

Massively Parallel Sequencing Analysis of Whole Mitochondrial DNA Sequences and Point Heteroplasmy in Koreans

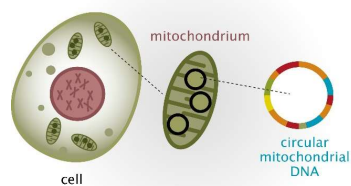
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1. Introduction

Mitochondrial DNA



- High copy number (10³–10⁴ copies/cell)
- Useful forensic tool for challenging samples



Hair shafts



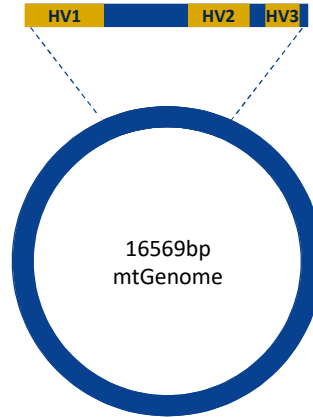
Skeletal remains

1. Introduction

Massively Parallel Sequencing



- High resolution based on read count
- High throughput data
- Cost-effective



Whole mtGenome!

1. Introduction

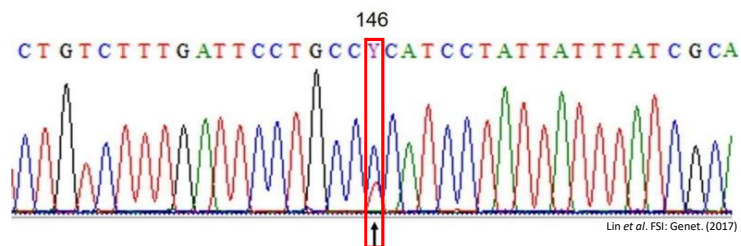
Point Heteroplasmy

Heteroplasmy:

The presence of more than one mtDNA type in an individual (Melton 2004)

Point Heteroplasmy:

The presence of two nucleotides at a single site.



1. Introduction

Objectives

- Compilation of whole mtGenome haplotype data for Koreans.
- Investigate point heteroplasmy distribution across Korean population.
- Compare haplotype diversity between control region and whole mtGenome using MPS.

2. Materials and Methods

➤ MPS workflow

Sample collection



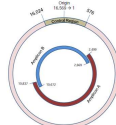
- 376 unrelated Koreans
- Peripheral Blood
- Buccal swab

DNA extraction



- QIAamp DNA Mini Kit

MPS Library prep.



- Long range PCR with 2 fragment
- Nextera XT DNA Library Prep Kit

MPS system



- MiSeq™ system
- MiSeq Kit v2 or v3

MPS analysis

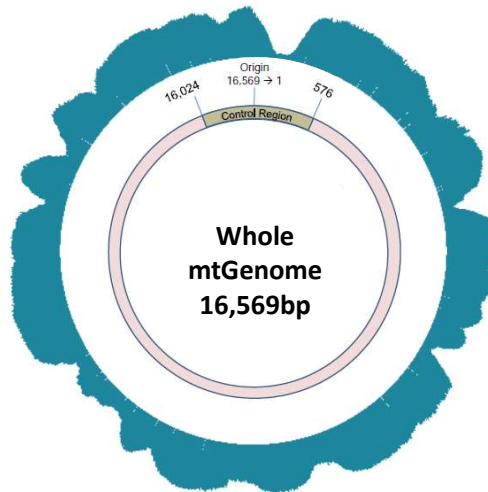


- GATK Mutect2

*This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University in Seoul, Korea.

3. Results and Discussions

➤ MPS coverage ($n=376$)



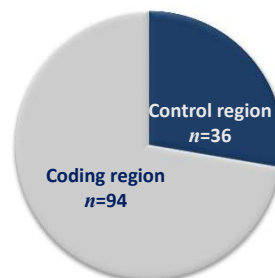
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3. Results and Discussions

➤ Observed point heteroplasmy of whole mtGenomes

- ✓ Variant calling threshold >100X
- ✓ Point heteroplasmy threshold > 400X
- ✓ Minor nucleotides of PHP were observed in > 5% of the total coverage

- 110 of the 376 donors
- Total 130 PHPs
- Up to 3 PHPs per donor
- Control region : Coding region = 36 : 94

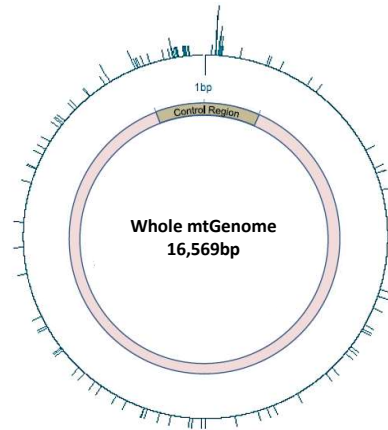


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3. Results and Discussions

➤ PHP distribution across the whole mtGenome of Koreans

Rank	Nucleotide position	Variant	Number of samples
1	152	Y	5
2	146	Y	4
3	204	Y	3
4	188	R	2
	234	R	2
	5020	Y	2
	5021	Y	2
	15119	R	2
	15530	Y	2
	16093	Y	2



3. Results and Discussions

➤ Haplotype diversity across the whole mtGenome of Koreans

Number of Haplotypes Matching	Control Region	Whole mtGenome
6	1	0
4	5	0
3	10	2
2	30	8
1 (Unique)	260(69.1%)	354(94.1%)
Total no. of haplotypes	306	364
Total no. of samples	376	376

3. Results and Discussions

➤ Haplotype diversity across the whole mtGenome of Koreans

Case I)

Control Region Haplotype	Matching haplotype
73G 194T 263G 315.1C 489C 523DEL 524DEL 16223T 16362C 16519C	6



Whole mtGenome Haplotype	Matching haplotype
73G 194T 263G 315.1C 489C16223T 16362C 16519C	3
73G 194T 263G 315.1C 489C ... 10373A ... 14325C 15331A 16223T 16362C 16519C	1
73G 194T 263G 315.1C 489C ... 6252G16223T 16362C 16519C	1
73G 194T 263G 315.1C 489C ... 12630A16223T 16362C 16519C	1

3. Results and Discussions

➤ Haplotype diversity across the whole mtGenome of Koreans

Case II)

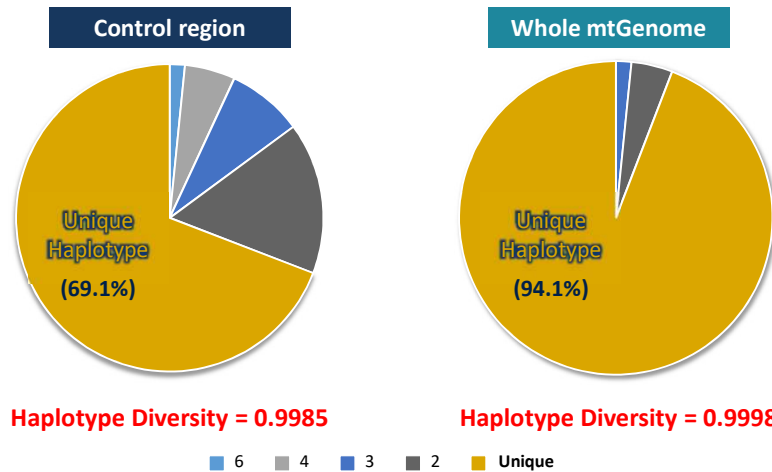
Control Region Haplotype	Matching haplotype
73G 150T 199C 263G 315.1C 489C 16129A 16183C 16189C 16223T 16297C 16298C	4



Whole mtGenome Haplotype	Matching haplotype
73G 150T 199C 263G 315.1C.....16183C 16189C 16223T 16297C 16298C	1
73G 150T 199C 263G 315.1C..... 15002A ...16183C 16189C 16223T 16297C 16298C	1
73G 150T 199C 263G 315.1C..... 5147A ... 15002A16183C 16189C 16223T 16297C 16298C	1
73G 150T 199C 263G 315.1C..... 13011T 14319C16183C 16189C 16223T 16297C 16298C	1

3. Results and Discussions

➤ Haplotype diversity across the whole mtGenome of Koreans



4. Conclusions

- We successfully analyzed the whole mtGenome variants from **376 Koreans** using MPS.
- We investigated point heteroplasmy (>400X, >5% of the total coverage) distribution and frequency across the Korean population.
- The number of **unique haplotype was increased in the whole mtGenome** (94.1%) than the control region(69.1%).
- The data on the whole mtGenome sequence of Korean population obtained from this study could be used as reference in forensic casework.

Acknowledgment



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Thank you!

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