Ejercicio GHEP-ISFG 2015

Resultados ADNmt

Cracovia, 31 de agosto de 2015

GOBIERNO DE ESPAÑA MINISTERIO DE JUSTICIA

INTCF CIENCIAS FORENSES

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Ejercicio Básico

M1, M2 y M3

M1: sangre de mujer /*blood from a female*
M2: saliva de varón /*saliva from a male*
M3: saliva de varón /*saliva from a male*

ADN mitocondrial/*Mitochondrial DNA*				
ITEM	**N**	**n**	**% Consenso**	**Haplotipo HV1 (16024-16365)**
M1	49	47	95,92%	16192T 16224C 16311C
M2	49	48	97,96%	16224C 16270T
M3	49	48	97,96%	16069T 16126C 16300G

ADN mitocondrial/*Mitochondrial DNA*				
ITEM	**N**	**n**	**% Consenso**	**Haplotipo HV2 (73-340)**
M1	49	44	89,80%	73G 146C 152C 263G
M2	49	46	93,88%	73G 150T 263G
M3	49	44	89,80%	73G 185A 228A 263G 295T

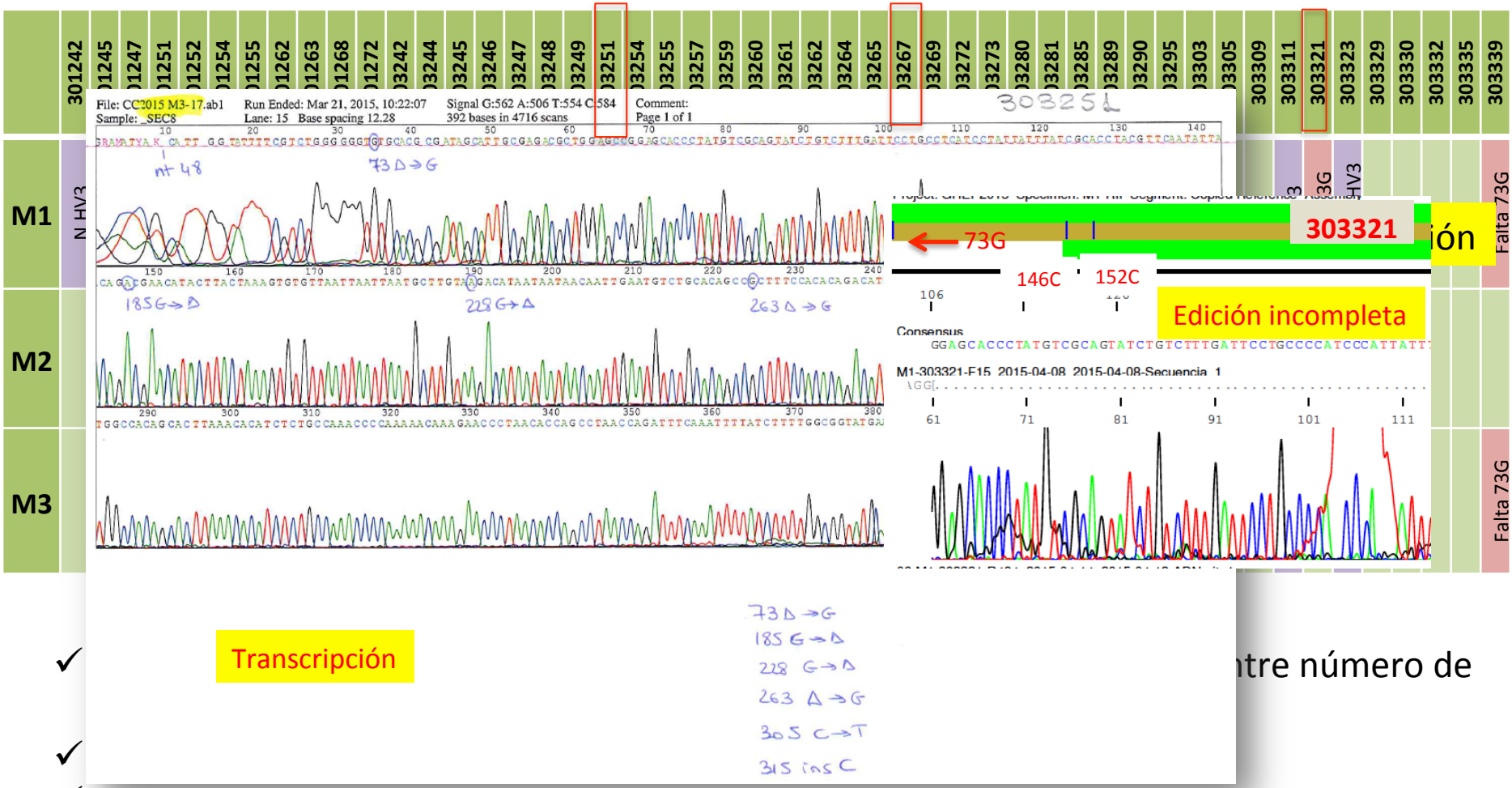
Edicion incompleta

ADN mitocondrial/*Mitochondrial DNA*				
ITEM	**N**	**n**	**% Consenso**	**Haplotipo HV3**
M1	25	19	76,00%	497T 524.1A 524.2C
M2	25	24	96,00%	517T
M3	26	25	88,46%	462T 489C 523DEL 524DEL

¿?

Nomenclatura “confusa” dinucleotido

Ejercicio Básico (M_1, M_2, M_3)



- ✓ ntre número de
- ✓
- ✓ **Transcripción:**
- ✓ **Nomenclatura:** p ej HVSIII, dinucleotido 523.1C 523.2A en lugar de 524.1A 524.2C

303267: 73G 185A 228A 263G 295T 315.1C **16069Y 16126Y 16300R** (mezcla exclusiva en HV2¿?)

Ejercicio Básico (M4)

ADNmt GHEP-ISFG 2015

M4: mezcla 2:1 (v/v) de saliva y semen (diluído ¼ en TE). Saliva del donante del ítem M2 más semen de un donante tipado previamente para STRs autosómicos y de CrY./*Mixture 2:1(v/v) of saliva from donor of item M2 and semen (1/4 diluted in TE) from a donor typed previously with autosomal STRs and YChr .*

No se evalúa el análisis de ADN mitocondrial en mezclas.
DNA mitochondrial analysis results in mixtures are not evaluated.

28 laboratorios

301242	301245	301251	301255	301263	301268	303242	303245	303246	303248	303254	303255	303259	303260	303261	303262	303264	303265	303272	303273	303285	303290	303303	303311	303323	303329	303335	303339
	Formato																			Falta 73G							Falta 73G

Resultados A-STRs y Amelogenina/*A-STR markers and Amelogenin Results*

Marcador/ *Marker*	M4T	Saliva varón *Male saliva*	Semen varón *Male semen*	% Labs mismo valor/*Labs same value*
AMELOGENINA	X-Y	X-Y	X-Y	95,77
D8S1179	10-12-14	10-14	10-12	94,37
D21S11	27-29-31.2	29	27-31.2	94,29
D7S820	10-12	10	10-12	95,16
CSF1PO	10-11	10	10-11	93,55
D3S1358	14-15-18	14-18	14-15	88,73
TH01	6-8-9.3	6-9.3	8-9.3	92,96
D13S317	10-11-13	11	10-13	95,16
D16S539	12-13	12-13	12	88,73
D2S1338	17-19-24-25	17-24	19-25	89,86
D19S433	13-14	14	13	95,59
VWA	14-16	14	14-16	91,30
TPOX	8-9	8	8-9	95,08
D18S51	12-14-15-16	12-14	15-16	88,73
D5S818	11-12-13	11-12	12-13	95,08
FGA	21-23	21-23	23	88,57
Penta_D	9-13	9	13	94,59
Penta_E	5-12-15-18	15-18	5-12	86,11
D10S1248	12-13-14-15	14-15	12-13	95,65
D22S1045	11-15	15	11-15	91,30
D2S441	10-11-15	10-15	11-15	91,49
D1S1656	15-15.3-16-18.3	15-15.3	16-18.3	89,66
D12S391	17-18.3-21-22	21-22	17-18.3	96,43
SE33_ACTBP2	18-27.2-28.2-30.2	18-27.2	28.2-30.2	96,30

303290: 75G 152T 265G 311.1C 312.1C 16224C 16270T

!!!+2 bases!!!

Donante 1-M2- (saliva)

73G 150T 263G 309.1C 315.1C 517T 16224C 16270T 16519C

Donante 2 (semen)

73G 152C 182T 185T 189G 195C 247A 263G 523DEL 524DEL 16092C 16126C 16187T 16189C 16223T 16264T 16270T 16278T 16293G 16311C

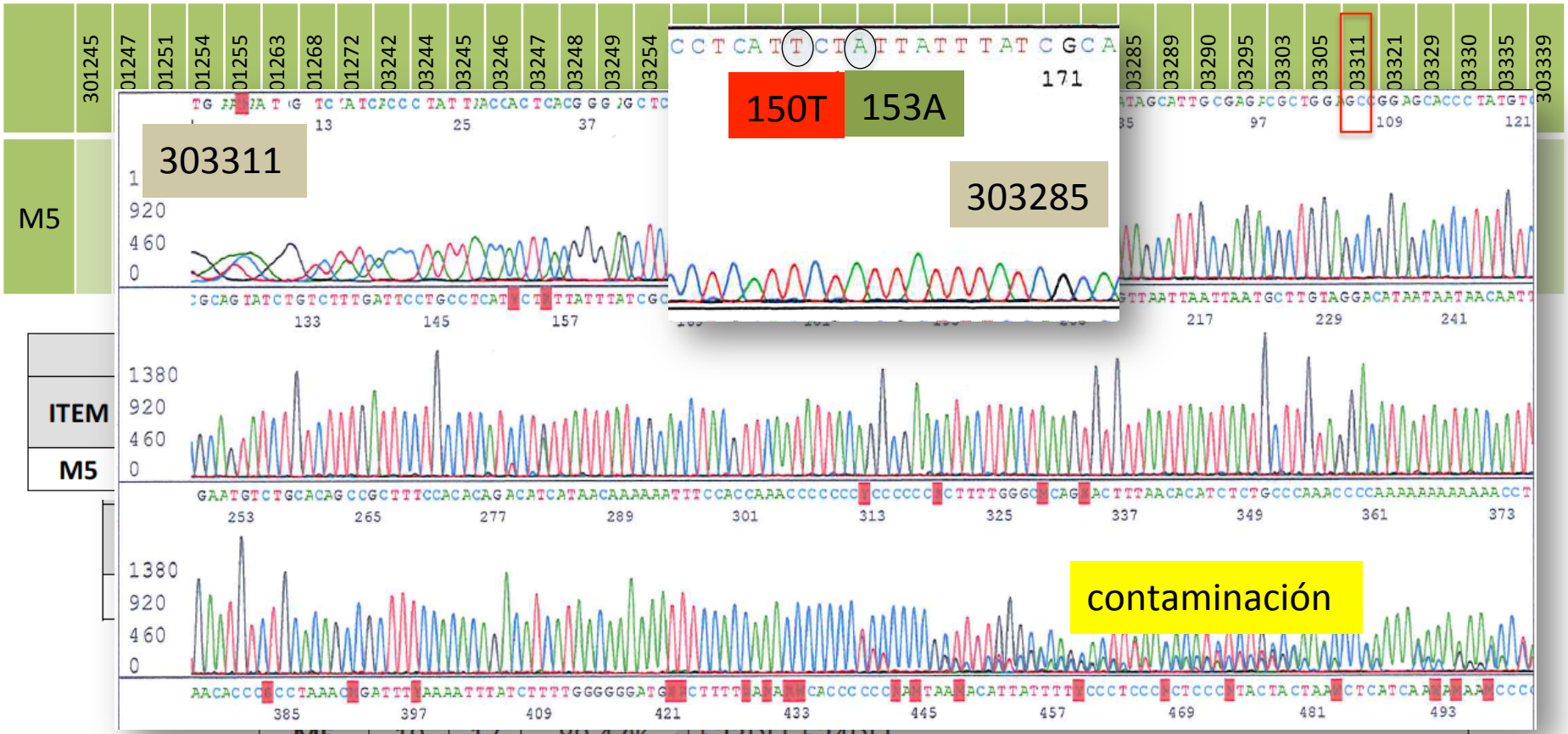
✓ 7 labs presentan datos de 2ª fracción que son idénticos a los de la 1ª fracción

Ejercicio Básico (M_5)

M5: cabellos de varón/ *hair from a male*

!!!+2 bases!!!

303290: 155G 265G 312.1C / CRS



- ✓ **Formato:** Presencia de comas, orden de bases (no creciente), espacio entre número de base y nucleotido…
- ✓ **Edición incompleta:** p ej: HV2 (no reportar 73 G)
- ✓ **Transcripción, contaminación**

Ejercicio avanzado

ADNmt GHEP-ISFG 2015

M6: 20 µl de mezcla de sangre de varón, saliva de varón (donante del item M5 del Ejercicio de 2013) y sangre de mujer en proporción 5:10:1 (v/v) sobre una tela de tapicería/ 20 µl *of a mixture of blood from a male, saliva from a male (donor of item M5 from the 2013 Exercise) and blood of a female in 5:10:1(v/v) on a tapestry fabric.* Los dos varones estaban emparentados: eran padre e hijo. *Both males were relatives: they were father and son.*

15 labs

	301245	301263	303242	303246	303254	303255	303259	303261	303262	303303	303305	303311	303323	303329	303330
M6	No repiten el resultado en ningún caso														

- ✓ Detección de posiciones mezcla

Resultados A-STRs y Amelogenina/*A-STR markers and Amelogenin Results*				
Marcador/*Marker*	M6	N	n	% Consenso/ *Consensus*
AMELOGENINA	X-Y	50	49	98,00
D8S1179	11-13-14	50	47	94,00
D21S11	28-29-30-33.2	50	44	88,00
D7S820	8-9-11-12	47	46	97,87
CSF1PO	10-11	47	46	97,87
D3S1358	15-17-18	50	48	96,00
TH01	6-8-9-9.3	50	48	96,00
D13S317	8-9-10-11-13	47	45	95,74
D16S539	9-10-11-12	50	49	98,00
D2S1338	17-18-20-21	50	47	94,00
D19S433	13-15-15.2	49	44	89,80
VWA	15-16-18-19	50	47	94,00
TPOX	8-9-12	47	46	97,87
D18S51	12-13-14-15-19	49	46	93,88

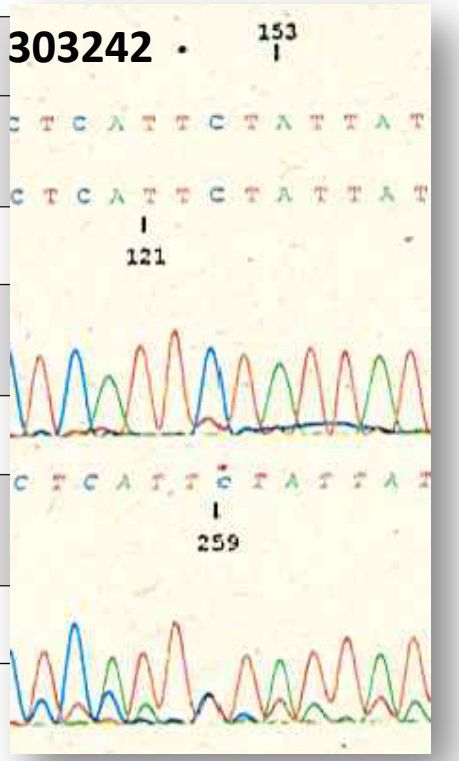
- ✓ Distintos equipos
  Distinta sensibilidad
- ✓ Distintos laboratorios
  Distintos criterios para asignar posiciones “mezcla”
- ✓ Distinta metodología de extracción
  Distintos resultados finales

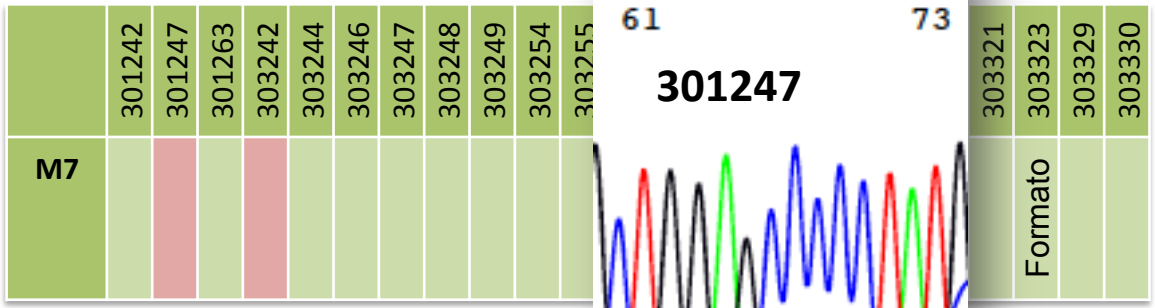
Ejercicio avanzado

M7: 20 µl de saliva de mujer en un trozo de leotardo

M7: ADN mitocondrial / *M7 Mitochondrial DNA*

Resultados de ADN mitocondrial/*Mitochondrial DNA results*				
Ítem/*Item*	**N**	**n**	**% Consenso *Consensus***	**Haplotipo/*Haplotype HV1 (16024-16365)***
M7	23	22	95,65	Sin discrepancias
	N	**n**	**% Consenso *Consensus***	**Haplotipo/*Haplotype HV2 (73-340)***
	23	19	82,61	96T 106DEL 107DEL 108DEL 109DEL 110DEL 111DEL 263G
	N	**n**	**% Consenso *Consensus***	**Haplotipo/*Haplotype HV3***
	13	13	100,00	Sin discrepancias





--ggagccggagcaccctatgtc-
105

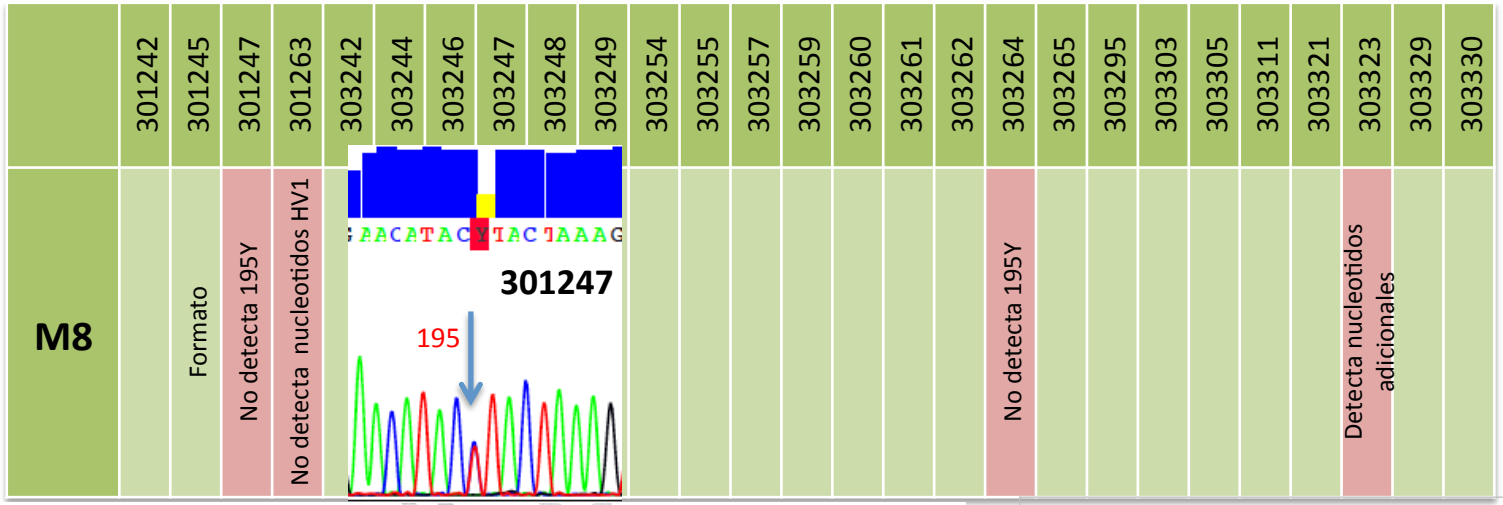
303242: 73G 150T 199C 204C 207A 263G 309.1C 315.1C 16179T 16182 DEL 16183 DEL 16193.1C 16223T 16239T 16311C 16320T 16362C

301247: 96T 105-113DEL 263G 315.1C

Ejercicio avanzado

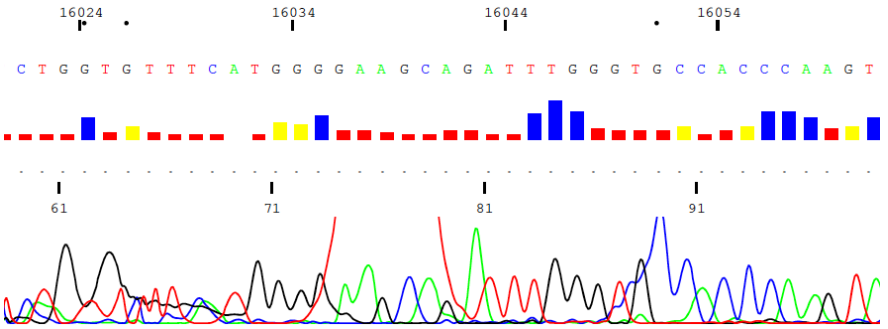
ADNmt GHEP-ISFG 2015

M8: 50 µl de semen de varón vasectomizado sobre un trozo de sábana/ *50 µl semen from a vasectomised male on a piece of sheet.*



Resultados de ADN mitocondrial/*Mitochondrial DNA Results*				
Ítem/*Item*	N	n	% Consenso *Consensus*	Haplotipo/*Haplotype HV1 (16024-16365)*
M8	27	25	92,59	16051G 16356C
	N	n	% Consenso *Consensus*	Haplotipo/*Haplotype HV2 (73-340)*
	27	26	96,30	73G 195Y 263G
	N	n	% Consenso *Consensus*	Haplotipo/*Haplotype HV3*
	15	14	93,33	499A

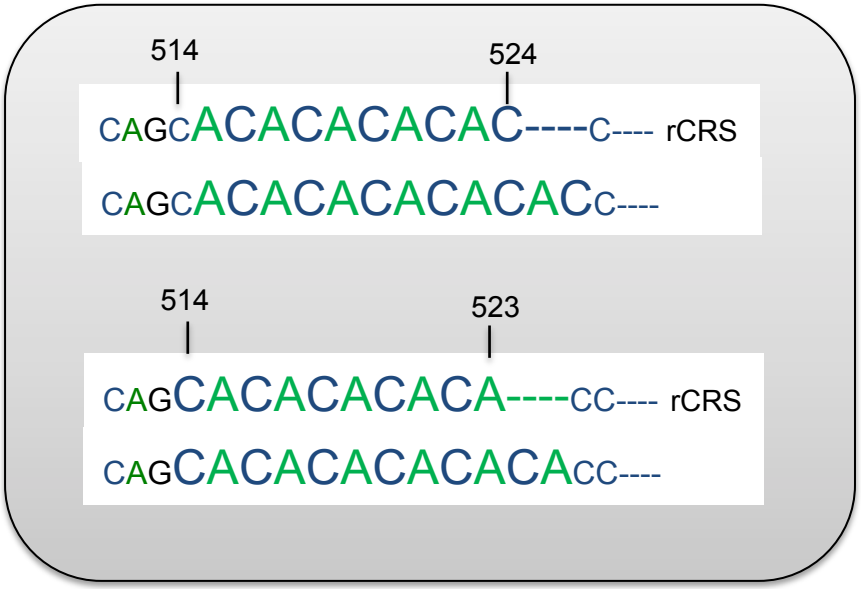
303323: 73G 195Y 263G 315.1C 16024G 16026G 16051G 16356C (solo lectura Forward ¿?

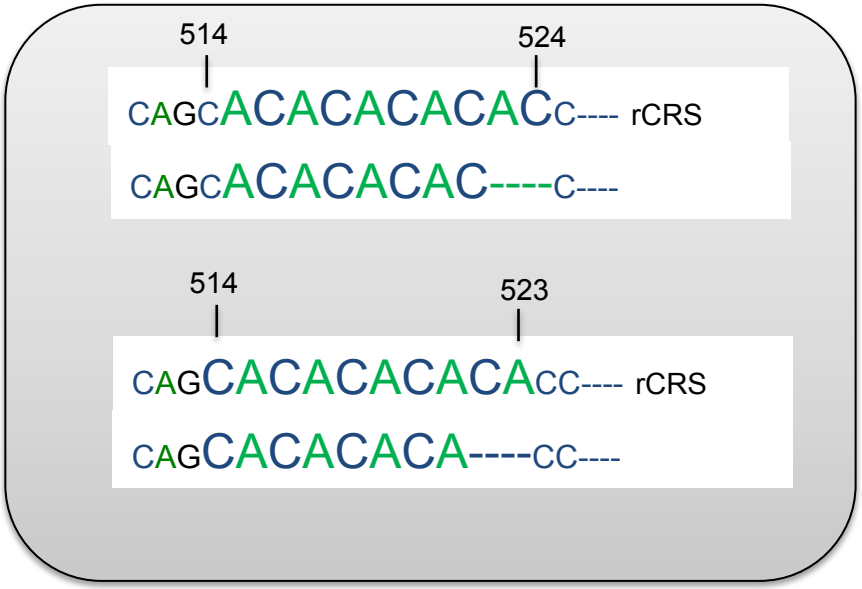


Conclusiones –Nomenclatura HVSIII-

Muestra	Hp consensuado (HV3)	Discrepan
M1	...524.1A 524.2C...	... 523.1C 523.2A ...

Muestra	Hp consensuado (HV3)	Discrepan
M3	...523DEL 524DEL...	... 522DEL 523DEL ...





¿Cuál es la unidad repetitiva CA o AC?

Conclusiones –Nomenclatura HVSIII-

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

DNA Commission of the International Society for Forensic Genetics:
Revised and extended guidelines for mitochondrial DNA typing

W. Parson [a,b,*], L. Gusmão [c,d], D.R. Hares [e], J.A. Irwin [e], W.R. Mayr [f], N. Morling [g], E. Pokorak
M. Prinz [h], A. Salas [i], P.M. Schneider [j], T.J. Parsons [k]

One consequence of reporting mtDNA sequences relative to a reference is that multiple alignments of the same string of nucleotides are feasible. For example, the insertion of a C in a homopolymeric tract of seven Cs (e.g. between positions 302 and 310) could be reported as 302.1C or 309.1C (or at any position in between). Because there is no simple molecular approach to determine the "correct" alignment, conventions are required to prevent identical sequences from being reported in different ways. The forensic community traditionally aligns insertions and deletions (indels) 3′ relative to the affected position in homopolymeric sequence tracts and other repeat regions (e.g. the AC repeat between 514 and 524; [2]). Although this convention is in contrast to medical genetics where indels are recorded 5′, the 3′ notation should be maintained to provide consistency with earlier data.

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Revised Cambridge Reference Sequence (rCRS) of the Human Mitochondrial DNA

The rCRS sequence is a fully corrected version of the original Ca
The rCRS is GenBank sequence NC_012920 gi:251831106

```
  1 gatcacaggt ctatcaccct attaaccact cacgggagct ctccatgcat ttggtatttt
 61 cgtctggggg gtatgcacgc gatagcattg cgagacgctg gagccggagc accctatgtc
121 gcagtatctg tctttgattc ctgcctcatc ctattattta tcgcacctac gttcaatatt
181 acaggcgaac atacttacta aagtgtgtta a   aat g   gga cataataata T
241 acaattgaat gtctgcacag ccActttcca     atc a   aaa atttccacca
301 aacccccct CCCCCgcttc tggccacagc acttaaacac atctctgcca aaccccaaaa
361 acaaagaacc ctaacaccag cctaaccaga tttcaaattt tatctttgg cggtatgcac
421 ttttaacagt caccccccaa ctaacacatt attctcccct cccctccca tactactaat
481 ctcatcaata caacccccgc ccatcctacc cagcacacac acaccgctgc taaccccata
541 ccccgaacca accaaacccc aaagacaccc cccacagttt atgtagctta cctcctcaaa
601 gcaatacact gaaaatgttt agacgggctc acatcacccc ataaacaaat aggtttggtc
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514 524

Conclusiones -mezclas-

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Forensic Science International 160 (2006) 157–167

Forensic Science International

www.elsevier.com/locate/forsciint

Results of the 2003–2004 GEP-ISFG collaborative study on mitochondrial DNA: Focus on the mtDNA profile of a mixed semen-saliva stain

Datos aparentemente contradictorios entre resultados de marcadores autosómicos STRs y mtDNA:

- ✓ ADNn: varón (semen)↑ y femenino (saliva)↓
- ✓ ADNmt: femenino exclusivo

M4 y M6 se comportan de manera diferente:

- ✓ M4: ADNmt (valor exclusivo)
- ✓ M6: ADNmt (mezcla)

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Available online at www.sciencedirect.com
ScienceDirect

Forensic Science International 168 (2007) 42–56

Forensic Science International

www.elsevier.com/locate/forsciint

Analysis of body fluid mixtures by mtDNA sequencing: An inter-laboratory study of the GEP-ISFG working group

Abstract

The mitochondrial DNA (mtDNA) working group of the GEP-ISFG (Spanish and Portuguese Group of the International Society for Forensic Genetics) carried out an inter-laboratory exercise consisting of the analysis of mtDNA sequencing patterns in mixed stains (saliva/semen and blood/semen). Mixtures were prepared with saliva or blood from a female donor and three different semen dilutions (pure, 1:10 and 1:20) in order to simulate forensic casework. All labs extracted the DNA by preferential lysis and amplified and sequenced the first mtDNA hypervariable region (HVS-I). Autosomal and Y-STR markers were also analysed in order to compare nuclear and mitochondrial results from the same DNA extracts. A mixed stain prepared using semen from a vasectomized individual was also analysed. The results were reasonably consistent among labs for the first fractions but not for the second ones, for which some laboratories reported contamination problems. In the first fractions, both the female and male haplotypes were generally detected in those samples prepared with undiluted semen. In contrast, most of the mixtures prepared with diluted semen only yielded the female haplotype, suggesting that the mtDNA copy number per cell is smaller in semen than in saliva or blood. Although the detection level of the male component decreased in accordance with the degree of semen dilution, it was found that the loss of signal was not consistently uniform throughout each electropherogram. Moreover, differences between mixtures prepared from different donors and different body fluids were also observed. We conclude that the particular characteristics of each mixed stain can deeply influence the interpretation of the mtDNA evidence in forensic mixtures (leading in some cases to false exclusions). In this sense, the implementation of preliminary tests with the

Conclusiones -mezclas-

Forensic Mitochondrial DNA Analysis: Current Practice and Future Potential

REFERENCE: Melton T, Holland C, Holland M: Forensic mitochondrial DNA analysis: Current practice and future potential; *Forensic Sci Rev* 24:101; 2012.

D. Mixture Interpretation

Interpreting mtDNA mixtures continues to present itself as one of the major challenges in forensic DNA analysis. A typical mixture in forensic casework samples may be defined as DNA originating from more than one individual. Possible explanations for a DNA mixture result can range from contamination of a sample during collection or DNA testing, to a "naturally occurring" mixture such as an intimate sample or a biological sample taken from an article of clothing worn or stained by more than one person. In STR analysis it is often possible and appropriate to deconvolute mixtures in order to determine the number of contributors and sometimes also determine the major and minor components of the mixture. With mtDNA mixtures, such deconvolution can prove to be difficult if not impossible using current methods. In addition, because mtDNA analysis is inherently more susceptible to contamination, many laboratories choose not to interpret mixtures at all, and simply categorize the result as inconclusive.

However, each polymorphism must also be considered individually. In general, Sanger sequencing and the current instruments and chemistry associated with that analytical approach allow for detection of a secondary minor nucleotide where the minor variant is at least 5–10% of the total component of the mixture, whether due to heteroplasmy or a mixture of mtDNAs from two or more individuals. Below that level, there is a possibility of mixed-base dropout (akin to allelic dropout in STR analysis) for the minor templates in one or more amplification products that constitute a full analysis. Therefore, interpretations of hair an judiciously.

sible. The report will state that when a mixture profile is obtained, the number of potential mtDNA types that may be derived from that mixture is equal to 2^n, where n is equal to the number of nucleotide positions at which two different nucleotides have been observed, and that all of these types are not equally probable. The report will state how many possible types there are for the mixture observed. Mixtures may be used with care to exclude an individual as contributor of a sample. Because of the phenomenon of base dropout, mixtures containing any of the substitutions characterizing the known comparison sample must be interpreted with extreme caution in order to conclude that there is an exclusion" (Mitotyping Technologies). Mixed

Conclusiones -mezclas-

Forensic Mitochondrial DNA Analysis: Current Practice and Future Potential

REFERENCE: Melton T, Holland C, Holland M: Forensic mitochondrial DNA analysis: Current practice and future potential; *Forensic Sci Rev* 24:101; 2012.

- ✓ Limitación de métodos de secuenciación convencionales.
- ✓ Falta de consenso en lecturas "forward" y "reverse"
- ✓ Mayor sensibilidad a procesos de contaminación.
- ✓ Modificaciones post-mortem en el ADNmt → "Falsas mezclas".

↓

Falsas exclusiones.

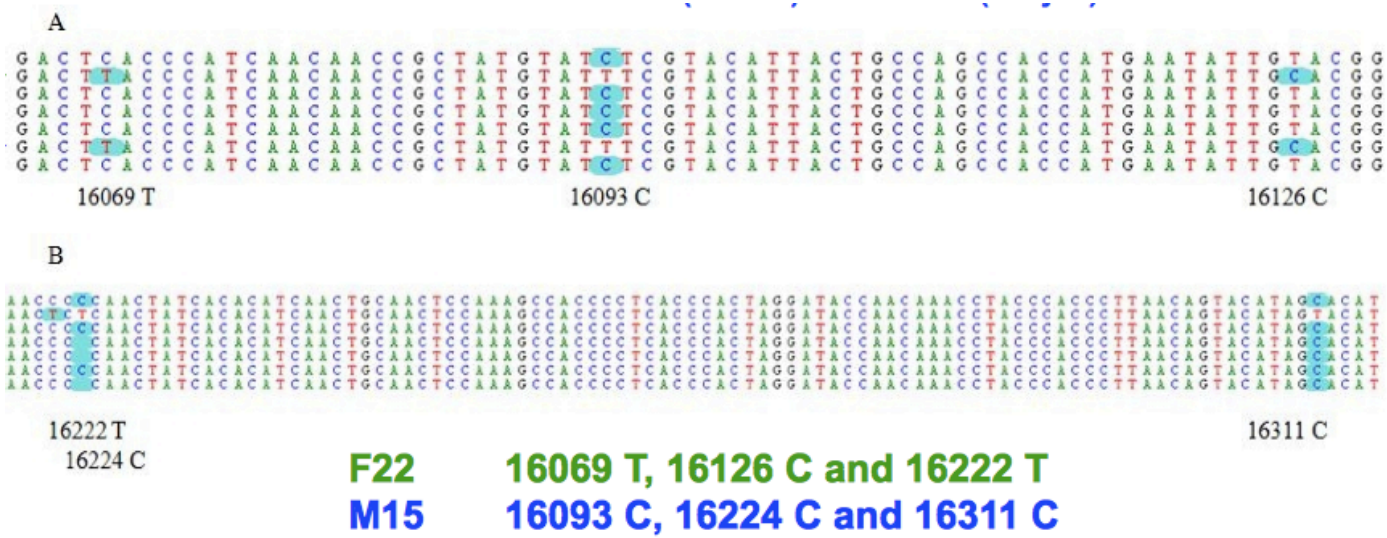
- ✓ Valoración estadística

Croatian Medical Journal
Medicinska Naklada

Second generation sequencing allows for mtDNA mixture deconvolution and high resolution detection of heteroplasmy

Mitchell M. Holland, Megan R. McQuillan, and Katherine A. O'Hanlon

Based on the results from this study, we would recommend reporting low-level variants when at least 40 reads have been generated, and when the ratio of forward to reverse reads is consistent with the total ratio. Therefore, for mixtures of 1:100, 1:250, 1:500, and 1:1000, the coverage would need to be at least 4000, 10 000, 20 000, and 40 000, respectively. This level of coverage is well within the capability of the GS Junior



Conclusiones finales

- ✓ Buenos resultados: Errores fácilmente evitables (transcripción / nomenclatura).
  - Dobles revisiones/Doble edición
  - Chequeo filogenético
  - Empleo de programas adecuados
  - Volcado de datos automático
- ✓ Mezclas:
  - Precaución en la emisión de conclusiones (pej falsas exclusiones)
  - Valorar el tipo de fluidos
- ✓ Nomenclatura (HVIII):
  - Poca trascendencia práctica

Gracias por su atención