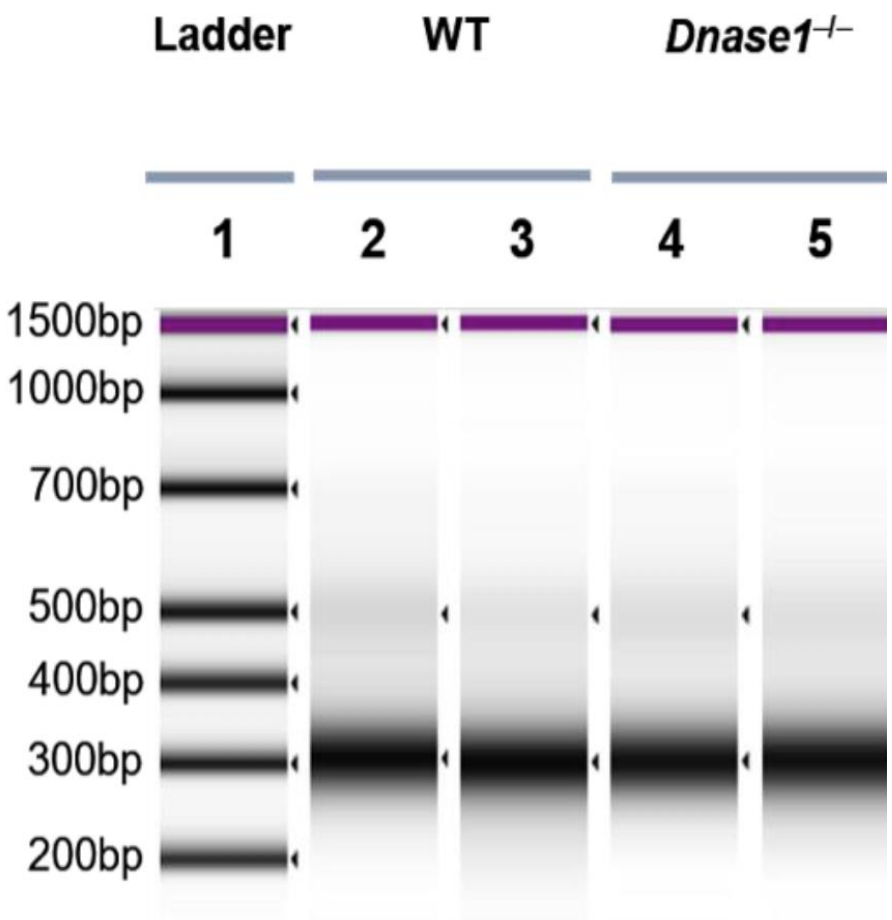
Cheng THT[1,2], Lui KO[1,2], Peng XL[1,2], Cheng SH[1,2], Jiang P[1,2], Chan KCA[1,2], Chiu RWK[1,2], Lo YMD[3,2].



Cheng et al. Clin Chem; 2018: 406

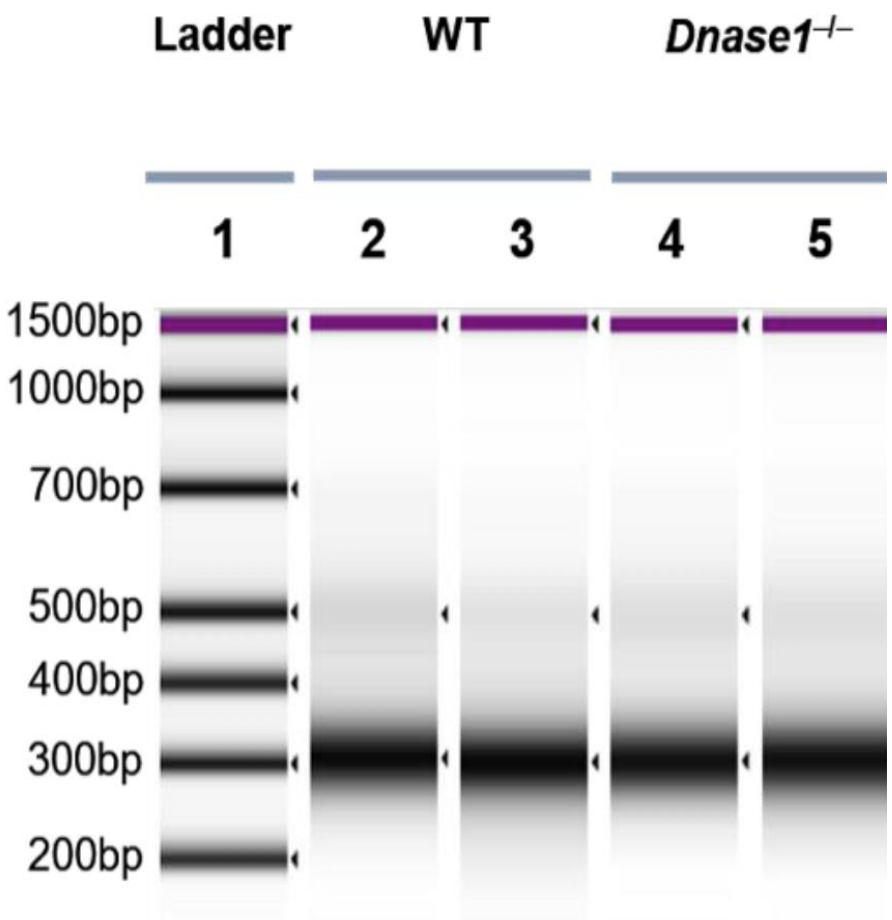


← Mononucleosomal DNA



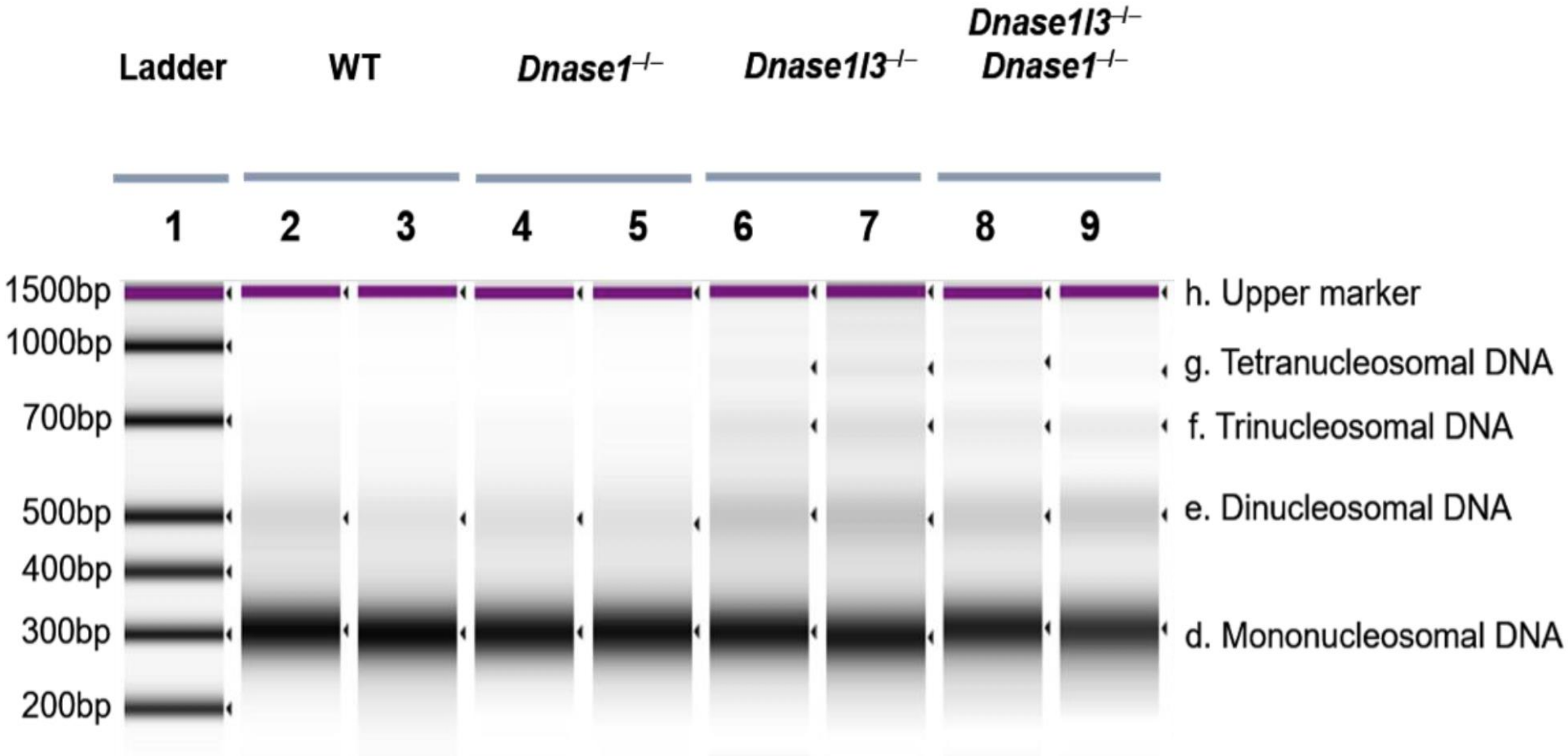
Another Nuclease?

← Mononucleosomal DNA

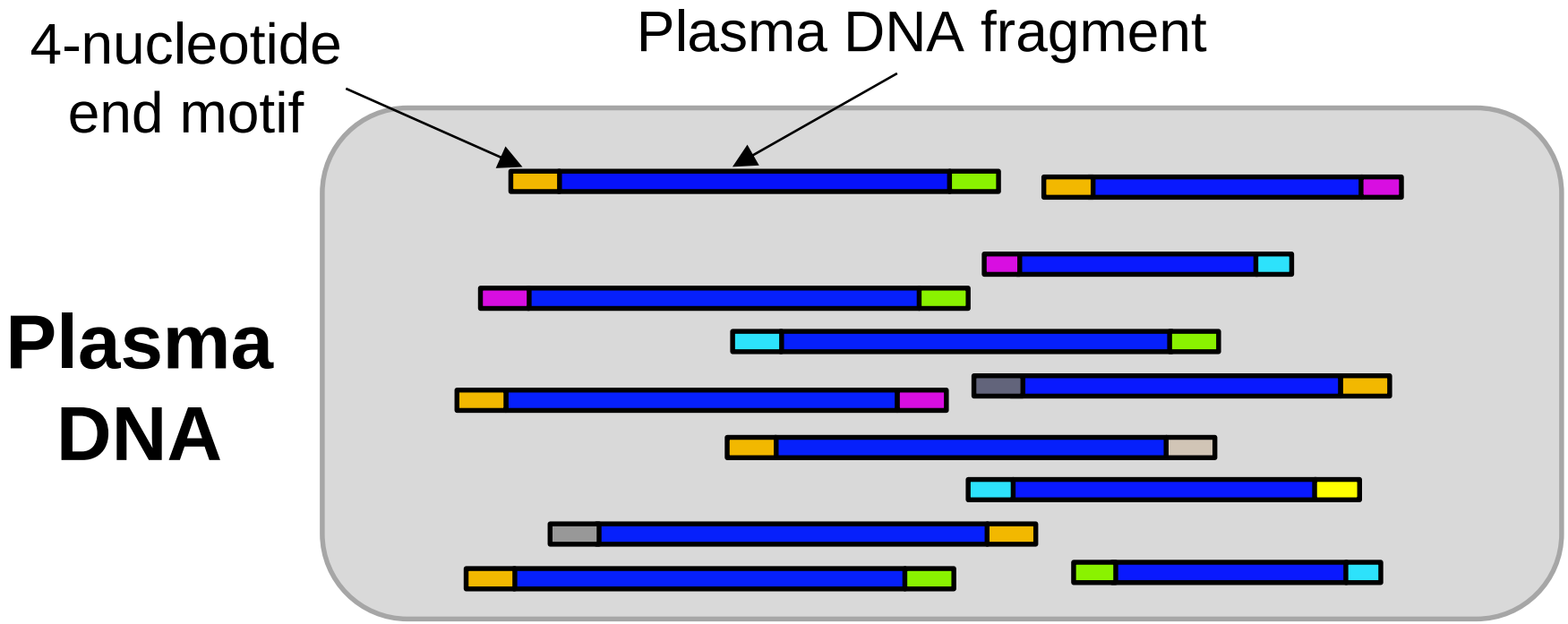


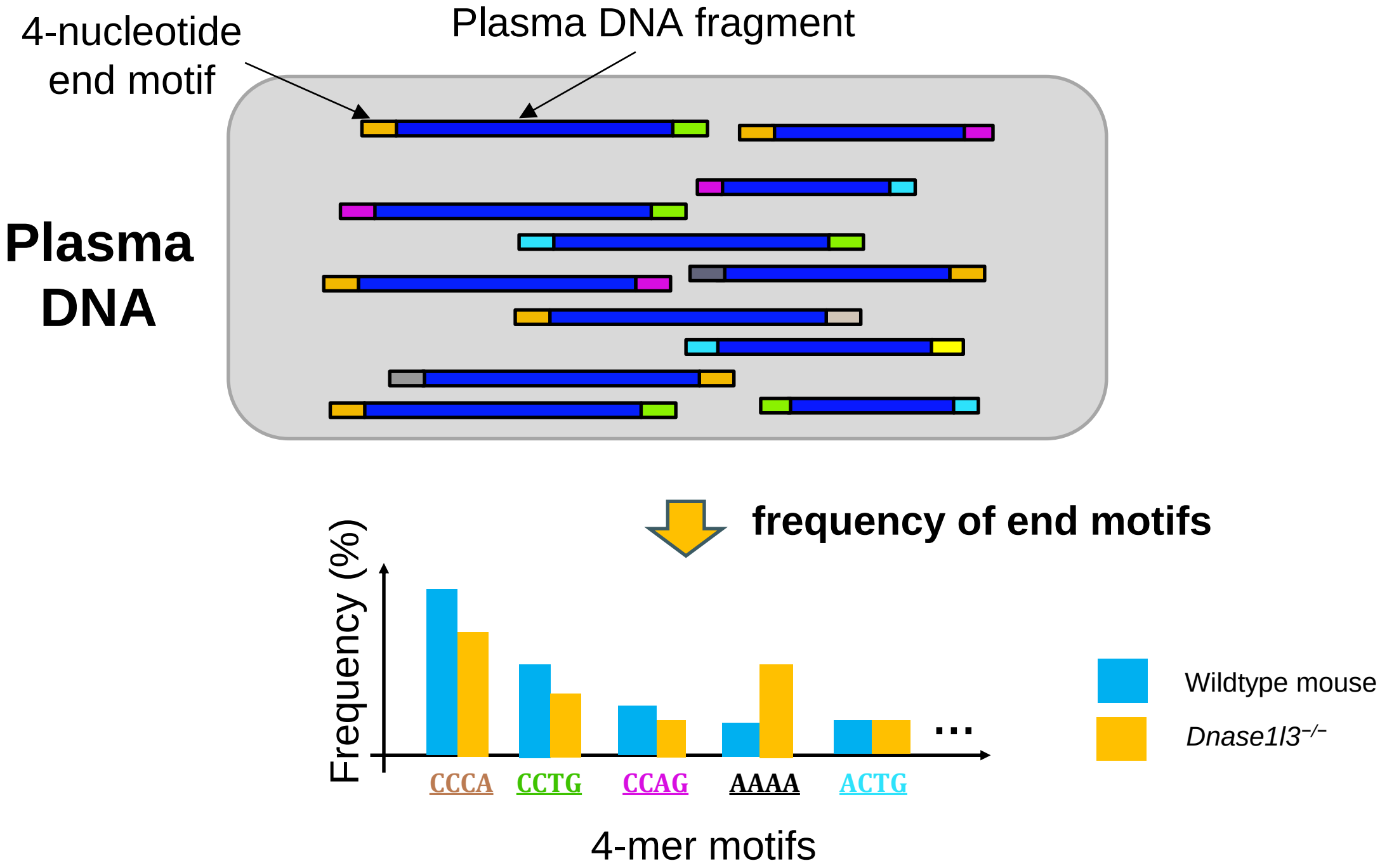
Another Nuclease?
DNASE 1-Like-3

← Mononucleosomal DNA



Serpas et al. PNAS 2019; 116: 641

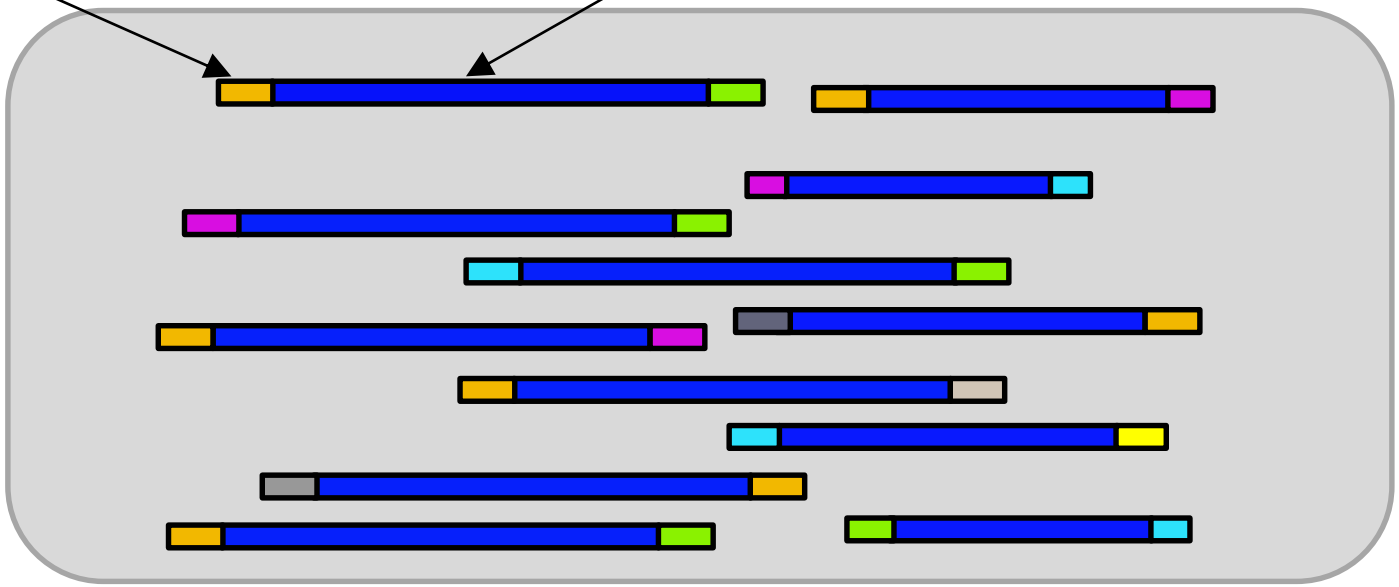

4-nucleotide
end motif
Plasma DNA fragment
Plasma
DNA


4-nucleotide
end motif
Plasma DNA fragment
Plasma
DNA
frequency of end motifs
Frequency (%)
CCCA
CCTG
CCAG
AAAA
ACTG
...
Wildtype mouse
Dnase1l3-/-
4-mer motifs

4-nucleotide end motif

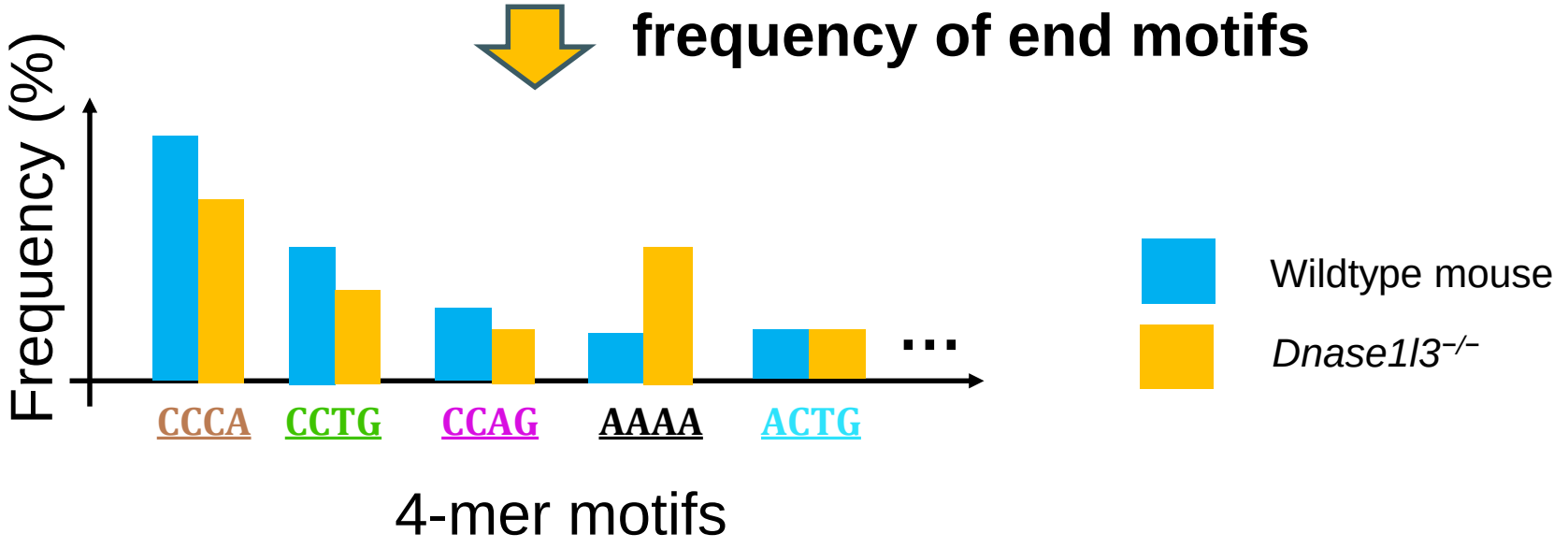
Plasma DNA fragment

Plasma DNA



1/256 (0.39%)

frequency of end motifs



Plasma DNA End Motifs

Motif	Motif frequency WT % (a)
CCCA	1.51
CCTG	1.45
CCAG	1.37
CCAA	1.12
CCAT	1.11
CCTC	1.10
CAAA	1.02
TGTG	0.98
TGTT	0.96
CCTA	0.87

Serpas et al. PNAS 2019; 116: 641

Plasma DNA End Motifs

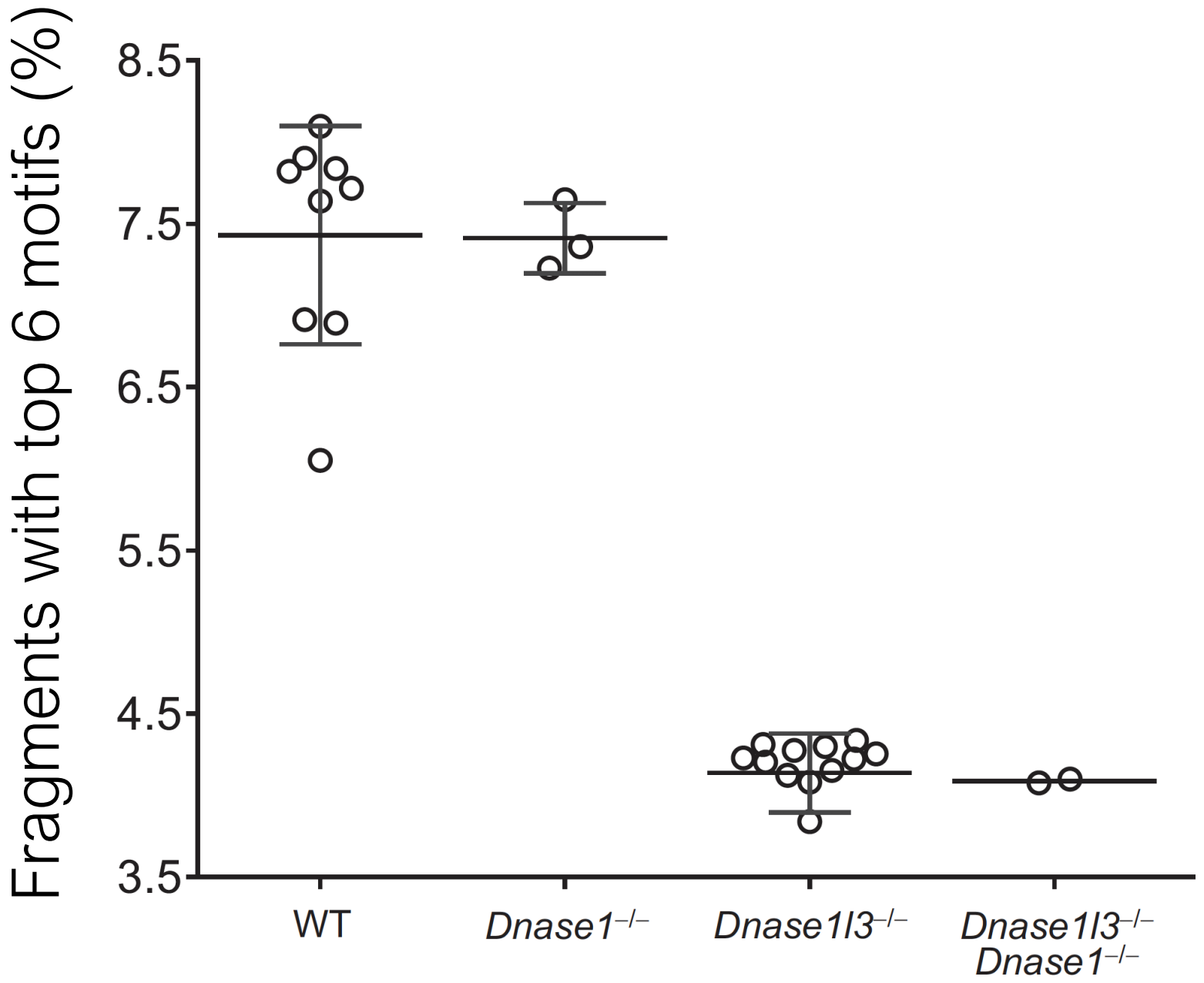
Motif	Motif frequency WT, % (a)	Motif frequency *Dnase1l3*$^{-/-}$, % (b)
CCCA	1.51	0.76
CCTG	1.45	0.80
CCAG	1.37	0.56
CCAA	1.12	0.51
CCAT	1.11	0.67
CCTC	1.10	0.93
CAAA	1.02	0.78
TGTG	0.98	0.51
TGTT	0.96	0.58
CCTA	0.87	0.52

Serpas et al. PNAS 2019; 116: 641

Plasma DNA End Motifs

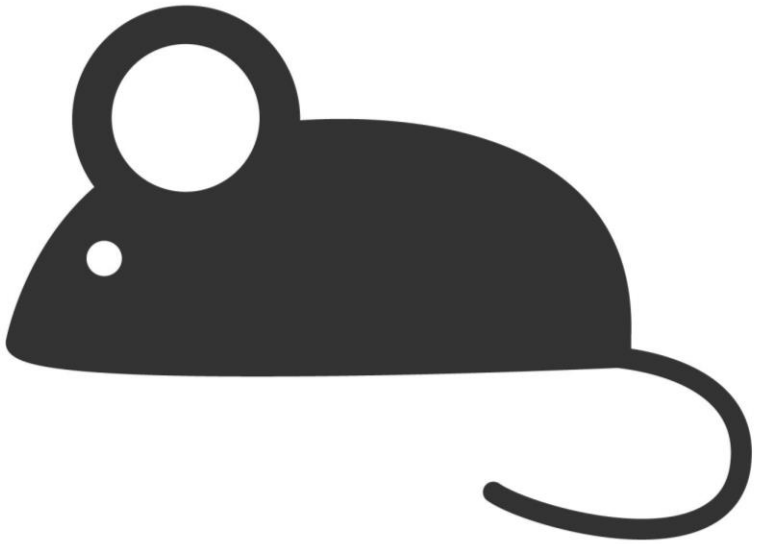
Motif	Motif frequency WT, % (a)	Motif frequency *Dnase1l3*$^{-/-}$, % (b)
CCCA	1.51	0.76
CCTG	1.45	0.80
CCAG	1.37	0.56
CCAA	1.12	0.51
CCAT	1.11	0.67
CCTC	1.10	0.93
CAAA	1.02	0.78
TGTG	0.98	0.51
TGTT	0.96	0.58
CCTA	0.87	0.52

Serpas et al. PNAS 2019; 116: 641


Fragments with top 6 motifs (%)
8.5
7.5
6.5
5.5
4.5
3.5
WT
Dnase1−/−
Dnase1l3−/−
Dnase1l3−/−
Dnase1−/−

Female: B6

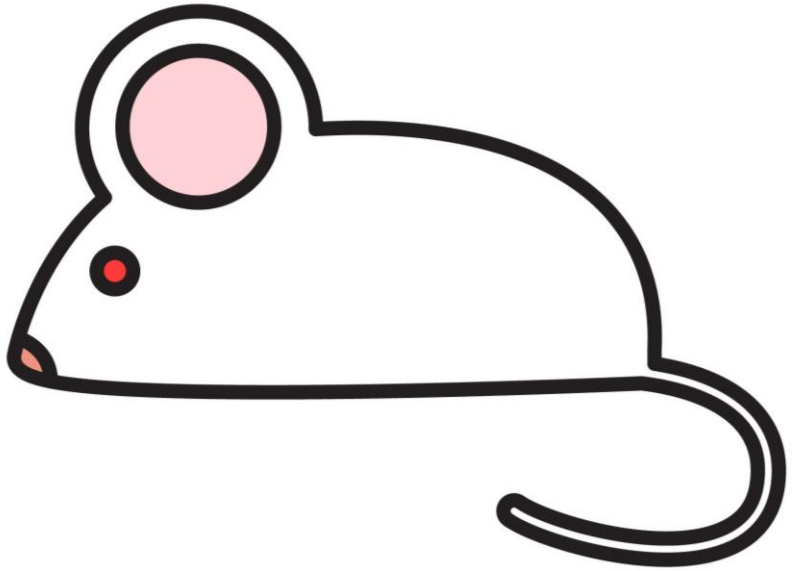
Dnase1l3$^{-/-}$



X

Male: Balb/C

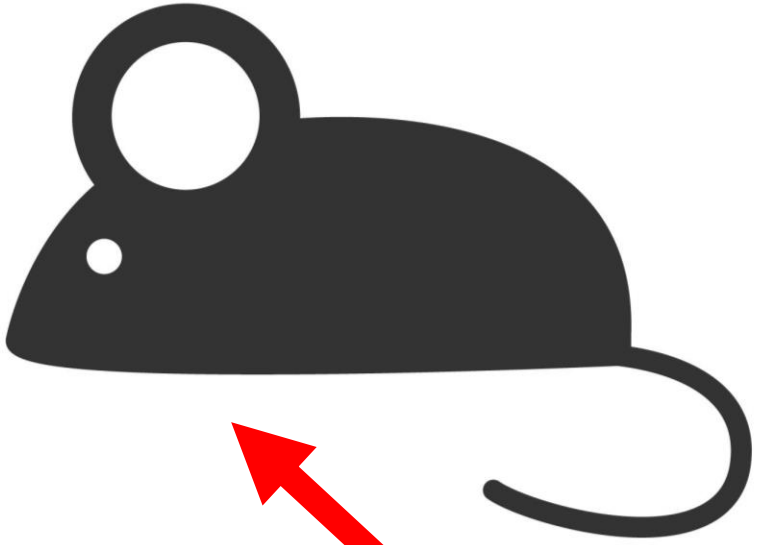
Dnase1l3$^{+/+}$



Fetuses: *Dnase1l3*$^{+/-}$

Female: B6

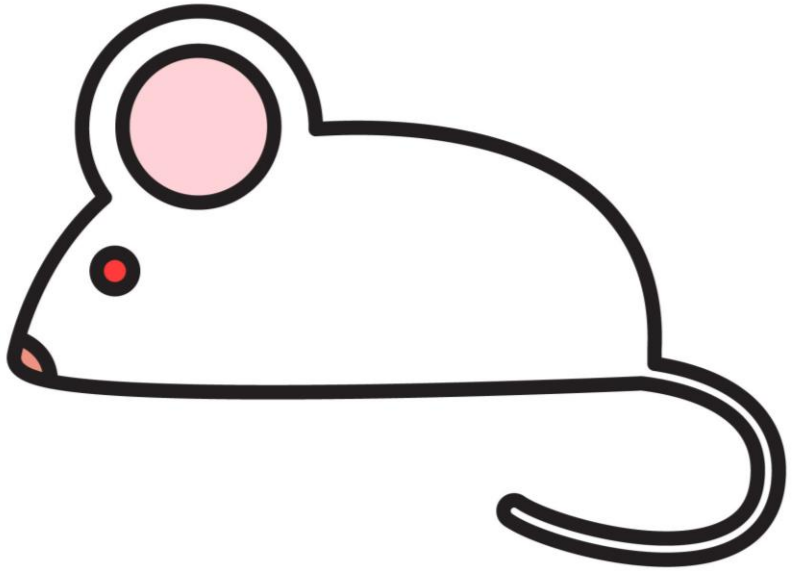
Dnase1l3$^{-/-}$



X

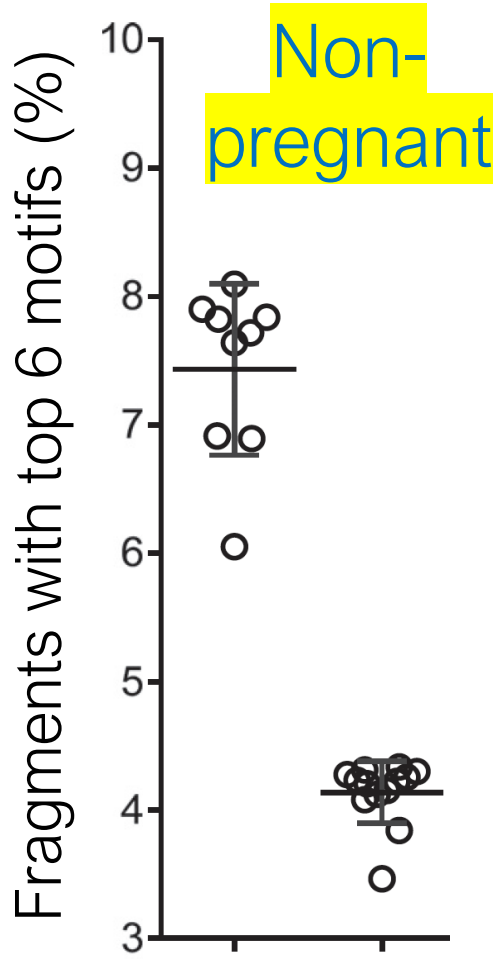
Male: Balb/C

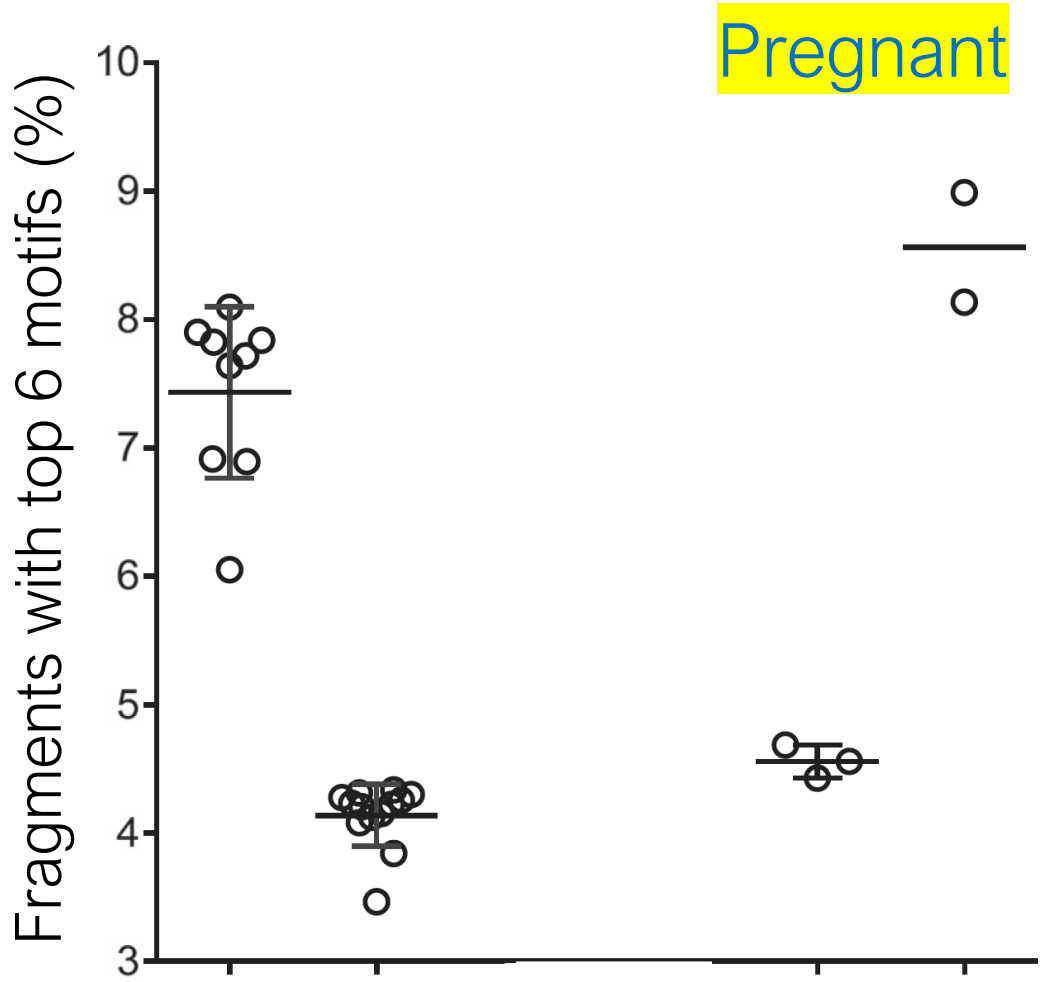
Dnase1l3$^{+/+}$



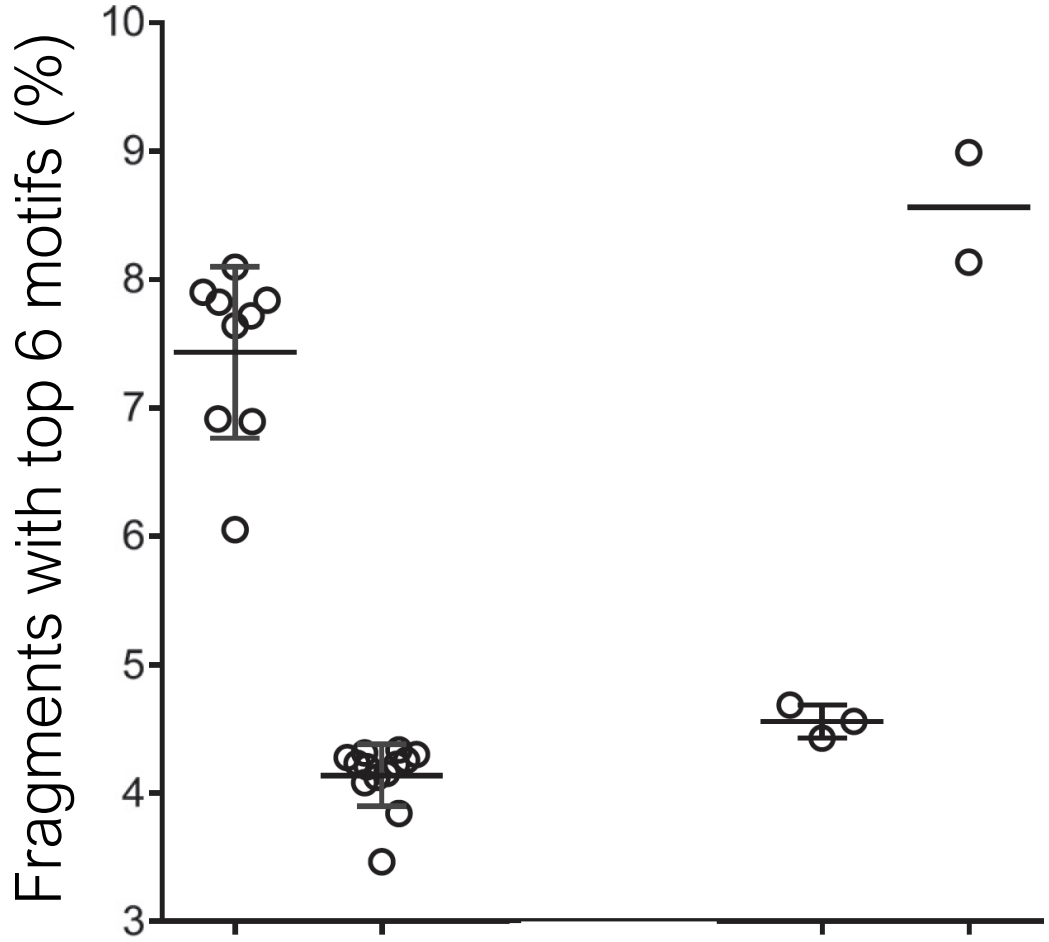
fetal DNA

Fetuses: *Dnase1l3*$^{+/-}$


Non-pregnant
Fragments with top 6 motifs (%)
10
9
8
7
6
5
4
3
+/+
-/-
Dnase1l3
Genotype


Pregnant
Fragments with top 6 motifs (%)
10
9
8
7
6
5
4
3
+/+
-/-
Mother -/- +/+
Fetuses -/- +/+
Dnase1l3
Genotype

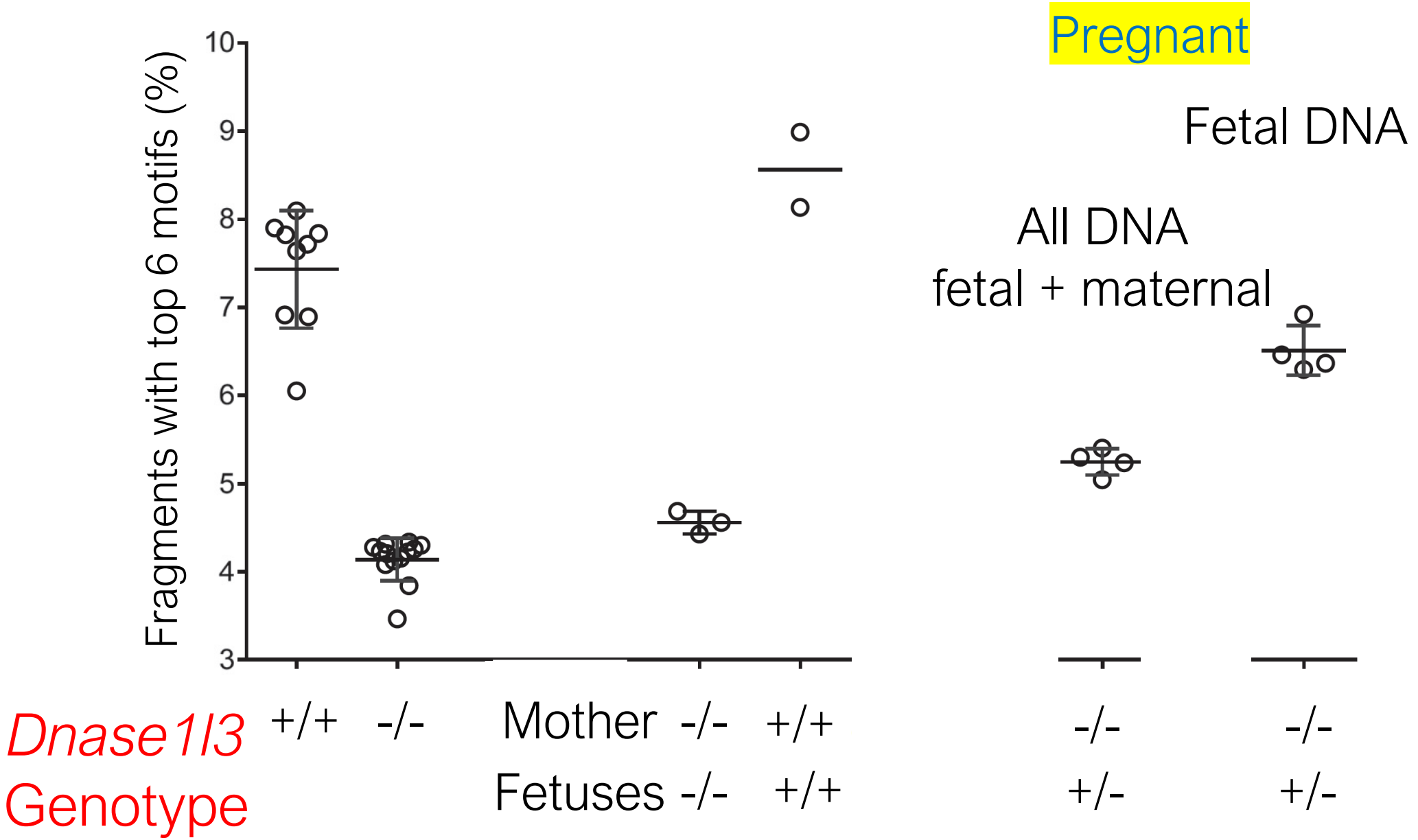
Pregnant



All DNA
fetal + maternal

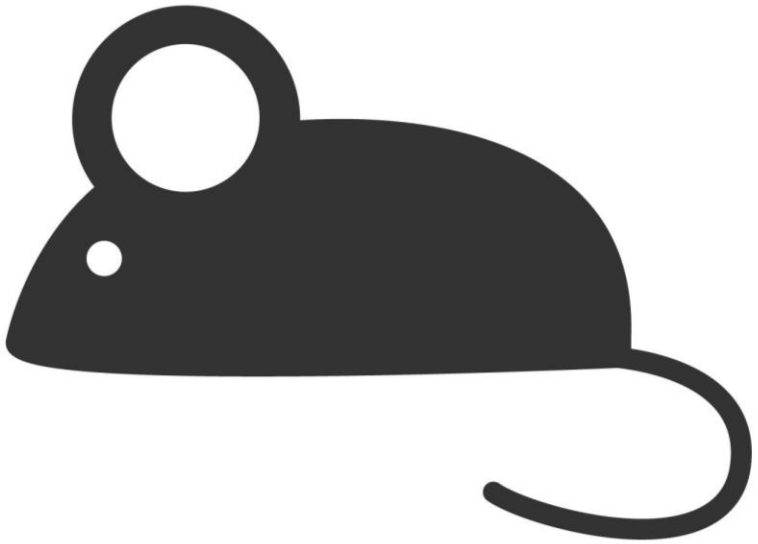
Dnase1l3 Genotype

	+/+	-/-		-/-	+/+	-/-
Mother						
Fetuses				-/-	+/+	+/-


Pregnant
Fetal DNA
All DNA
fetal + maternal
Fragments with top 6 motifs (%)
10
9
8
7
6
5
4
3
Dnase1l3
Genotype
+/+
-/-
Mother -/- +/+
Fetuses -/- +/+
-/-
+/-
-/-
+/-

Female: B6

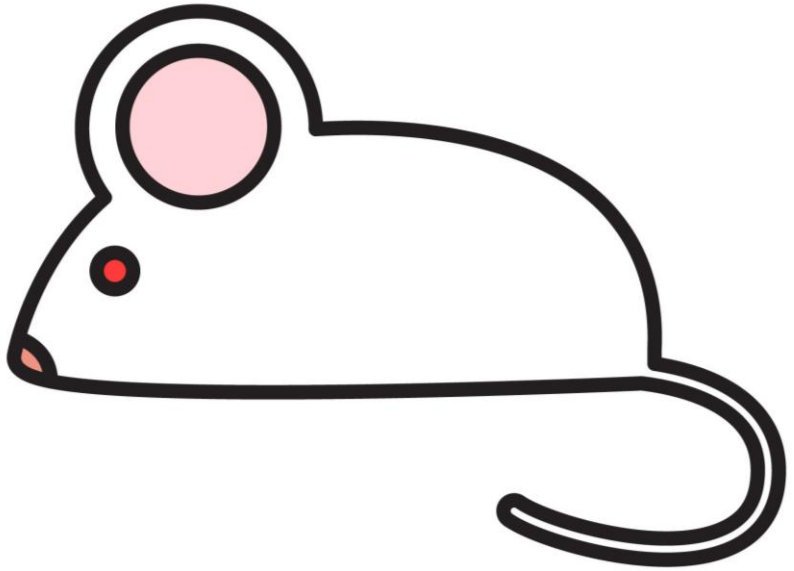
Dnase1l3$^{-/-}$



X

Male: Balb/C

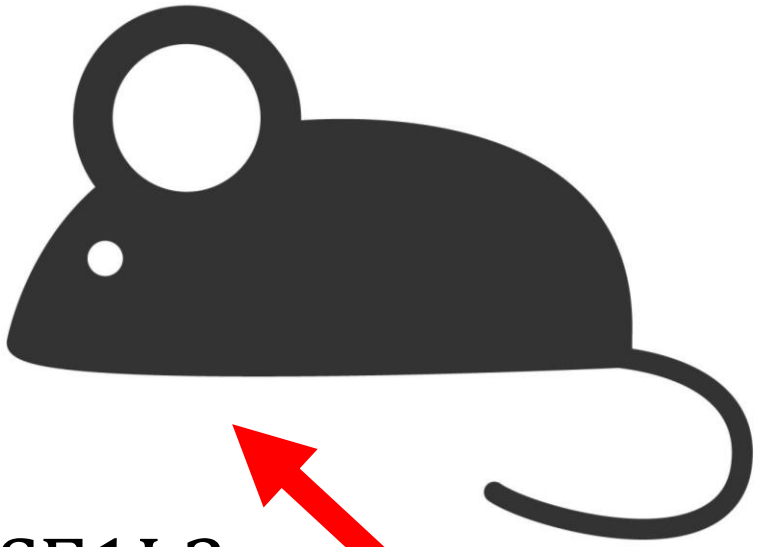
Dnase1l3$^{+/+}$



Fetuses: *Dnase1l3*$^{+/-}$

Female: B6

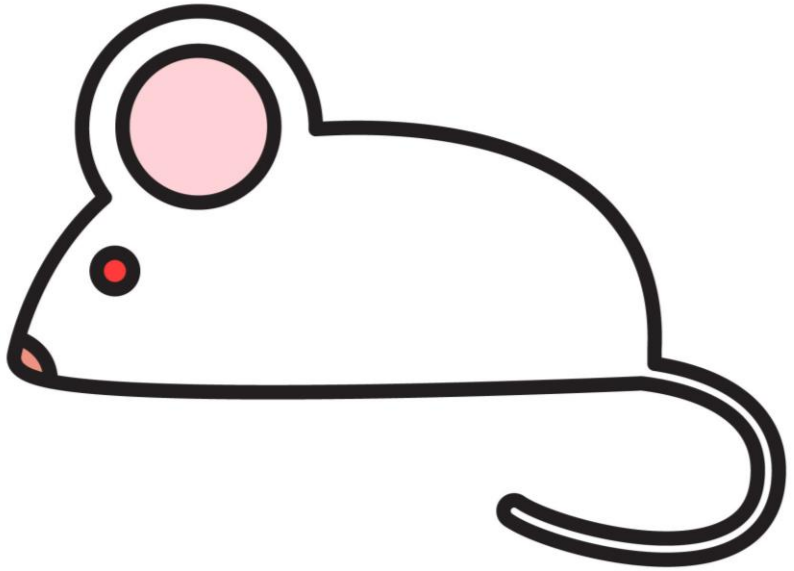
Dnase1l3$^{-/-}$



X

Male: Balb/C

Dnase1l3$^{+/+}$



DNASE1L3 enzyme + ‘Locally cut’ fetal DNA

Fetuses: *Dnase1l3*$^{+/-}$

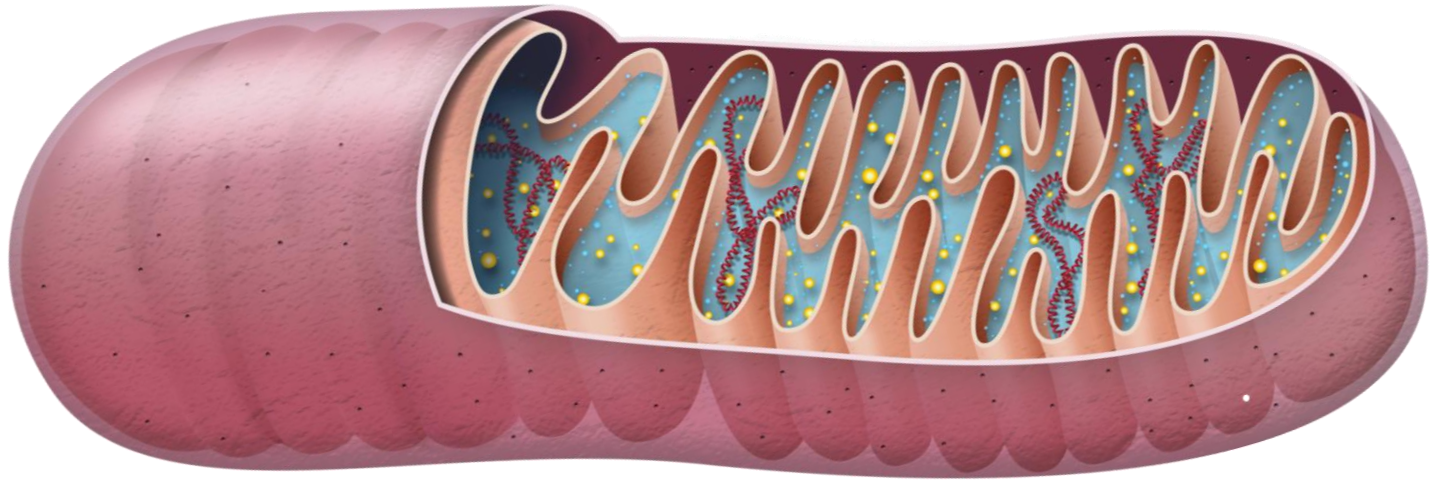
DNASE1L3

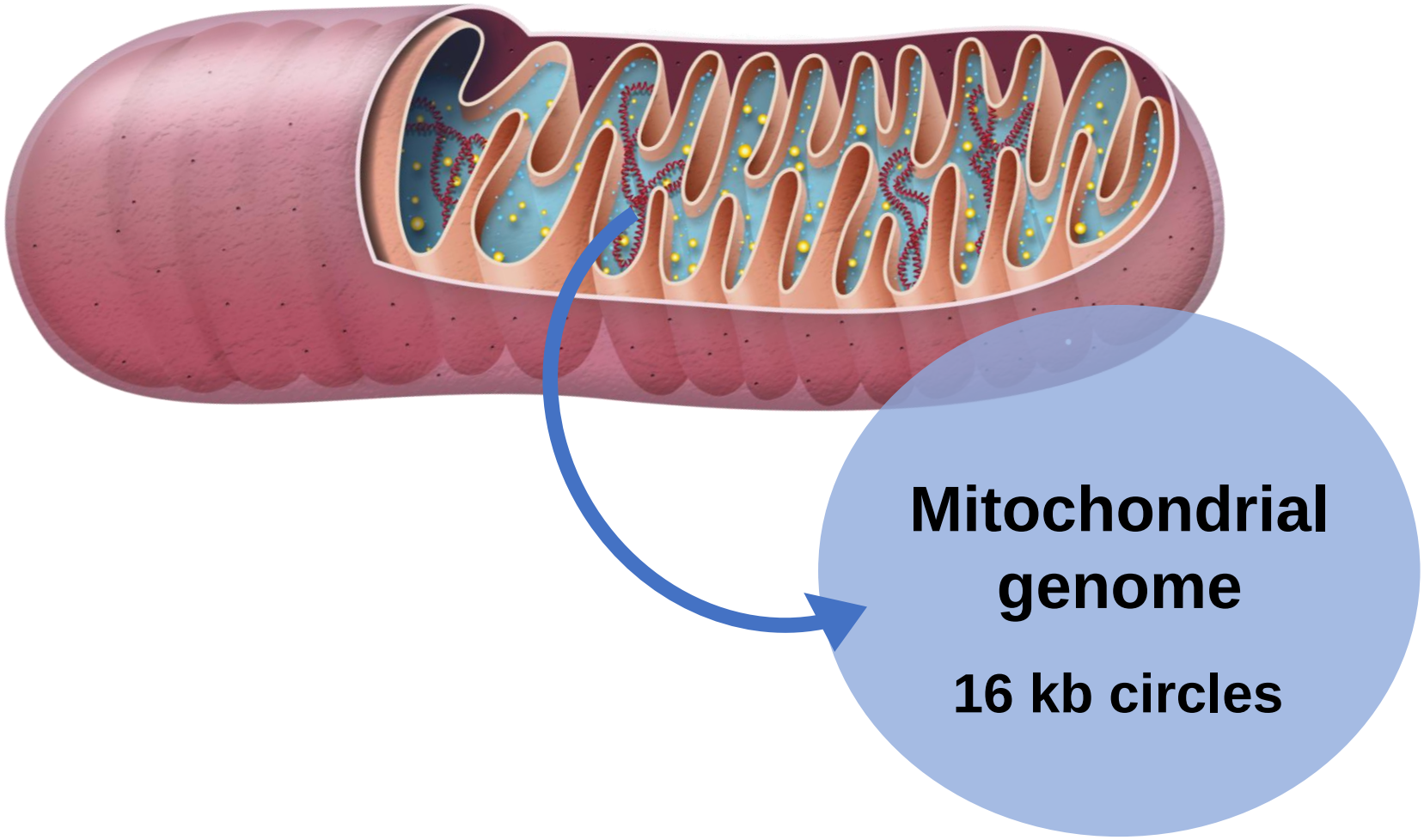
- First link of nuclease biology and circulating nucleic acids
- Alterations with physiologic and pathologic changes
- Role of other nucleases
- Diagnostic role of plasma DNA end motifs

Fragmentation Patterns

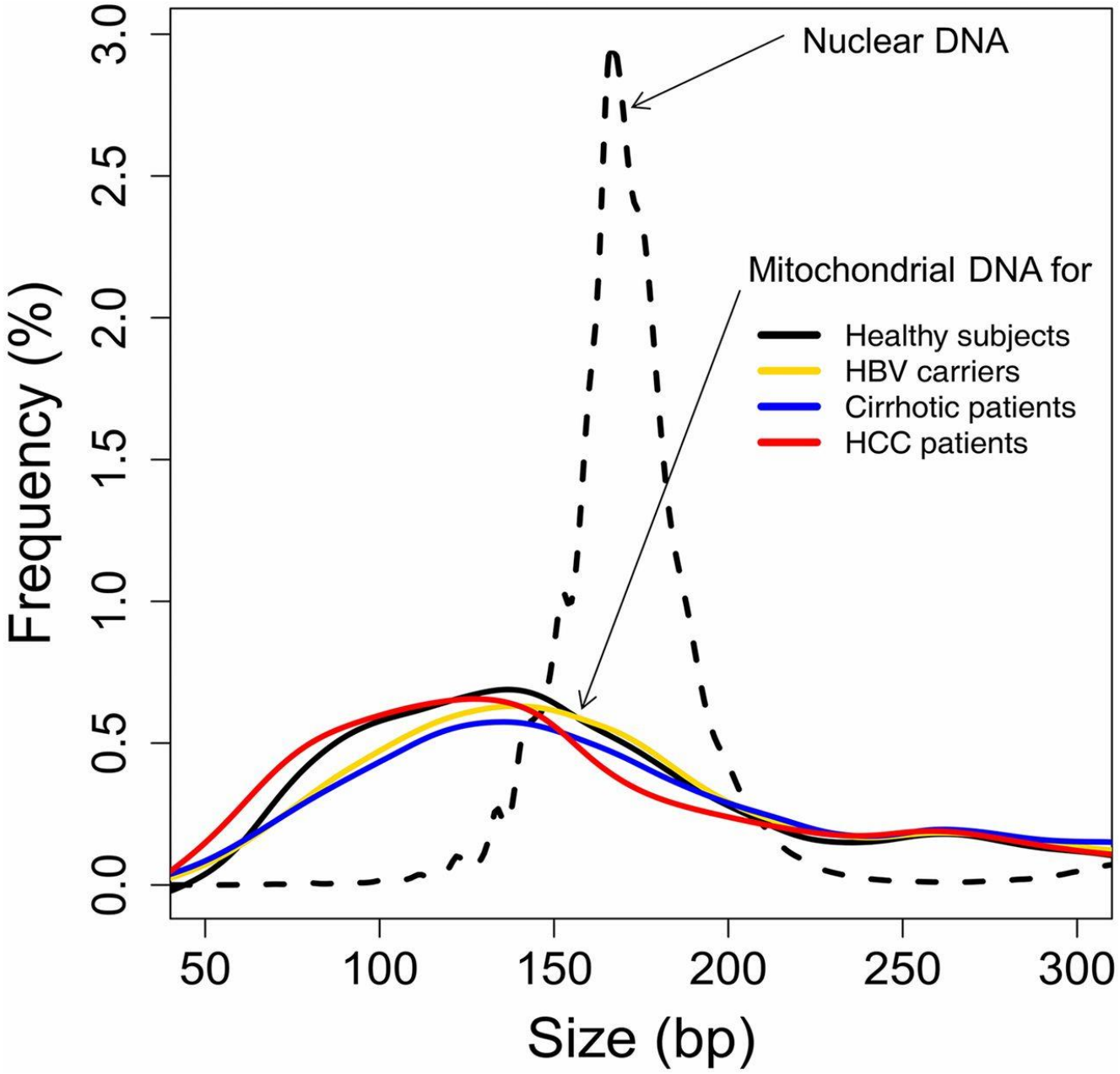
“Fragmentomics”


“Integrity”




Mitochondrial genome
16 kb circles

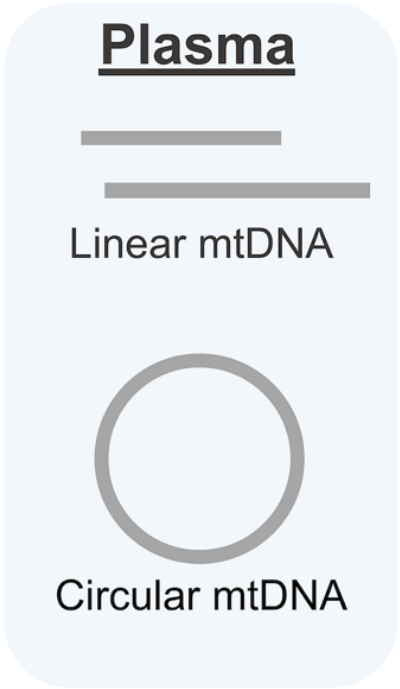
Size profile of circulating DNA



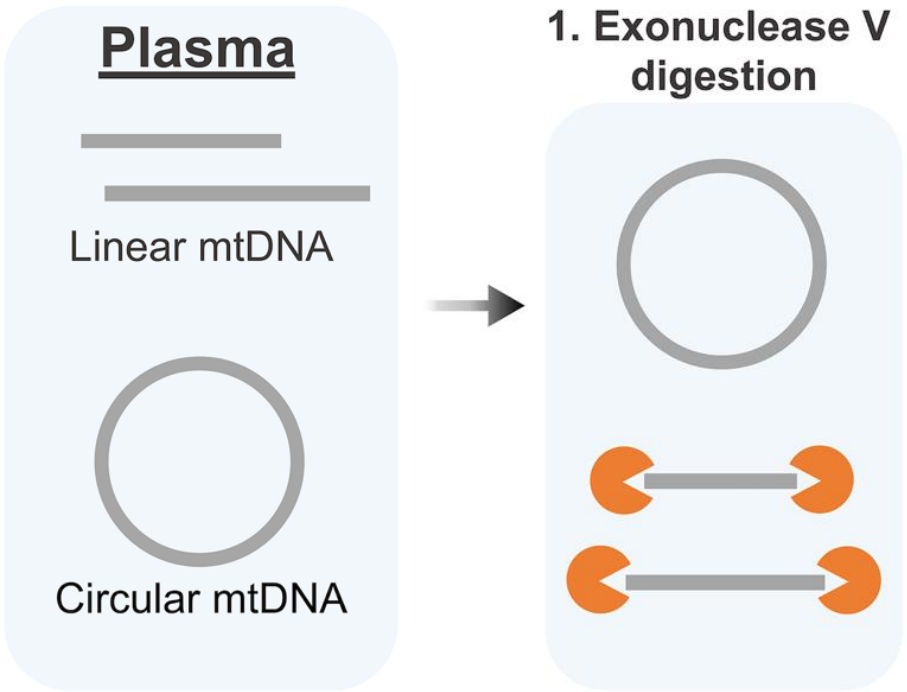
Jiang *et al. PNAS* 2015.

Circular?

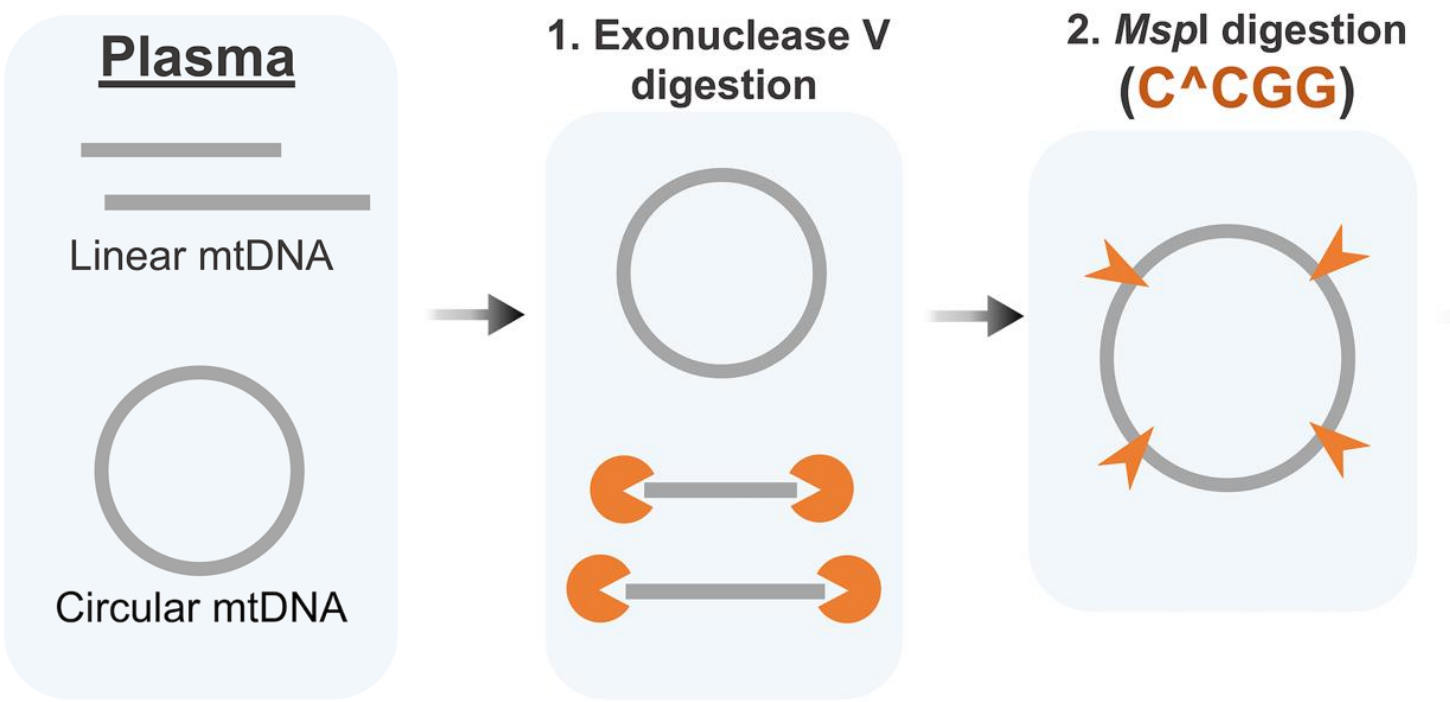
Search for circular mtDNA in plasma



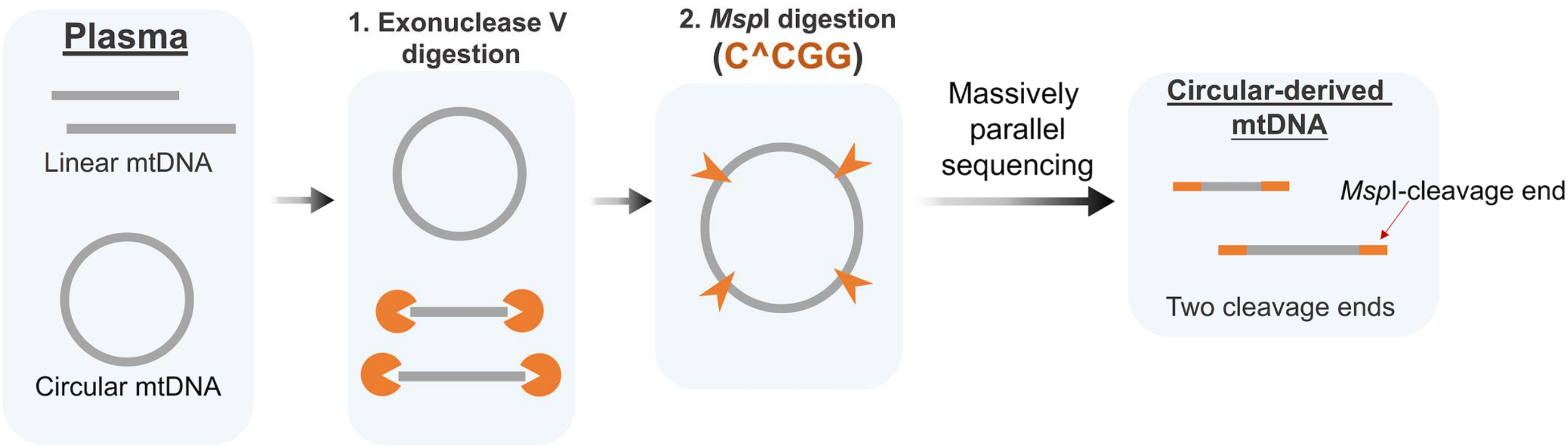
Search for circular mtDNA in plasma



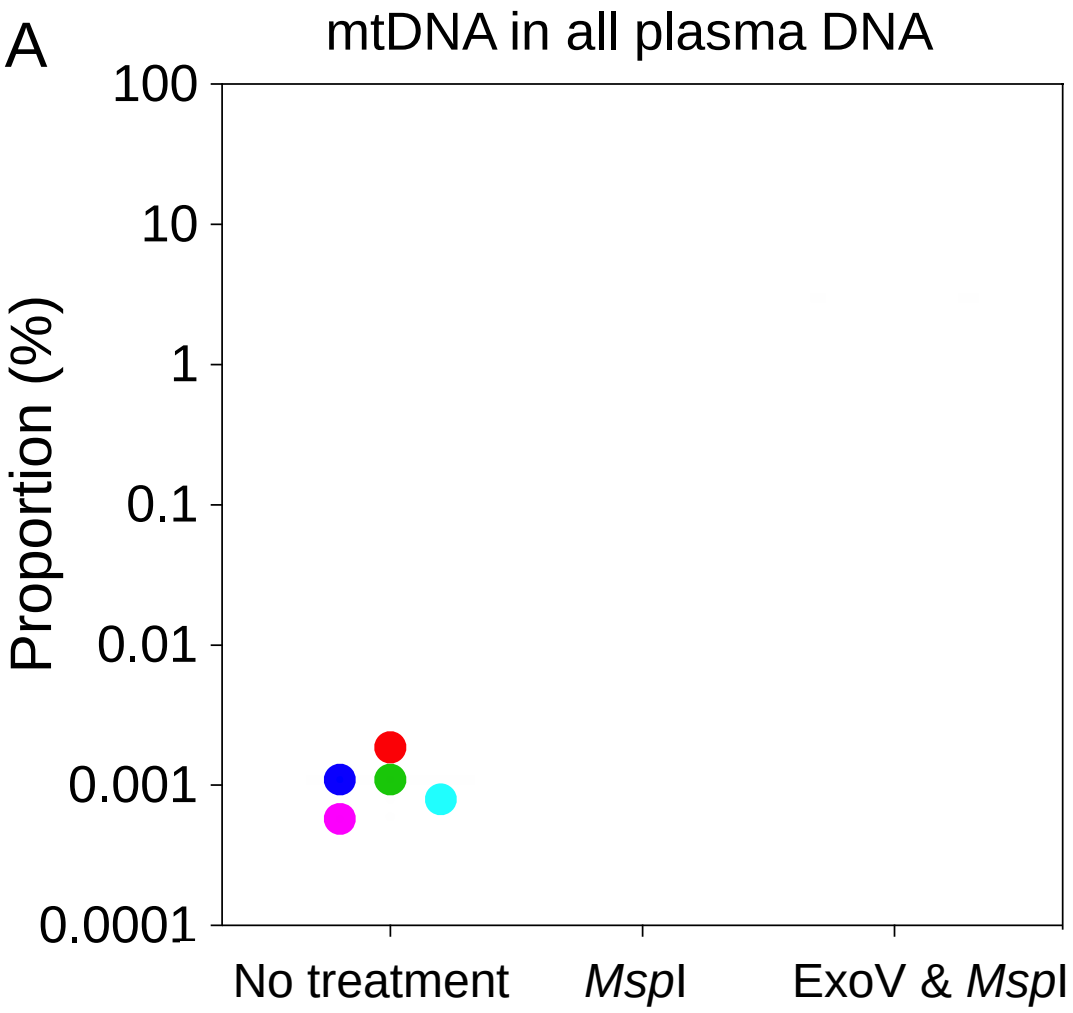
Search for circular mtDNA in plasma



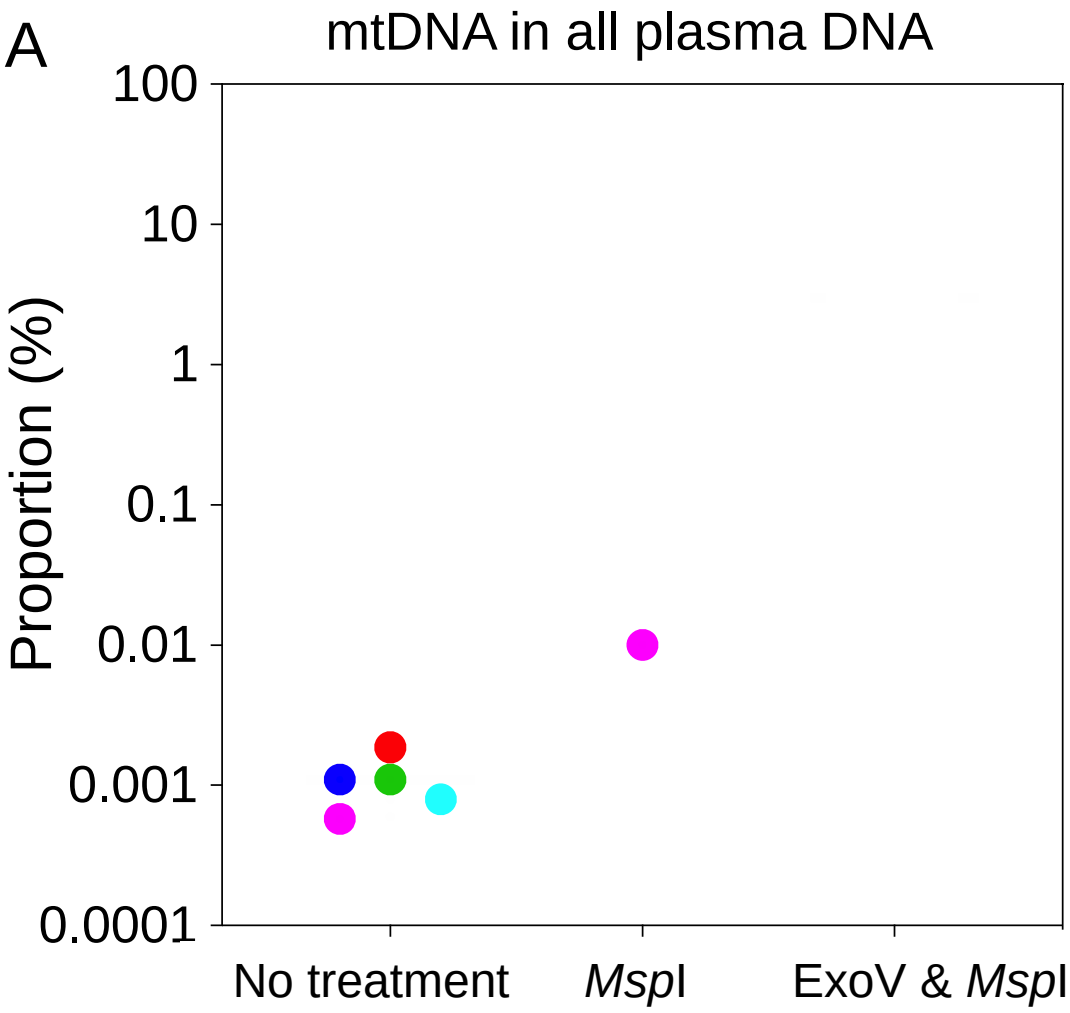
Search for circular mtDNA in plasma



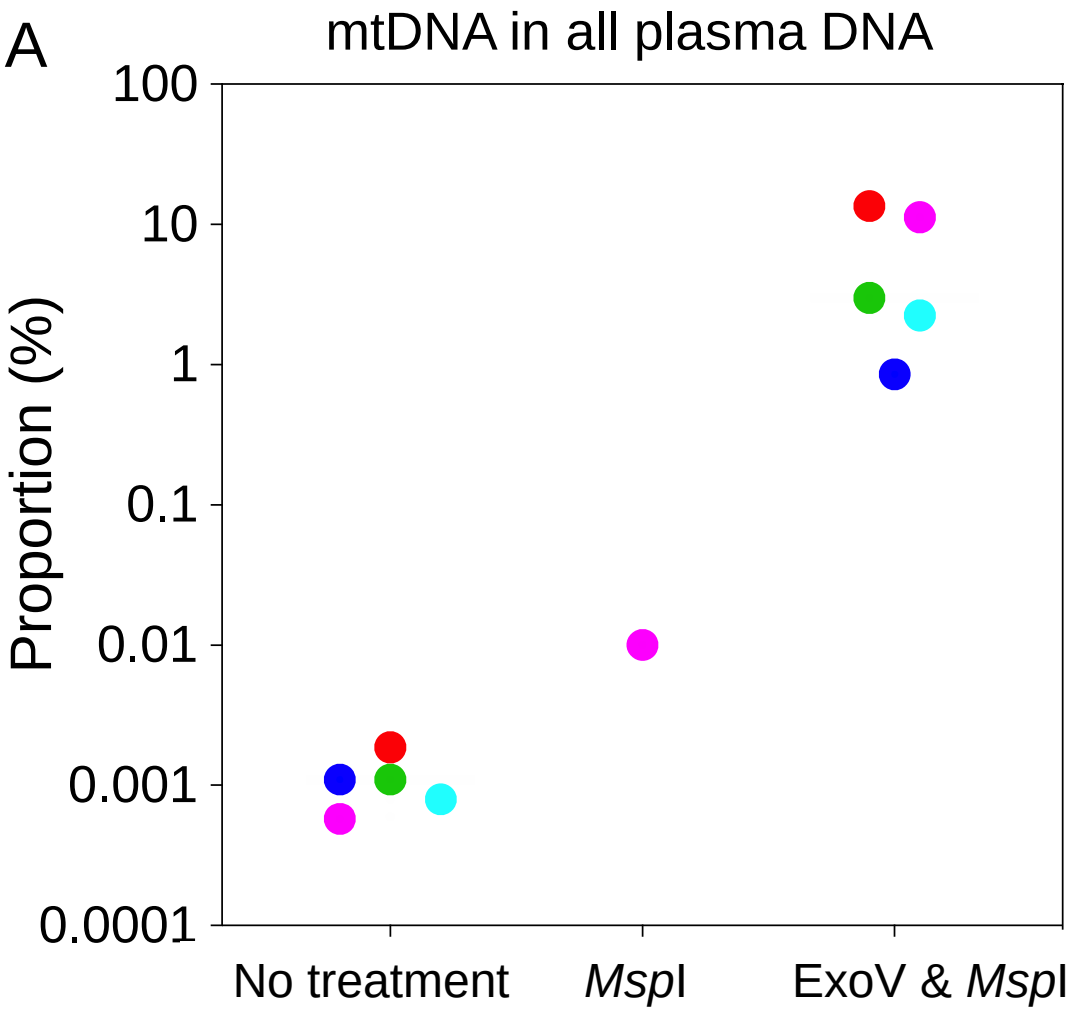
*Exo*V & *Msp*I digestion in pregnant women



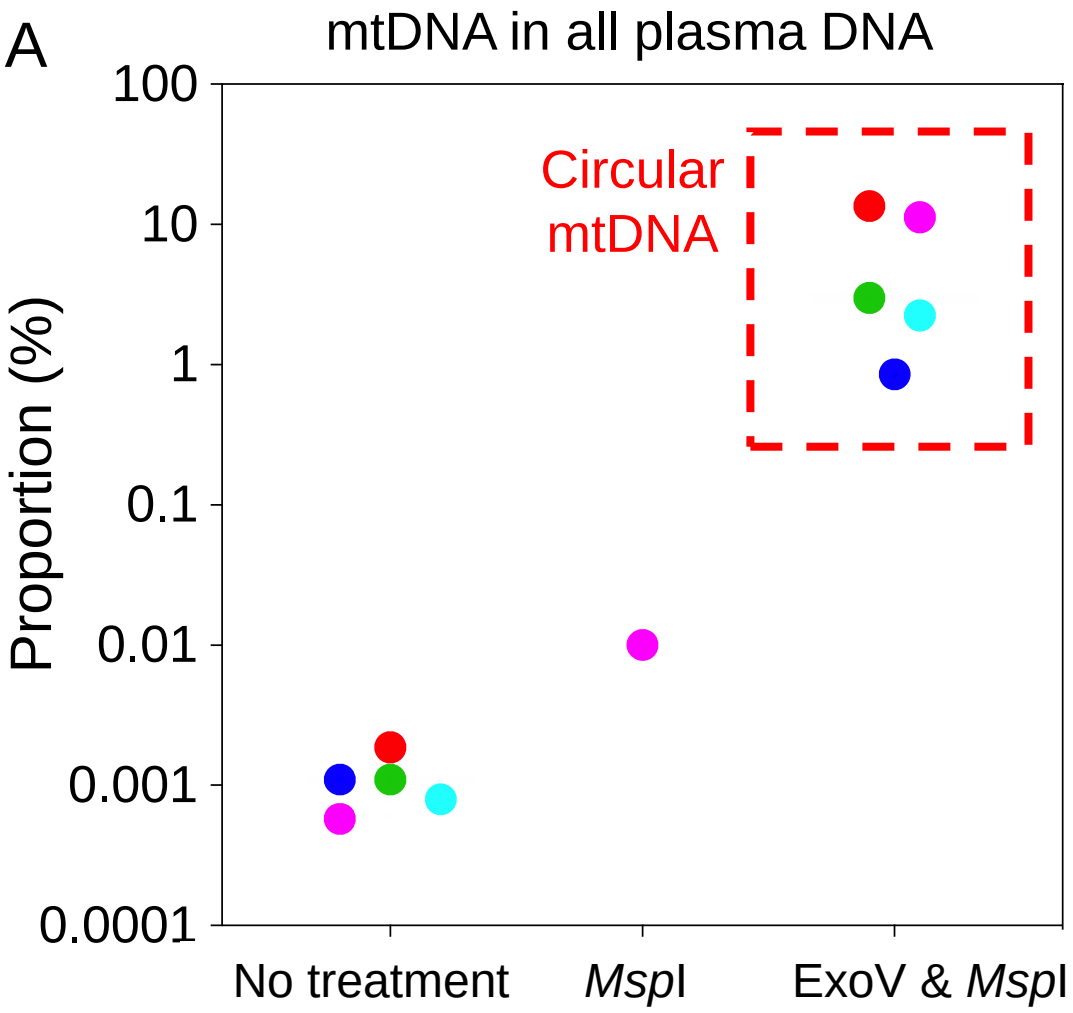
*Exo*V & *Msp*I digestion in pregnant women



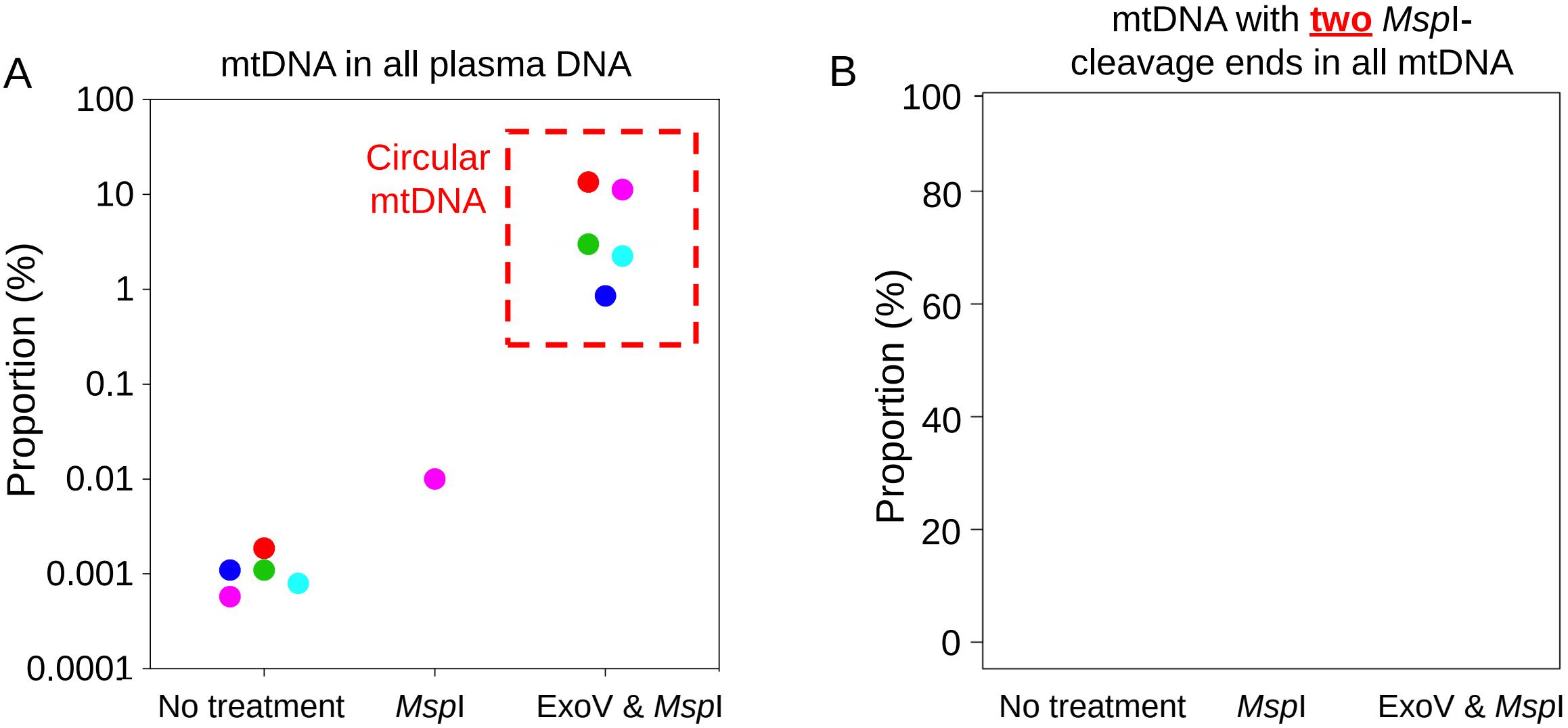
*Exo*V & *Msp*I digestion in pregnant women



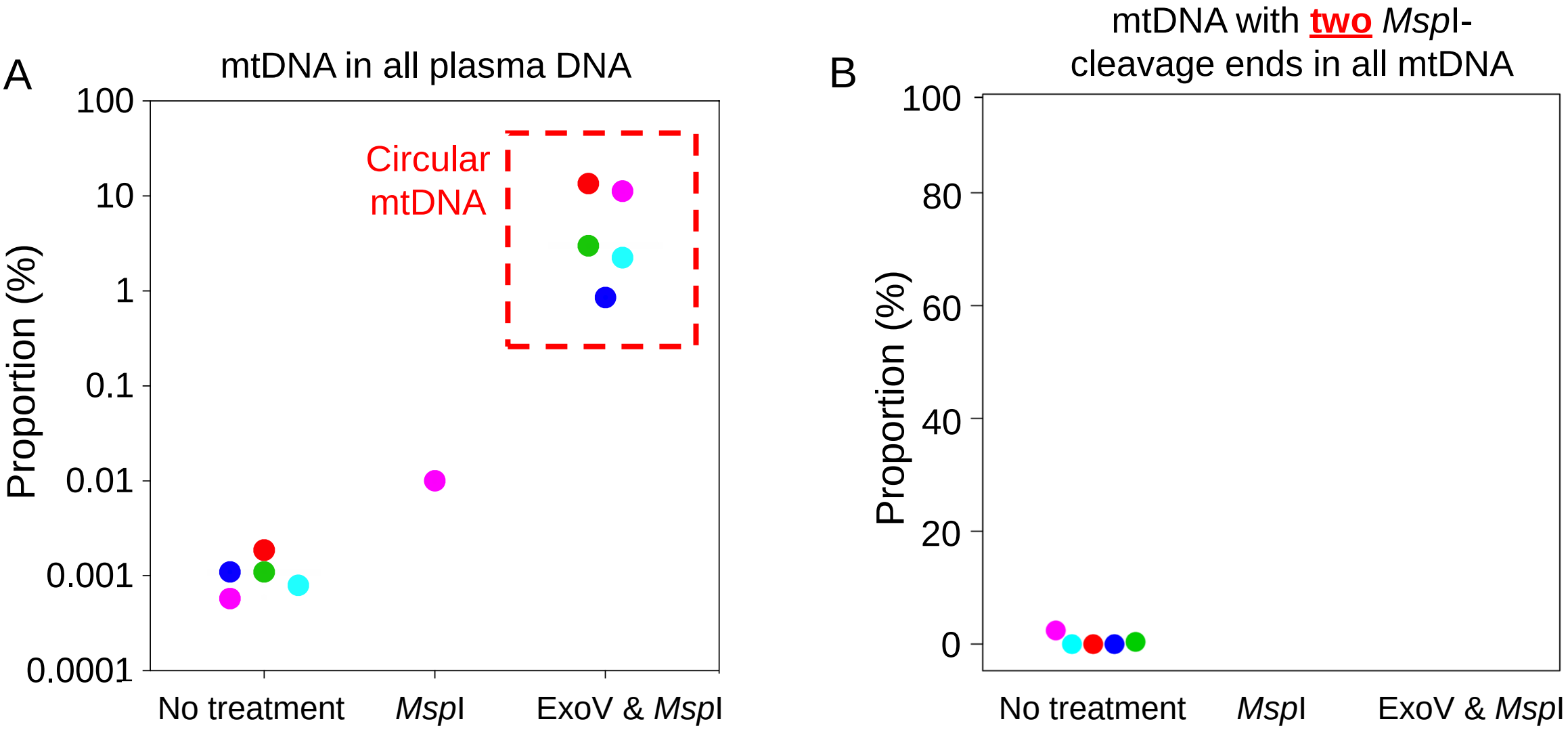
*Exo*V & *Msp*I digestion in pregnant women



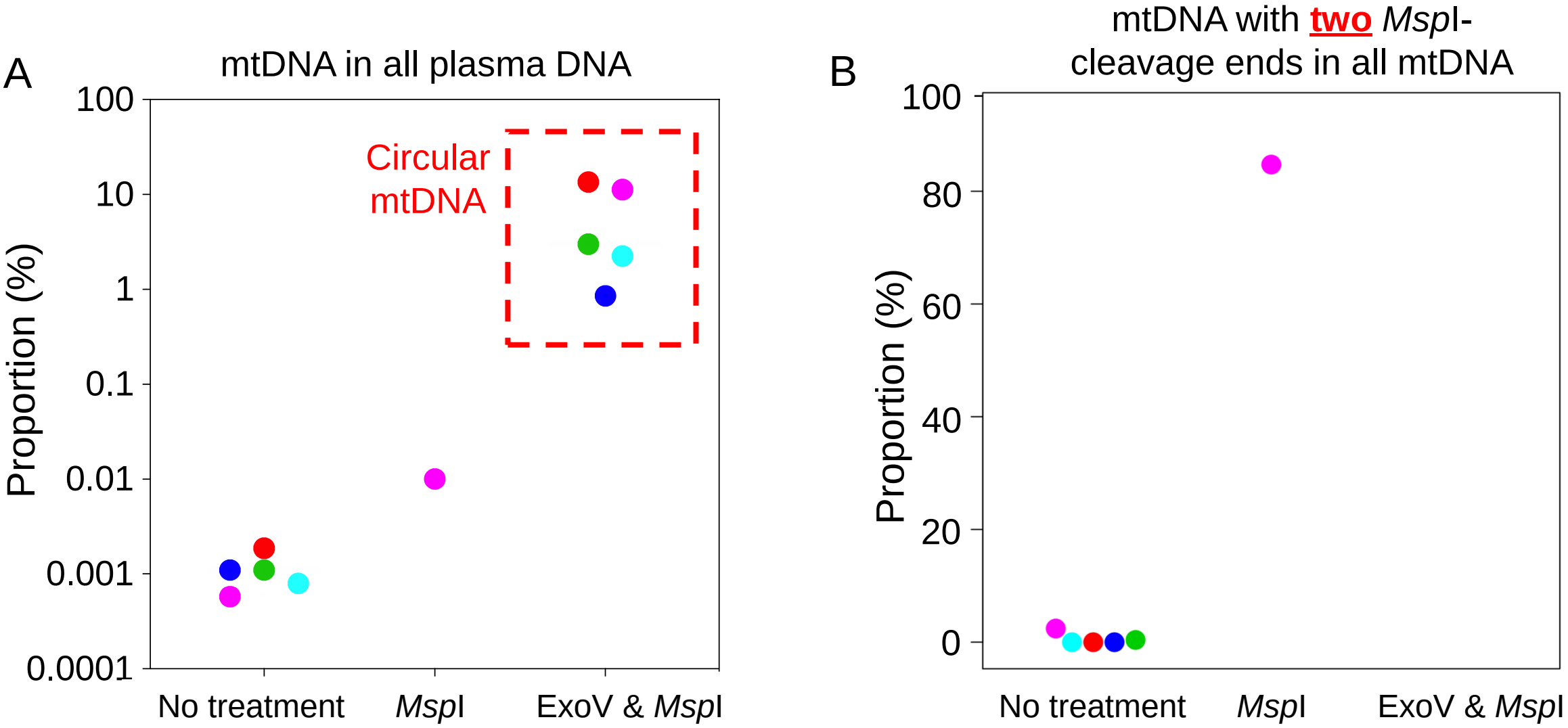
*Exo*V & *Msp*I digestion in pregnant women



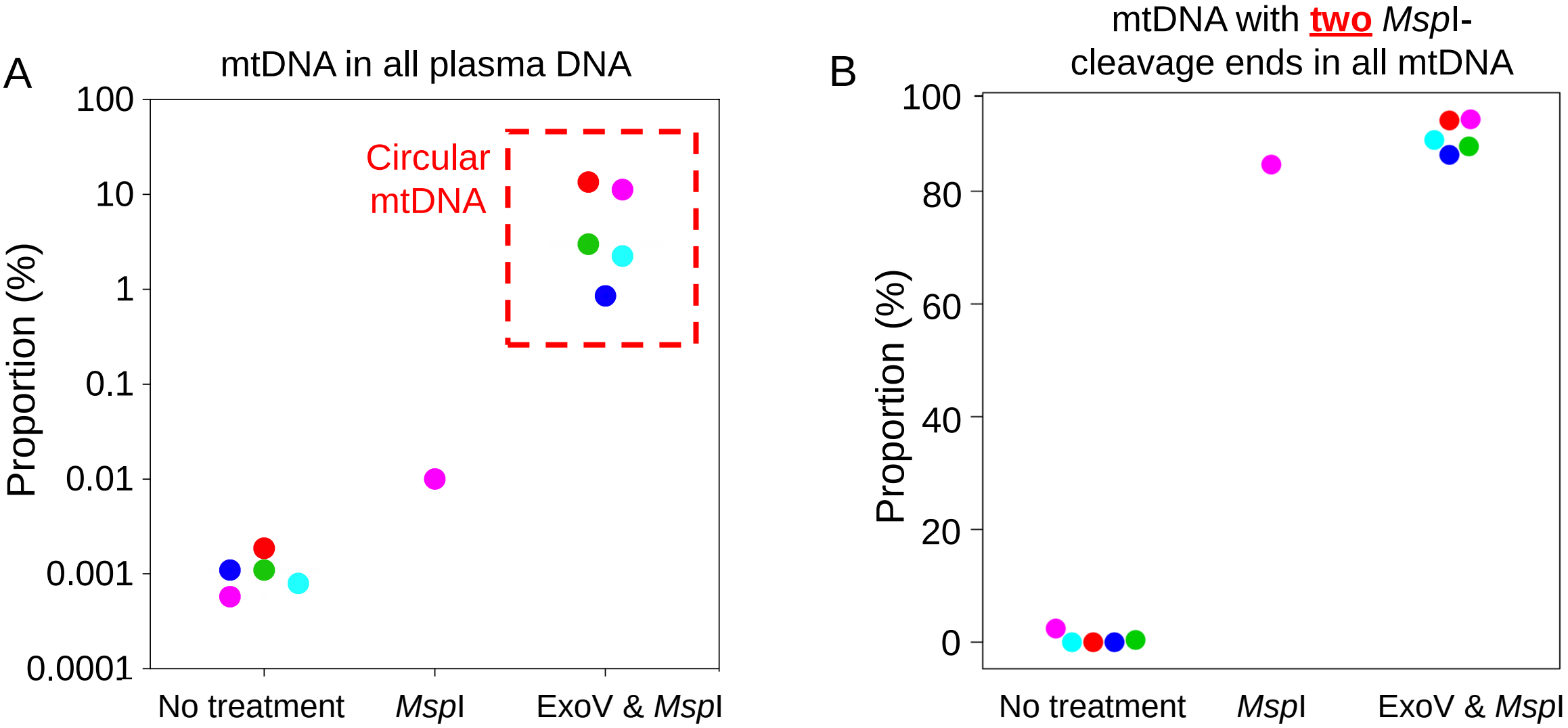
*Exo*V & *Msp*I digestion in pregnant women



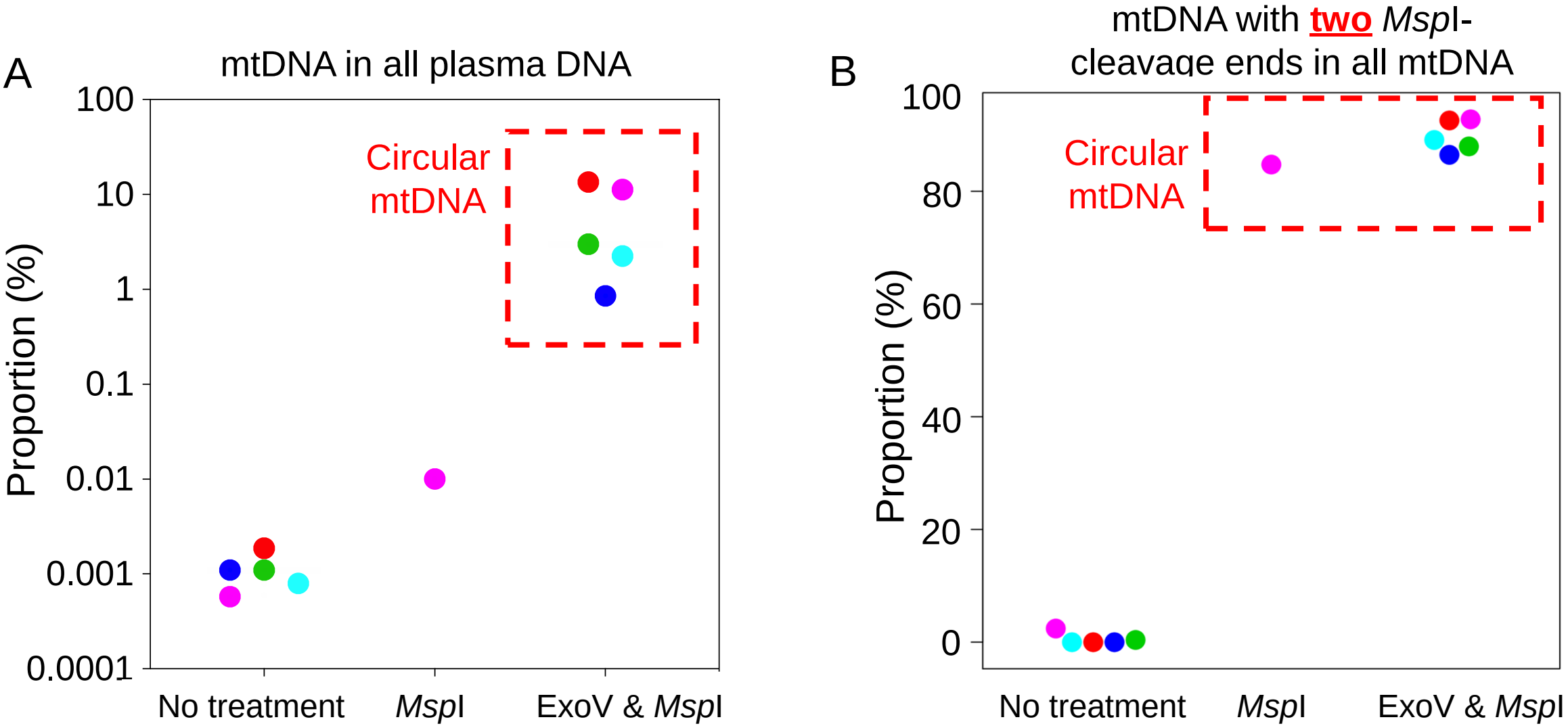
*Exo*V & *Msp*I digestion in pregnant women



*Exo*V & *Msp*I digestion in pregnant women



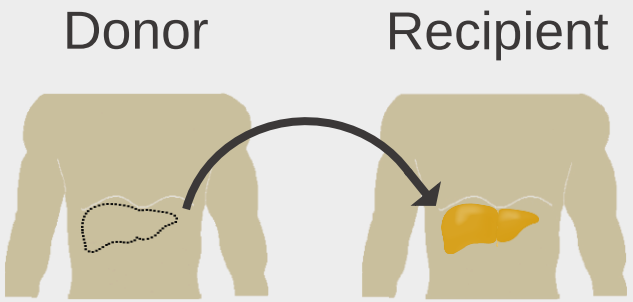
*Exo*V & *Msp*I digestion in pregnant women



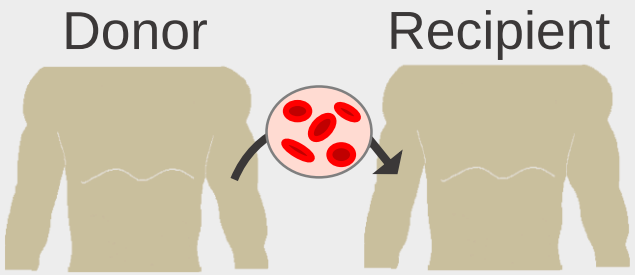
Tissue of Origin?

mtDNA in transplantation

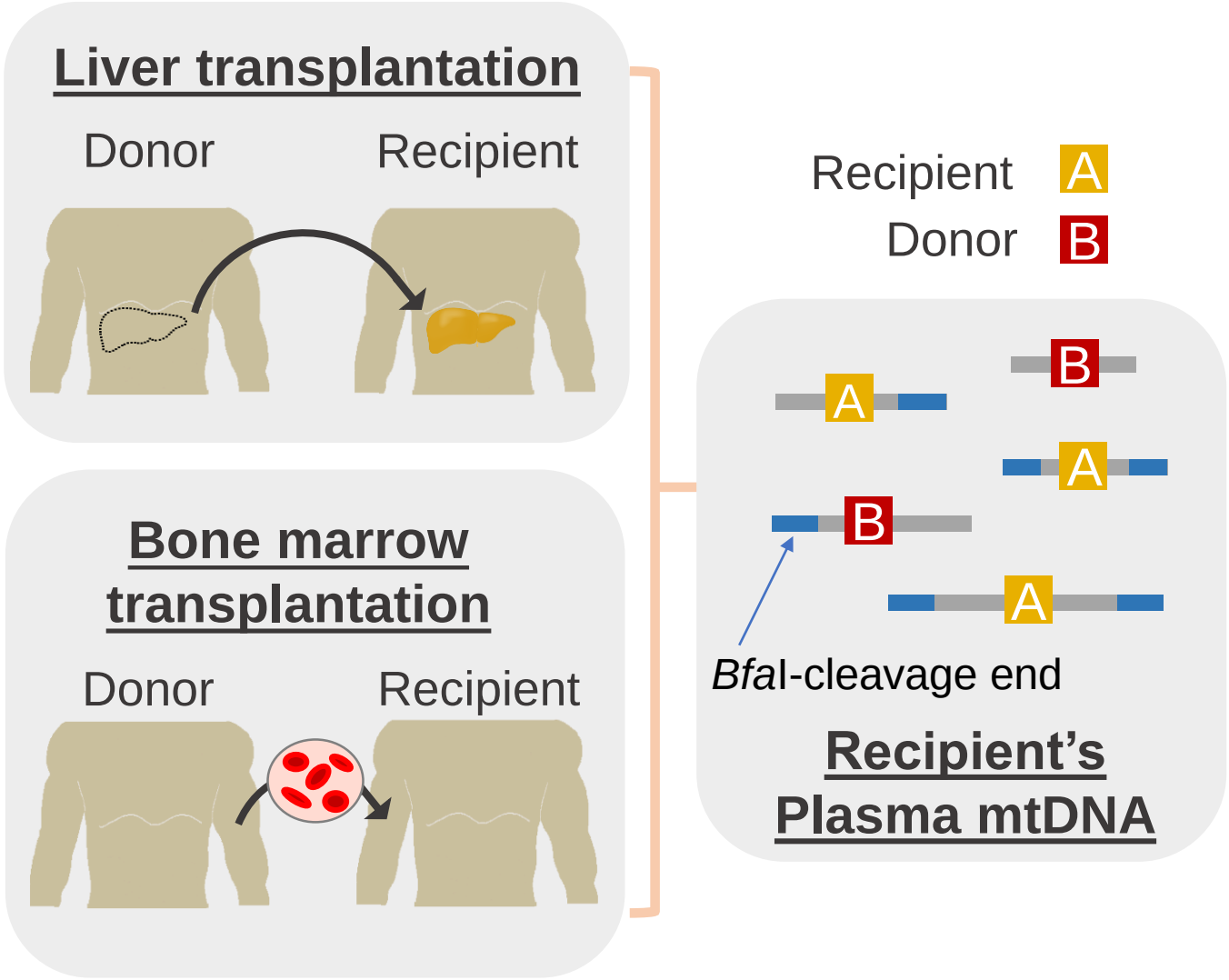
Liver transplantation



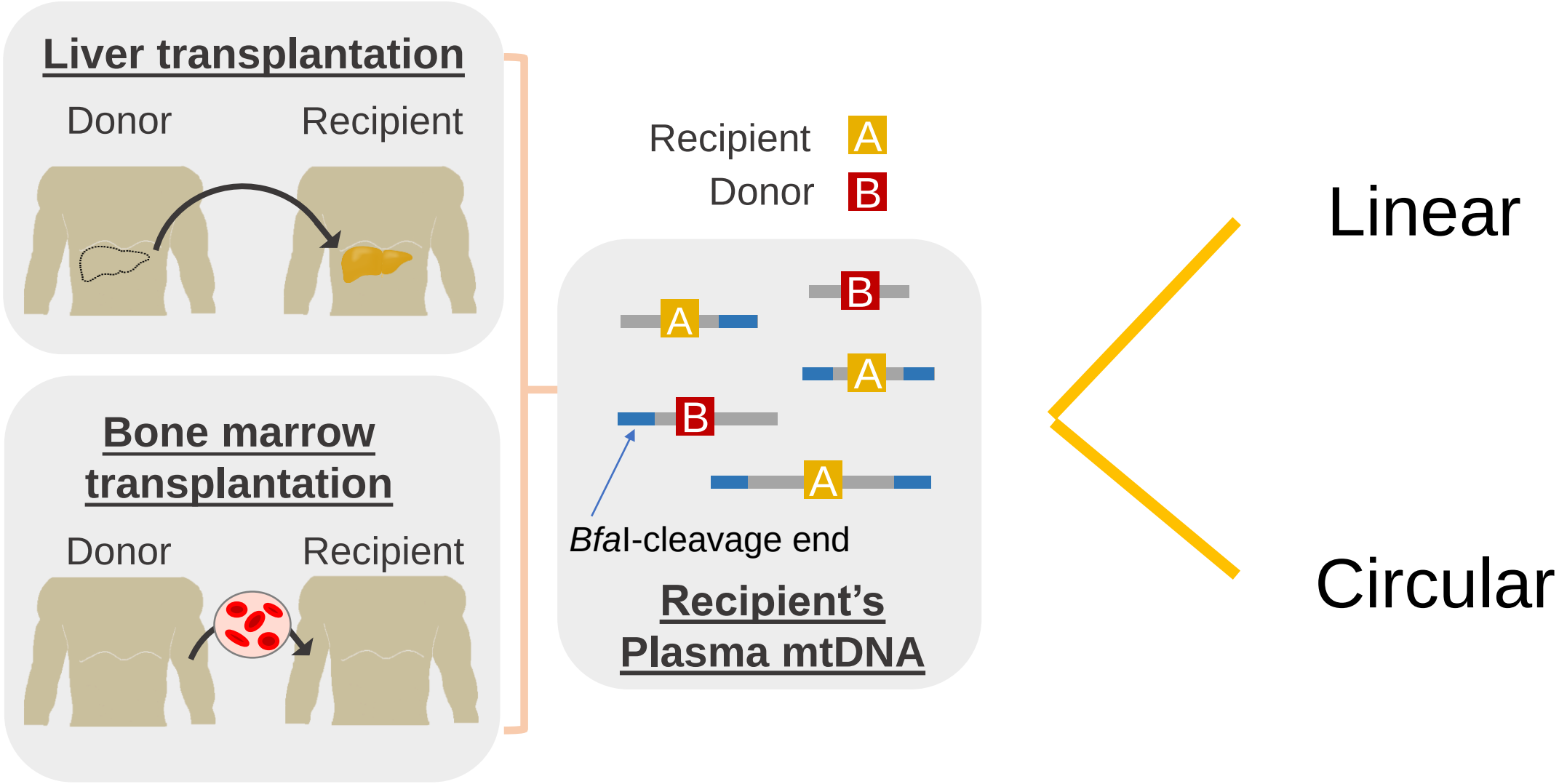
Bone marrow transplantation



mtDNA in transplantation



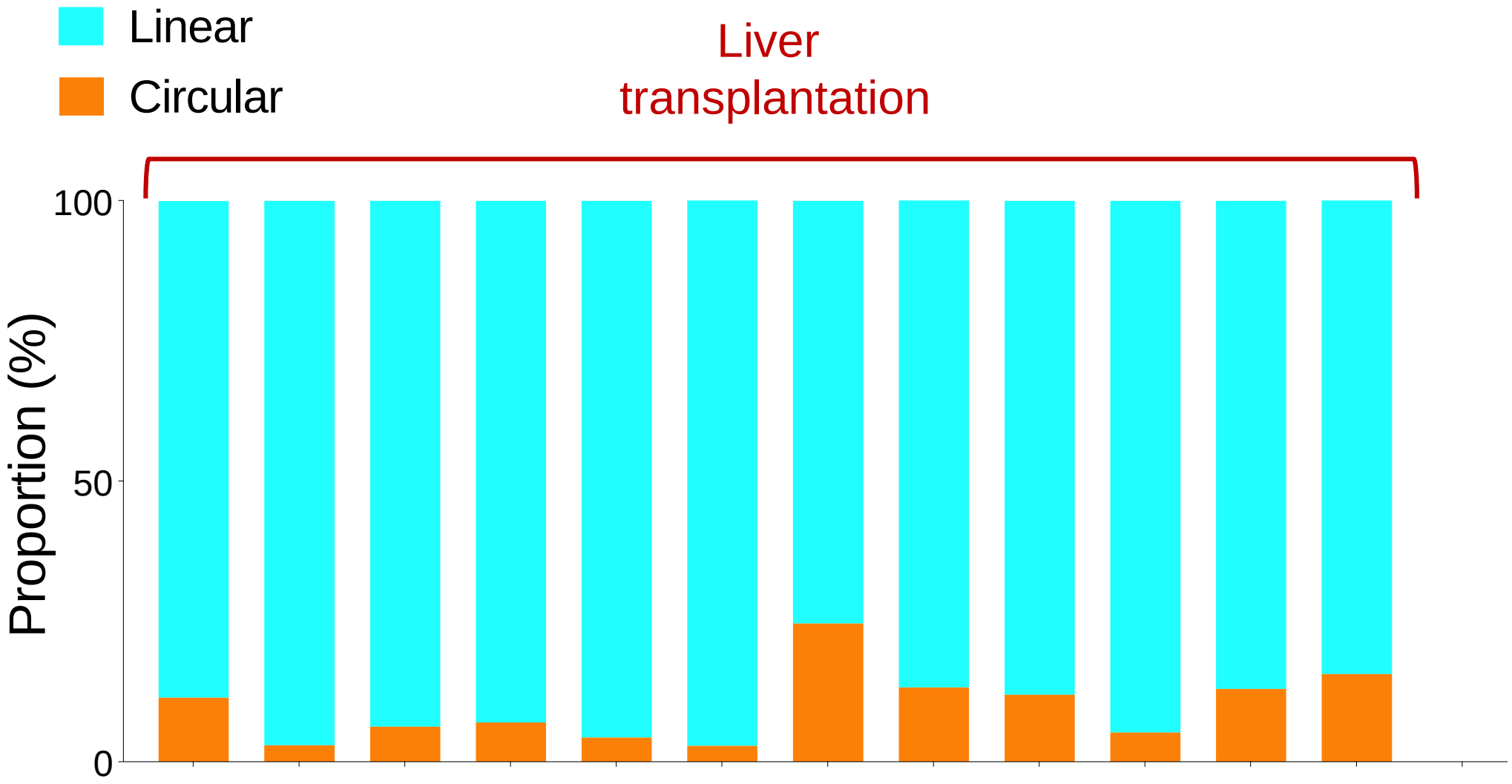
mtDNA in transplantation



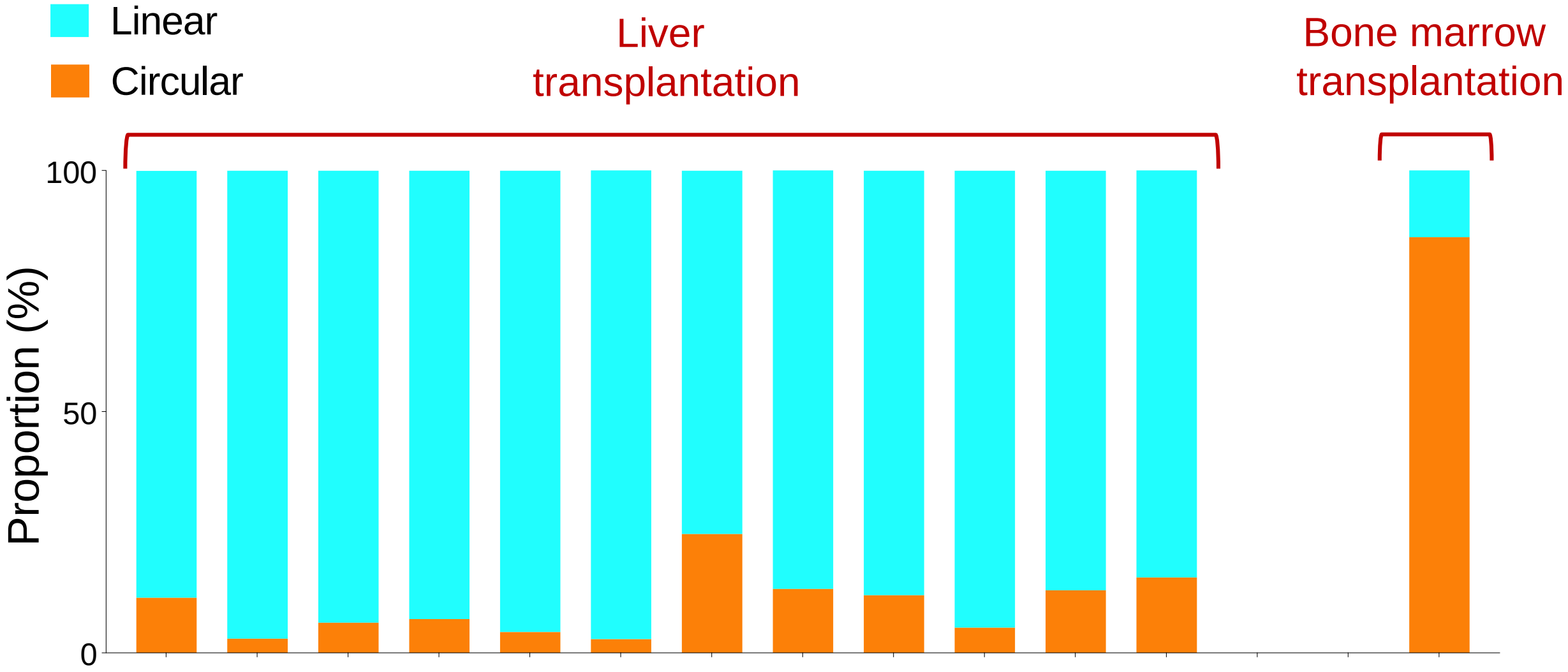
mtDNA of donor origin

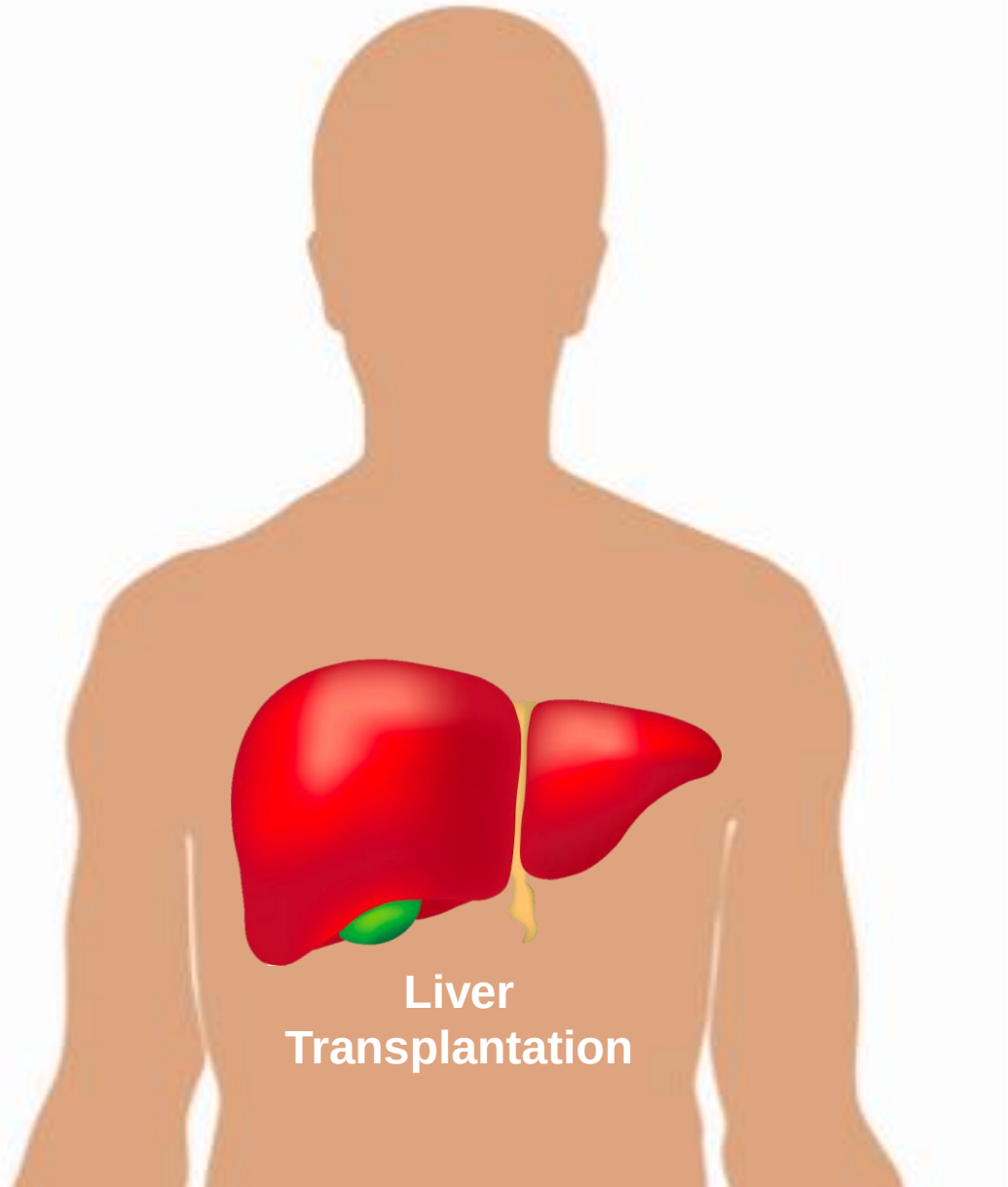
- Linear
- Circular

mtDNA of donor origin



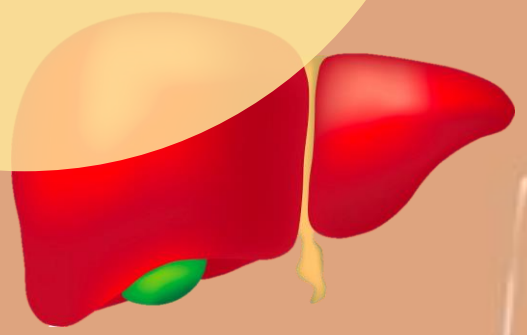
mtDNA of donor origin




Liver
Transplantation

Liver mtDNA

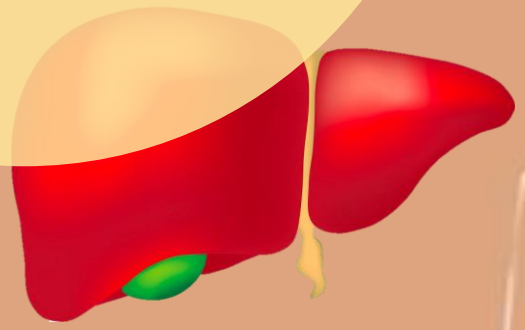
Linear



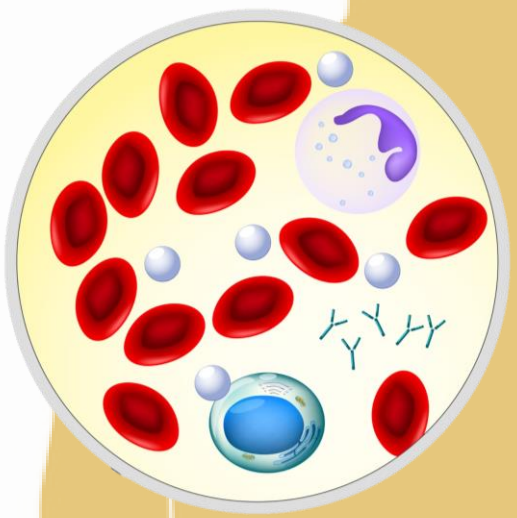
Liver Transplantation

Liver mtDNA

Linear



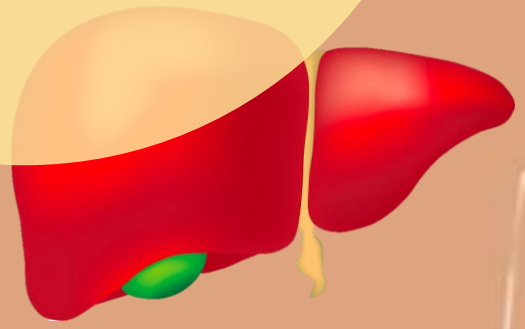
Liver Transplantation



Bone marrow Transplantation

Liver mtDNA

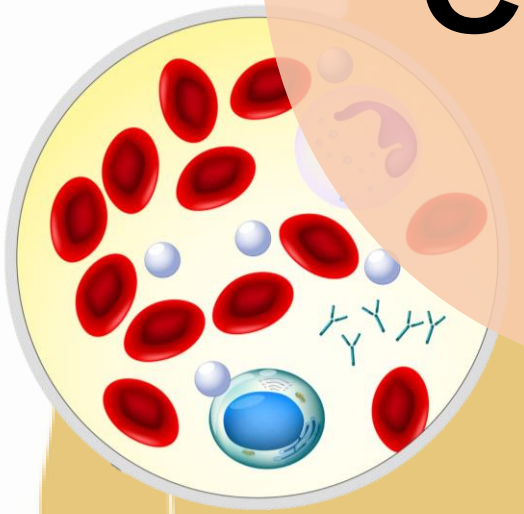
Linear



Liver Transplantation

Hematopoietic mtDNA

Circular

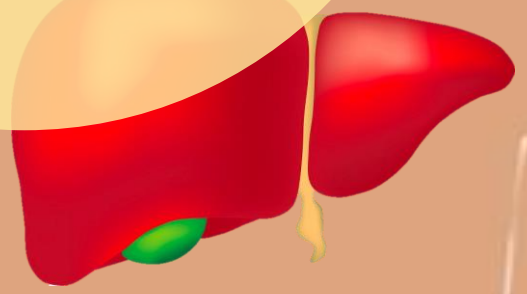


Bone marrow Transplantation

Circulating mitochondria
Other particles

Liver mtDNA

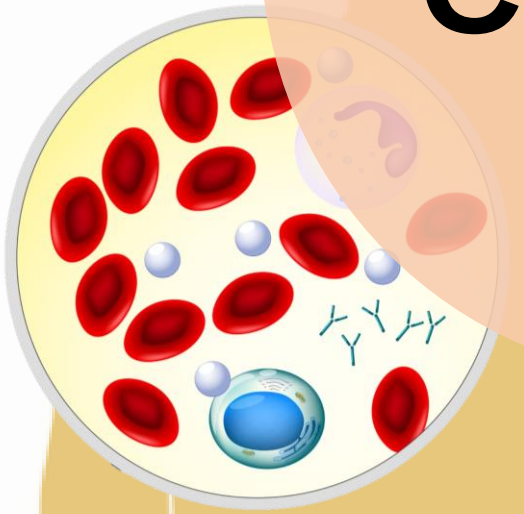
Linear



Liver Transplantation

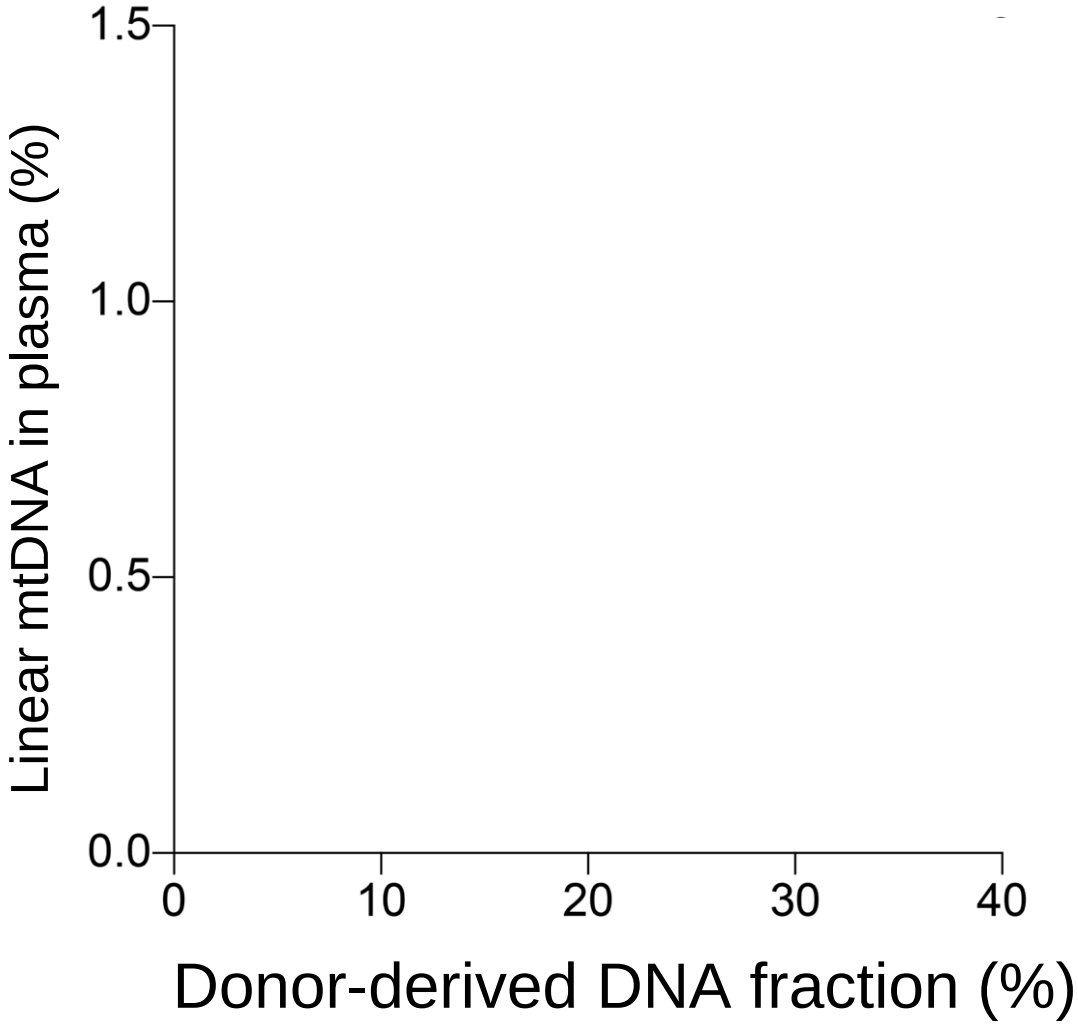
Hematopoietic mtDNA

Circular

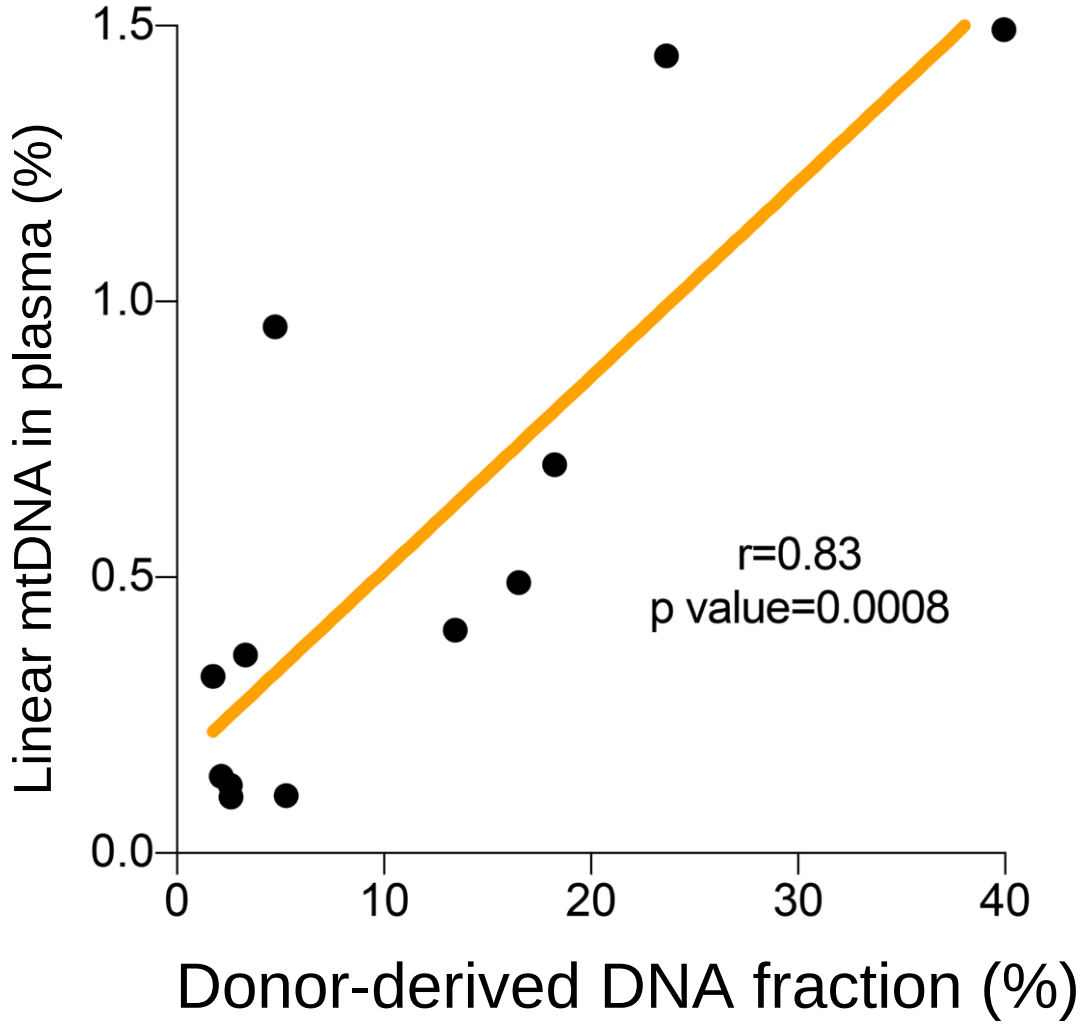


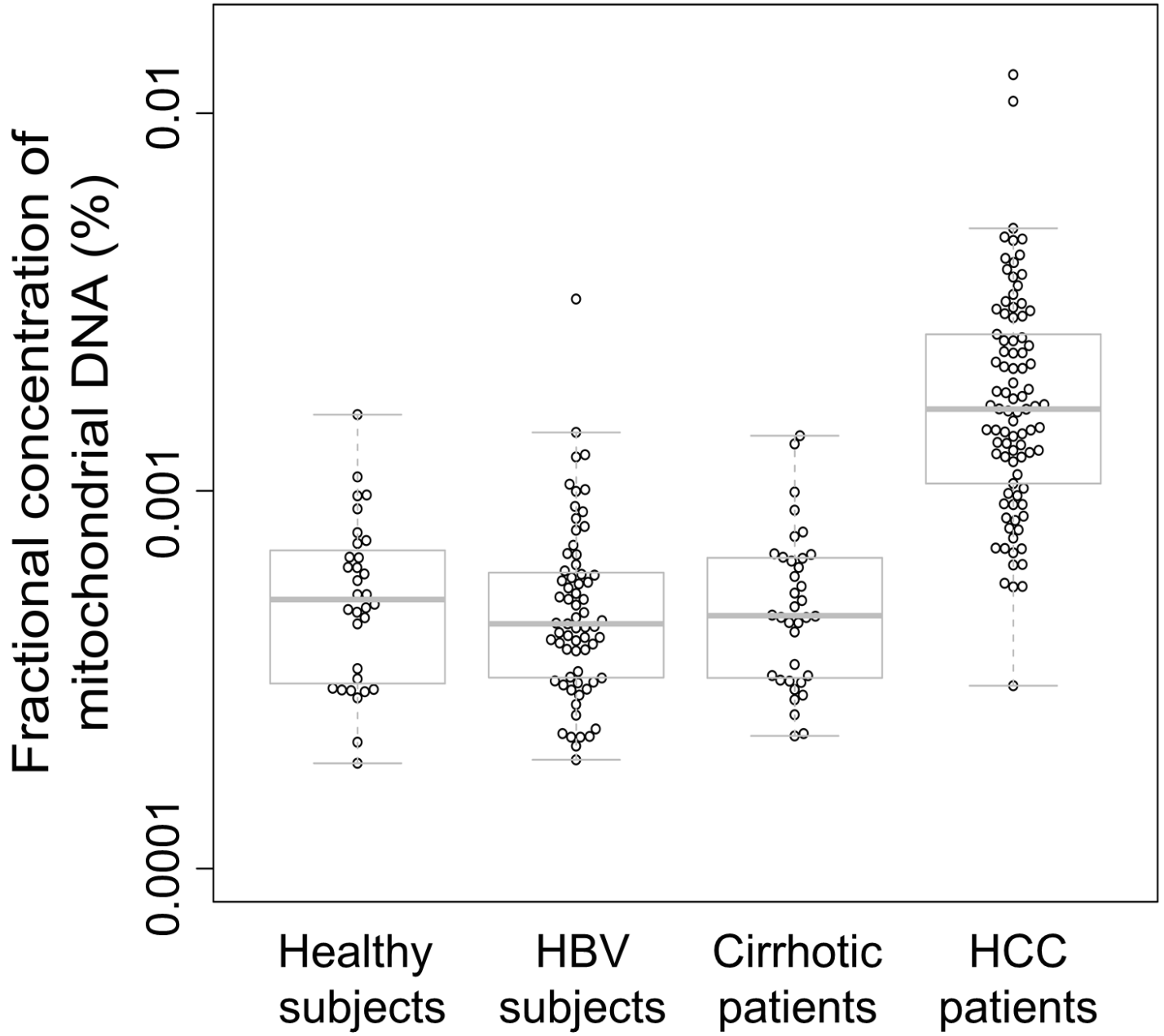
Bone marrow Transplantation

Liver Transplantation

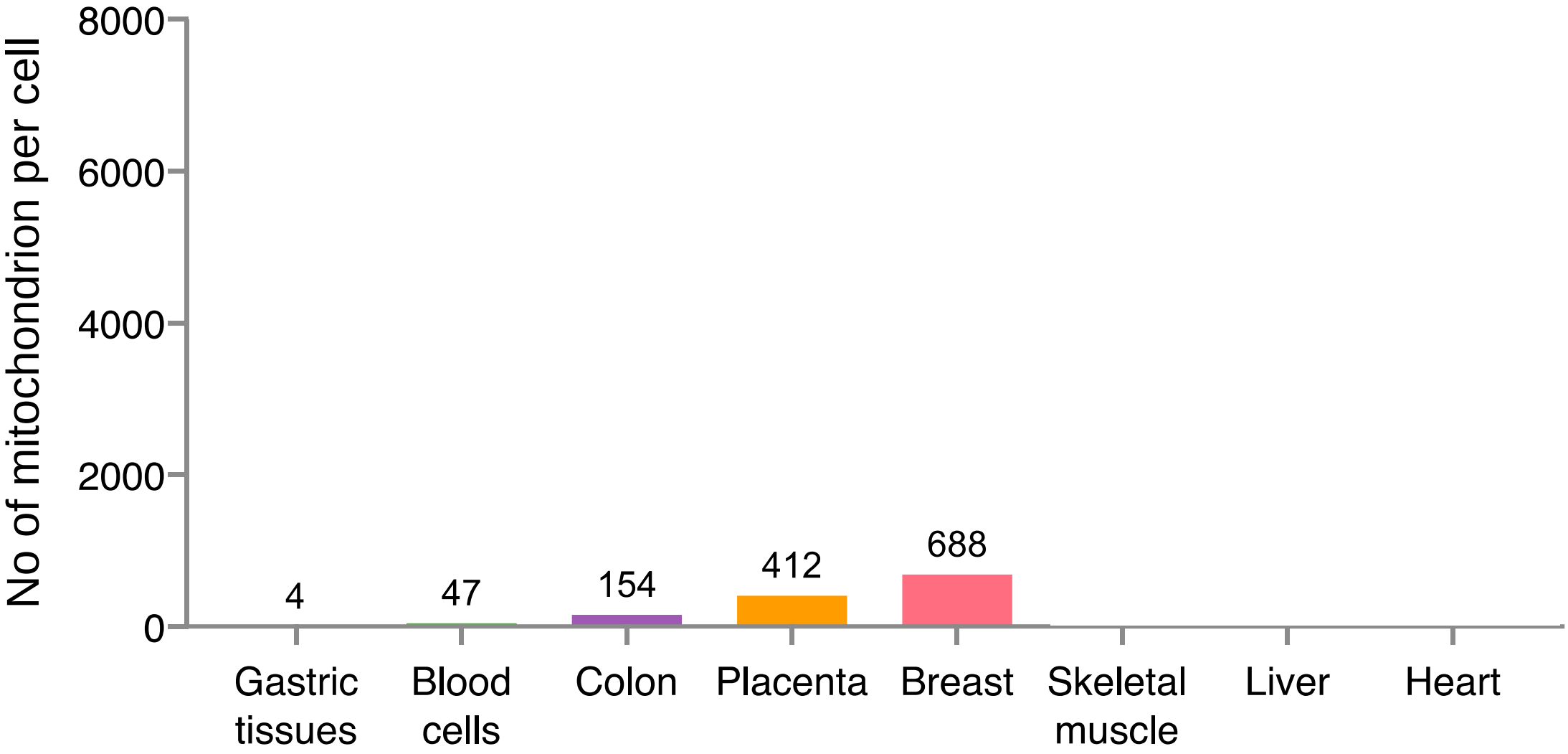


Liver Transplantation

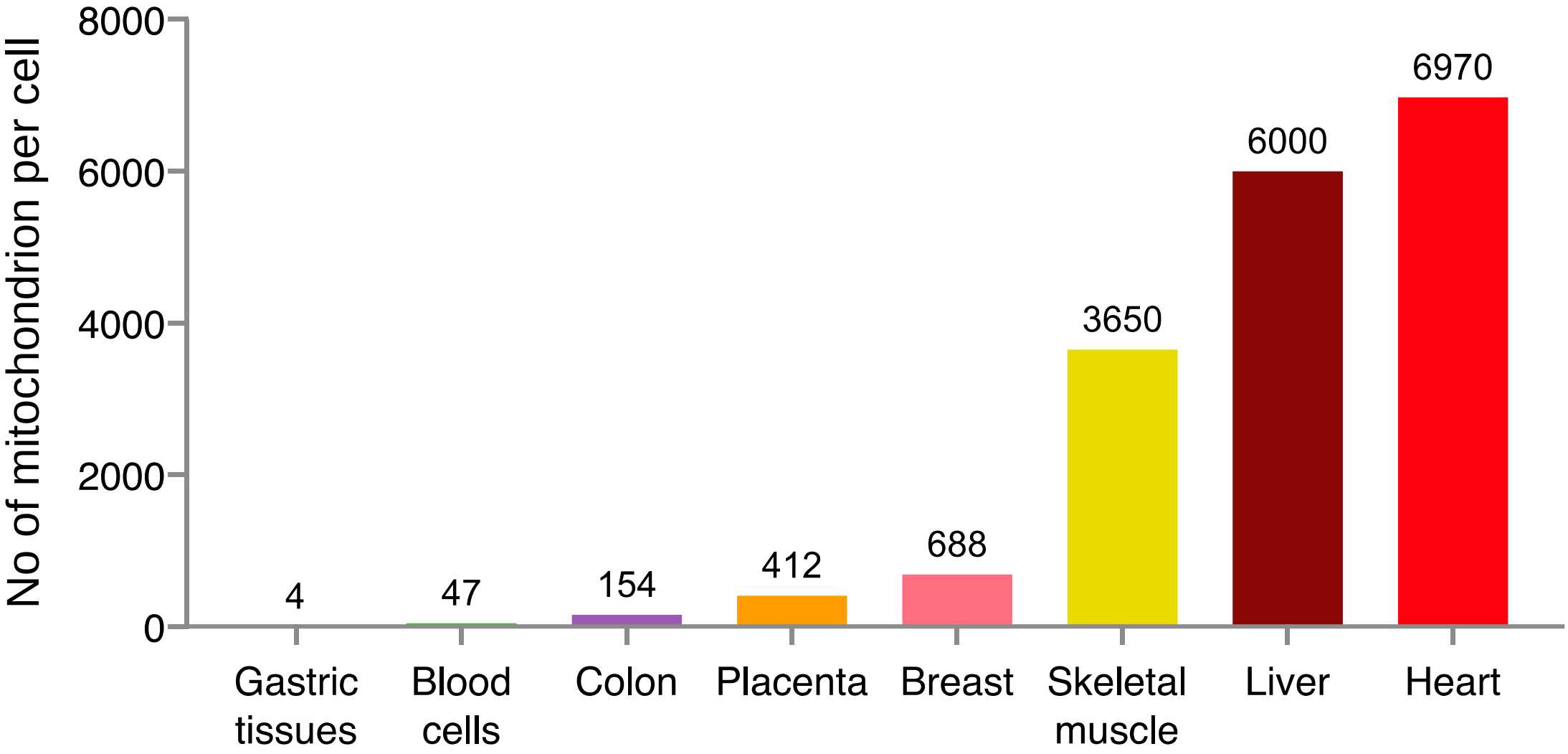



Fractional concentration of mitochondrial DNA (%)
0.01
0.001
0.0001
Healthy subjects
HBV subjects
Cirrhotic patients
HCC patients

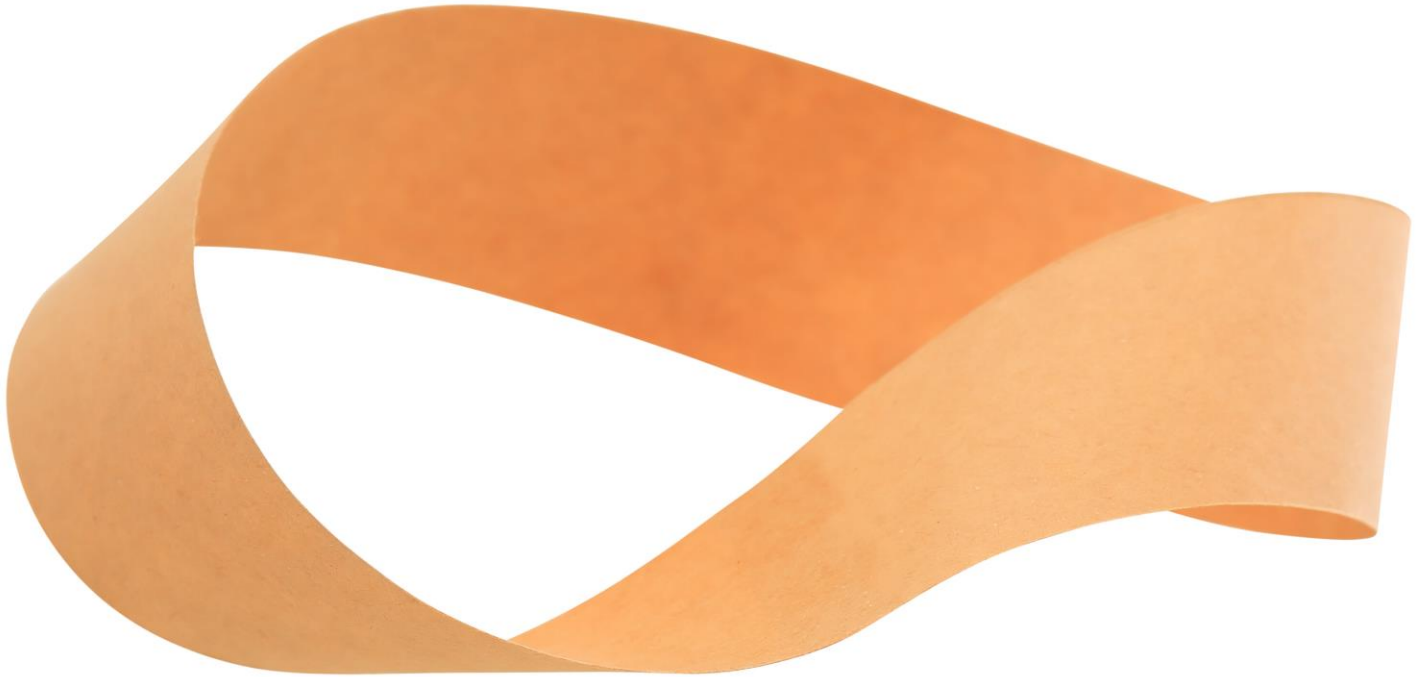
Number of mitochondria per cell



Number of mitochondria per cell



Plasma DNA Topologics



Summary

- Plasma nucleic acids represent a treasure trove for molecular diagnostics
- NIPT: global adoption and clinical paradigm shift
- Fragmentomics: fragment size, preferred ends, end motifs
- Plasma DNA topologics: circular and linear mtDNA in plasma
- Link to nuclease biology

Thank You!

香港中文大學醫學院

Faculty of Medicine

The Chinese University of Hong Kong

Secondary Genomic Findings Following Non-Invasive Prenatal Genetic Testing: Maternal Malignancy

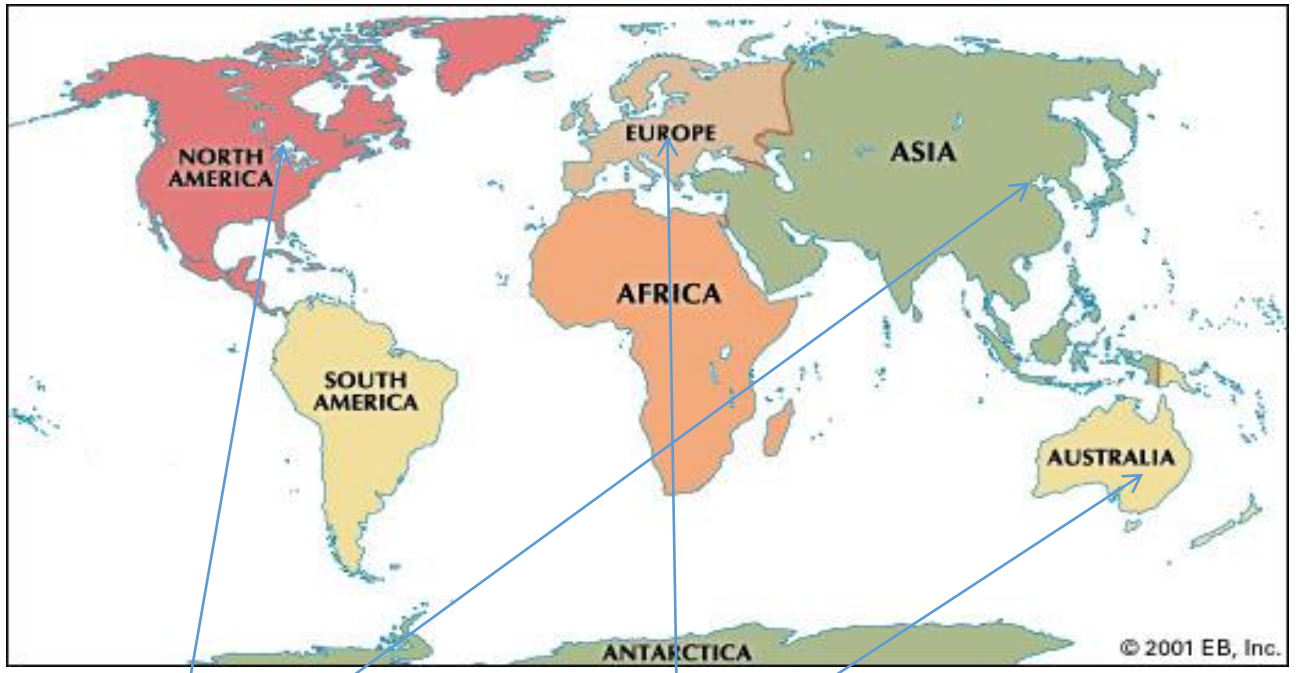
Diana W. Bianchi, M.D.

@DianaBianchiMD

NIH Eunice Kennedy Shriver National Institute of Child Health and Human Development

#1 Implementation of Genomic Medicine That Has Transformed Medical Care

> 10 million clinical tests to date



2011 2012

2013

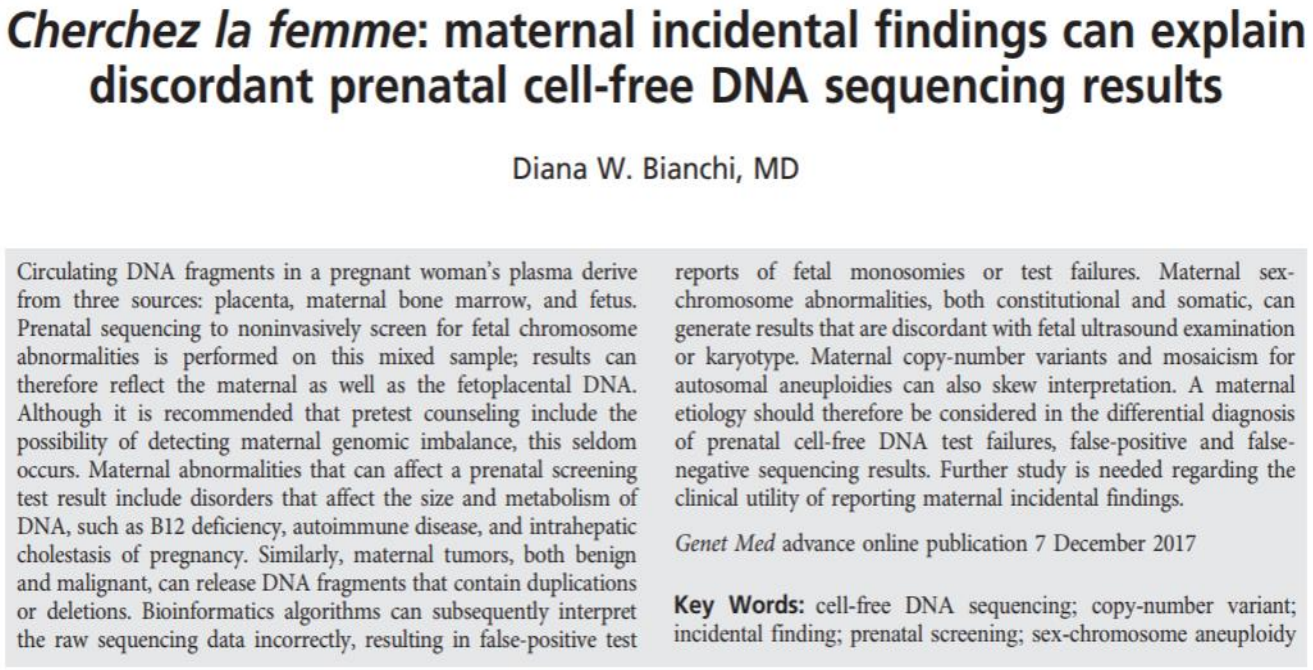
CLINICAL OPINION www.AJOG.org

GENETICS

Is it time to sound an alarm about false-positive cell-free DNA testing for fetal aneuploidy?

Michael T. Mennuti, MD; Athena M. Cherry, PhD; Jennifer J. D. Morrissette, PhD; Lorraine Dugoff, MD

Maternal Secondary Genomic Findings



Cherchez la femme: maternal incidental findings can explain discordant prenatal cell-free DNA sequencing results

Diana W. Bianchi, MD

Circulating DNA fragments in a pregnant woman's plasma derive from three sources: placenta, maternal bone marrow, and fetus. Prenatal sequencing to noninvasively screen for fetal chromosome abnormalities is performed on this mixed sample; results can therefore reflect the maternal as well as the fetoplacental DNA. Although it is recommended that pretest counseling include the possibility of detecting maternal genomic imbalance, this seldom occurs. Maternal abnormalities that can affect a prenatal screening test result include disorders that affect the size and metabolism of DNA, such as B12 deficiency, autoimmune disease, and intrahepatic cholestasis of pregnancy. Similarly, maternal tumors, both benign and malignant, can release DNA fragments that contain duplications or deletions. Bioinformatics algorithms can subsequently interpret the raw sequencing data incorrectly, resulting in false-positive test reports of fetal monosomies or test failures. Maternal sex-chromosome abnormalities, both constitutional and somatic, can generate results that are discordant with fetal ultrasound examination or karyotype. Maternal copy-number variants and mosaicism for autosomal aneuploidies can also skew interpretation. A maternal etiology should therefore be considered in the differential diagnosis of prenatal cell-free DNA test failures, false-positive and false-negative sequencing results. Further study is needed regarding the clinical utility of reporting maternal incidental findings.

Genet Med advance online publication 7 December 2017

Key Words: cell-free DNA sequencing; copy-number variant; incidental finding; prenatal screening; sex-chromosome aneuploidy

Genet Med 2017;
doi.10.1038/gim.2017.219

- Fetal sex discordance due to maternal transplant from XY donor or maternal DSD
- Vitamin B12 deficiency
- Autoimmune disease
- Copy number variants
  - 22q11.2 deletion
- Mosaic autosomal aneuploidies
  - Trisomy 8
- Sex chromosome aneuploidies
  - 45,X/47,XXX

When a Fetus's Test Finds a Mother's Cancer:
Mothers-to-be expecting to learn about chromosomal defects from a noninvasive prenatal test sometimes instead learn they may have cancer.
Anna Nowogrodzki *MIT Technology Review* July 2015

Details of Case 1

- 37 yo G2P1 woman, prior history negative
- NIPT x 2 13 wks: T13, M18, repeated 17 wks
- Amniocentesis: 46, XY, normal CMA
- 19 wks: normal fetal U/S, Son healthy, Apgars 8/9
- 3rd Tri: abdominal pain, fatigue, vaginal bleeding
- NIPT sent to another lab: nonreportable
- Pelvic fractures post delivery, bx of cervical mass and bone: metastatic neuroendocrine carcinoma
- Patient recently passed away, 7 years post dx

Maternal Malignancies

- Cancer during pregnancy is rare, occurs 1 in 1000 cases
- Most common malignancies in pregnant women are:
  - Breast and cervical cancers
  - Hodgkin and non-Hodgkin lymphomas, leukemia
  - Malignant melanoma
  - Ovarian and colorectal cancer
- In some cancers, tumor DNA is shed into the circulation
- This results in genomic imbalances that can be detected by NIPT
- Incidence of cancer as detected by NIPT is 1 in 10,000 cases

Which Cell Types Are Analyzed With NIPT?

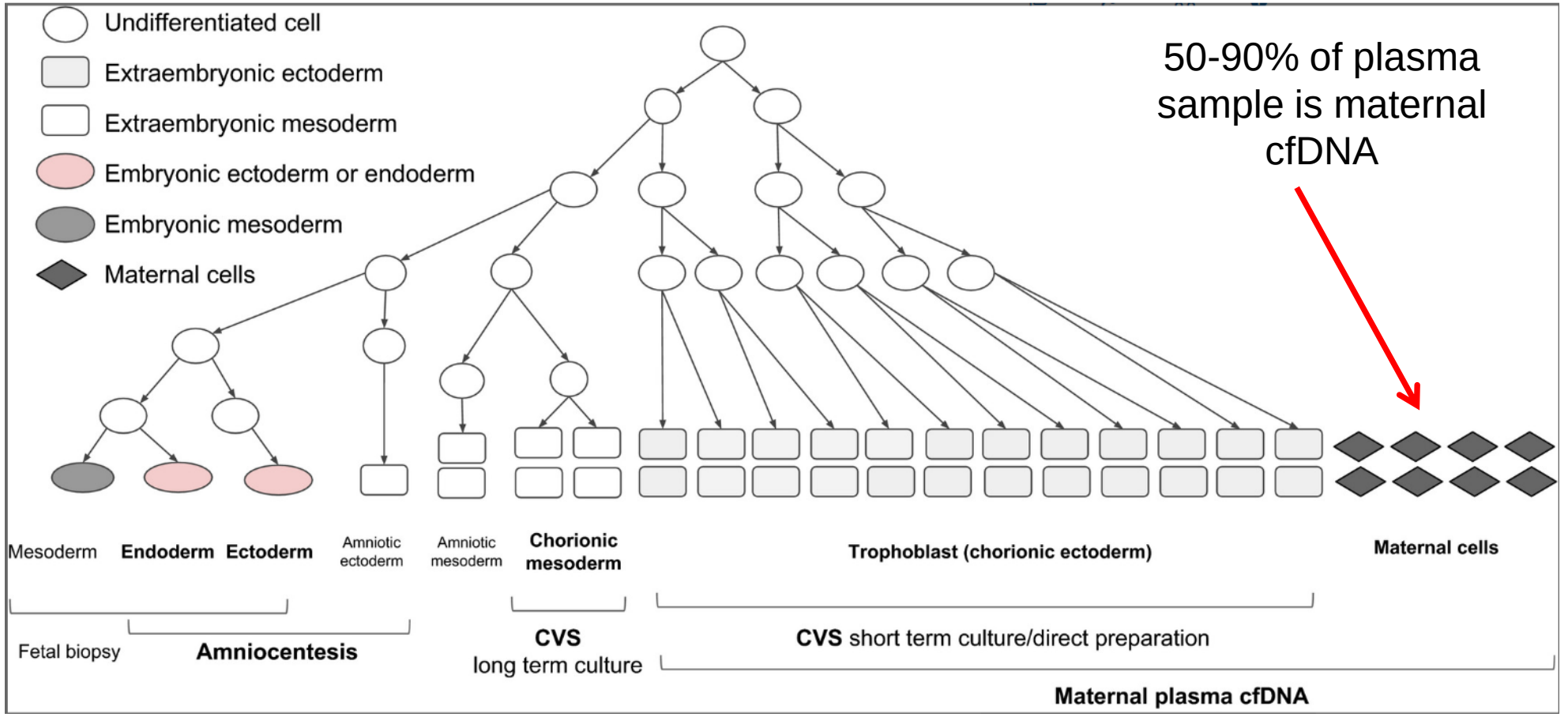
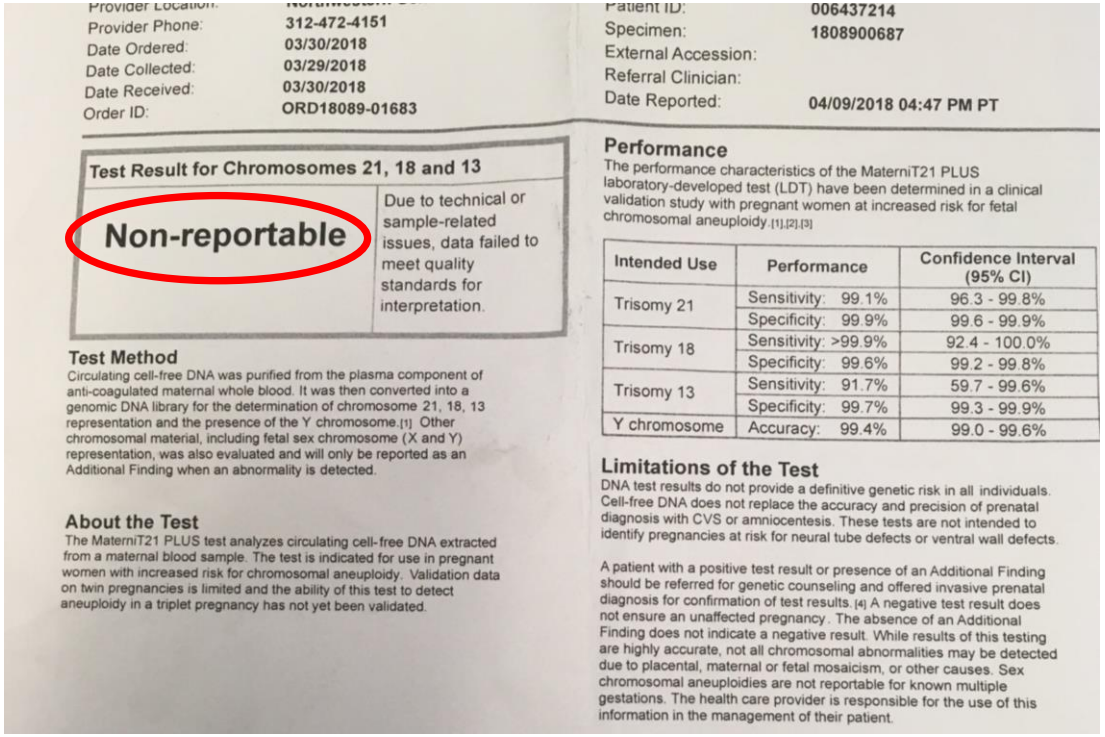


Figure from Rieder et al., *Aust NZ J Obstet Gynaecol* 2018,
Modified from Bianchi et al. *Am J Hum Genet* 1993

Many Presentations of Maternal Cancer

Unusual or Discordant Results/Test Failures/No Results



Provider Location:
Provider Phone: 312-472-4151
Date Ordered: 03/30/2018
Date Collected: 03/29/2018
Date Received: 03/30/2018
Order ID: ORD18089-01683

Patient ID: 006437214
Specimen: 1808900687
External Accession:
Referral Clinician:
Date Reported: 04/09/2018 04:47 PM PT

Test Result for Chromosomes 21, 18 and 13

Non-reportable

Due to technical or sample-related issues, data failed to meet quality standards for interpretation.

Test Method

Circulating cell-free DNA was purified from the plasma component of anti-coagulated maternal whole blood. It was then converted into a genomic DNA library for the determination of chromosome 21, 18, 13 representation and the presence of the Y chromosome.[1] Other chromosomal material, including fetal sex chromosome (X and Y) representation, was also evaluated and will only be reported as an Additional Finding when an abnormality is detected.

About the Test

The MaterniT21 PLUS test analyzes circulating cell-free DNA extracted from a maternal blood sample. The test is indicated for use in pregnant women with increased risk for chromosomal aneuploidy. Validation data on twin pregnancies is limited and the ability of this test to detect aneuploidy in a triplet pregnancy has not yet been validated.

Performance

The performance characteristics of the MaterniT21 PLUS laboratory-developed test (LDT) have been determined in a clinical validation study with pregnant women at increased risk for fetal chromosomal aneuploidy.[1],[2],[3]

Intended Use	Performance	Confidence Interval (95% CI)
Trisomy 21	Sensitivity: 99.1%	96.3 - 99.8%
	Specificity: 99.9%	99.6 - 99.9%
Trisomy 18	Sensitivity: >99.9%	92.4 - 100.0%
	Specificity: 99.6%	99.2 - 99.8%
Trisomy 13	Sensitivity: 91.7%	59.7 - 99.6%
	Specificity: 99.7%	99.3 - 99.9%
Y chromosome	Accuracy: 99.4%	99.0 - 99.6%

Limitations of the Test

DNA test results do not provide a definitive genetic risk in all individuals. Cell-free DNA does not replace the accuracy and precision of prenatal diagnosis with CVS or amniocentesis. These tests are not intended to identify pregnancies at risk for neural tube defects or ventral wall defects.

A patient with a positive test result or presence of an Additional Finding should be referred for genetic counseling and offered invasive prenatal diagnosis for confirmation of test results.[4] A negative test result does not ensure an unaffected pregnancy. The absence of an Additional Finding does not indicate a negative result. While results of this testing are highly accurate, not all chromosomal abnormalities may be detected due to placental, maternal or fetal mosaicism, or other causes. Sex chromosomal aneuploidies are not reportable for known multiple gestations. The health care provider is responsible for the use of this information in the management of their patient.

Table 2. Association of Maternal Cancers With Different Types of Aneuploidies Detected at Noninvasive Prenatal Testing

Type of Aneuploidy Detected by NIPT	Total No. of Samples	No. of Known Maternal Cancers (%) [95% CI]
Single trisomy[a]	2650	2 (0.08) [0-0.27]
Single SCA[b]	950	0 (0) [0-0.39]
Single trisomy + SCA	30	0 (0) [0-11.5]
Single monosomy	88	1 (1.14) [0-6.1]
Multiple aneuploidy[c]	39	7 (17.9) [7.5-33.5]
Total abnormal NIPT	3757	10 (0.26) [0.12-0.48]

Snyder et al. *Prenat Diagn* 2016;
Bianchi et al. *JAMA* 2015

Maternal Cancer as a Mechanism for False Positive Results

- Test the hypothesis that NIPT acts as a liquid biopsy
- Clinical laboratory database of 125,426 test results
- Physicians voluntarily informed laboratory of maternal cancer diagnosis as explanation for test failure or discordant results
- Patients re-contacted, gave consent, sequencing results re-analyzed

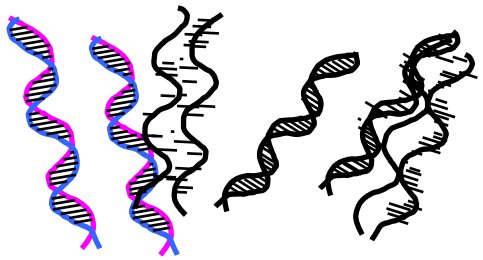
Bianchi et al. *JAMA* 2015; 314:162-169

Retrospective Maternal Cancer Study

Bianchi et al., *JAMA* 2015; 314:162-169

Case Number	1	2	3	4	5	6	7	8
Maternal Age	37	36	33	36	23	37	39	39
GA (weeks)	13	12	13	20	20	12	11	10
Aneuploidy Detection by NIPT	M18, T13	M18	T13	M21, M18, M13	T21, M18, T13	T18	M18, XXY	T21, T18, T13, MX
Fetal Karyotype / Birth Outcome	46,XY Term	ND Term	46,XY Term	46,XY Term	46,XY PE 29 wks	46,XX Term	46,XY 35 wks	46,XX 32 wks
Cancer Type	Neuro-endocrine	B cell lym-phoma	Colo-rectal	Hodgkin lym-phoma	ALL (T cell)	B cell lym-phoma	B cell lym-phoma	Anal
Stage at Dx	IV	IVB	IIIC	IIA	NA	IV	II	IIIB
Time to Dx (weeks)	28	13	39	3 to MRI 29 to Bx	3	~20	~10	8
Postnatal NIPT	ND	ND	T13, M18	M13, 18, 21, MX	ND	ND	ND	ND

WGS: Sequence, Align, Count



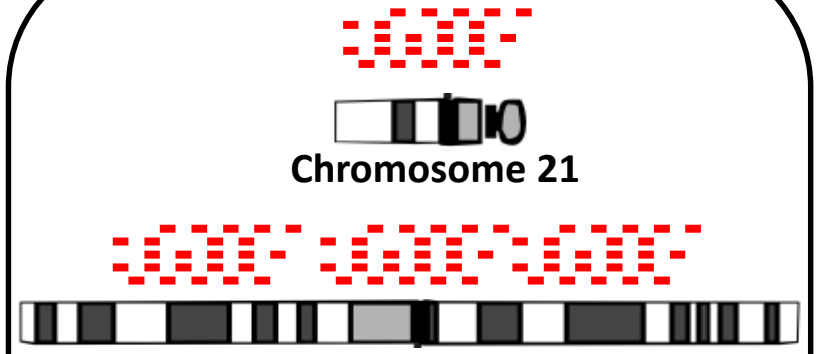
Cell-free DNA in Maternal Plasma

~150-200 bp fragments from both pregnant woman and her fetus

CCCTTAGCGCTTTAACGTACGTAAAA
CCCTTAGCGCTTTAACGTACGTAAAA
ACGGGGTCAAAGGTTCCCACACGTCC
GACTTAAAATCGGAATCGATGCCCAA
GACTTAAAATCGGAATCGATGCCCAA
ACGGGGTCAAAGGTTCCCACACGTCC
CCCTTAGCGCTTTAACGTACGTAAAA
CCCTTAGCGCTTTAACGTACGTAAAA
ACGGGGTCAAAGGTTCCCACACGTCC

Total DNA is sequenced

25-36 base pair reads



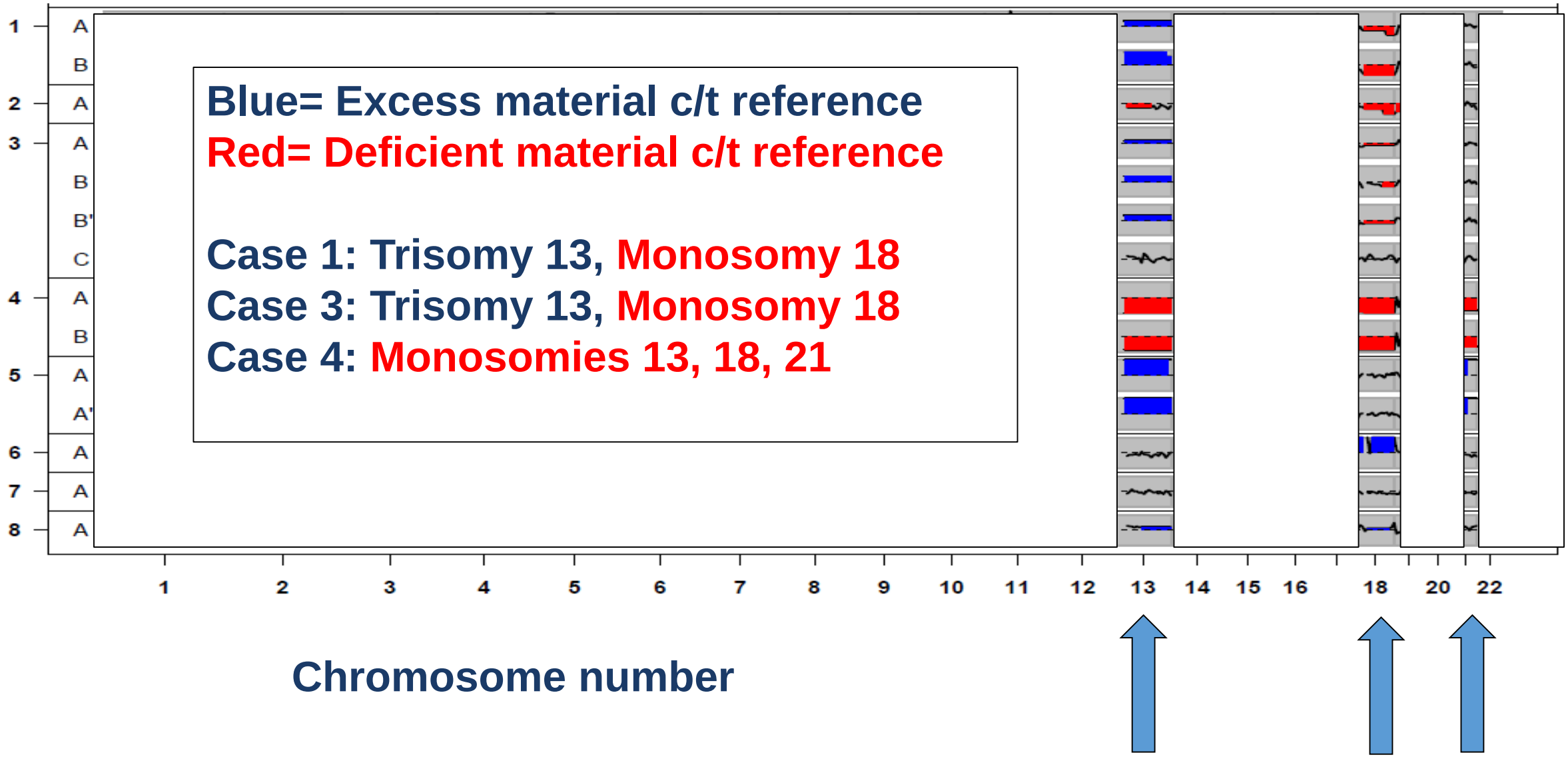
Alignment of reads

Measure counts relative to a reference

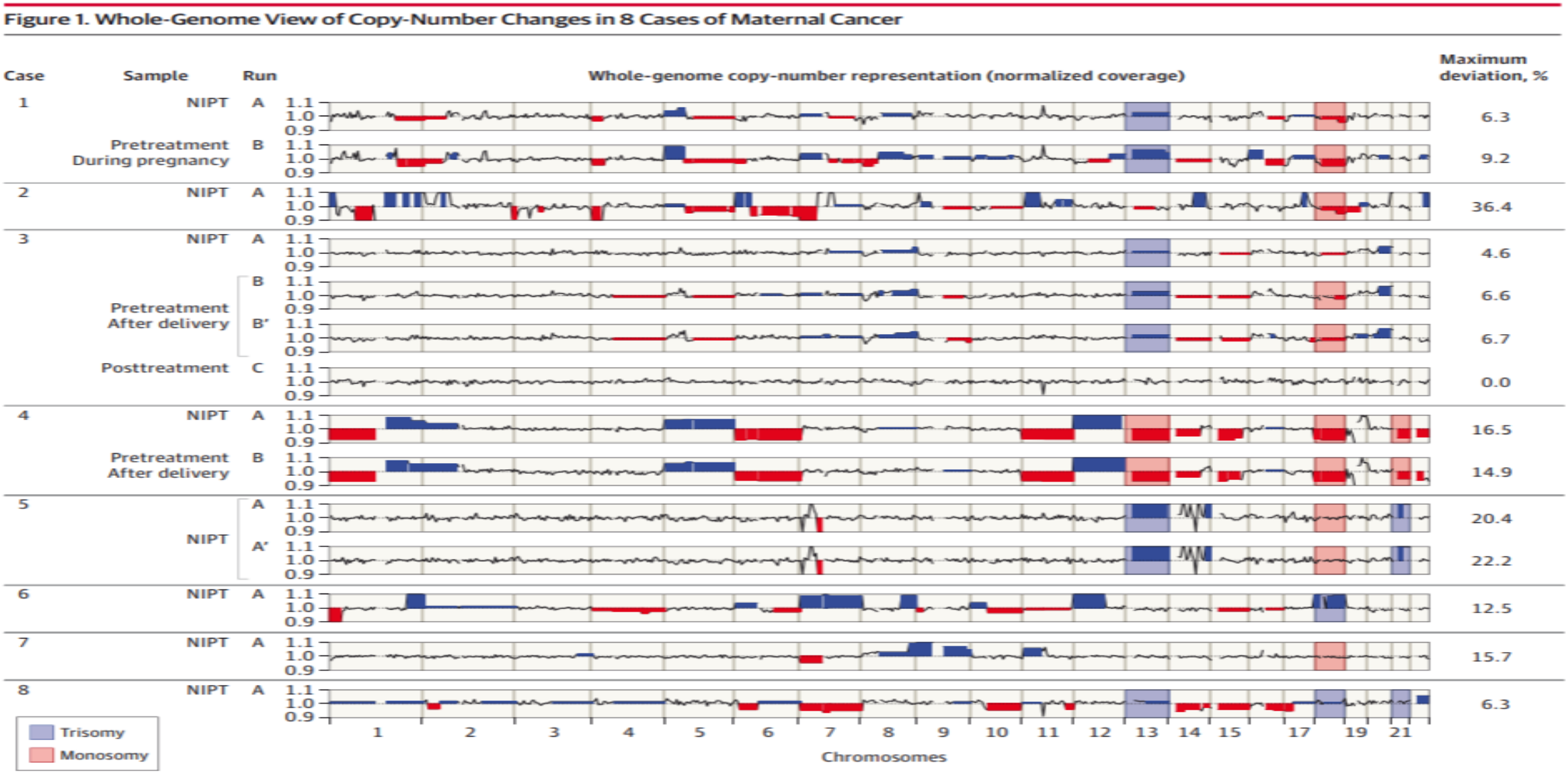
“Masking” Sequencing Data

Partial Genome View Seen in CLIA Lab

Bianchi et al. *JAMA* 2015; 314:162-169

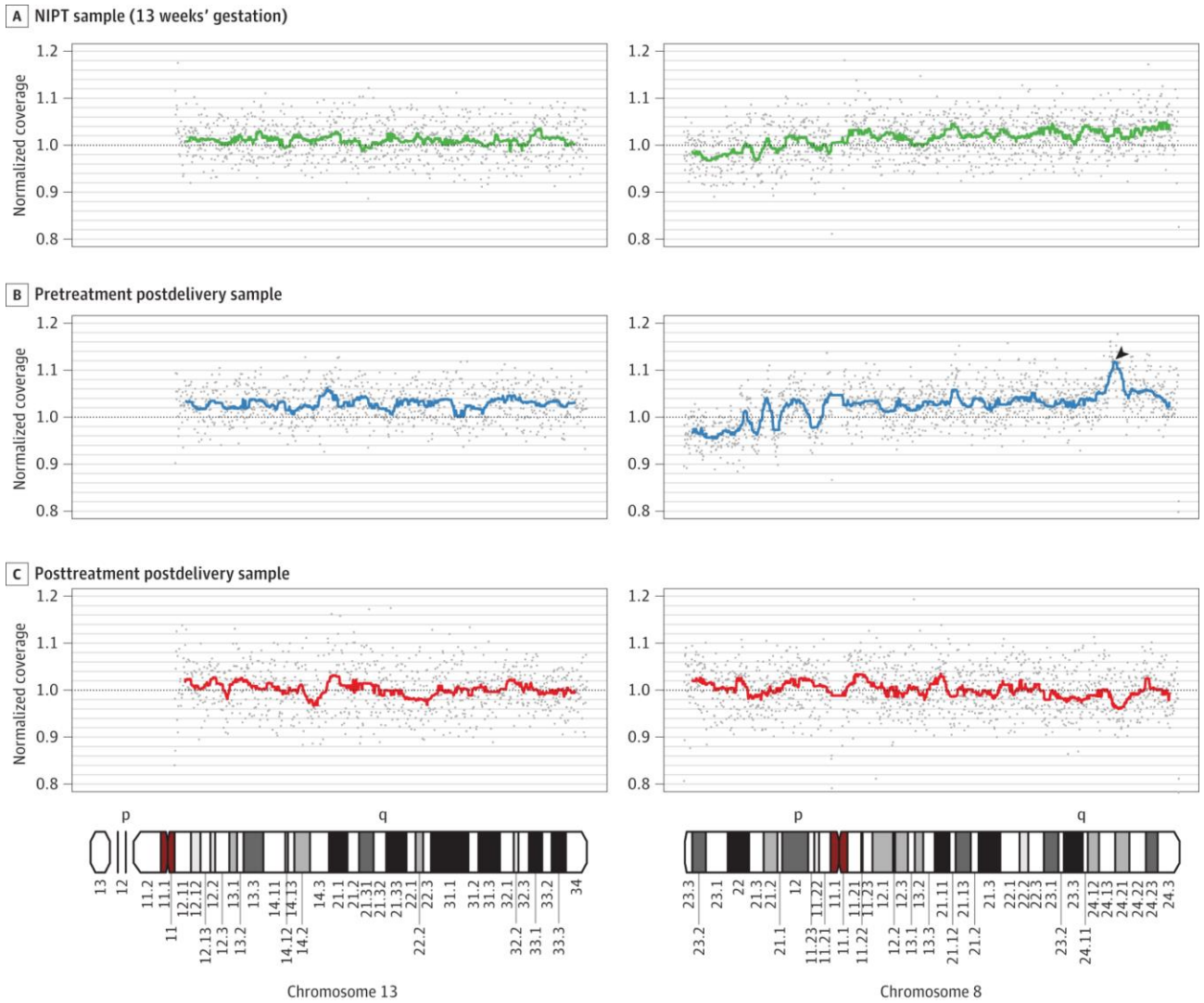


Whole Genome (Unmasked) View



Bianchi et al. *JAMA* 2015; 314:162-169

Close-Up View of JAMA Colon Cancer Case



- Bioinformatics algorithm reported trisomy 13
- Postpartum sample reported as T13, monosomy 18
- Real issue is trisomy 8. Chr 8 used as reference for chr 18.
- Post-treatment sample shows normalized profile

Machine Learning to Determine Which Women Have Cancer

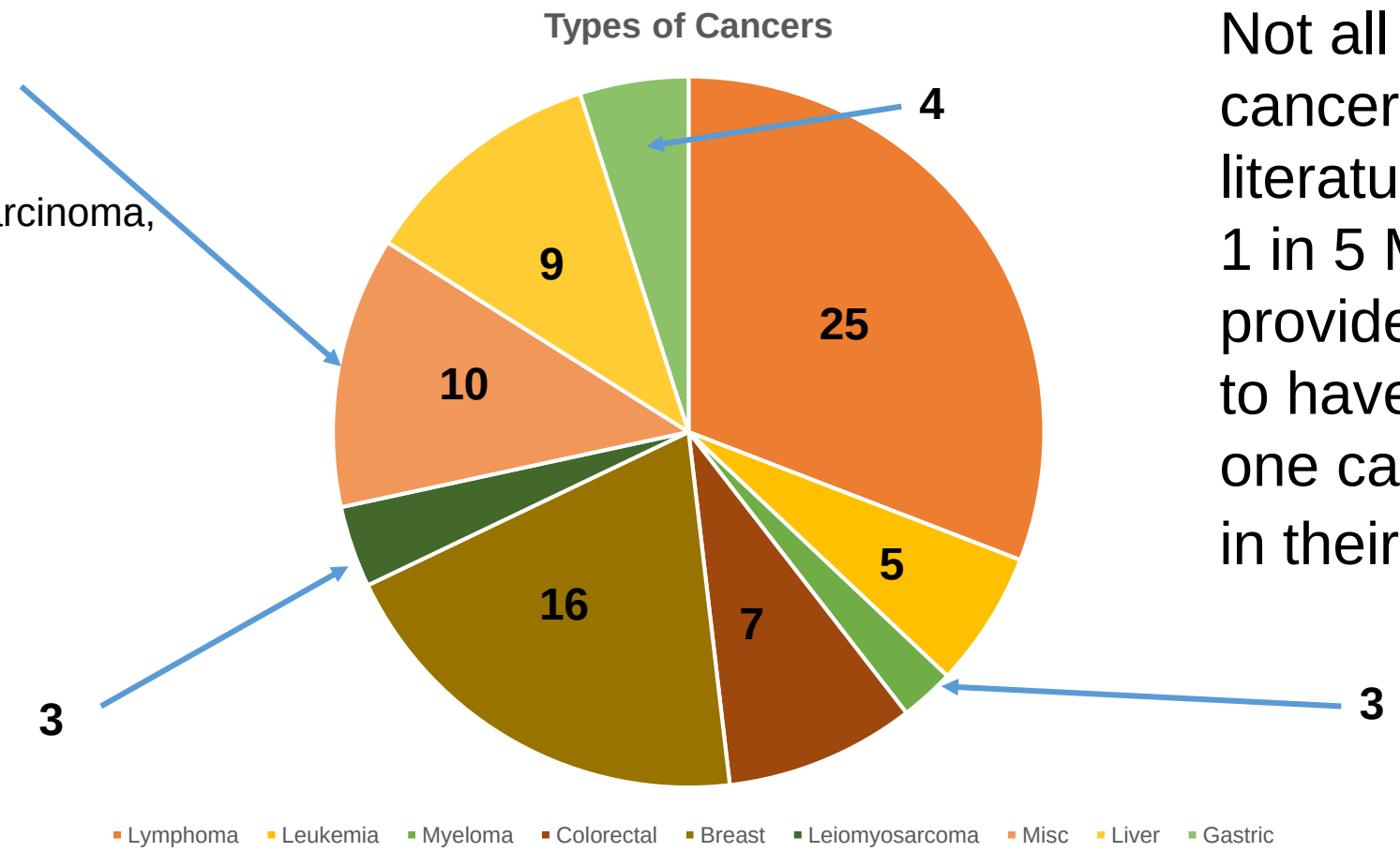
Ji et al. *Genet Med* April 12 2019

- 1.93 million cfDNA prenatal samples
- 639 had multiple aneuploidies (0.03%)
  - PPV of multiple aneuploidies alone=7.6%
- Retrospective study, 41 known to have cancer
- 34/41 (83%) correctly called with improved algorithm, 422/501 could be excluded
- Plasma tumor markers improved PPV to 75%
  - CA15-3, CA19-9, CA12-5, CEA, AFP

Secondary Maternal Cancers Reported as of Nov 2019

N=81 (in literature)

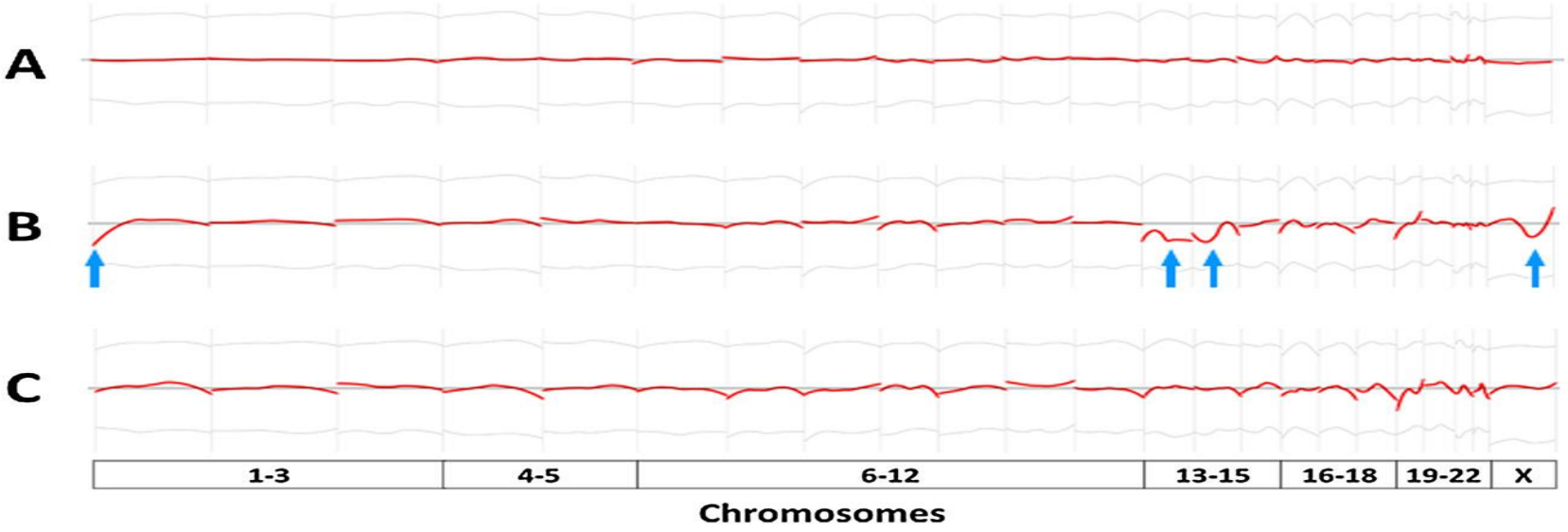
<u>Miscellaneous</u>
1 each:
Neuroendocrine
Teratoma,
Nasopharyngeal carcinoma,
Cervical,
Lung,
Dysgerminoma
Etc.



Not all maternal cancers reported in literature.
1 in 5 MFM providers estimated to have had at least one case of cancer in their practice.

Case 2

- 40 yo woman underwent cf DNA screening 14.5 wks
- Very negative chr 13 z-score, suggested monosomy 13
- Genome-wide analysis showed M13 and decreased sequence tags mapping to 1p, 14, Xq
- Amniocentesis: 46, XX



Dharajiya et al. *Prenat Diagn* 2015

Uterine Fibroids

- In one study, half of the abnormal NIPT results suggestive of cancer were due to uterine fibroids
- Fibroids are present in ~7% of African American women
- Why don’t we see more genome-wide dysregulation in women with fibroids?
- Are the cases shedding DNA undergoing malignant change?

Dharajiya et al., *Clin Chem* 2017

Follow-Up Management of NIPT Results Suggestive of Cancer?

Current Commentary

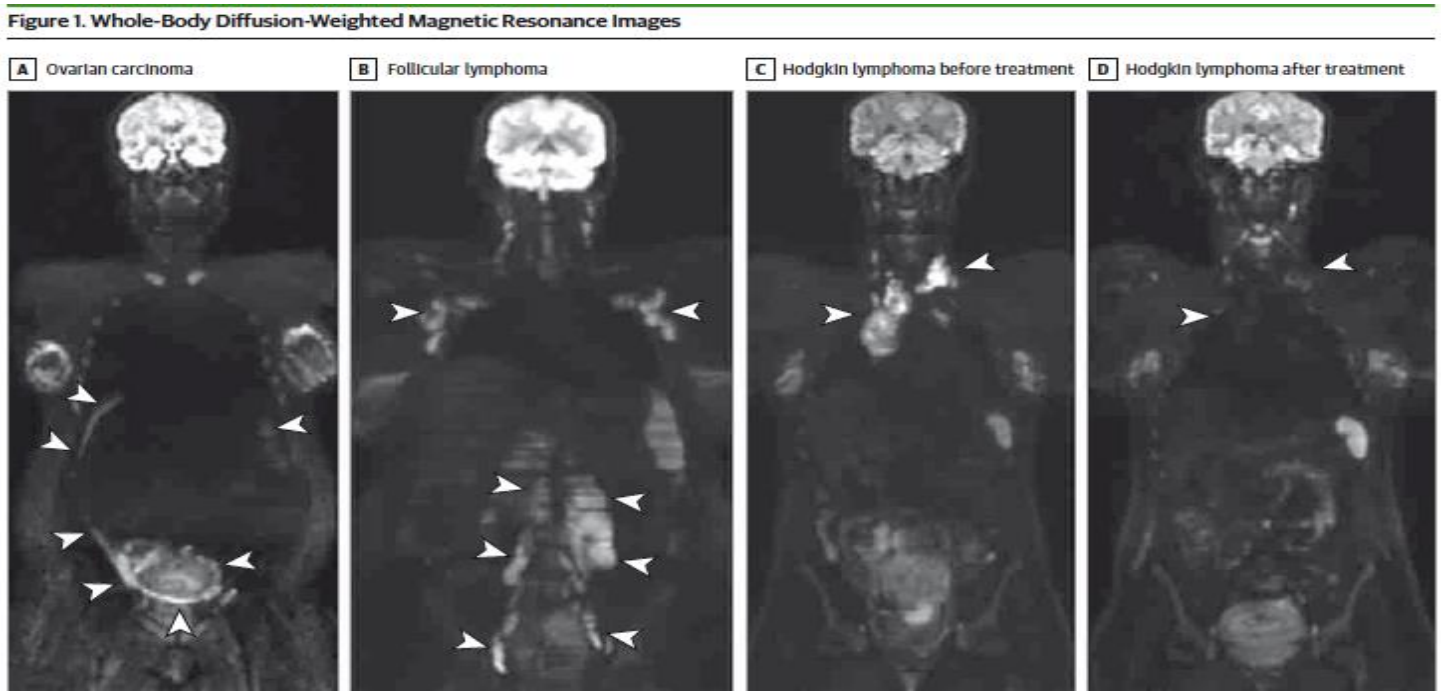
Maternal Malignancy Evaluation After Discordant Cell-Free DNA Results

Laura M. Carlson, MD, Emily Hardisty, MS, Catherine C. Coombs, MD, and Neeta L. Vora, MD

Box 1. Stepwise Evaluation of the Patient With More Than One Aneuploidy Detected on Cell-Free DNA

1. Discuss results with performing laboratory
   ↓
2. History and physical examination with laboratory evaluation
   - Complete blood count with peripheral smear
   - Metabolic panel
   - Pap test
   - Fecal occult blood
   ↓
3. Chest radiograph
   ↓
4. Magnetic resonance image of the chest, abdomen, and pelvis
   ↓
5. Consider annual complete blood count for surveillance

Obstet Gynecol 2018



Amant et al. *JAMA Oncology* 2015; 1:814-819

ISPD Debate: Should Results be Reported?

Received: 27 September 2018 | Revised: 8 October 2018 | Accepted: 10 October 2018

DOI: 10.1002/pd.5379

ISPD 2018 MEETING ISSUE

WILEY PRENATAL DIAGNOSIS

Current controversies in prenatal diagnosis 2: NIPT results suggesting maternal cancer should always be disclosed

Peter Benn[1] | Sharon E. Plon[2] | Diana W. Bianchi[3]

Stakeholder Perspectives

What Do Genetic Counselors Say?

(Giles et al. *Prenat Diagn* 2017; 37:126-32)

- **Survey of >300 US genetic counselors**
- **95% were aware NIPT results could suggest neoplasm**
- **Only 29% communicate this in the pre-test setting**
- **77% reported that they would disclose results**
- **Post-test recommendations highly variable**
- **91% said that institutional or national guidelines were needed for patient management**

Introducing the IDENTIFY Study

Incidental DEtection of maternal Neoplasia Through non-Invasive cell-Free dna analYsis

IDENTIFY: Follow-Up of cfDNA Patterns Suggestive of Malignancy

Prenatal Genomics and Therapy Lab, NHGRI

Women's Malignancy Branch, NCI

Complete work-up is free for women in the US

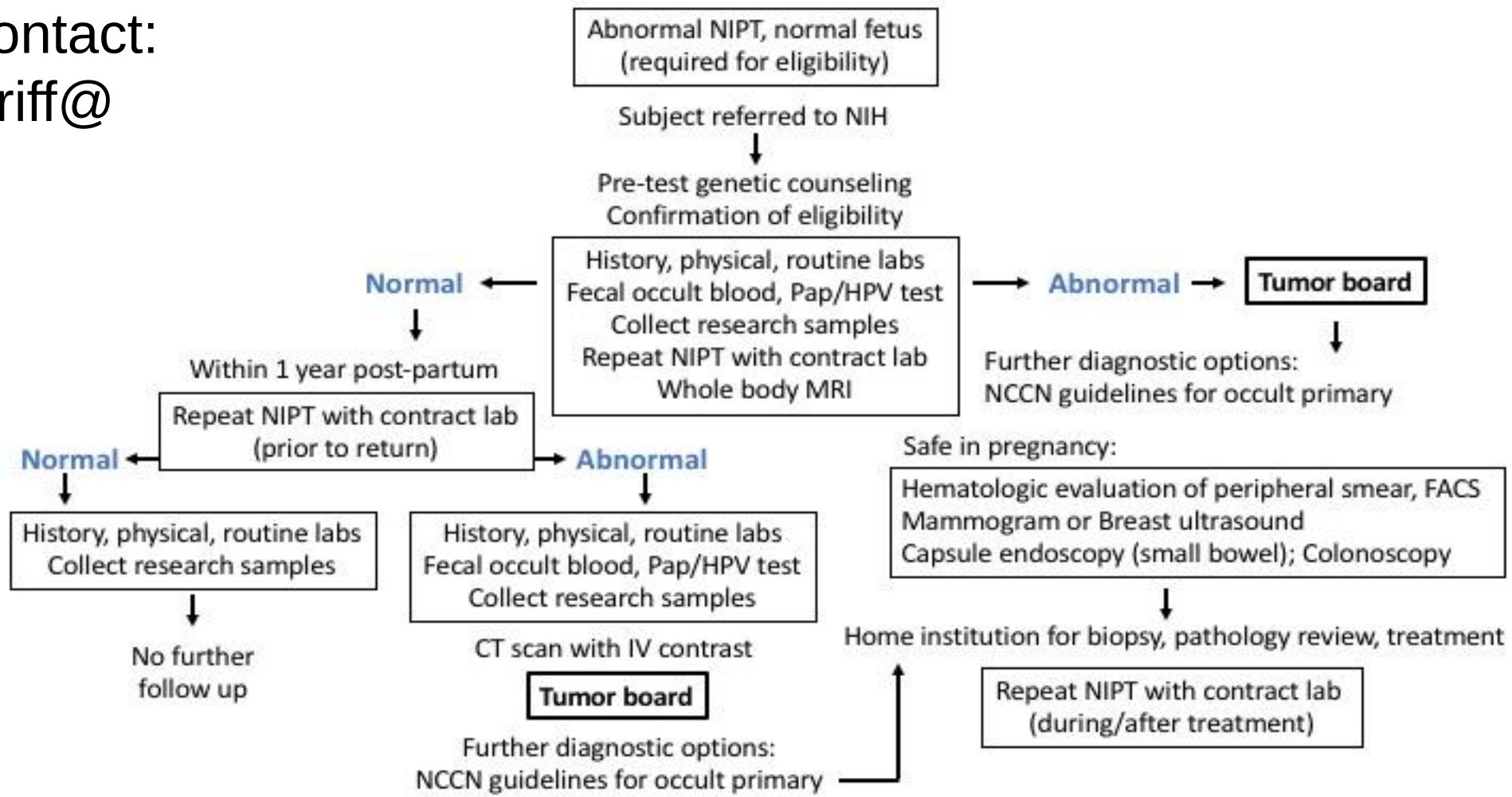
Can study women from outside of US, but have to get to US port of entry

NIH U.S. National Library of Medicine

ClinicalTrials.gov NCT 04049604

IDENTIFY Study Protocol

Study Contact:
Amy.Turriff@
nih.gov



Summary

- Maternal cancer is a rare, but increasingly recognized explanation for false positive cf DNA results (<1% of positive reports)
- Most suspicious is pattern of multiple aneuploidies, with genome-wide imbalance
- Not all cases are cancer: consider leiomyomas
- Need more evidence to determine management recommendations and whether early treatment saves lives
- Please refer cases to our NIH Clinical Center study!

Thank you!

Q&A with Speakers

Dennis Lo, MD

Diana Bianchi, MD

Bruce Korf, MD, PhD
Moderator

AJHG

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Thank You for Attending

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