

**Table S1. General Aspects. Participants Characterization.**

	GHEP-MIX1 (N = 32)		GHEP-MIX2 (N = 24)		GHEP-MIX3 (N = 17)	
	n (labs)	%	n (labs)	%	n (labs)	%
<b>1. Kind of cases that your laboratory examine</b>						
Forensic	3	9.4	3	12.5	0	0.0
Paternity	5	15.6	3	12.5	2	11.8
Both	24	75.0	18	75.0	15	88.2
<b>2. Send DNA profile to Criminal DataBase:</b>						
National	7	21.9	8	33.3	5	29.4
Own	2	6.3	2	8.3	2	11.8
Both	4	12.5	4	16.7	6	35.3
No	19	59.4	11	45.8	4	23.5
<b>3. Report results about mixture profiles of autosomal markers:</b>						
Yes	14	43.8	16	66.7	12	70.6
No	9	28.1	4	16.7	2	11.8
Only if there is reference sample	9	28.1	5	20.8	3	17.6
<b>4. In case of obtaining a mixture profiles:</b>						
They are sent to Criminal DataBases	3	9.4	5	20.8	2	11.8
They are not sent to Criminal DataBases	14	43.8	8	33.3	1	5.9
They are only registered on the reports	15	46.9	10	41.7	8	47.1
They are registered on both reports and Criminal DataBases	N/D	N/D	N/D	N/D	6	35.3
<b>5. When mixture profiles are interpreted, allelic assignment has been carried out by procedure:</b>						
Automatic	3	9.4	5	20.8	6	35.3
Manual	18	56.3	12	50.0	2	11.8
Both	10	31.3	6	25.0	9	52.9
<b>6. Your laboratory's mixture interpretation criterias have been validated:</b>						
Yes	5	15.6	3	12.5	4	23.5
No	26	81.3	21	87.5	5	29.4
Validation in progress	N/D	N/D	N/D	N/D	8	47.1

N/D = No Data

**Table S2. Technical Aspects. Edition Tools.**

	GHEP-MIX1 (N = 32)		GHEP-MIX2 (N = 24)		GHEP-MIX3 (N = 17)	
	n (labs)	%	n (labs)	%	n (labs)	%
<b>7. Which edition software have you used:</b>						
GeneMapper	29	90.6	23	95.8	17	100.0
GeneScan	2	6.3	0	0.0	0	0.0
Genotyper	1	3.1	0	0.0	0	0.0
PeakScanner	0	0.0	1	4.2	0	0.0
<b>8. Which criterias do you use to define a profile as a mixture:</b>						
> 4 STRs with >3 alleles	2	6.3	2	8.3	0	0.0
> 3 STRs with >3 alleles	2	6.3	1	4.2	0	0.0
> 2 STRs with >3 alleles	6	18.8	4	16.7	3	17.6
Alleles Imbalance	0	0.0	1	4.2	0	0.0
Amelogenin Imbalance (exclusively)	0	0.0	0	0.0	0	0.0
> 4 STRs with >3 alleles and Alleles Imbalance	2	6.3	1	4.2	0	0.0
> 3 STRs with >3 alleles and Alleles Imbalance	4	12.5	3	12.5	2	11.8
> 2 STRs with >3 alleles and Alleles Imbalance	10	31.3	5	20.8	11	64.7
> 3 STRs with >3 alleles and Amelogenin Imbalance	1	3.1	0	0.0	0	0.0
> 2 STRs with >3 alleles and Amelogenin Imbalance	1	3.1	0	0.0	0	0.0
Others	4	12.5	7	29.2	1	5.9
<b>9. In the present exercise, the alleles have been assigned when the peak is higher than:</b>						
150 RFUs	1	3.1	2	8.3	0	0.0
100 RFUs	10	31.3	4	16.7	1	5.9
50 RFUs	21	65.6	18	75.0	15	88.2
Others: 40 RFUs	0	0.0	0	0.0	1	5.9

**Table S2. Technical Aspects. Edition Tools.**

	GHEP-MIX1 (N = 32)		GHEP-MIX2 (N = 24)		GHEP-MIX3 (N = 17)	
	n (labs)	%	n (labs)	%	n (labs)	%
<b>10. The "stutter" positions (n-4/n+4) have been assigned as possible alleles:</b>						
Every	0	0.0	0	0.0	1	5.9
> 15% from principal allele	8	25.0	7	29.2	2	11.8
> 150 RFUs	0	0.0	0	0.0	0	0.0
> 100 RFUs	0	0.0	1	4.2	0	0.0
> 50 RFUs	0	0.0	1	4.2	0	0.0
The assignment has been variable depending on which STR marker is taken into account	13	40.6	9	37.5	8	47.1
> 15% from principal allele and > 150 RFUs	2	6.3	0	0.0	0	0.0
> 15% from principal allele and > 100 RFUs	2	6.3	0	0.0	0	0.0
> 15% from principal allele and > 50 RFUs	1	3.1	4	16.7	2	11.8
The assignment has been variable depending on which STR marker is taken into account, and > 150 RFUs	0	0.0	0	0.0	0	0.0
The assignment has been variable depending on which STR marker is taken into account, and > 100 RFUs	0	0.0	0	0.0	1	5.9
The assignment has been variable depending on which STR marker is taken into account, and > 50 RFUs	3	9.4	1	4.2	3	17.6
The assignment has been variable depending on which STR marker is taken into account, and > 15% from principal allele	3	9.4	1	4.2	0	0.0
<b>11. Which is / are the principal obstacle/s that your laboratory have to face up to interpret a mixture profiles?</b>						
Lack of an unique criterion on the laboratory	13	40.6	2	8.3	3	17.6
Lack of training	16	50.0	6	25.0	3	17.6
Lack of an unique criterion on the laboratory and Lack of training	1	3.1	6	25.0	5	29.4
There are not any obstacle	2	6.3	3	12.5	0	0.0
Others	N/D	N/D	7	29.2	6	35.3

N/D = No Data

**Table S3. Statistical Aspects.**

	<b>GHEP-MIX1 (N = 32)</b>		<b>GHEP-MIX2 (N = 24)</b>		<b>GHEP-MIX3 (N = 17)</b>	
	<b>n (labs)</b>	<b>%</b>	<b>n (labs)</b>	<b>%</b>	<b>n (labs)</b>	<b>%</b>
<b>13. LR calculation is carried out by using:</b>						
Automatic (software)	N/D	N/D	11	45.8	8	47.1
Manual	N/D	N/D	7	29.2	5	29.4
Both	N/D	N/D	6	25.0	4	23.5

N/D = No Data

**Table S4. Samples Discrepancies.**

		GHEP-MIX1 (N = 32)					GHEP-MIX2 (N = 24)					GHEP-MIX3 (N = 17)					Total (N = 73)										
		M1 (1:5)		M2 (1:10)		M3 (1:1)		M4 (1:5)		Total		M1 (1:5)		M2 (2:1:1)		Total		M1 (1:5)		M2 (1:10)		M3 (1:3:7)		Total		n	%
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%		
Group A	-A	31	37.3	21	13.0	0	0.0	0	0.0	52	19.8	4	44.4	1	6.3	5	20.0	2	12.5	82	61.7	44	59.5	128	57.4	185	36.3
	+A	10	12.0	69	42.6	5	71.4	4	40.0	88	33.6	4	44.4	15	93.8	19	76.0	7	43.8	7	5.3	25	33.8	39	17.5	146	28.6
Group B	-B	1	1.2	70	43.2	0	0.0	0	0.0	71	27.1	0	0.0	0	0.0	0	0.0	4	25.0	44	33.1	2	2.7	50	22.4	121	23.7
	+B	40	48.2	2	1.2	1	14.3	6	60.0	49	18.7	0	0.0	0	0.0	0	0.0	2	12.5	0	0.0	0	0.0	2	0.9	51	10.0
Group C	C	1	1.2	0	0.0	1	14.3	0	0.0	2	0.8	1	11.1	0	0.0	1	4.0	1	6.3	0	0.0	3	4.1	4	1.8	7	1.4
Total		83	31.7	162	61.8	7	2.7	10	3.8	262	discrepancies	9	36.0	16	64.0	25	discrepancies	16	7.2	133	59.6	74	33.2	223	discrepancies	510	discrepancies

**Group A:** those which take place at a stutter position (n-4, n+4 or n-3, n+3).

-A: No report of a real allele which is located at a stutter position, which represents a false allelic loss in the profile.

+A: Report of a non-existent allele (actual stutter peak), which represents a false allelic gain in the genetic profile.

**Group B:** those which occur in a position non-stutter.

-B: No report of a real allele in a non-stutter position, which represents a false allelic loss in the genetic profile.

+B: Report of a non-existent allele in a non-stutter position, which represents a false allelic gain in the genetic profile.

**Group C:** discrepancies attributable to an incorrect transcription.

**Table S5. Statistical Treatment. Locked Hypothesis. (GHEP-MIX2)**

**Case 1**

$$LR = (H_p:S+U)/(H_d:2U)$$

	LR <sub>partial</sub>	LR <sub>partial</sub>	LR <sub>partial</sub>	LR <sub>partial</sub>	LR <sub>partial</sub>	LR <sub>partial</sub>
D21S11	3.51	3.51	--	3.4	1.51	72.62
D7S820	4.85	4.85	--	4.6	0.73	28.82
D16S539	15.49	15.49	--	11.7	2.97	52.25
D2S1338	4.93	4.95	--	4.5	3.51	77.13
TPOX	4.87	4.91	--	4.6	10.27	12.92
D5S818	1.71	1.71	--	1.6	2.68	4.15
LR <sub>total</sub>	1.08E+04	1.10E+04	--	6.06E+03	3.16E+02	4.52E+08

N	23					
n (labs)	18	1	1	1	1	1
%	78.3	4.3	4.3	4.3	4.3	4.3
n (labs)				3		
%				13.0		

	Suspect	Mixture	H <sub>p</sub> /H <sub>d</sub>
D21S11	29-31.2	29-30-31.2-32.2	1/12 f <sub>29</sub> f <sub>31.2</sub>
D7S820	8-12	8-11-12	(f <sub>11</sub> +2f <sub>8</sub> +2f <sub>12</sub> )/12f <sub>8</sub> f <sub>12</sub> (f <sub>11</sub> +f <sub>8</sub> +f <sub>12</sub> )
D16S539	11-14	11-12-14	(f <sub>12</sub> +2f <sub>11</sub> +2f <sub>14</sub> )/12f <sub>11</sub> f <sub>14</sub> (f <sub>12</sub> +f <sub>11</sub> +f <sub>14</sub> )
D2S1338	17-19	17-19-25	(f <sub>25</sub> +2f <sub>17</sub> +2f <sub>19</sub> )/12f <sub>17</sub> f <sub>19</sub> (f <sub>25</sub> +f <sub>17</sub> +f <sub>19</sub> )
TPOX	8-9	8-9	(f <sub>8</sub> +f <sub>9</sub> ) <sup>2</sup> /2f <sub>8</sub> f <sub>9</sub> (2f <sub>8</sub> <sup>2</sup> +3f <sub>8</sub> f <sub>9</sub> +2f <sub>9</sub> <sup>2</sup> )
D5S818	11	11-12	(2f <sub>11</sub> +f <sub>12</sub> )/2f <sub>11</sub> (2f <sub>11</sub> <sup>2</sup> +3f <sub>11</sub> f <sub>12</sub> +2f <sub>12</sub> <sup>2</sup> )

**Case 2**

$$LR = (H_p:S+V)/(H_d:V+U)$$

	LR <sub>partial</sub>	LR <sub>partial</sub>	LR <sub>partial</sub>	LR <sub>partial</sub>	LR <sub>partial</sub>	LR <sub>partial</sub>
D21S11	21.03	21.04	20.72	--	20.1	9.03
D7S820	6.91	6.91	6.91	--	6.5	4.40
D16S539	61.60	61.60	29.29	--	47.6	17.84
D2S1338	5.46	5.53	5.31	--	5.1	21.03
TPOX	8.14	8.14	8.14	--	7.6	61.60
D5S818	1.90	1.90	1.90	--	1.8	16.08
LR <sub>total</sub>	7.56E+05	7.65E+05	3.45E+05	--	4.34E+05	1.48E+07

N	23					
n (labs)	17	1	1	1	1	1
%	73.9	4.3	4.3	4.3	4.3	4.3
n (labs)	2		3			
%	8.7		13.0			

	Suspect	Victim	Mixture	H <sub>p</sub> /H <sub>d</sub>
D21S11	29-31.2	30-32.2	29-30-31.2-32.2	1/2f <sub>29</sub> f <sub>31.2</sub>
D7S820	8-12	8-11	8-11-12	1/f <sub>12</sub> (f <sub>12</sub> +2f <sub>8</sub> +2f <sub>11</sub> )
D16S539	11-14	12	11-12-14	1/2f <sub>11</sub> f <sub>14</sub>
D2S1338	17-19	19-25	17-19-25	1/f <sub>17</sub> (f <sub>17</sub> +2f <sub>19</sub> +2f <sub>25</sub> )
TPOX	8-9	8	8-9	1/f <sub>9</sub> (f <sub>9</sub> +2f <sub>8</sub> )
D5S818	11	11-12	11-12	1/(f <sub>11</sub> +f <sub>12</sub> ) <sup>2</sup>

**Table S6. Statistical Treatment. Open Hypothesis. (GHEP-MIX3)**

Case 1 (N = 17)		
Hypothesis Couples (LR = $H_p/H_d$ )	n (labs)	%
$2S/2U$	5	29.4
$(2S/2U) + [(S1+U)/2U] + [(S2+U)/2U]$	11	64.7
$[(S1+U)/2U] + [(S2+U)/2U]$	1	5.9

$H_p$  = Prosecution hypothesis  
 $H_d$  = Defence hypothesis  
**S** = Suspect  
**V** = Victim  
**U** = Unknown

Case 2 (N = 17)		
Hypothesis Couples (LR = $H_p/H_d$ )	n (labs)	%
$(S+V)/(V+U)$	8	47.1
$[(S+V)/(V+U)] + [(S+V)/2U]$	2	11.8
$[(S+V)/(V+U)] + [(S+V)/2U] + [(S+V)/(S+U)]$	1	5.9
$(S+V)/2U$	3	17.6
$[(S+V)/(V+U)] + [(S+V)/2U] + [(V+U)/2U]$	1	5.9
$[(S+V)/(V+U)] + [(S+V)/2U] + [(S+U)/2U]$	1	5.9
$[(S+U)/2U] + [(V+U)/2U]$	1	5.9

**Table S7. Statistical Treatment. Open Hypothesis. LRs combined of Case 1. (GHEP-MIX3)**

$H_p/H_d = S1+S2 / 2U$			
LR <sub>combined</sub>	n (labs)	% <sub>partial</sub>	% <sub>total</sub>
6.17E+10	1	6.3	5.9
1.12E+11	9	56.3	52.9
3.25E+11	4	25.0	23.5
2.01E+12	1	6.3	5.9
2.64E+17	1	6.3	5.9
<b>min.</b>	<b>6.17E+10</b>		
<b>Max.</b>	<b>2.64E+17</b>		

<b>N<sub>partial</sub></b>	16
<b>N<sub>total</sub></b>	17

$H_p/H_d = S1+U / 2U$			
LR <sub>combined</sub>	n (labs)	% <sub>partial</sub>	% <sub>total</sub>
1.48E+05	10	83.3	58.8
1.55E+06	1	8.3	5.9
6.96E+08	1	8.3	5.9
<b>min.</b>	<b>1.48E+05</b>		
<b>Max.</b>	<b>6.96E+08</b>		

<b>N<sub>partial</sub></b>	12
<b>N<sub>total</sub></b>	17

$H_p$  = Prosecution hypothesis  
 $H_d$  = Defence hypothesis

$H_p/H_d = S2+U / 2U$			
LR <sub>combined</sub>	n (labs)	% <sub>partial</sub>	% <sub>total</sub>
7.74E+02	1	8.3	5.9
1.98E+03	1	8.3	5.9
3.14E+03	6	50.0	35.3
3.86E+03	1	8.3	5.9
8.73E+03	1	8.3	5.9
9.16E+03	1	8.3	5.9
3.80E+08	1	8.3	5.9
<b>min.</b>	<b>7.74E+02</b>		
<b>Max.</b>	<b>3.80E+08</b>		

<b>N<sub>partial</sub></b>	12
<b>N<sub>total</sub></b>	17



**Table S8. Statistical Treatment. Open Hypothesis. LR<sub>s</sub> combined of Case 2. (GHEP-MIX3)**

$H_p/H_d = S+V / V+U$			
LR <sub>combined</sub>	n (labs)	% <sub>partial</sub>	% <sub>total</sub>
3.30E+07	1	7.7	5.9
3.52E+07	11	84.6	64.7
6.96E+08	1	7.7	5.9

<b>min.</b>	<b>3.30E+07</b>
<b>Max.</b>	<b>6.96E+08</b>

N <sub>partial</sub>	13
N <sub>total</sub>	17

$H_p/H_d = S+V / 2U$			
LR <sub>combined</sub>	n (labs)	% <sub>partial</sub>	% <sub>total</sub>
1.12E+11	7	87.5	41.2
2.01E+12	1	12.5	5.9

<b>min.</b>	<b>1.12E+11</b>
<b>Max.</b>	<b>2.01E+12</b>

N <sub>partial</sub>	8
N <sub>total</sub>	17

$H_p/H_d = S+V / S+U$			
LR <sub>combined</sub>	n (labs)	% <sub>partial</sub>	% <sub>total</sub>
7.52E+05	1	100.0	5.9

<b>min.</b>	<b>7.52E+05</b>
<b>Max.</b>	<b>7.52E+05</b>

N <sub>partial</sub>	1
N <sub>total</sub>	17

$H_p/H_d = S+U / 2U$			
LR <sub>combined</sub>	n (labs)	% <sub>partial</sub>	% <sub>total</sub>
1.48E+05	2	100.0	11.8

<b>min.</b>	<b>1.48E+05</b>
<b>Max.</b>	<b>1.48E+05</b>

N <sub>partial</sub>	2
N <sub>total</sub>	17

$H_p/H_d = V+U / 2U$			
LR <sub>combined</sub>	n (labs)	% <sub>partial</sub>	% <sub>total</sub>
3.14E+03	2	100.0	11.8

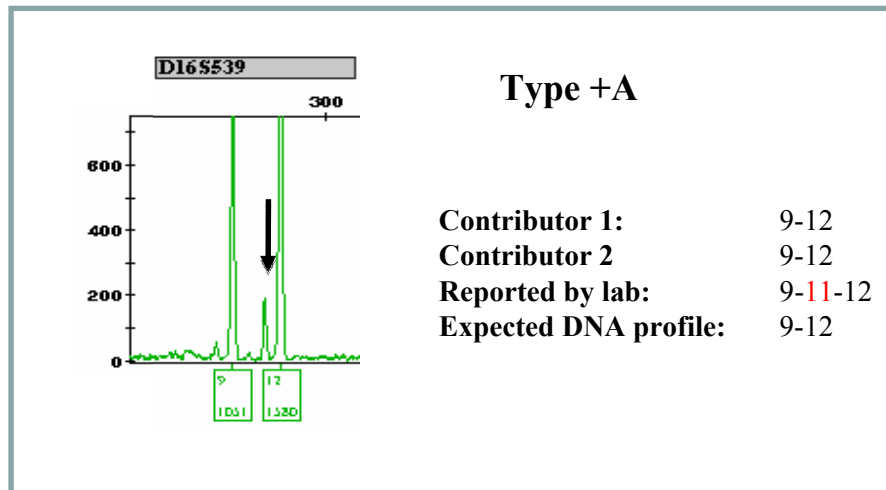
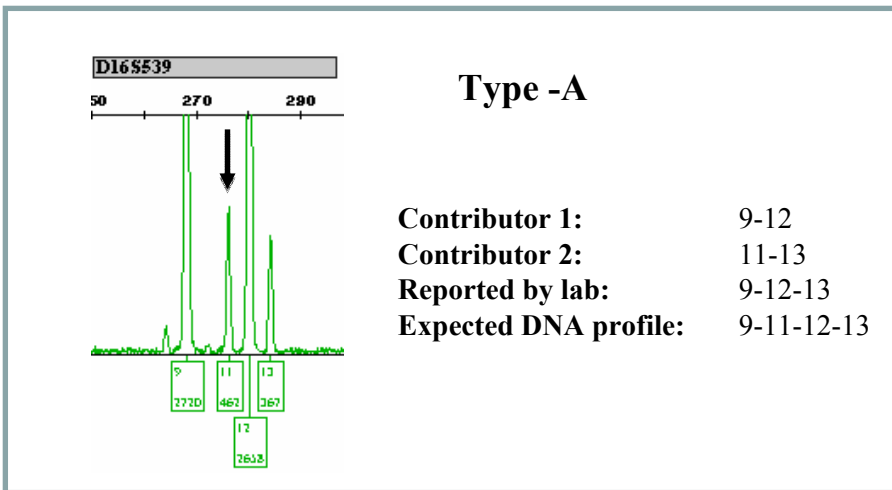
<b>min.</b>	<b>3.14E+03</b>
<b>Max.</b>	<b>3.14E+03</b>

N <sub>partial</sub>	2
N <sub>total</sub>	17

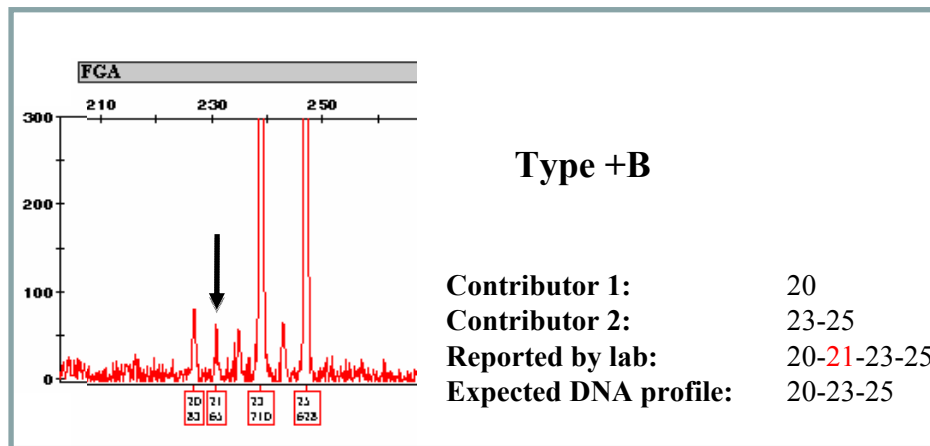
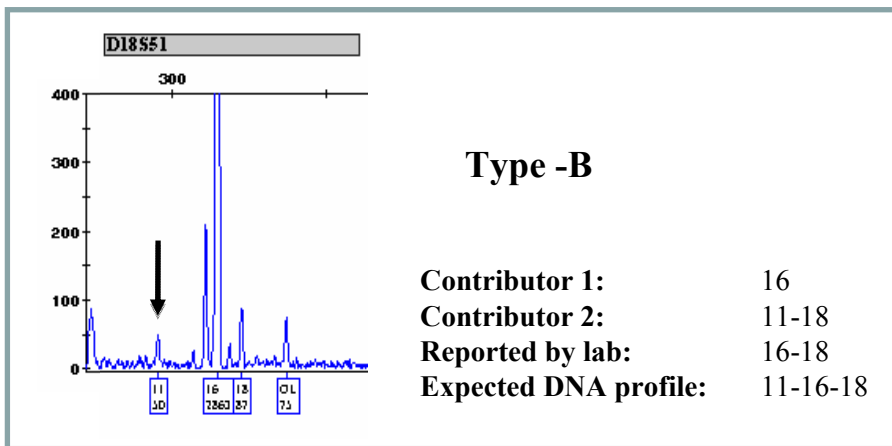
H<sub>p</sub> = Prosecution hypothesis  
H<sub>d</sub> = Defence hypothesis

## Classification of discrepancies

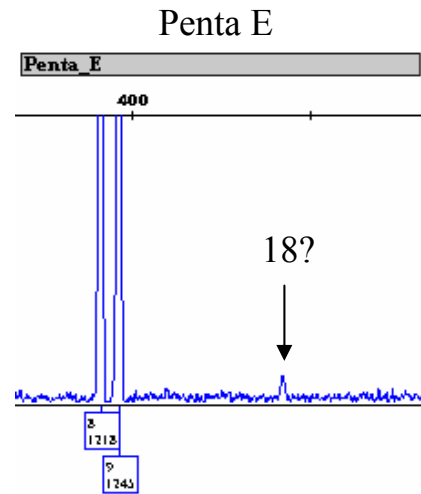
**Group A:** those which take place at a stutter position (n-4, n+4 or n-3, n+3)



**Group B:** those which occur in a non-stutter position.



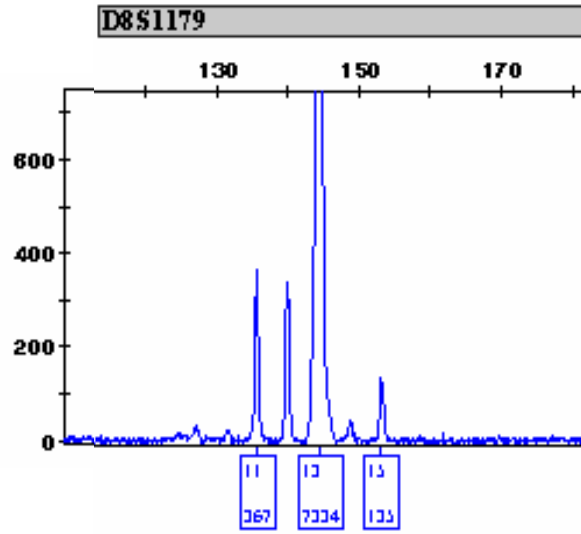
**GHEP-MIX01**  
**-M2 (1:10)-**



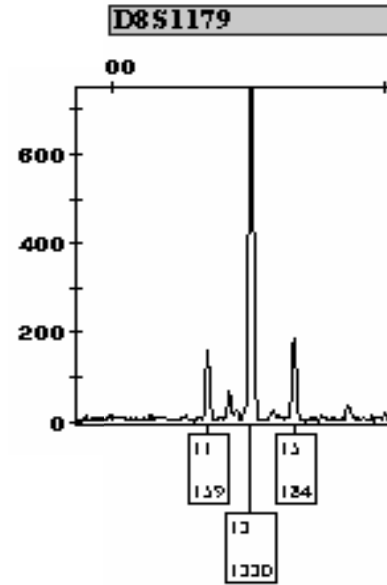
**Powerplex®**

Penta E	
Contributor A (minor)	Contributor B (major)
9-18	8-9

**GHEP-MIX01**  
**-M1 (1:5)-**



**Identifiler®**



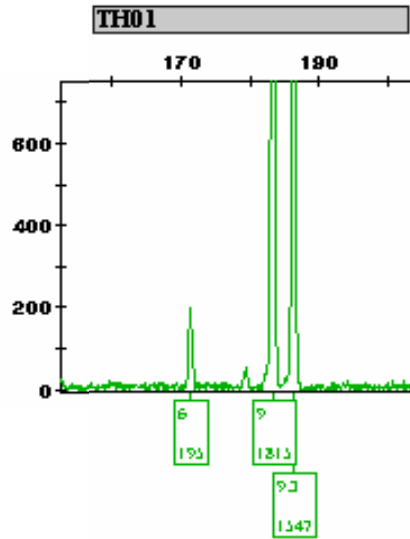
**Powerplex®**

D8S1179				
11-13	11-13-15	(11)-13-(15)	11-12-13	11-12-13-15
3	26		1	2

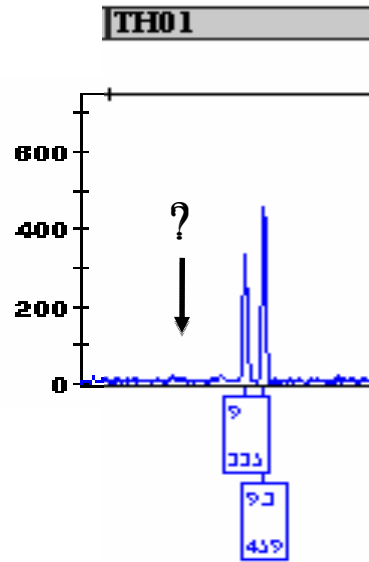
D8S1179	
Contributor A (minor component)	Contributor B (major component)
11-15	13

# GHEP-MIX01

## -M1 (1:5)-

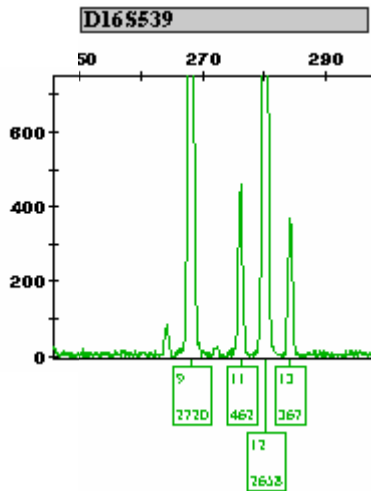


Identifiler®

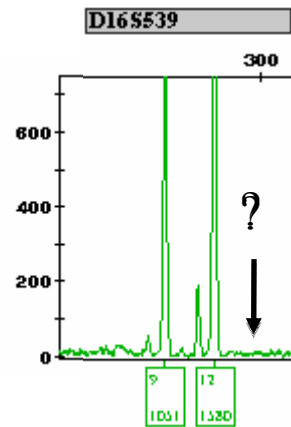


Powerplex®

TH01	
Contributor A (minor component)	Contributor B (major component)
6-9	9-9.3



Identifiler®

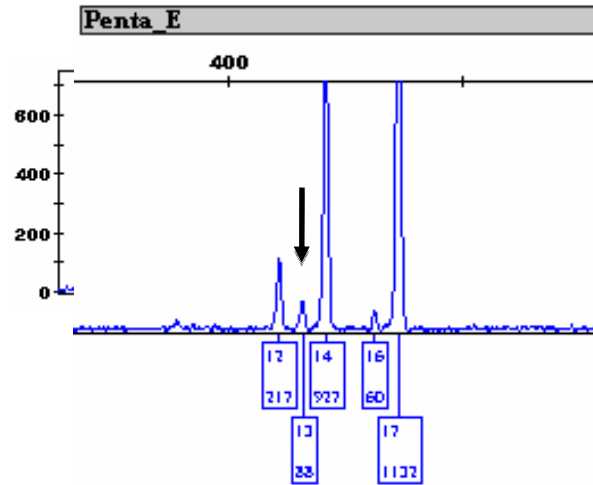


Powerplex®

D16S539	
Contributor A (minor component)	Contributor B (major component)
11-13	9-12

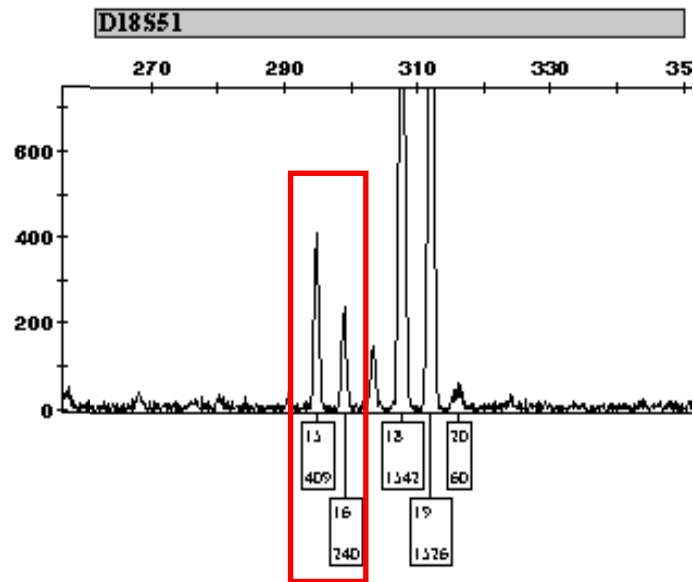
# GHEP-MIX01

**-M1 (1:5)-**

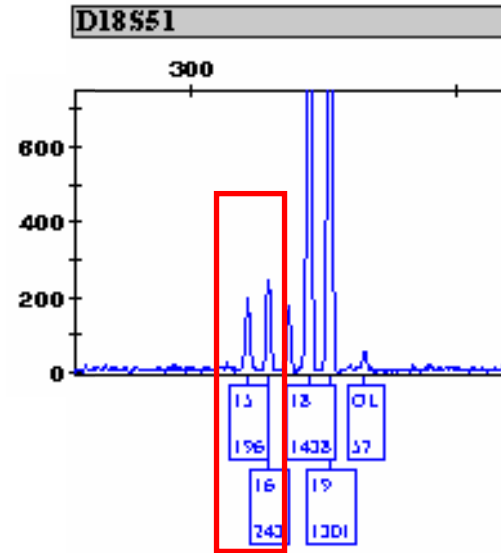


Penta E	
Contributor A (minor component)	Contributor B (major component)
12-13	14-17

**GHEP-MIX01**  
**-M1 (1:5)-**



**Identifiler®**

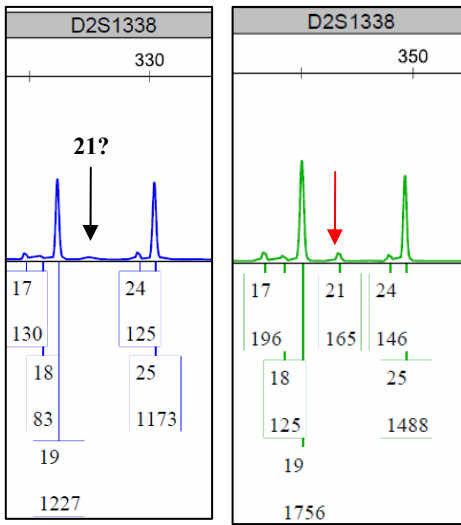


**Powerplex®**

# GHEP-MIX03

**-M2 (1:10)-**

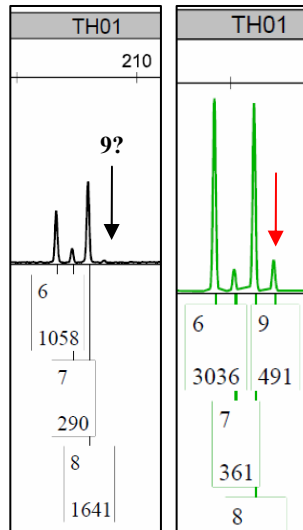
D2S1338



NGM®

Identifiler Plus®

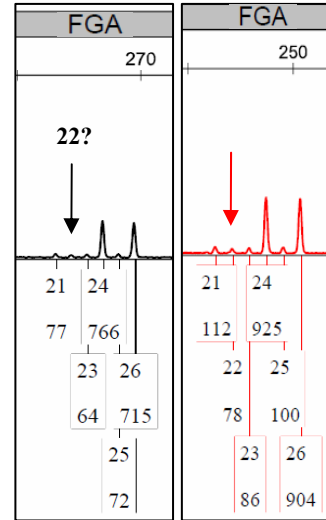
TH01



NGM®

Identifiler Plus®

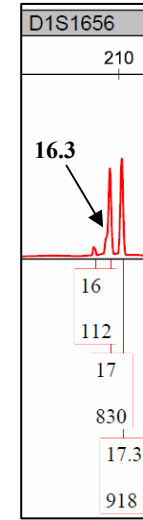
FGA



NGM®

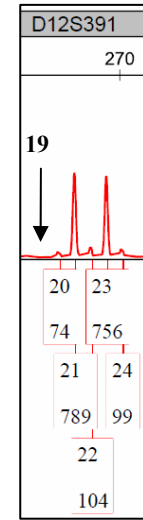
Identifiler Plus®

D1S1656



NGM®

D12S391



NGM®



# GHEP-MIX03

## -M2 (1:10)-

