ทฤษฎี และสิ่งที่ต้องรู้เกี่ยวกับไมโตคอนเดรีย

บุษบา ฤกษ์อำนวยโชค

Outline :

- บทนำ
- ความรู้ทั่วไปเกี่ยวกับไมโตคอนเดรีย
- การใช้ไมโตคอนเดรียในงานด้านนิติเวชศาสตร์
- การแปลผลการตรวจไมโตคอนเดรียทางนิติเวชศาสตร์

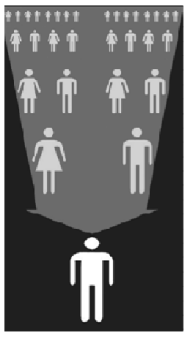
บทนำ

Conventional STR typing systems do not work in every instance :

Ancient DNA specimens / highly degraded samples often fail to produce results with nuclear DNA.

Because of high copies of mtDNA, the probability of obtaining mtDNA result is higher than nuclear DNA

บทนำ



Autosomal DNA marker

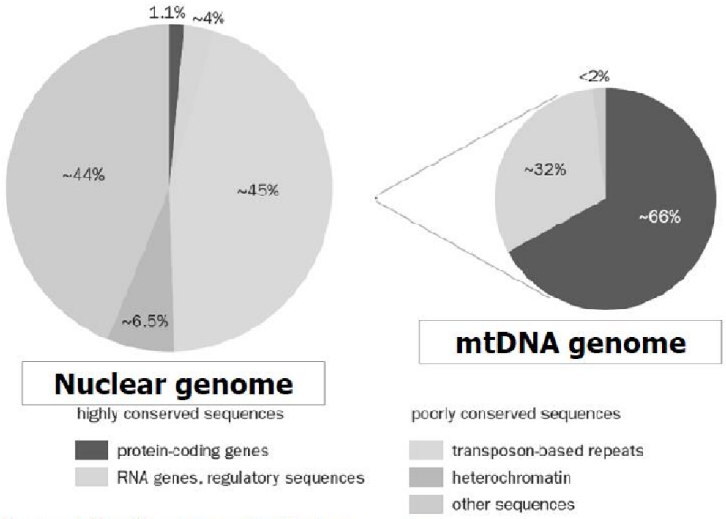
Mendelian transmission

บทนำ

Lineage marker

- **Y chromosomal DNA**
- **mtDNA**

Pass down from generation to generation without changing (except mutation)



From: http://www.garlandscience.com

ความรู้ทั่วไปเกี่ยวกับไมโตคอนเดรีย 1

Comparison of nuclear DNA and mtDNA

characteristics	nuclear DNA	mtDNA
Size of genome	~ 3.3 Billion bp	~ 16,596 bp
Copies per cell	2 (1 alleles from each parent)	can be >1,000
Percent of total DNA per cell	99.75%	0.25% content
Structure	linear, packaged in chromosome	circular

Butler JM 2012

ความรู้ทั่วไปเกี่ยวกับไมโตคอนเดรีย 2

Comparison of nuclear DNA and mtDNA

characteristics	nuclear DNA	mtDNA
Inherited from	Father and mother	mother
Chromosomal pairing	Diploid	Haploid
Generational recombination	Yes	No
Replication repair	Yes	No

Butler JM 2012

ความรู้ทั่วไปเกี่ยวกับไมโตคอนเดรีย 3

Comparison of nuclear DNA and mtDNA

characteristics	nuclear DNA	mtDNA
unique	Unique to individual (except identical twins)	Not unique to individual (same as m)
Mutation rate	Low	At least 5-10 times nDNA
Reference sequence	Described in 2001 by the Human Genome Project	Described in 1981 by Anderson et al.

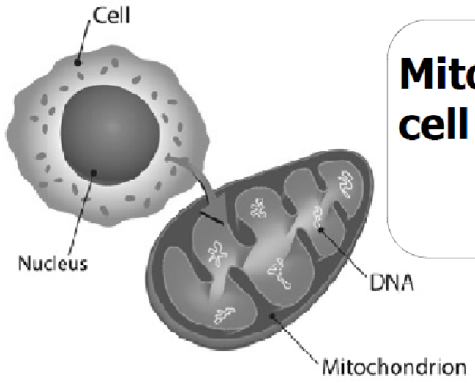
Butler JM 2012

Comparison of nuclear DNA and mtDNA		
characteristics	**nuclear DNA**	**mtDNA**
Occurrence in human genome	~ 1 in every 15 Kb	~ 1 in every 1 Kb
General informativeness	high	Low, only 20-30% as informative as STRs
Marker type	Di-, tri-, tetranucleotide repeat markers	Bi-allelic markers
Number of alleles per marker	Typically > 5	Typically 2
Detection method	Gel/CE	Sequencing, microarray

Butler JM 2001

Average mtDNA/mitochondria : 4-5 copies

Satoh & Kuroiwa 1991



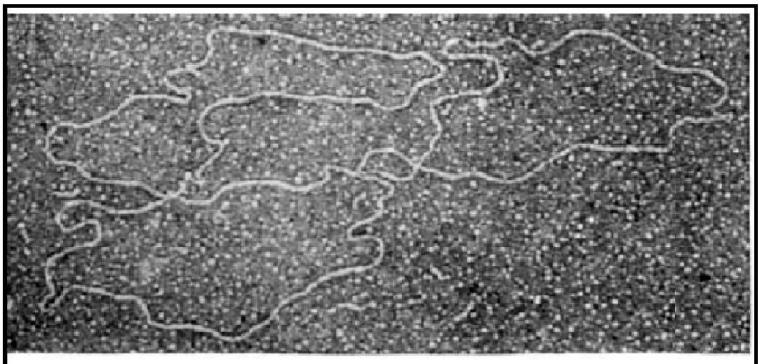
Mitochondria/ cell : hundreds

Robin & Wong 1988

Average mtDNA/ cell : ~ 500 copies

Satoh & Kuroiwa 1991

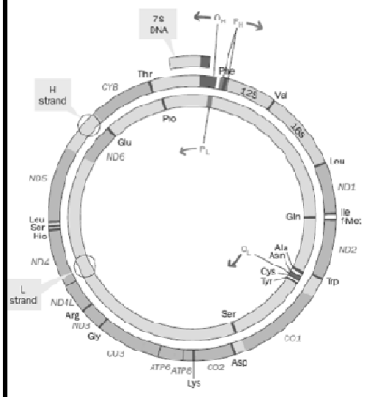
Greater success with biological samples that may be damaged with heat and humidity than nDNA



Mitochondria have circular genomes : A scanning electron micrograph of mitochondrial DNA.

Hudson, B. *et al.* 1967

The mitochondrial genome is densely packed with genetic information

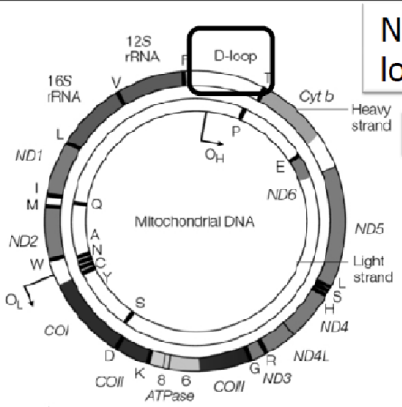


2 mtDNA strands have significantly different base composition :

- heavy (H) strand is richer in G
- light(L) strand is richer in C

mtDNA ~ 16,569 bp

mtGenome contains for 37 gene products

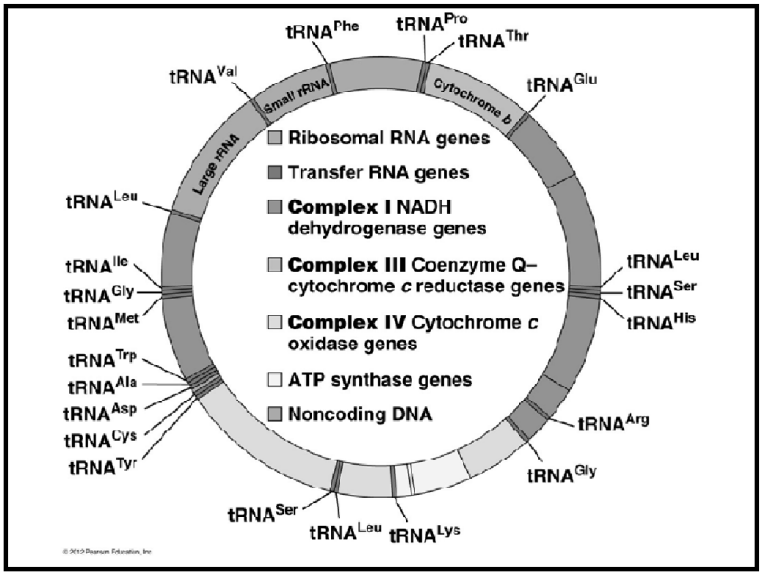


Non-coding region (D-loop) or control region

Coding region encode

- 13 proteins
- 22 tRNAs
- 2 rRNAs

Taylor et al 2005



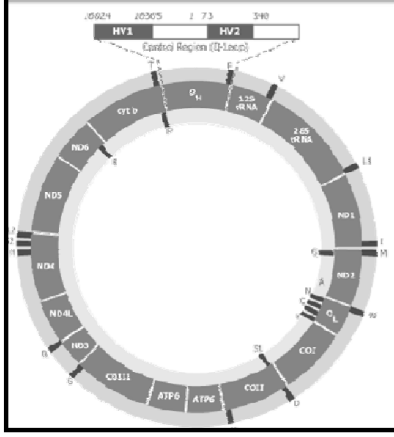
1.The coding region :

responsible for the production of various biological molecules involved in the process of energy production in the cell.

2.The non-coding region :

responsible for regulation of the mtDNA molecule.

Non-coding region (D-loop) or control region



- Does not code for gene product
- polymorphism between individuals

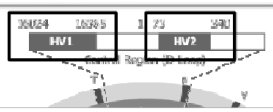
Greenberg et al. 1983

Two regions of mtDNA within the non-coding (control) region are highly polymorphic, or variable, within the human population

Hypervariable Regions

Hypervariable Regions

16,024 -16,365



73-340

1. Hypervariable Region I (HV1)
   position 16,024 ~ 16,365
   ~342 bp length
2. Hypervariable Region II (HV2)
   position 73 ~ 340
   ~ 268 bp length

Hypervariable Regions

Forensic mtDNA examinations are performed using these two regions (HV1 and HV2) because of the high degree of variability found among individuals.

HV3 shows less polymorphic than HV1 and HV2 but may resolve some cases where additional discrimination is desired.

Human mtDNA reference sequences

First sequenced in 1981 in the laboratory of Frederick Sanger in Cambridge, England (Anderson et al. 1981)

- **Anderson sequence/**
- **Cambridge reference sequence**

The sequence was derived primarily from a single individual of Europe descent and it also contained somw HeLa and bovine sequences to fill in gaps resulting from incomplete sequencing procedure (Anderson et al.1981)

Revised Cambridge reference sequences (rCRS)

In 1999, the original placental material used by Anderson and co-workers to generate the CRS was re-sequenced (Andrews et al.1999)

With the improvements in DNA sequencing technology, the reanalysis effort confirmed all of the original nucleotides published but 11 positions were corrected.

Other interesting differences between mtDNA and nDNA

- mtDNA uses a different genetic code than nDNA (Scheffler 1999)

mtDNA : UGA --- tryptophan
nDNA : UGA --- stop codon

mtDNA : AUA --- methionine
nDNA : AUA --- isoleucine

Other interesting differences between mtDNA and nDNA

- Fewer DNA repair mechanisms exist in mitochondria

⇩

Higher mutation rate than nDNA

⇩

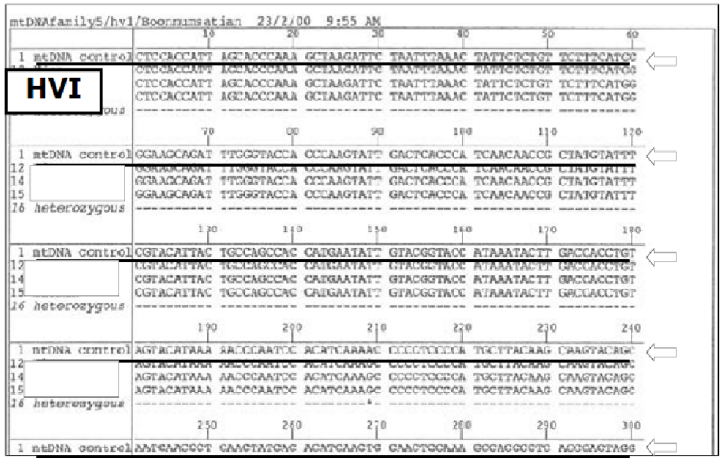
More variability in samples from identical maternal lineages

Other interesting differences between mtDNA and nDNA

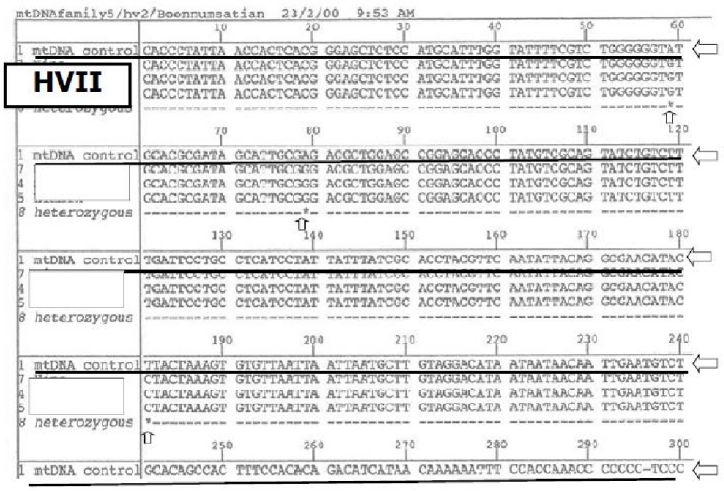
- Circular nature of mtDNA makes it less susceptible than nDNA to exonuclease that break down DNA molecules

- The encapsulation of mtDNA in a two-walled organelle enhances mtDNA survival rate.

Revised Cambridge reference sequences (rCRS)



Revised Cambridge reference sequences (rCRS)



Revised Cambridge reference sequences (rCRS)

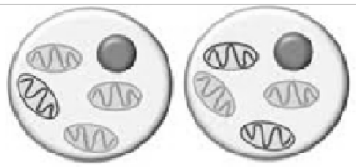
Each base pair in this sequence is assigned a number.

Deviations from this reference sequence are recorded as the number of the position demonstrating a difference and a letter designation of the different base.

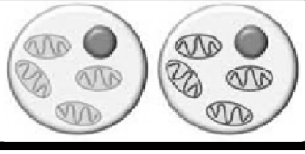
Example : a transition from A to G at Position 263 would be recorded as 263 G

Heteroplasmy

The presence of a mixture of more than one type of an mitochondrial DNA genome within a cell or individual.



Homoplasmy

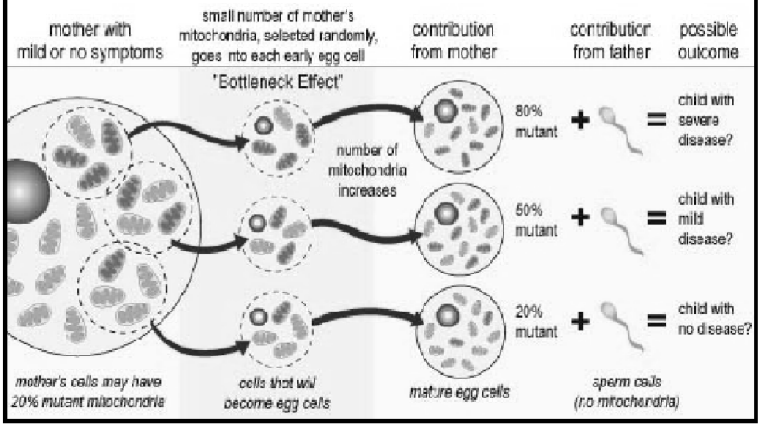


Two forms of heteroplasmy in mtDNA :

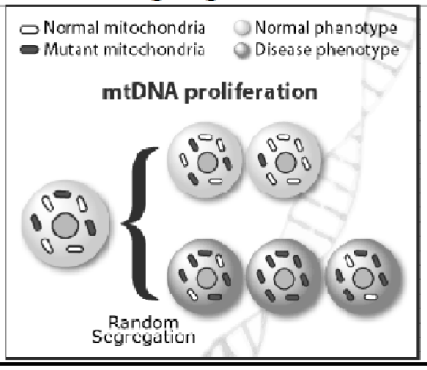
1. Sequence heteroplasmy **(point heteroplasmy)** is the occurrence of more than one base at a particular position or positions in the mtDNA sequence.

2. Length heteroplasmy is the occurrence of more than one length of a stretch of the same base in a mtDNA sequence.

During cell division, the multiple mtDNA molecules in a dividing cell segregate in a random way to the two daughter cells.



At cell division, the mitochondria and their genomes are randomly distributed to the daughter cells, a process known as replicative segregation.



Causes of Heteroplasmy

- Molecular events not quite understood
- Most probable explanation: mutations in female germ line followed by subsequent differentiation during embryonic development
- HVI and HVII (Hypervariable sequences) are typically analyzed for forensic use
- Hotspots are prone to heteroplasmy

Heteroplasmy and Human Hair

- Human hair has high frequency of heteroplasmy
- Example: hair from same individual may have different mtDNA
- Although hair contains heteroplasmy, other tissues from same individual may not, i.e. blood, muscle, skin

1st hair

2nd hair

3rd hair

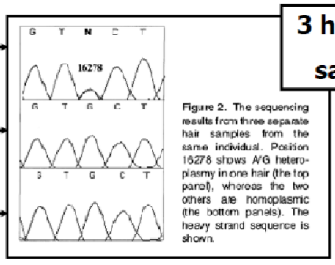


Figure 2. The sequencing results from three separate hair samples from the same individual. Position 16278 shows A/G heteroplasmy in one hair (the top panel), whereas the two others are homoplasmic (the bottom panels). The heavy strand sequence is shown.

3 hair from the same person

Het+

Het-

Heteroplasmy complicates forensic mtDNA testing

May not always be detected depending on the tissue type and analysis procedure

- May be difficult to form a basis of exclusion
- In case of non-mutation, sequence frequency is determined from a mtDNA database

Heteroplasmy Benefits Forensic mtDNA Testing

- Rarity of a particular heteroplasmy may increase discriminating power

Table 2. Actual casework example in which heteroplasmy was found

Sample	mtDNA position 16090
Q hair	T/C
R blood	T
R hair 1	T
R hair 2	T
R hair 3	T
R hair 4	T/C

Q, questioned
R, reference

Summary of heteroplasmy

- An individual may have different versions of mtDNA
- Heteroplasmy may complicate mtDNA testing, but may also make it more efficient
- Tissue type is important when analyzing mtDNA
- Standardization is needed in mtDNA testing procedures

การใช้ไมโตคอนเดรียในงานด้านนิติเวชศาสตร์

Reasons for using mtDNA analysis

- These markers occur in males and females and are inherited through the maternal line.
- Forensic Casework
  - ** Analyze sample materials that are not suitable for nuclear DNA
  - ** Test hair strands without the root
  - ** Test highly degraded tissue
  - ** Test skeletal remains Maternity Testing
- Sibling Studies (from the same mother)
- Grandmaternity (from the same grandmother)
- Maternal kinship analysis

Forensic application :

Mitochondrial DNA analysis is an appropriate method for:

- **Charred remains**
- **Degraded specimens**
- **Old skeletal and fingernail samples**
- **Hair shafts**

การแปลผลการตรวจ ไมโตคอนเดรียทางนิติเวชศาสตร์

Sequencing Process

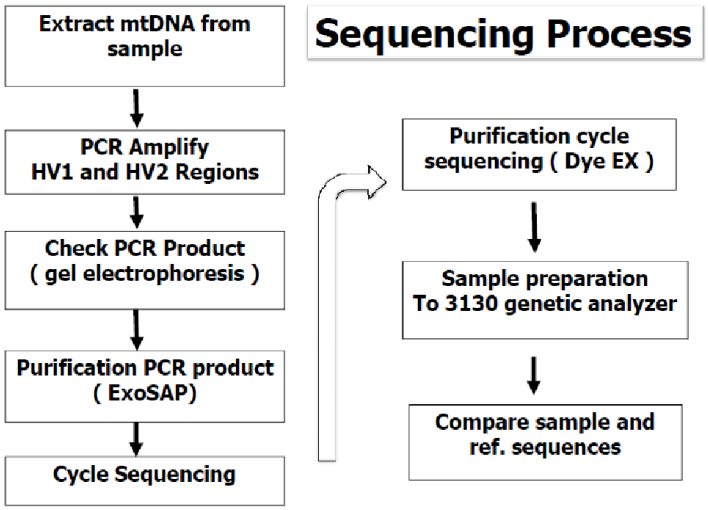
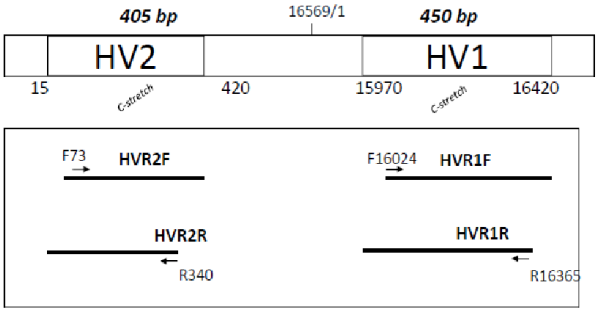
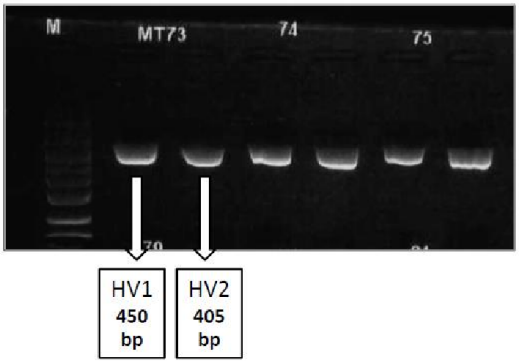


Figure 10.4, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

PCR Amplification HV1 and HV2 Regions

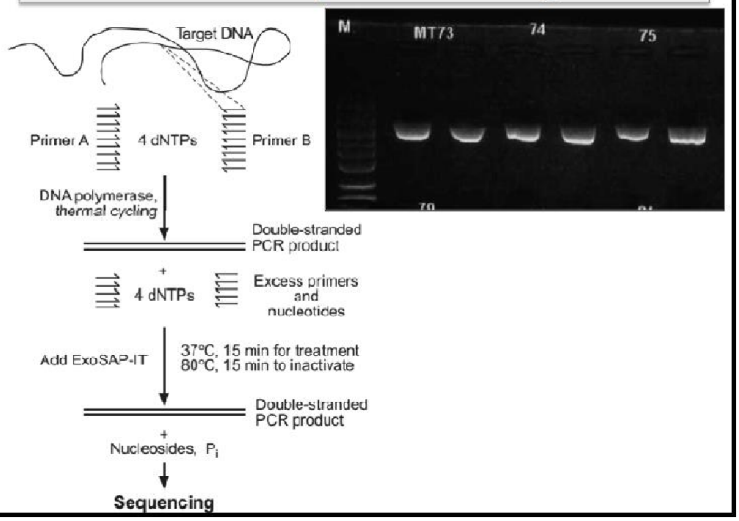


Check PCR Products using agarose gel electrophoresis

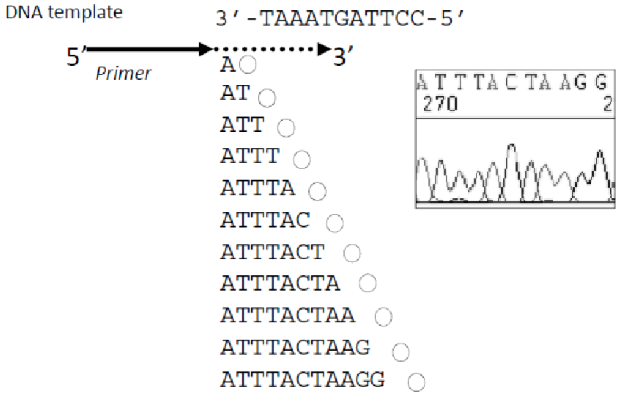


HV1 450 bp | HV2 405 bp

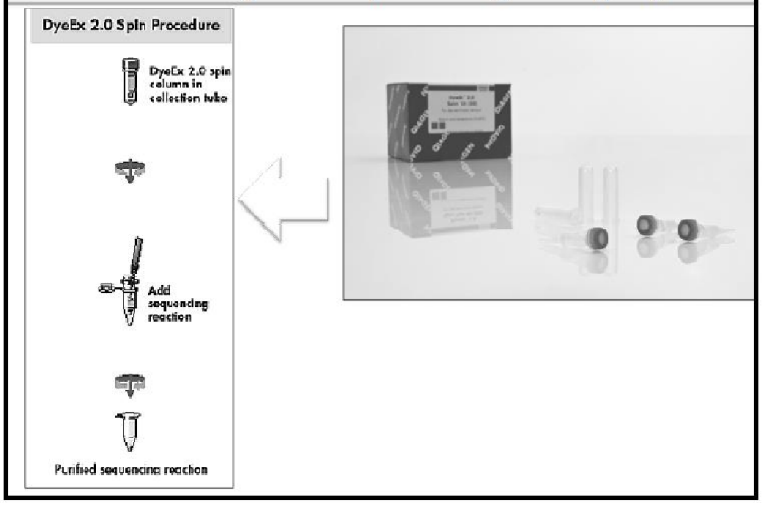
Purification PCR products using ExoSAP



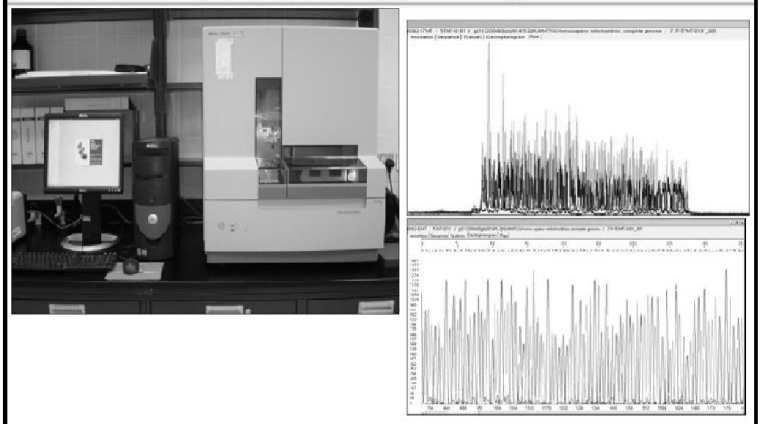
Cycle Sequencing



Purification cycle sequencing using Dye EX



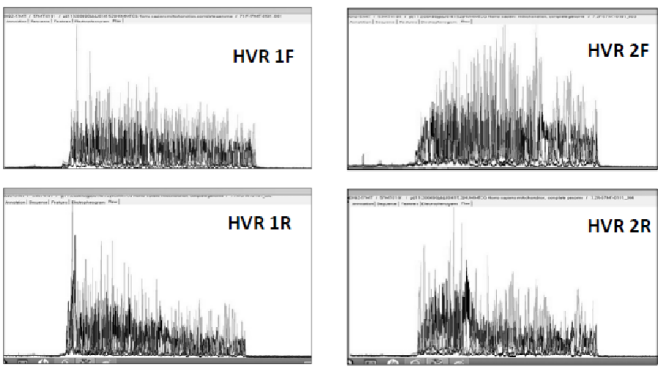
Sample preparation to 3130 genetic analyzer



Compare sample and reference sequences

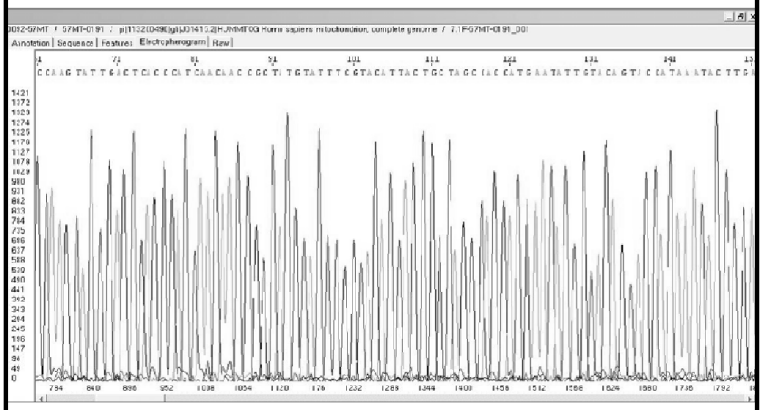
Raw data from Genetic Analyzer

HVR1 **HVR2**



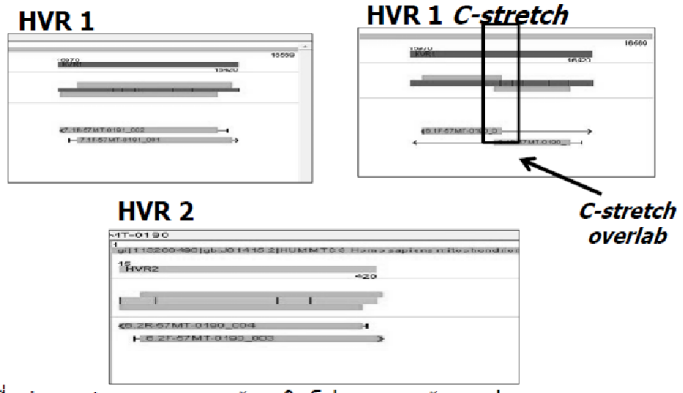
Compare sample and reference sequences

Electropherogram from Genetic Analyzer



Compare sample and reference sequences

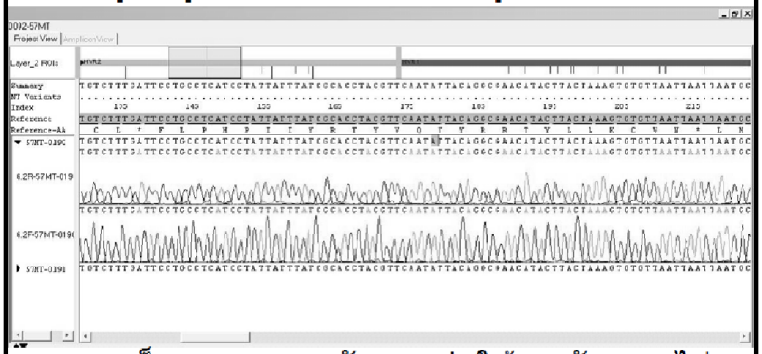
SeqScape v2.5 When Analyzed import sequence



เมื่อ import sequence เข้ามาในโปรแกรมแล้ว analyze sequence

Compare sample and reference sequences

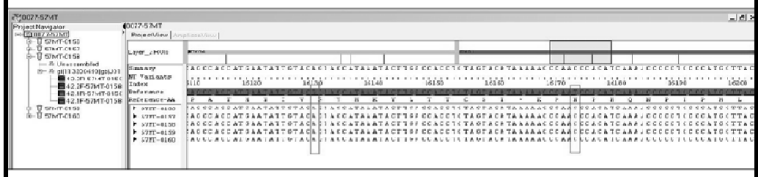
SeqScape v2.5 : Edit Sequence



ตรวจเช็ค sequence กับ peak ให้ตรงกัน หากไม่ตรงกันให้ทำการแก้ไข sequence ให้ตรงกับ peak

Compare sample and reference sequences

SeqScape v2.5: (A) mtDNA sequences aligned with rCRS



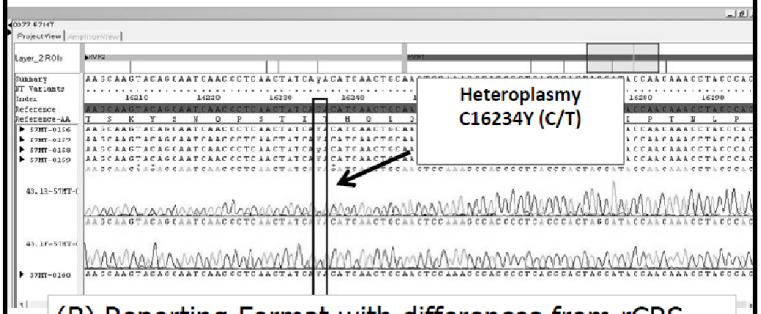
(B) Reporting Format with Differences from rCRS

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
16129A	16129A	16129A	16129A	16129A
16172C	16172C	16172C	16172C	16172C

Compare sample and reference sequences

SeqScape v2.5: (A) mtDNA sequences aligned with rCRS

Heteroplasmy

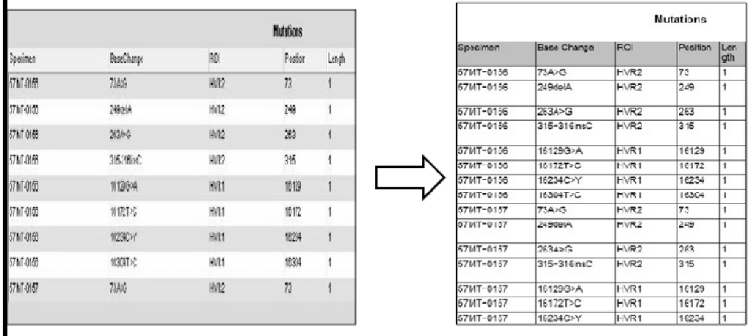


(B) Reporting Format with differences from rCRS

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
16234C/T	16234C/T	16234C/T	16234C/T	16234C/T

Compare sample and reference sequences

SeqScape v2.5: Export Report



Mutations

Specimen	Base Change	RCI	Position	Length
57MT-0156	73A>G	HVR2	73	1
57MT-0156	249delA	HVR2	249	1
57MT-0156	263A>G	HVR2	263	1
57MT-0156	315-316insC	HVR2	315	1
57MT-0156	16129G>A	HVR1	16129	1
57MT-0156	16172T>C	HVR1	16172	1
57MT-0156	16234C>Y	HVR1	16234	1
57MT-0156	16304T>C	HVR1	16304	1
57MT-0157	73A>G	HVR2	73	1
57MT-0157	249delA	HVR2	249	1
57MT-0157	263A>G	HVR2	263	1
57MT-0157	315-316insC	HVR2	315	1
57MT-0157	16129G>A	HVR1	16129	1
57MT-0157	16172T>C	HVR1	16172	1
57MT-0157	16234C>Y	HVR1	16234	1

การแปลผลการตรวจวิเคราะห์

ผลการตรวจวิเคราะห์ : ไม่ขัดแย้ง (Identical) หมายถึง ลำดับนิวคลีโอไทด์ของบุคคลที่นำมาเปรียบเทียบกันจะมีชนิดและตำแหน่งนิวคลีโอไทด์ที่แตกต่างจากลำดับนิวคลีโอไทด์อ้างอิง (rCRS, 1999) เหมือนกันทุกตำแหน่ง

การแปลผลการตรวจวิเคราะห์

ผลการตรวจวิเคราะห์ : ขัดแย้ง (Unidentical)
หมายถึง ลำดับนิวคลีโอไทด์ของบุคคลที่นำมาเปรียบเทียบกันจะมีชนิดและตำแหน่งนิวคลีโอไทด์ที่แตกต่างจากลำดับนิวคลีโอไทด์อ้างอิง (rCRS, 1999) ไม่เหมือนกัน 2 ตำแหน่งขึ้นไป

การแปลผลการตรวจวิเคราะห์

ผลการตรวจวิเคราะห์ : ไม่สามารถสรุปผลได้ (Inconclusive) หมายถึง ลำดับนิวคลีโอไทด์ของบุคคลที่นำมาเปรียบเทียบกันจะมีชนิดและตำแหน่งนิวคลีโอไทด์ที่แตกต่างจากลำดับนิวคลีโอไทด์อ้างอิง (rCRS, 1999) ไม่เหมือนกันเพียง 1 ตำแหน่ง

ข้อจำกัดในการรายงานผล

การแปลผลการตรวจวิเคราะห์ทั้งสามกรณีข้างต้น จะไม่นำนิวคลีโอไทด์ในบางตำแหน่งมาแปลผลร่วมกับนิวคลีโอไทด์ที่ตำแหน่งอื่นๆ ดังนี้

- ตำแหน่งนิวคลีโอไทด์ที่ **16193** ของ **HVR I** ซึ่งเป็นบริเวณ **C- Stretch** ที่ไม่สามารถระบุจำนวนนิวคลีโอไทด์ **C** ที่แน่นอนได้

- ตำแหน่งนิวคลีโอไทด์ที่ **309** ของ **HVR II** ที่มี **insertion** ของนิวคลีโอไทด์ **C** จำนวนไม่เท่ากัน

ข้อเสนอแนะในการรายงานผล

กรณีที่ผลเป็น Inconclusive

ให้ผู้ปฏิบัติงานดำเนินการตรวจวิเคราะห์ซ้ำอีกครั้ง โดยเริ่มต้นตั้งแต่ขั้นตอนการสกัดสารพันธุกรรมดีเอ็นเอจากตัวอย่างเดิมจนเสร็จสิ้นขบวนการ หรือทำการวิเคราะห์โดยเพิ่ม **region** ในการทดสอบเพิ่มขึ้น หากได้ผลการตรวจวิเคราะห์ไม่แตกต่างจากเดิม จะแปลผลการตรวจวิเคราะห์ว่า “ไม่สามารถสรุปผลการตรวจวิเคราะห์ได้”