Technology Transition Workshop| *Christiane Honisch, Ph.D.*

The MassARRAY® System: Multi-Applications in Forensics

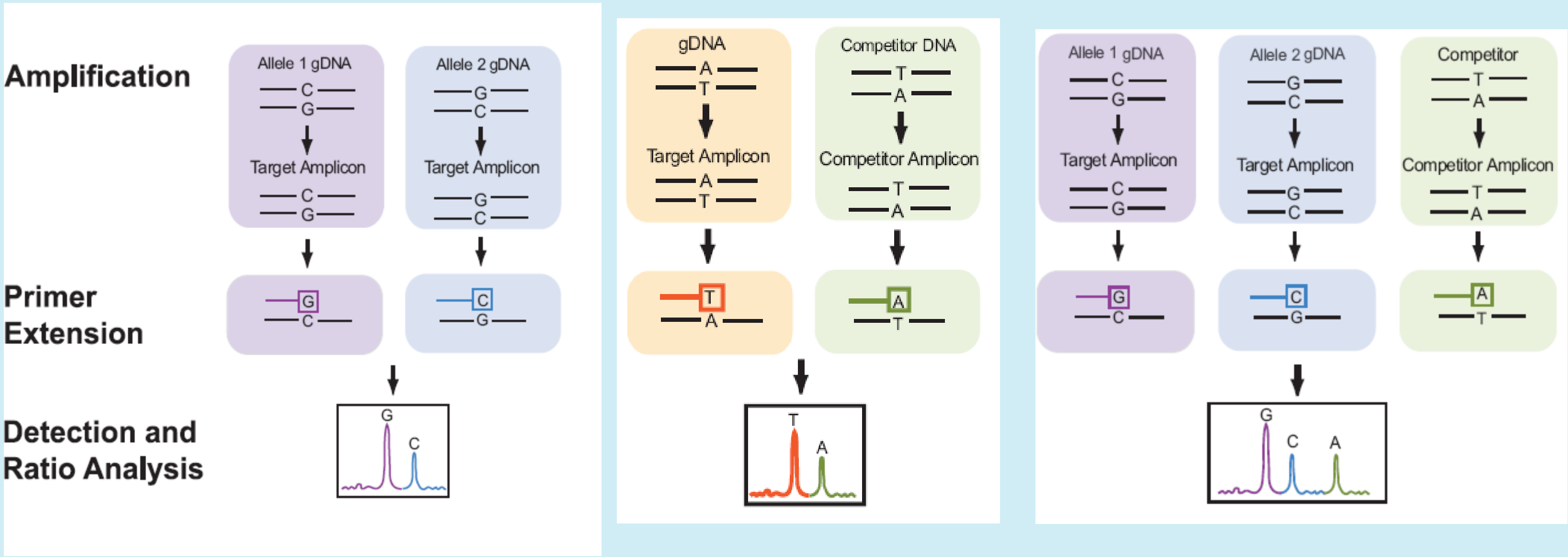
iPLEX® Genotyping

Qualitative and quantitative applications

SNP allele ratio

Absolute copy number

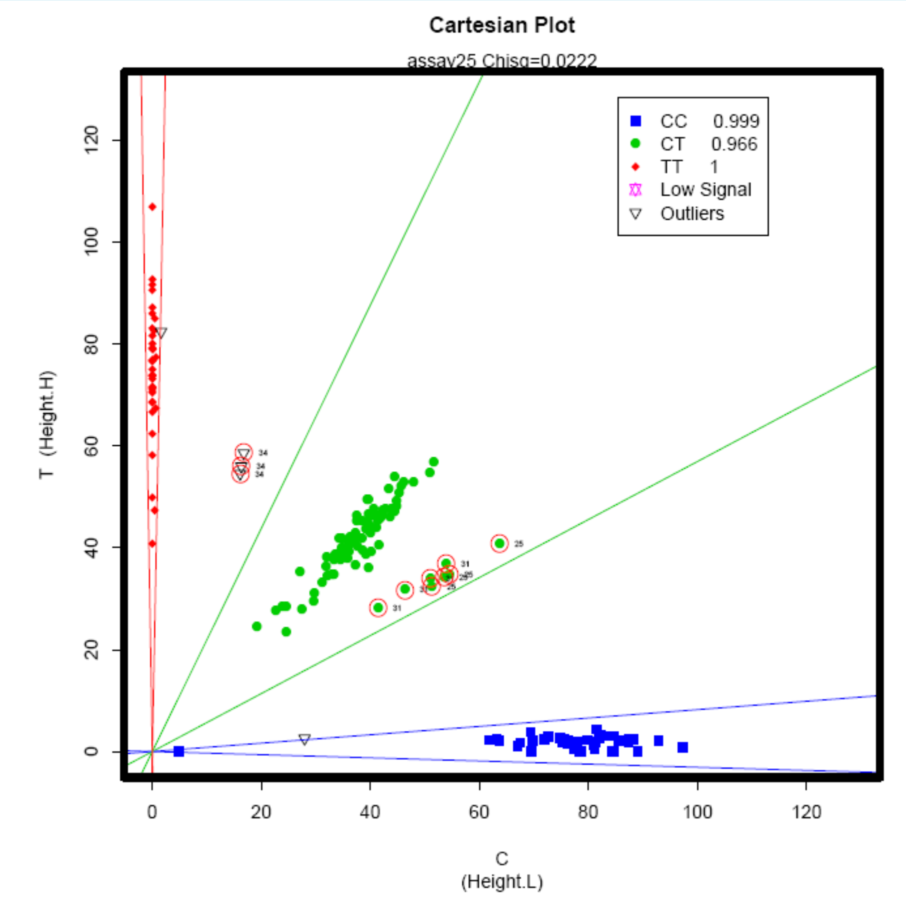
Allele specific copy number



- **Automated flexible assay designs and multiplexing (re-plexing, super-plexing)**
- **Short amplicon length**
- **Sensitivity**

iPLEX*® *Genotyping

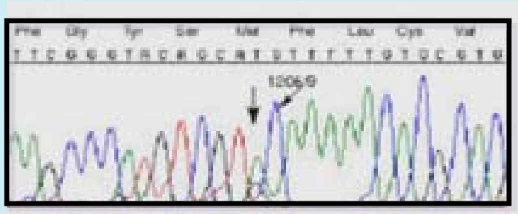
SNP allele ratios



iPLEX® Genotyping

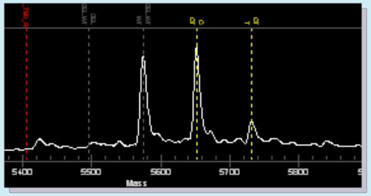
Sensitive detection of low frequency mutations

- **Compared to dideoxy sequencing, the MassARRAY® System provides a significant advantage for the detection of low frequency mutations – e.g., in admixed samples of tumor and healthy tissue**
- **This has been demonstrated in several publications:**
  - **Thomas R. K. et al. (2007) Nature Genetics 39 (3): 347-351**
  - **Vivante A. et al. (2007) Leukemia 21: 1318-21**



Sequencing trace

50 % heterozygote



MassARRAY® spectrum

16 % Mutant

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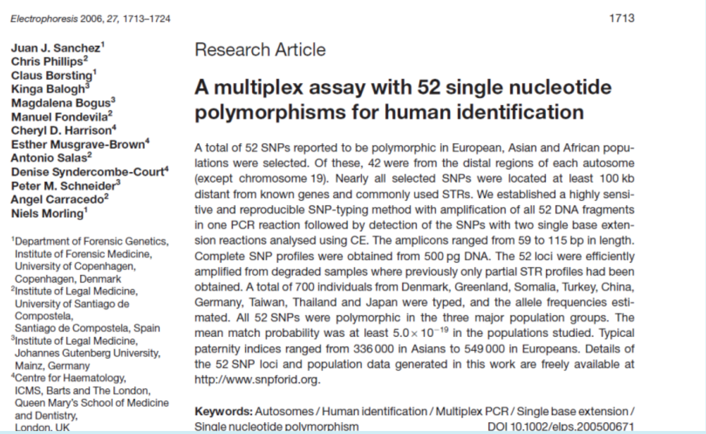
iPLEX® Genotyping

A multiplexed human SNP identification panel

- **48-plex MassARRAY® iPLEX® SNP panel based on validated SNPs:**
  - **Source of SNPs is publication by the SNPforID Consortium**



Electrophoresis 2006, *27*, 1713–1724 1713

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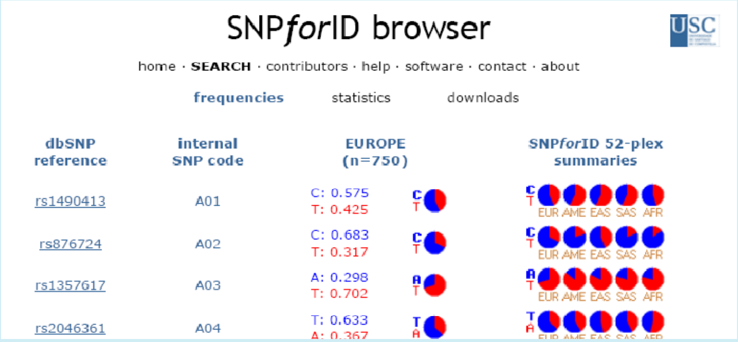
Research Article

A multiplex assay with 52 single nucleotide polymorphisms for human identification

A total of 52 SNPs reported to be polymorphic in European, Asian and African populations were selected. Of these, 42 were from the distal regions of each autosome (except chromosome 19). Nearly all selected SNPs were located at least 100 kb distant from known genes and commonly used STRs. We established a highly sensitive and reproducible SNP-typing method with amplification of all 52 DNA fragments in one PCR reaction followed by detection of the SNPs with two single base extension reactions analysed using CE. The amplicons ranged from 59 to 115 bp in length. Complete SNP profiles were obtained from 500 pg DNA. The 52 loci were efficiently amplified from degraded samples where previously only partial STR profiles had been obtained. A total of 700 individuals from Denmark, Greenland, Somalia, Turkey, China, Germany, Taiwan, Thailand and Japan were typed, and the allele frequencies estimated. All 52 SNPs were polymorphic in the three major population groups. The mean match probability was at least 5.0×10^{-19} in the populations studied. Typical paternity indices ranged from 336 000 in Asians to 549 000 in Europeans. Details of the 52 SNP loci and population data generated in this work are freely available at http://www.snpforid.org.

Keywords: Autosomes / Human identification / Multiplex PCR / Single base extension / Single nucleotide polymorphism DOI 10.1002/elps.200500671



SNP*for*ID browser

dbSNP reference	internal SNP code	EUROPE (n=750)	SNP*for*ID 52-plex summaries
rs1490413	A01	C: 0.575 T: 0.425	EUR AME EAS SAS AFR
rs876724	A02	C: 0.683 T: 0.317	EUR AME EAS SAS AFR
rs1357617	A03	A: 0.298 T: 0.702	EUR AME EAS SAS AFR
rs2046361	A04	T: 0.633 A: 0.367	EUR AME EAS SAS AFR

Image courtesy of http://www.snpforid.org/

- **Validation of the 48-plex on HapMap samples**
  - **Over 99% call rate in cell line samples (HapMap sample plate)**

Sanchez, J. et al. *Electrophoresis* (2006) ***27(9)*** *1713-1724*

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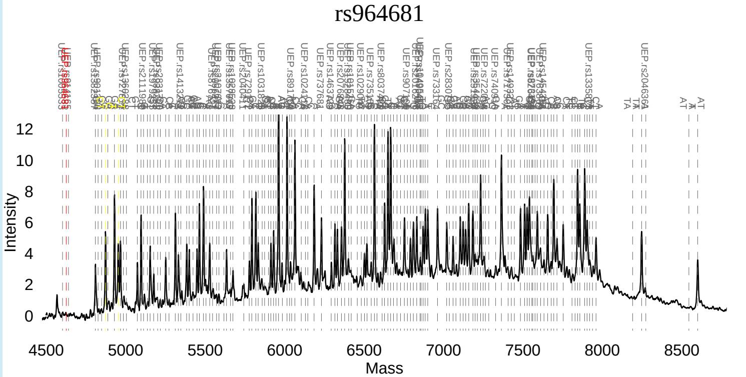
iPLEX® Genotyping

A multiplexed human SNP identification panel

- **Next steps required:**
  - **Validation outside SQNM labs, including validation on lower quality samples**
  - **Inclusion of an XY assay**
- **Further panels that could be useful:**
  - **Ancestry panels**
  - **Population admixture panels**
  - **Y-chromosome panel**



MALDI-TOF MS 48-plex spectrum

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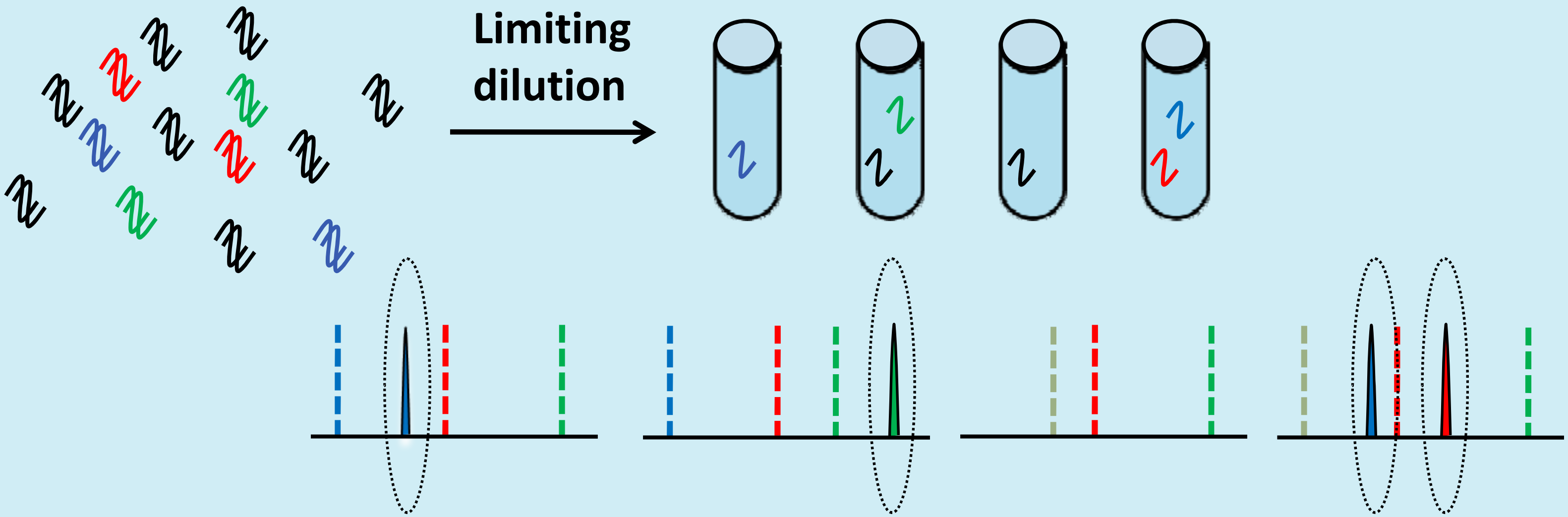
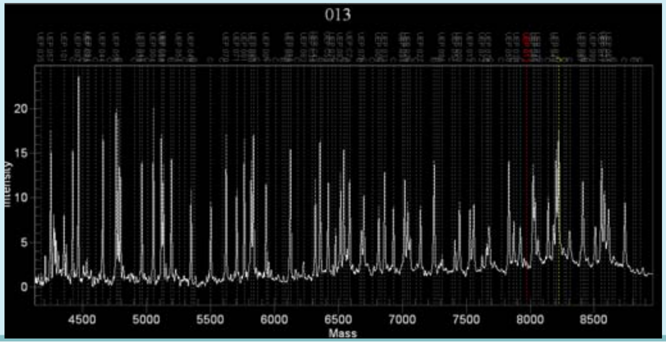
iPLEX® Genotyping

High multiplexing – low sensitivity



Raw data generated using a 59-plex PCR



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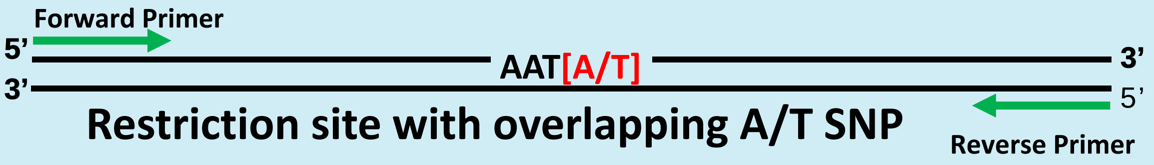
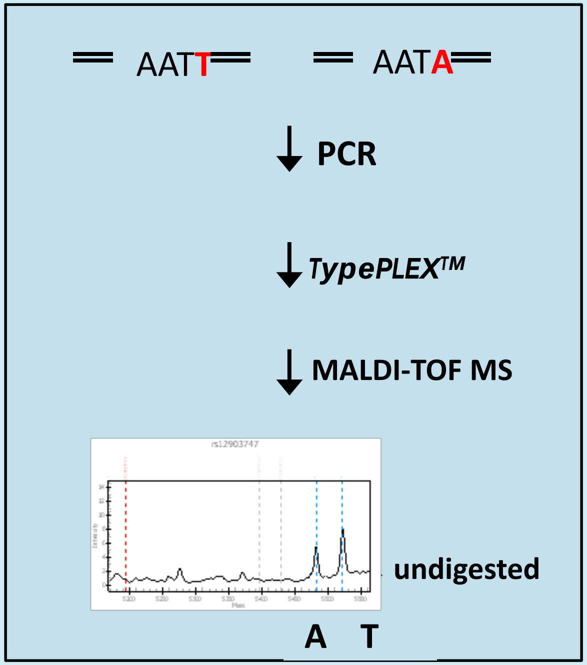
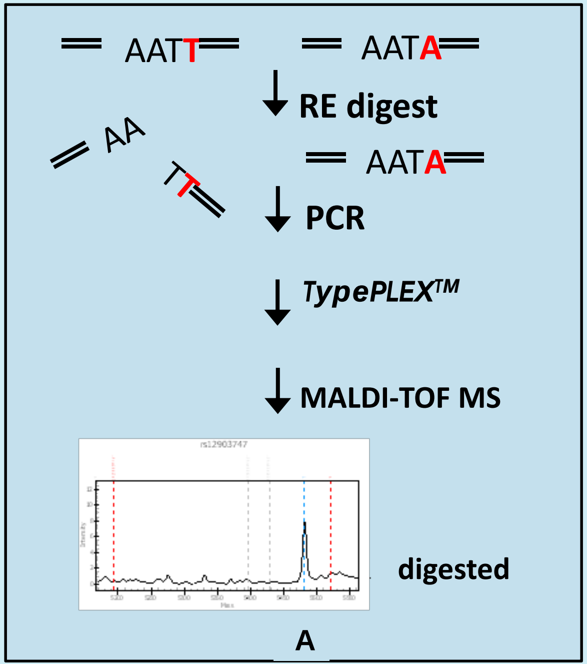
iPLEX® Genotyping

Restriction enzyme enhanced detection of SNP alleles



Restriction site with overlapping A/T SNP





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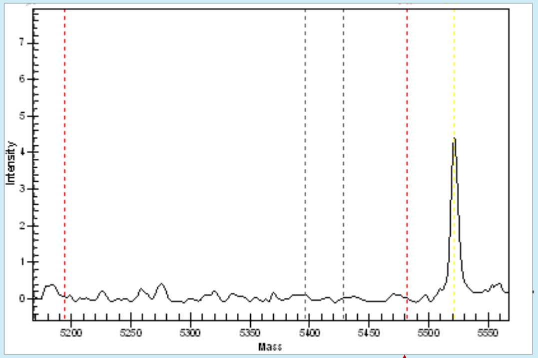
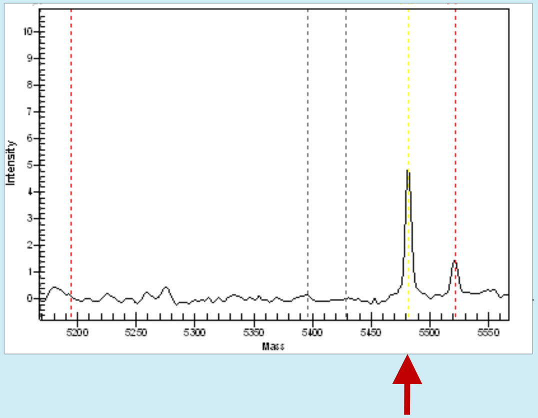
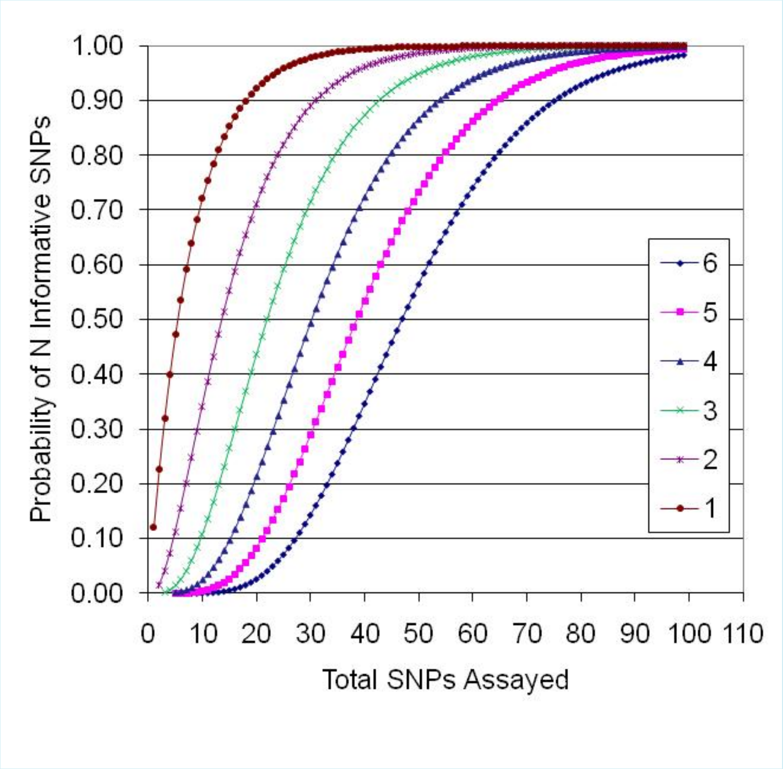
Informative genotype combinations



Compare Allele Ratios





iPLEX® Genotyping

The potential for SNP profiling

- **Identification**
- **Automated and flexible assay designs (re-plexing and super-plexing)**
- **Multiplexing (short amplicon length)**
- **Qualitative and quantitative results**
- **Sensitive detection of low frequency mutations**

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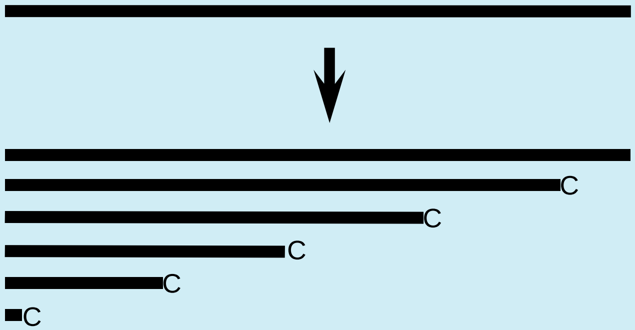
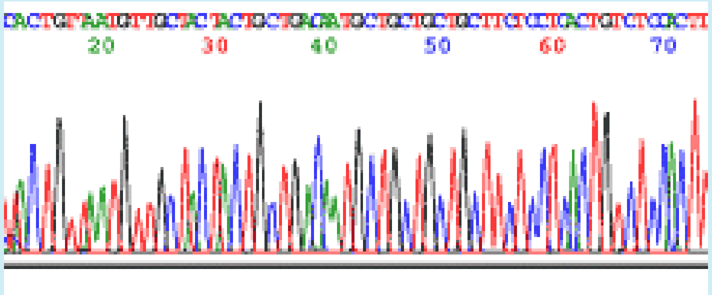
iSEQ™ Comparative Sequence Analysis

The potential for mitochondrial DNA profiling

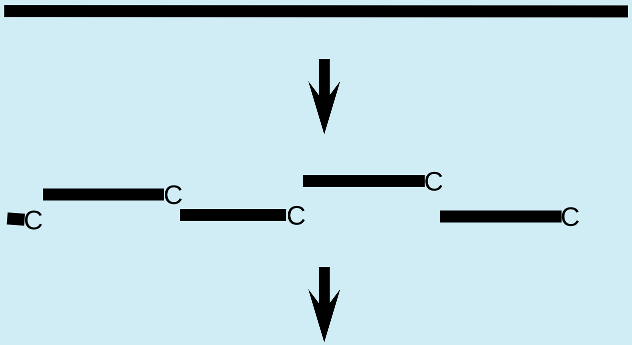
Dideoxy sequencing



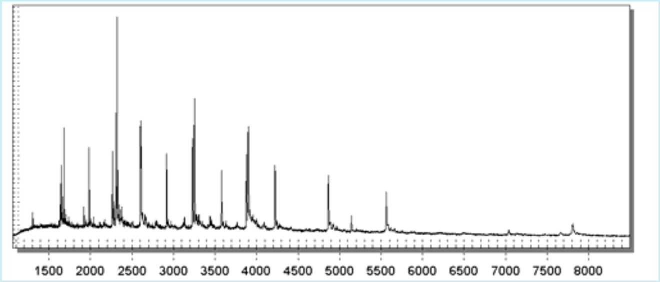
Capillary or gel-based separation



Base-specific cleavage



MALDI-TOF mass spectrometry



Similar to tryptic protein digestion

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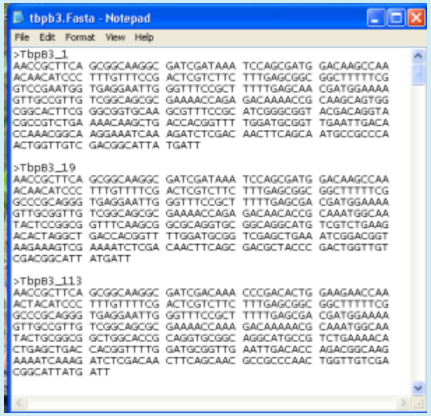
iSEQ™ Comparative Sequence Analysis

The potential for mitochondrial DNA profiling

Data import



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GTCCGAATGG TGAGGAATTG GGTTTCCGCT TTTTGAGCAA CGATGGAAAA
GTTGCCGTTG TCGGCAGCGC GAAAACCAGA GACAAAACCG CAAGCAGTGG
CGGCACTTCG GGCGGTGCAA GCGTTTCCGC ATCGGGCGGT ACGACAGGTA
CGCCGTCTGA AAACAAGCTG ACCACGGTTT TGGATGCGGT TGAATTGACA
CCAAACGGCA AGGAAATCAA AGATCTCGAC AACTTCAGCA ATGCCGCCCA
ACTGGTTGTC GACGGCATTA TGATT

>TbpB3_19
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ACAACATCCC TTTGTTTTCG ACTCGTCTTC TTTGAGCGGC GGCTTTTTCG
GCCCGCAGGG TGAGGAATTG GGTTTCCGCT TTTTGAGCGA CGATGGAAAA
GTTGCGGTTG TCGGCAGCGC GAAAACCAGA GACAACACCG CAAATGGCAA
TACTCCGGCG GTTTCAAGCG GCGCAGGTGC GGCAGGCATG TCGTCTGAAG
ACACTAGGCT GACCACGGTT TTGGATGCGG TCGAGCTGAA ATCGGACGGT
AAGAAAGTCG AAAATCTCGA CAACTTCAGC GACGCTACCC GACTGGTTGT
CGACGGCATT ATGATT

>TbpB3_113
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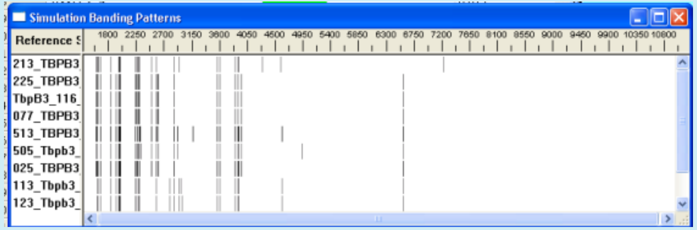
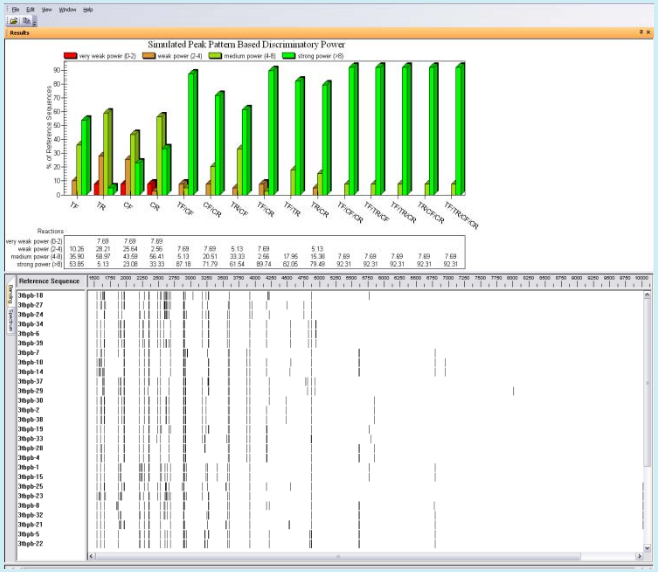
GCCCGCAGGG TGAGGAATTG GGTTTCCGCT TTTTGAGCGA CGATGGAAAA
GTTGCCGTTG TCGGCAGCGC GAAAACCAAA GACAAAAACG CAAATGGCAA
TACTGCGGCG GCTGGCACCG CAGGTGCGGC AGGCATGCCG TCTGAAAACA
CTGAGCTGAC CACGGTTTTG GATGCGGTTG AATTGACACC AGACGGCAAG
AAAATCAAAG ATCTCGACAA CTTCAGCAAC GCCGCCCAAC TGGTTGTCGA
CGGCATTATG ATT
```

Simulated banding pattern



Peak pattern simulation



Reactions	TF	TR	CF	CR	TF/CF	CF/CR	TR/CF	TF/CR	TF/TR	TR/CR	TF/CF/CR	TF/TR/CF	TF/TR/CR	TR/CF/CR	TF/TR/CF/CR
very weak power (0-2)		7.69	7.69	7.69											
weak power (2-4)	10.26	28.21	25.64	2.56	7.69	7.69	5.13	7.69		5.13					
medium power (4-8)	35.90	58.97	43.59	56.41	5.13	20.51	33.33	2.56	17.95	15.38	7.69	7.69	7.69	7.69	7.69
strong power (>8)	53.85	5.13	23.08	33.33	87.18	71.79	61.54	89.74	82.05	79.49	92.31	92.31	92.31	92.31	92.31

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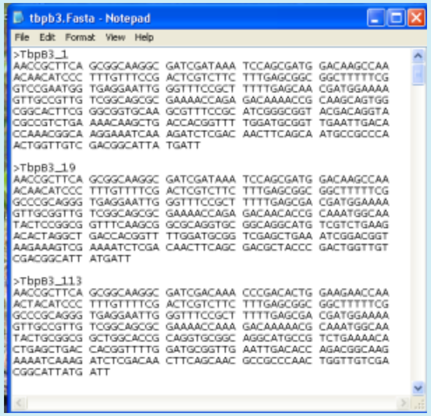
iSEQ™ Comparative Sequence Analysis

The potential for mitochondrial DNA profiling

Data import



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>TbpB3_1
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ACAACATCCC TTTGTTTCCG ACTCGTCTTC TTTGAGCGGC GGCTTTTTCG
GTCCGAATGG TGAGGAATTG GGTTTCCGCT TTTTGAGCAA CGATGGAAAA
GTTGCCGTTG TCGGCAGCGC GAAAACCAGA GACAAAACCG CAAGCAGTGG
CGGCACTTCG GGCGGTGCAA GCGTTTCCGC ATCGGGCGGT ACGACAGGTA
CGCCGTCTGA AAACAAGCTG ACCACGGTTT TGGATGCGGT TGAATTGACA
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ACTGGTTGTC GACGGCATTA TGATT

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GTTGCGGTTG TCGGCAGCGC GAAAACCAGA GACAACACCG CAAATGGCAA
TACTCCGGCG GTTTCAAGCG GCGCAGGTGC GGCAGGCATG TCGTCTGAAG
ACACTAGGCT GACCACGGTT TTGGATGCGG TCGAGCTGAA ATCGGACGGT
AAGAAAGTCG AAAATCTCGA CAACTTCAGC GACGCTACCC GACTGGTTGT
CGACGGCATT ATGATT

>TbpB3_113
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GTTGCCGTTG TCGGCAGCGC GAAAACCAAA GACAAAAACG CAAATGGCAA
TACTGCGGCG GCTGGCACCG CAGGTGCGGC AGGCATGCCG TCTGAAAACA
CTGAGCTGAC CACGGTTTTG GATGCGGTTG AATTGACACC AGACGGCAAG
AAAATCAAAG ATCTCGACAA CTTCAGCAAC GCCGCCCAAC TGGTTGTCGA
CGGCATTATG ATT
```
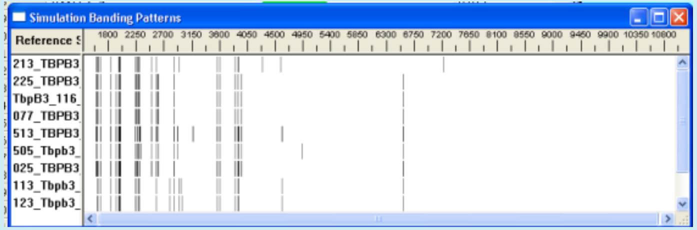
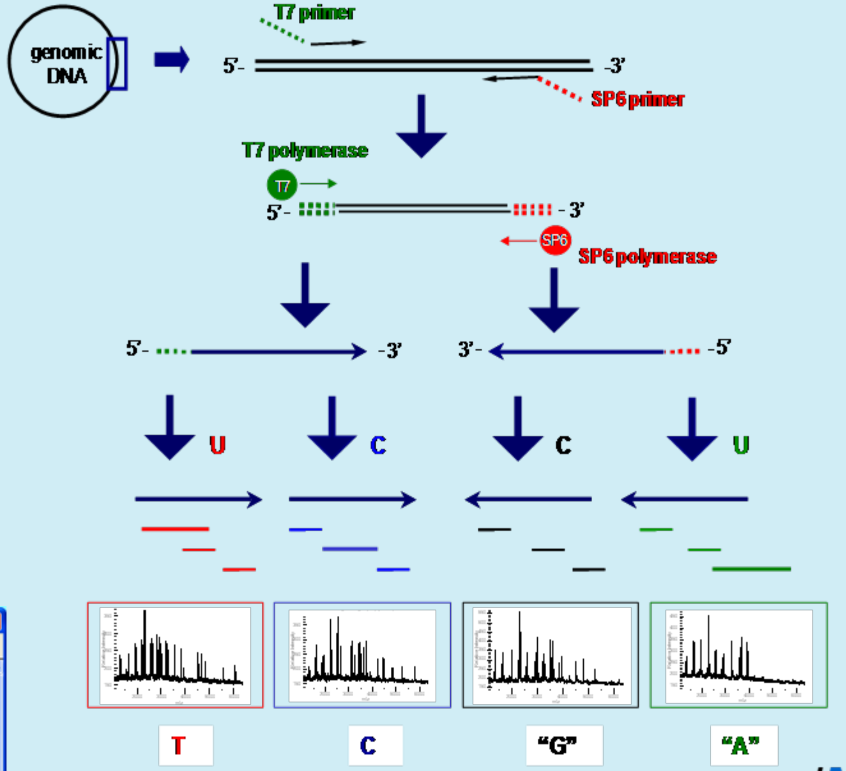

Simulated banding pattern



Homogeneous assay format



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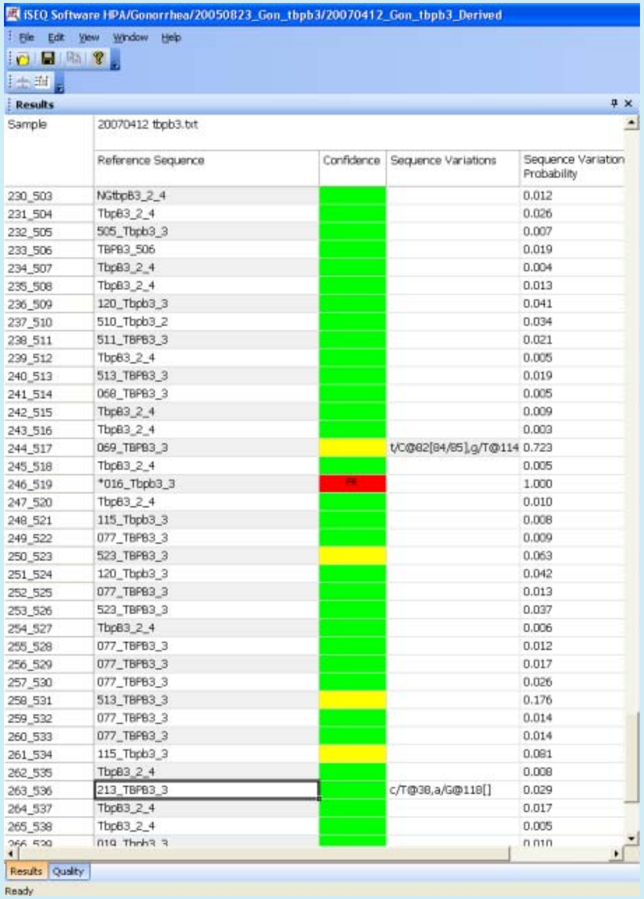
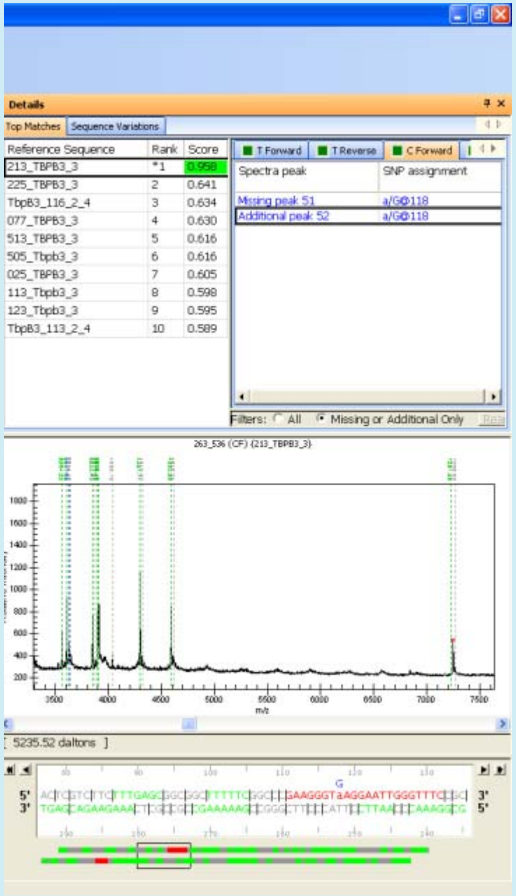
iSEQ™ Comparative Sequence Analysis

The potential for mitochondrial DNA profiling



Sample: 20070412 tbpb3.txt

	Reference Sequence	Confidence	Sequence Variations	Sequence Variation Probability
230_503	NGtbpB3_2_4	🟩		0.012
231_504	TbpB3_2_4	🟩		0.026
232_505	505_Tbpb3_3	🟩		0.007
233_506	TBPB3_506	🟩		0.019
234_507	TbpB3_2_4	🟩		0.004
235_508	TbpB3_2_4	🟩		0.013
236_509	120_Tbpb3_3	🟩		0.041
237_510	510_Tbpb3_2	🟩		0.034
238_511	511_TBPB3_3	🟩		0.021
239_512	TbpB3_2_4	🟩		0.005
240_513	513_TBPB3_3	🟩		0.019
241_514	068_TBPB3_3	🟩		0.005
242_515	TbpB3_2_4	🟩		0.009
243_516	TbpB3_2_4	🟩		0.003
244_517	069_TBPB3_3	🟨	t/C@82[84/85],g/T@114	0.723
245_518	TbpB3_2_4	🟩		0.005
246_519	*016_Tbpb3_3	🟥		1.000
247_520	TbpB3_2_4	🟩		0.010
248_521	115_Tbpb3_3	🟩		0.008
249_522	077_TBPB3_3	🟩		0.009
250_523	523_TBPB3_3	🟨		0.063
251_524	120_Tbpb3_3	🟩		0.042
252_525	077_TBPB3_3	🟩		0.013
253_526	523_TBPB3_3	🟩		0.037
254_527	TbpB3_2_4	🟩		0.006
255_528	077_TBPB3_3	🟩		0.012
256_529	077_TBPB3_3	🟩		0.017
257_530	077_TBPB3_3	🟩		0.026
258_531	513_TBPB3_3	🟨		0.176
259_532	077_TBPB3_3	🟩		0.014
260_533	077_TBPB3_3	🟩		0.014
261_534	115_Tbpb3_3	🟨		0.081
262_535	TbpB3_2_4	🟩		0.008
263_536	213_TBPB3_3	🟩	c/T@38,a/G@118[]	0.029
264_537	TbpB3_2_4	🟩		0.017
265_538	TbpB3_2_4	🟩		0.005
266_539	019_Tbpb3_3	🟩		0.010



Reference Sequence	Rank	Score
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225_TBPB3_3	2	0.641
TbpB3_116_2_4	3	0.634
077_TBPB3_3	4	0.630
513_TBPB3_3	5	0.616
505_Tbpb3_3	6	0.616
025_TBPB3_3	7	0.605
113_Tbpb3_3	8	0.598
123_Tbpb3_3	9	0.595
TbpB3_113_2_4	10	0.589

Spectra peak	SNP assignment
Missing peak 51	a/G@118
Additional peak 52	a/G@118

Filters: ○ All ● Missing or Additional Only

- **Identification**
- **SNP discovery**

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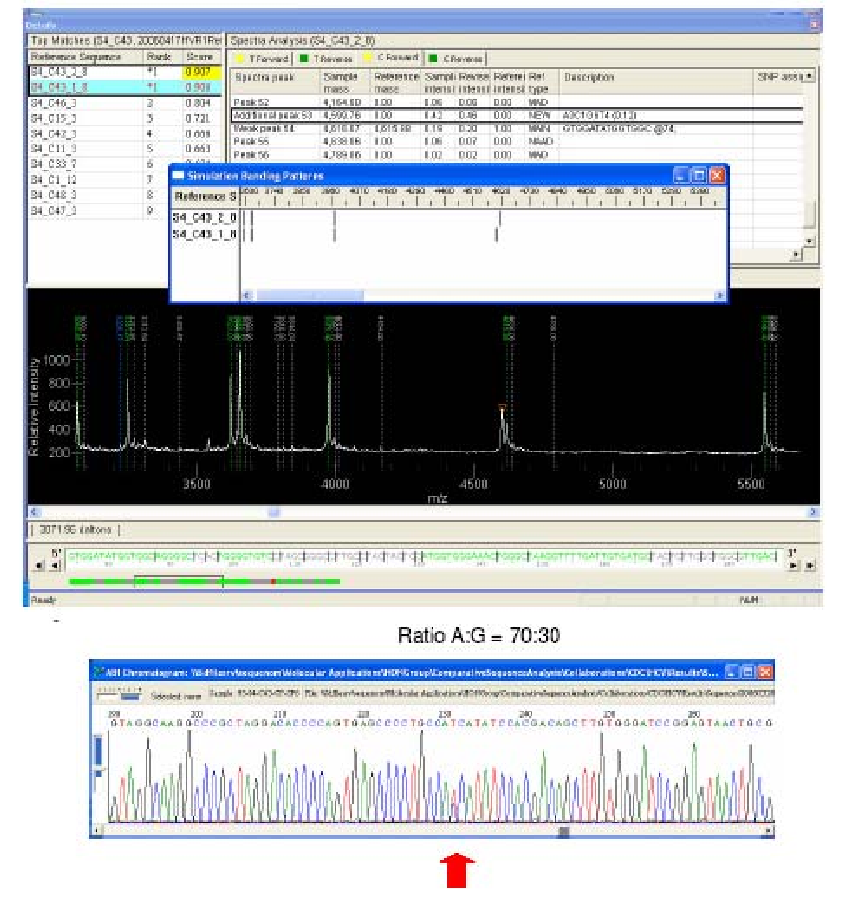
iSEQ™ Comparative Sequence Analysis

The potential for mitochondrial DNA profiling



Ratio A:G = 70:30

- **Mixture analysis**

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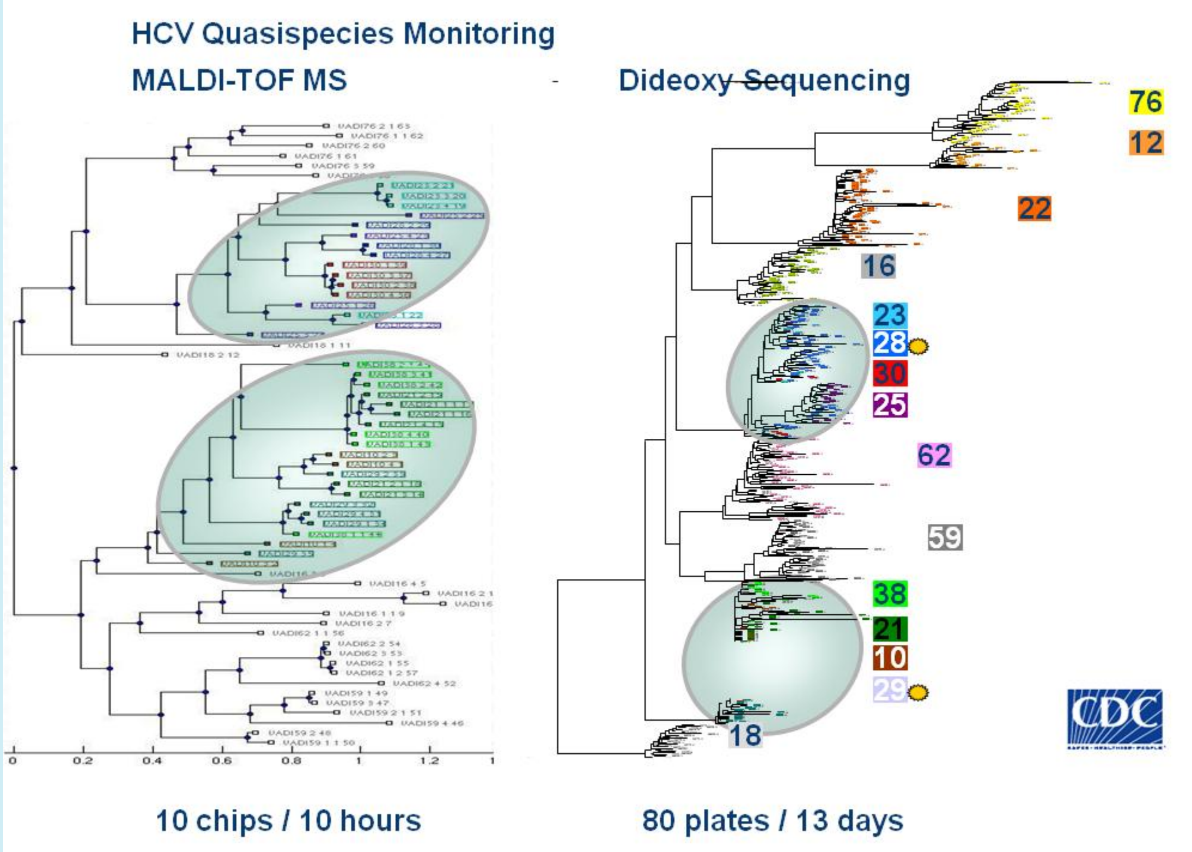
The potential for mitochondrial DNA profiling



10 chips / 10 hours

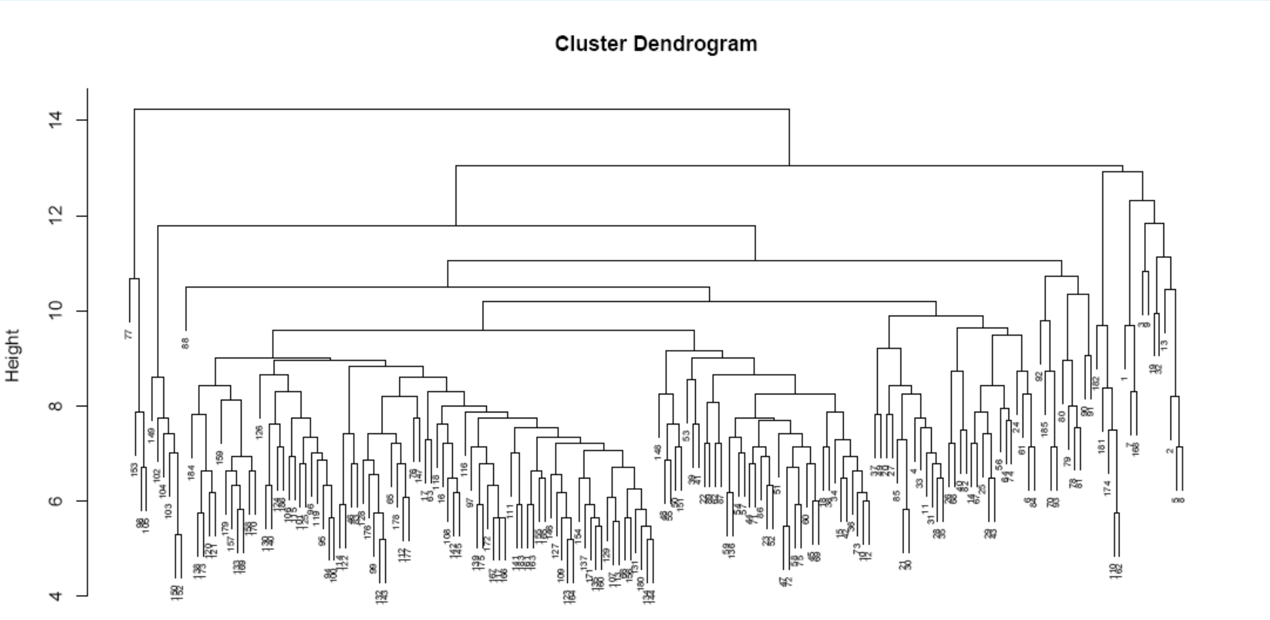
80 plates / 13 days

- **Tracking of directionality**

iSEQ™ Comparative Sequence Analysis

Screening for sequence variations in mitochondrial DNA

Sample pattern clustering



HV1 and HV2

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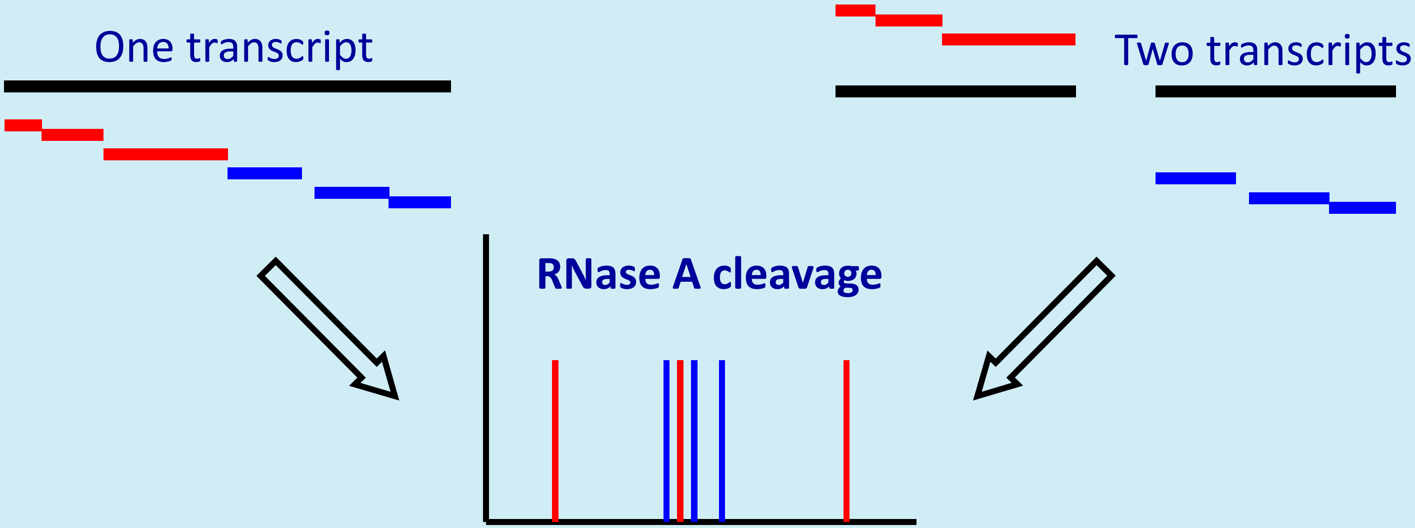
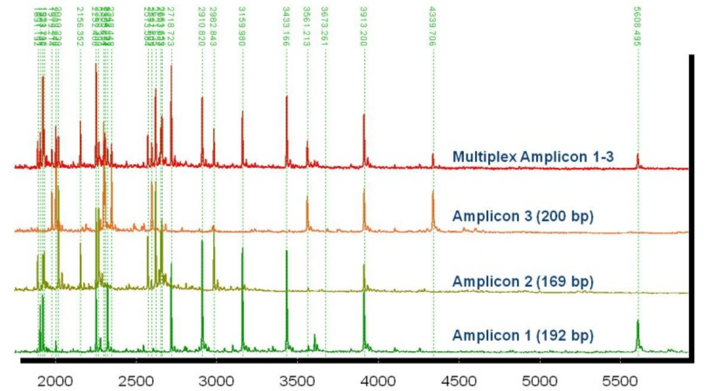
Multiplexing





iSEQ™ Comparative Sequence Analysis

The potential for mitochondrial DNA profiling

- **Identification**
- **SNPs and length variants can be detected**
- **Mixture analysis**
- **Tracking of directionality**
- **Multiplexing of different sequence contexts**

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Questions?

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Contact Information

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San Diego, CA 92121
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chonisch@sequenom.com

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