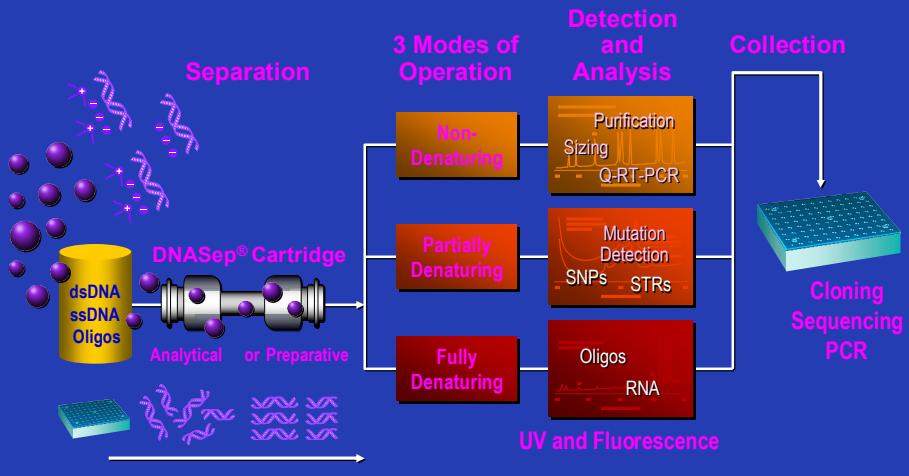
Automated, High-Accuracy Screening of Mutations and Polymorphisms in the Human Genome by Denaturing High Performance Liquid Chromatography (DHPLC)

WAVE® System Capabilities and Applications



Characteristics of the WAVE® System

- Rapid separation of DNA molecules on the robust DNASep® Cartridge
- Peltier-cooled, dual 96-well PCR plate Autosampler
- Fully-automated system
- UV and/or fluorescence detection of DNA
- Computer-controlled automated data acquisition
- Advanced predictive molecular biology applications software

System Components

- Autosampler – Peltier controlled
- Pump
- Column Oven
- DNASep Column
- Inline Degasser
- UV or FL Detectors
- Computer System and Monitor
- Color Printer
- Fragment Collector – Optional
- Accelerator - Optional

The DNASep® Cartridge

The DNASep cartridge is the "heart" of the WAVE® Nucleic Acid Fragment Analysis System.

Robust, nonporous, alkylated poly(styrene-divinylbenzene) matrix

Buffer System:

Buffer A: aqueous 0.1 M triethylammonium acetate (TEAA), pH 7.0

Buffer B: aqueous 0.1 M TEAA, 25 % (v/v) acetonitrile, pH 7.0

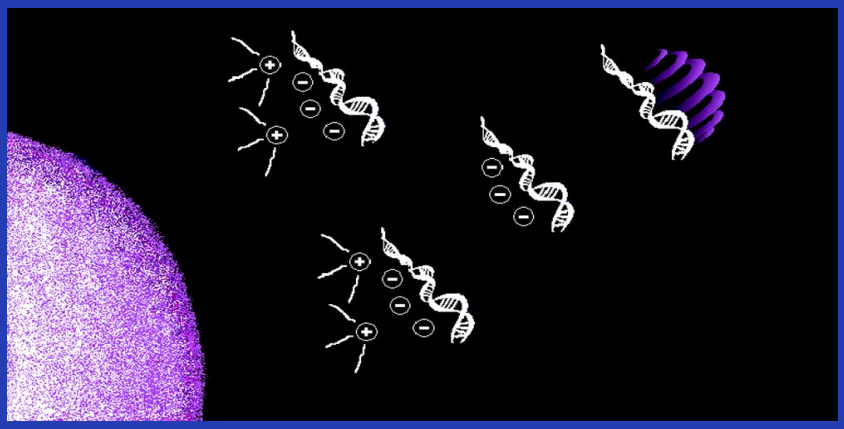
Analytical cartridge:	4.6 x 50 mm
Preparative cartridge:	7.8 x 50 mm

Polymeric DNASep Column:

	DNASep Column	Other HPLC column
◆ Base Material:	Polymer (patented)	Silica
◆ Stationary Phase	C18	C18
	Chemical bonding	Absorption
◆ Life time	6000/injection	1000/3000/injection
◆ Temperature	>80 oC	<65 oC
◆ Organic Solvent	100%	not recommended

DNASep® Cartridge Surface Chemistry

The positive charges of triethylammonium ions interact electrostatically with the negative charges on DNA. The alkyl groups of the triethylammonium ions interact with the hydrophobic surface of the alkylated DNASep® matrix.



Three Modes of Operation

Non-denaturing	Partially denaturing	Fully denaturing
		
Double-stranded DNA – Sizing – Quantification – Purification	**Heteroduplex analysis** – Mutation detection	**ss Nucleic Acids** – Oligonucleotides – ss DNA – RNA

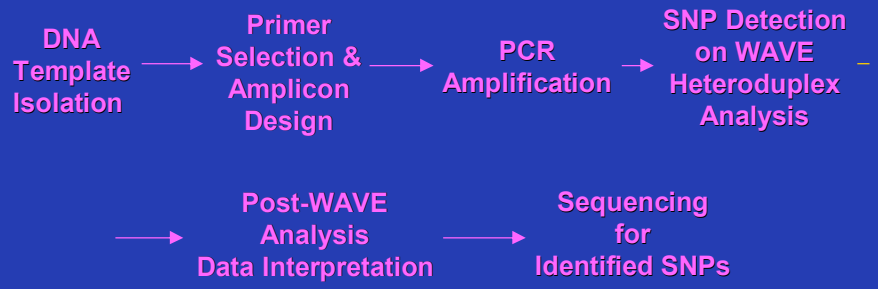
Three Modes of Operation

- Non denaturing: 50 °C (Size Dependent, Sequence Independent)
  - Sizing of double stranded DNA(up to 2000bp)
  - MSI, LOH
  - PCR quality check and purification
  - Quantitative Analysis (Q-RT-PCR)
- Partially denaturing: 52-78 °C (Size and Sequence Dependent)
  - Mutation Detection
  - SNP discovery
- Fully denaturing: 78-80 °C (Size and Sequence Dependent)
  - Oligo quality and purification analysis
  - RNA analysis (with RNASep column)

Principles of DNA Separation

- Sequence independent separation of double-stranded DNA fragments based on length
- Separation of DNA fragments according to their degree of denaturation (TMHA) - Separation of co-migrating fragments
- Analysis of nucleic acids under denaturing conditions (oligonucleotides, single-stranded DNA, RNA) – Separation dependent on single-stranded DNA based on length and sequence

General Protocol of WAVE DHPLC for SNP and Genetic Variation Study



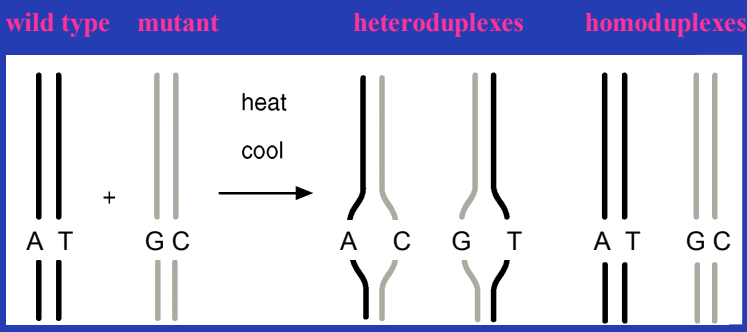
Genotyping and Scoring for SNPs and Mutations with DHPLC:

Search for the presence and absence of known Mutations and Polymorphisms

- Heteroduplex Analysis
- Primer Extension Assay

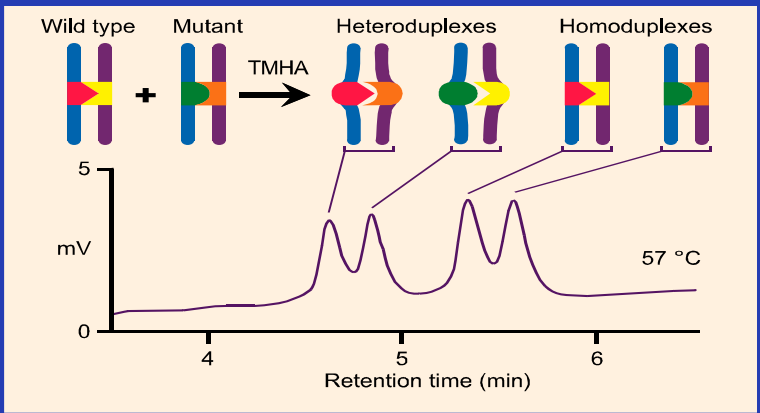
TMHA - Heteroduplex Analysis

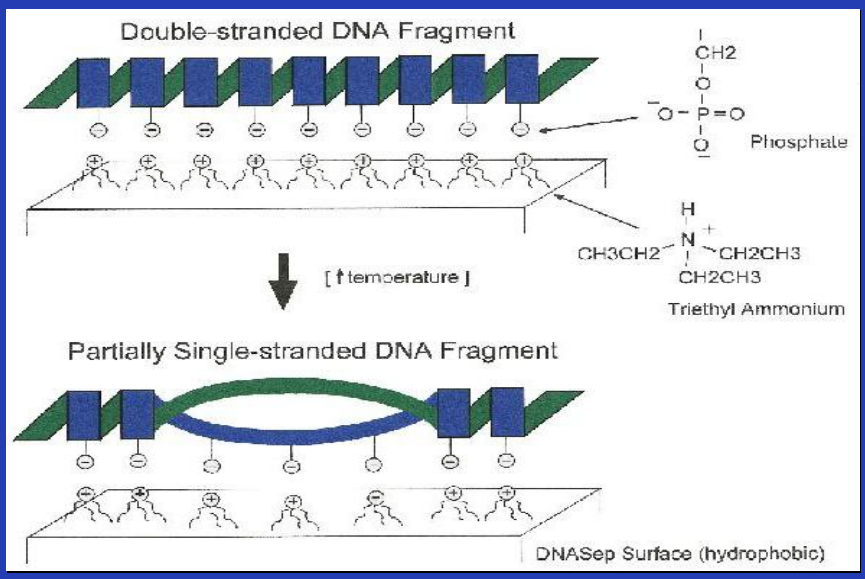
PCR products of wild-type and mutant alleles, differing by as little as a single base pair, are denatured by heating and re-annealed by slow cooling. The resultant wild-type and mutant homoduplexes melt at higher temperatures than the mismatch containing wild-type/mutant heteroduplexes. The difference in melting temperature between homo- and heteroduplexes is the basis for the identification of mutations by DNA chromatography.



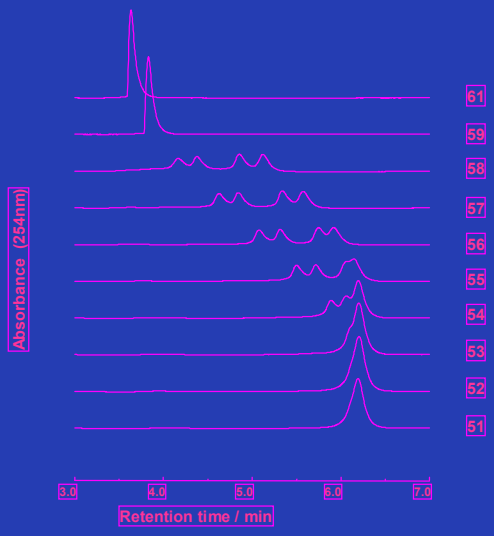
Examples of Partial Denaturing Operation for Mutation Detection

(Temperature Modulated Heteroduplex Analysis) TMHA





Resolution of Homo- and Heteroduplexes at Different Temperatures



Human Y chromosome STS, sY81 (DYS271) with A-to-G mutation at position 168 of a 209-bp fragment

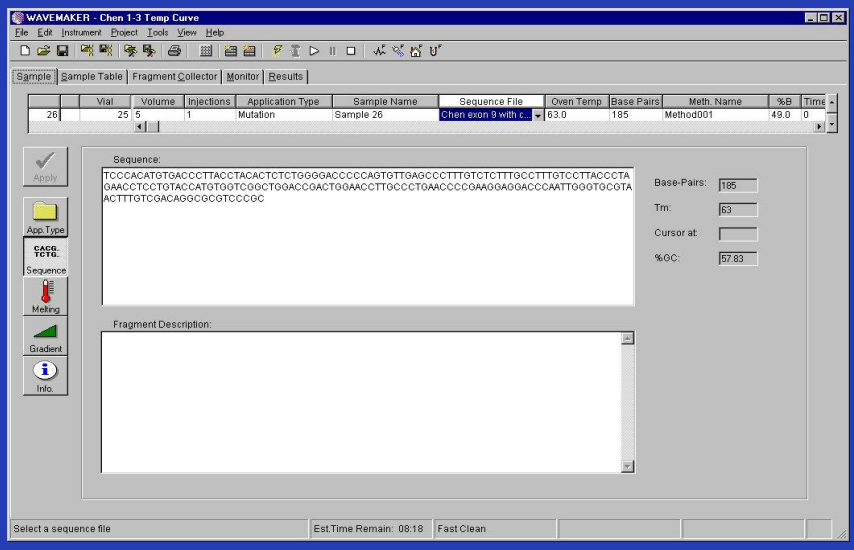
Temperature Modulated Heteroduplex Analysis (TMHA)

Heteroduplexes are resolved ahead of homoduplexes at temperatures from 54 to 58°C.

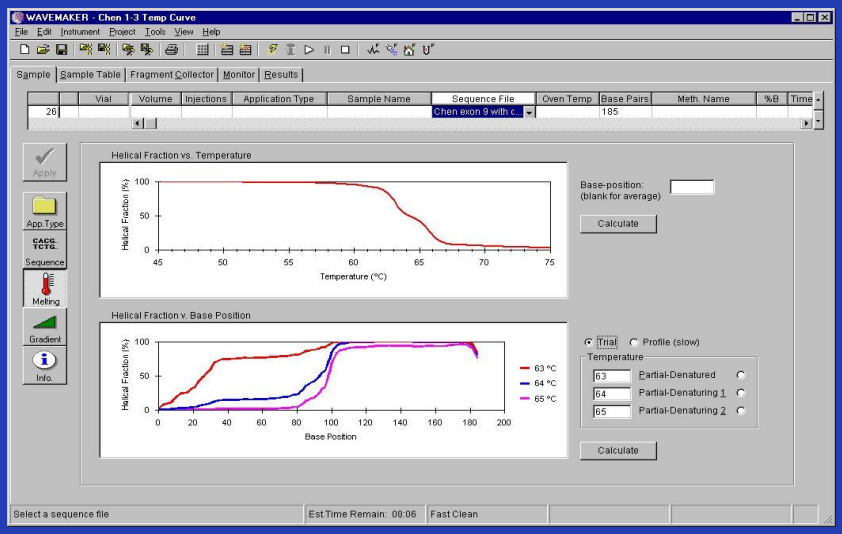
WAVEMAKER SOFTAWRE

From DNA Sequence to Gradient and Method

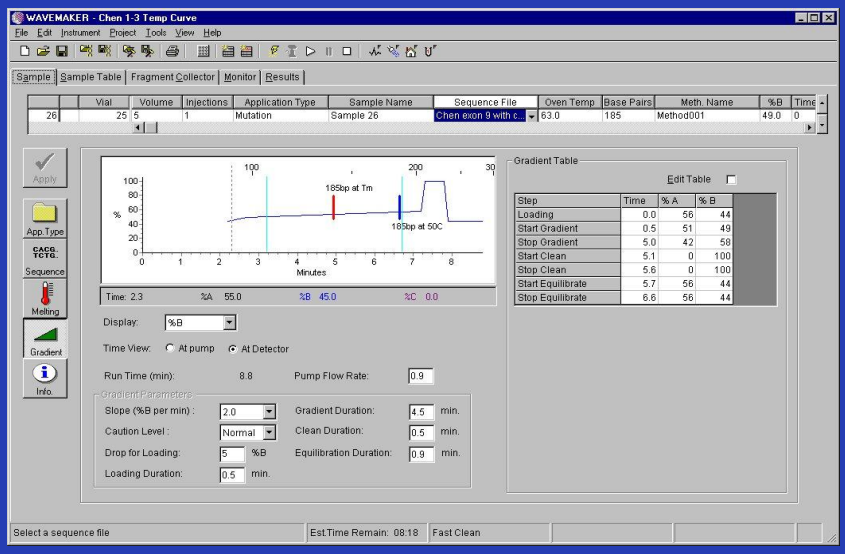
DNA Sequence Page



Melting Profiles Page

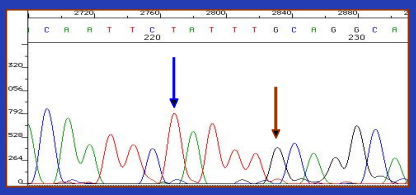


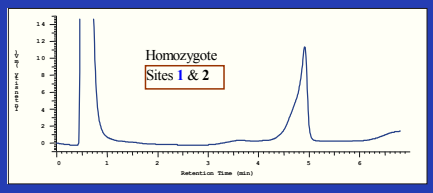
Gradient Template Page

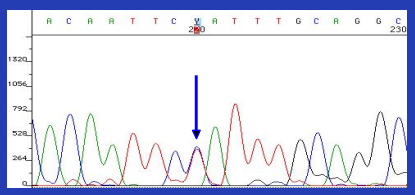


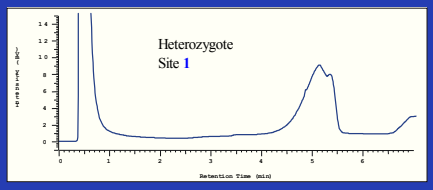
Heteroduplex Analysis

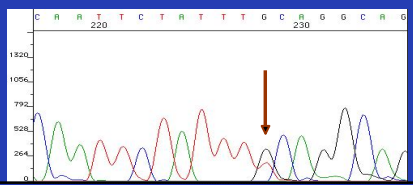
Genotyping with DHPLC

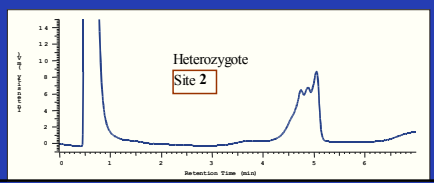






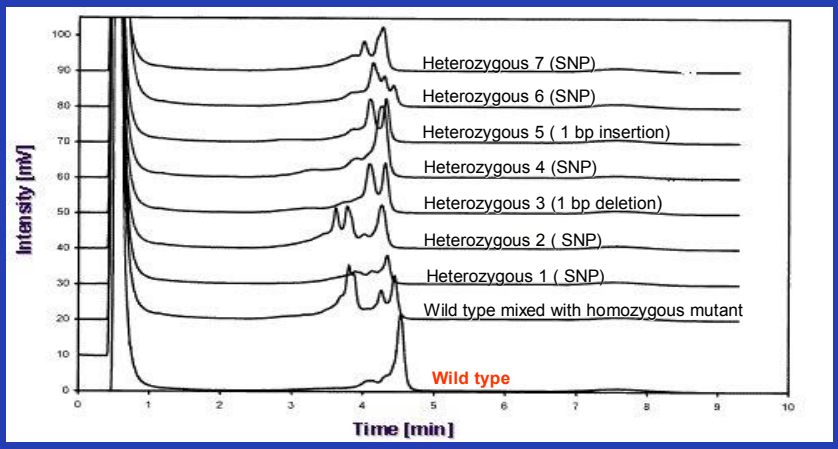




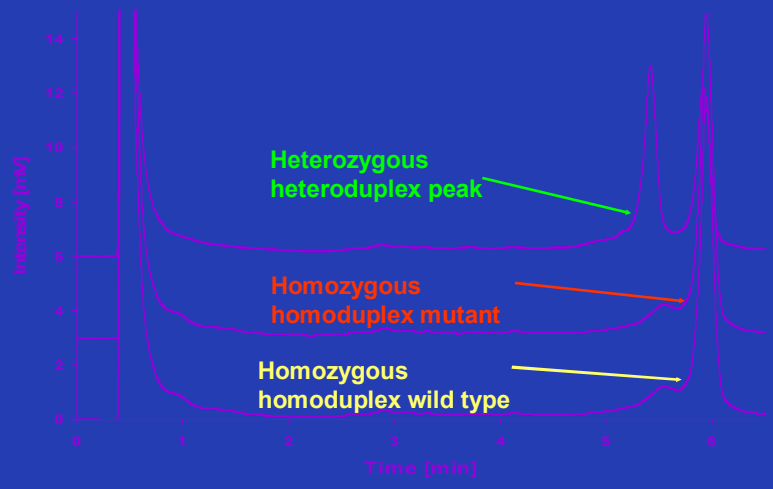


Mutation Detection

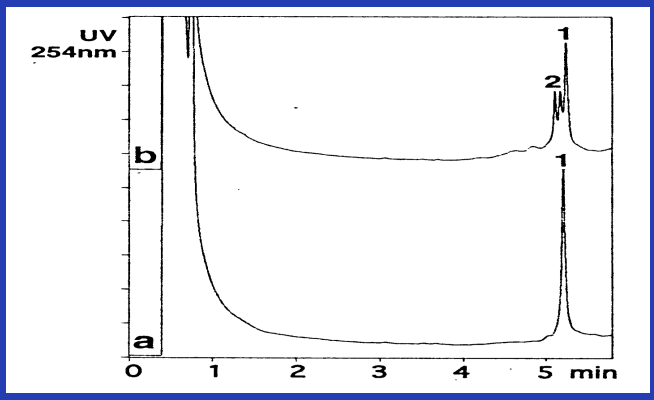
Eight different polymorphisms including a deletion, insertion and a homozygous change in one amplicon.



Mutation Detection in p53 Exon 8-9



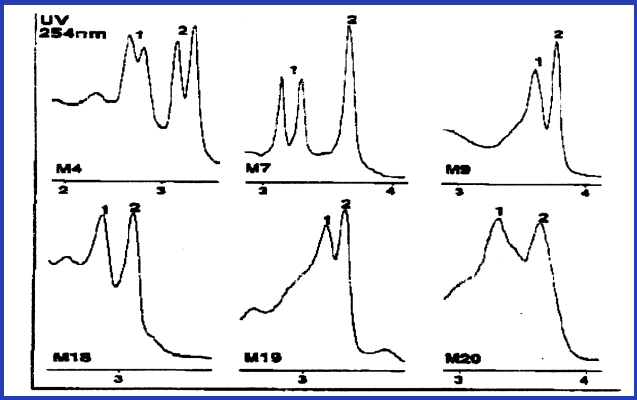
Application to SNP Discovery



Discovery of a Y chromosome-specific SNP in Native Americans

From Underhill, *et al*, *PNAS USA*, 1996, **93**, 196-200

Application to SNP Discovery



Discovery of numerous (19) Y chromosome SNPs by DHPLC

From Underhill, *et al*, *Genome Research*, 1997, 7, 996-1005

Low Level Mosaicism Detectable by DHPLC But Not by Direct Sequencing

Alistair C. Jones, Julian R. Sampson,* and Jeremy P. Cheadle

Institute of Medical Genetics, University of Wales College of Medicine, Cardiff,

UK

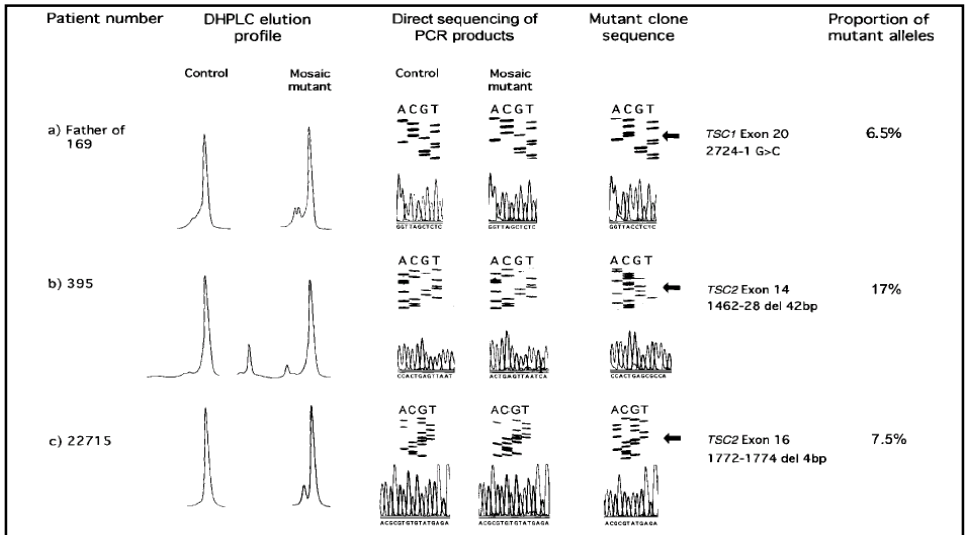


FIGURE 1. Mosaicism detected by DHPLC but not by direct sequencing of PCR products. Primer sequences, PCR and DHPLC conditions have been described previously [Jones et al., 2000]. PCR products were purified using the Qiagen (Crawley, UK) PCR purification kit. Manual sequencing was performed using the Thermo Sequenase cycle sequencing kit (Amersham-Pharmacia Biotech, Amersham, UK) and automated sequencing was carried using the Big-Dye Terminator cycle sequencing kit (P.E. Biosystems, Warrington, UK) with products analyzed on an ABI-377. Both forward and reverse PCR primers were used for manual and automated sequencing. All sequences except those in **b** are shown in the forward direction. (**a**) Father of case 169: despite the mutant DHPLC elution profile observed for exon 20 of *TSC1*, direct manually and automatically generated sequence of the PCR product was indistinguishable from the control and the mutation 2724-1G>C was only demonstrated after cloning (8/124 clones). (**b**) Patient 395: the aberrant DHPLC profile for exon 14 of *TSC2* corresponded to the mutation 1462-28del 42bp that was only revealed following cloning (18/104 clones). (**c**) Patient 22715: the aberrant DHPLC profile for exon 16 of *TSC2* corresponded to the mutation 1772-1774 del 4bp that was only revealed following cloning (7/93 clones). Arrows indicate the positions of mutations in the sequence.

DHPLC mutation analysis of the hereditary nonpolyposis colon cancer (HNPCC) genes

hMLH1 and hMSH2

Elke Holinski-Feder , Y. Muller-Koch , W. Friedl , G. Moeslein ,
G. Keller , J. Plaschke , W. Ballhausen , M. Gross ,
K. Baldwin-Jedele , M. Jungck , E. Mangold , H. Vogelsang ,
H.-K. Schackert , P. Lohse , J. Murken , Th. Meitinger

Table 2
Recommended temperatures and buffer gradients for mutation analysis of exons 1–19 for hMLH1 and exon 1–16 for hMSH2

Exon	Temperature (°C)															
	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
hMSH1																
1											(57–65)		(56–64)		(56–64)	
2			(54–62)		(54–62)		(48–56)									
3					(55–63)			(54–62)			(49–57)					
4		(55–63)			(51–59)			(50–58)								
5		(55–63)			(54–62)			(51–59)								
6				(57–65)		(56–64)		(52–60)								
7		(57–65)			(55–63)			(52–60)								
8			(53–61)		(49–57)		(47–55)									
9			(54–62)		(54–62)		(49–57)									
10			(53–61)		(52–60)			(46–54)								
11		(54–62)		(49–57)		(47–55)										
12						(57–65)		(54–62)			(48–56)					
13						(57–65)		(54–62)			(50–58)					
14					(58–66)		(56–64)		(51–59)							
15				(56–64)			(55–63)				(48–56)					
16				(55–63)		(52–60)		(48–56)								
hMLH1																
1												(56–64)		(48–64)		(48–6
2		(52–64)					(52–64)		(52–64)							
3				(49–61)		(49–61)			(49–61)							
4		(52–64)				(52–64)		(52–64)								
5				(49–61)		(49–61)										
6	(52–64)					(52–64)										
7					(50–58)		(48–56)									
8			(52–64)		(52–64)				(52–64)							
9							(54–62)		(50–58)							
10								(52–64)								
11						(58–66)					(58–66)					
12 F2										(49–61)		(49–61)				
12 F3																
13				(58–66)					(58–66)							
14								(54–66)		(54–66)						
15																
16									(54–66)							
17								(56–64)		(56–64)		(56–64)				
18				(52–64)					(52–64)							
19									(56–64)							

Mutation analysis of the entire mitochondrial genome using denaturing high performance liquid chromatography

Bianca J.C. van den Bosch, Rene F.M. de Coo[1], Hans R. Scholte[2], Jeroen G. Nijland, Ruud van den Bogaard[3], Marianne de Visser[4], Christine E. M. de Die-Sumlders and Hubert J. M. Smeets

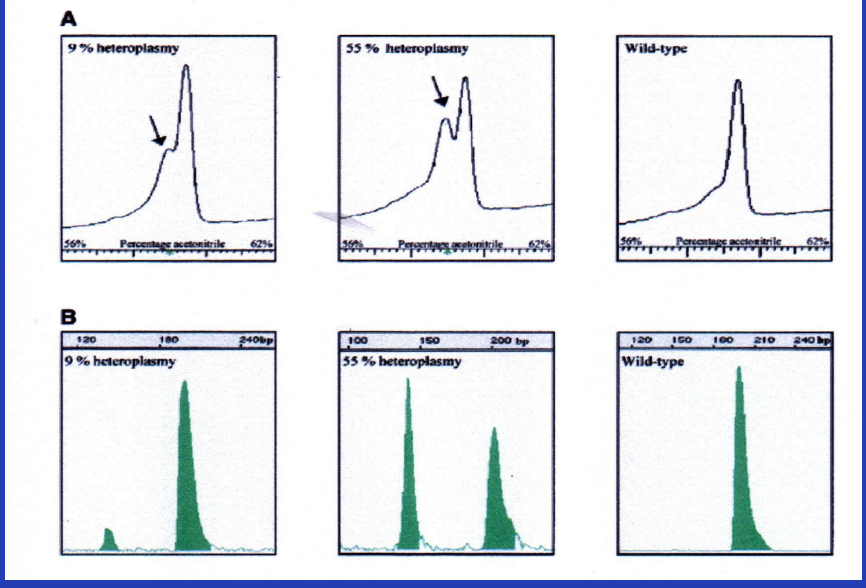
Department of Molecular Cell Biology and Genetics, Maastrict University, PO Box 1475, 6201 BL Maastricht, The Netherlands, [1] Department of Child Neurology, Unviersity Hospital Rotterdam, Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands, [2]Department of Biochemistry, Erasmu University Rotterdam, POBox 1738, 3000 DR Rotterdam, The Netherlands, [3]Department of Clinical Genetics, Academic Medical Center and [4]Department of Neurology, Academic Medical Center, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands

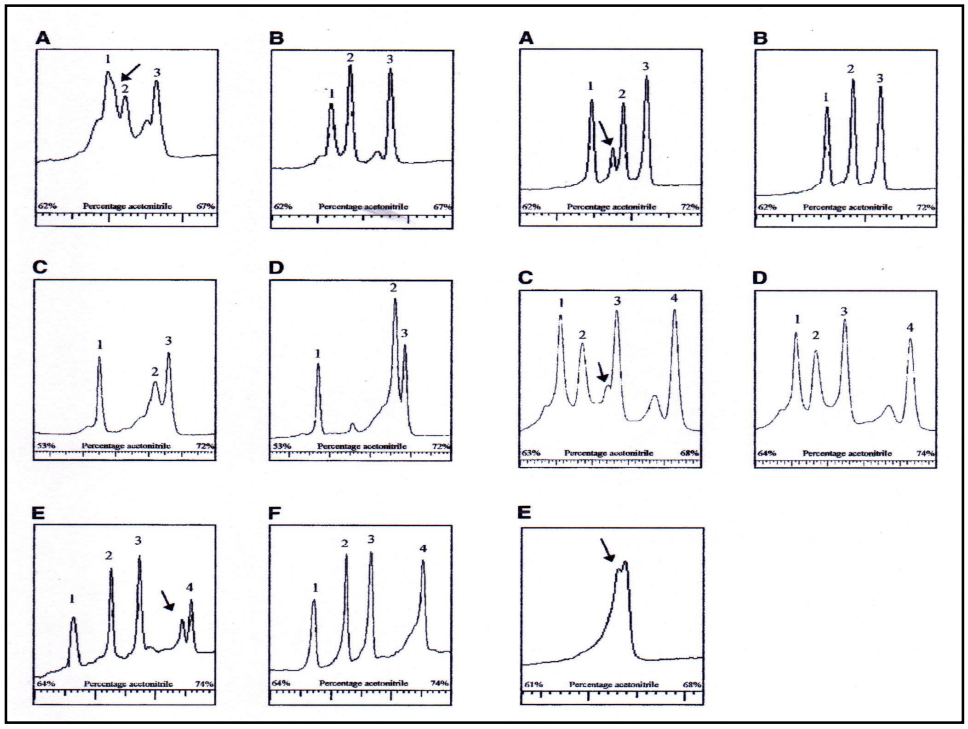
Table 2. Primer sequences used to amplify the mtDNA in 13 fragments of 1–2 kb in length

Fragment	Position	Length (bp)	Sequence forward primer	Sequence reverse primer
1	656–2490	1835	5′-TGG TCC TAG CCT TTC TAT TAG C-3′	5′-GGG TAA GAT TTG CCG AGT TCC-3′
2	2433–4224	1792	5′-CAG GCA TGC TCA TAA GGA AAG G-3′	5′-GGA GAC ATA TCA TAT AAG TAA TGC-3′
3	4152–5735	1584	5′-CGA CCA ACT CAT ACA CCT CC-3′	5′-GAG AAG TAG ATT GAA GCC AG-3′
4	5470–6908	1439	5′-CGC TAC TCC TAC CTA TCT CC-3′	5′-AGA TCA TTT CAT ATT GCT TCC GT-3′
5	6789–8000	1212	5′-GGA ATA GAC GTA GAC ACA CGA G-3′	5′-CAA CGT CAA GGA GTC GCA GGT-3′
6	7699–8738	1040	5′-CCT GTA TGC CCT TTT CCT AAC- 3′	5′-ATA AGA GAT CAG GTT CGT CCT T-3′
7	8577–10407	1831	5′-ACC CGC CGC AGT ACT GAT CAT-3′	5′-CCA ATT CGG TTC AGT CTA ATC C-3′
8	10233–11249	1017	5′-GCT ATT ACC TTC TTA TTA TTT GAT C-3′	5′-GTG CGA TGA GTA GGG GAA GG-3′
9	10866–12420	1554	5′-TCA TCC CTC TAC TAT TTT TTA ACC-3′	5′-TTT GTT AGG GTT AAC GAG GG-3
10	12175–13708	1534	5′-TGA CAA CAG AGG CTT ACG ACC-3′	5′-CCA GGC GTT TAA TGG GGT TTA GT-3′
11	13354–14458	1105	5′-TTT ATG TGC TCC GGG TCC ATC AT-3′	5′-GAT GGC TAT TGA GGA GTA TCC T-3′
12	14399–15593	1195	5′-ACA CTC ACC AAG ACC TCA ACC-3′	5′-ATC GGA GAA TTG TGT AGG CGA AT-3′
13	15498–711	1783	5′-GCG ACC CAG ACA ATT ATA CCC T-3′	5′-AAC GGG GAT GCT TGC ATG TGT-3′

Table 3. Enzymes used for restriction digestion (in a volume of 50 µl) and DHPLC analysis temperatures

Fragment	Enzyme	Restriction site	Enzyme used (U)	Incubation temperature (°C)	Fragments after restriction	Temperatures for DHPLC analysis (°C)
1	*Bfa*I	C↓TAG	10	37	132 187 260 365 401 484	55, 57, 58, 59, 60
2	*Nla*III	CATG↓	10	37	120 295 382 460 527	55, 58, 59, 60, 61
3	*Hpa*II	C↓CGG	5	37	135 396 493 560	55, 56, 58, 59, 60
4	*Hae*III	GG↓CC	5	37	123 190 233 369 524	55, 57, 59, 60, 61
5	*Hae*III	GG↓CC	5	37	113 170 240 300 389	55, 57, 58, 59, 60
6	*Alu*I	AG↓CT	10	37	229 377 434	54, 57, 58, 59, 60
7	*Taq*I	T↓CGA	5	65	159 227 270 308 381 486	50, 55, 58, 59, 60
8	*Hin*fI	G↓ANTC	15	37	141 205 278 393	53, 56, 57, 58, 60
9	*Hph*I	GGTGA$(N)_8$↓	7.5	37	284 330 413 528	55, 57, 58, 59, 60
10	*Sfa*NI	GCATC$(N)_5$↓	0.8	37	292 347 410 485	55, 57, 58, 59, 60
11	*Hae*III	GG↓CC	5	37	500 593	57, 58, 59, 60, 61
12	*Dpn*II	GA↓TC	5	37	191 235 297 472	55, 57, 59, 60, 61
13	*Alu*I	AG↓CT	10	37	90 130 218 280 482 548	53, 57, 58, 59, 60





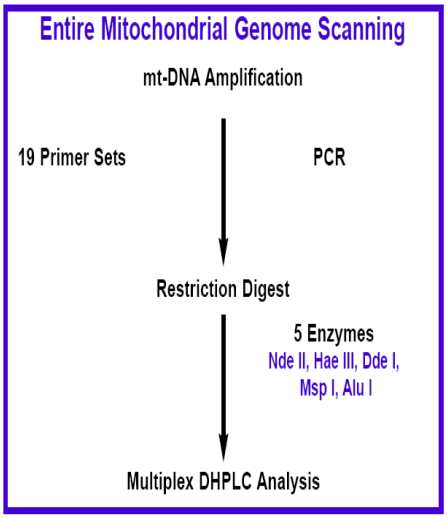


Figure 1: Schematic representation of the MitoScreen Assay Kit Protocol.

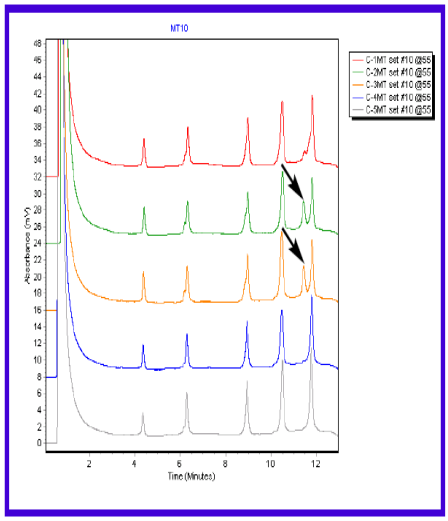
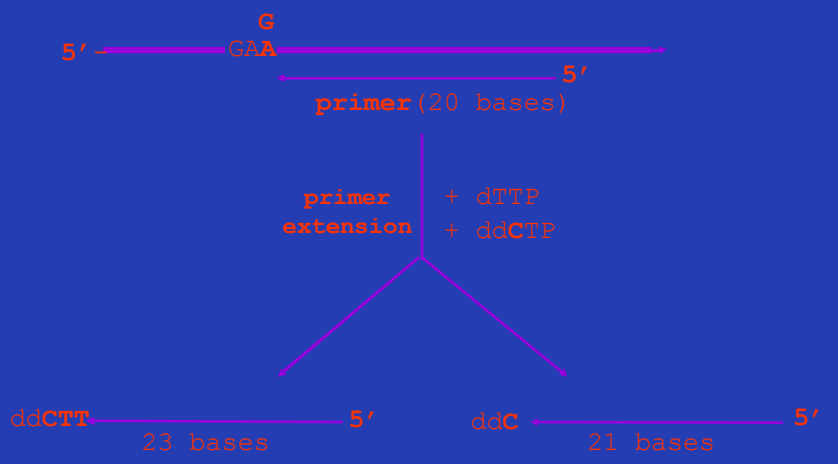


Figure 2: Analysis of PCR fragment MT10 in different pooled DNA samples at 55°C. A heteroplasmic polymorphism is clearly seen in the 5th fragment* of MT10 in DNA samples 2 and 3 as indicated by the arrows.
*Fragment 5 is a 504 bp fragment corresponding to nucleotides 7204–7707 of the mtDNA genome sequence.

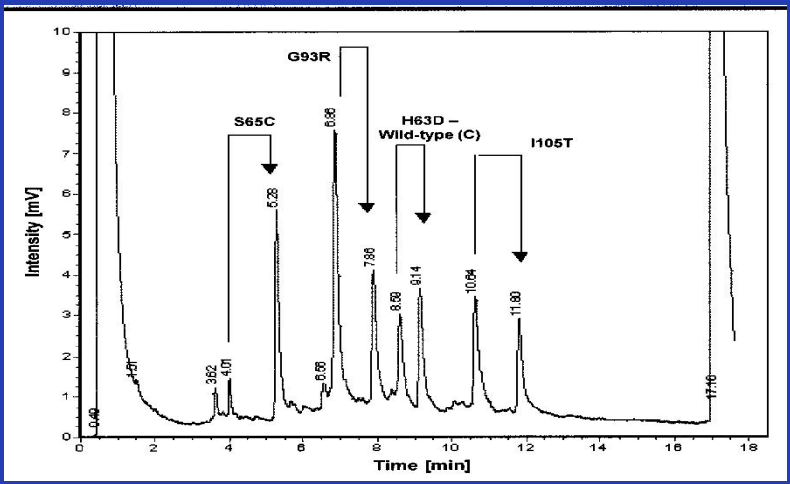
Primer extension schematic



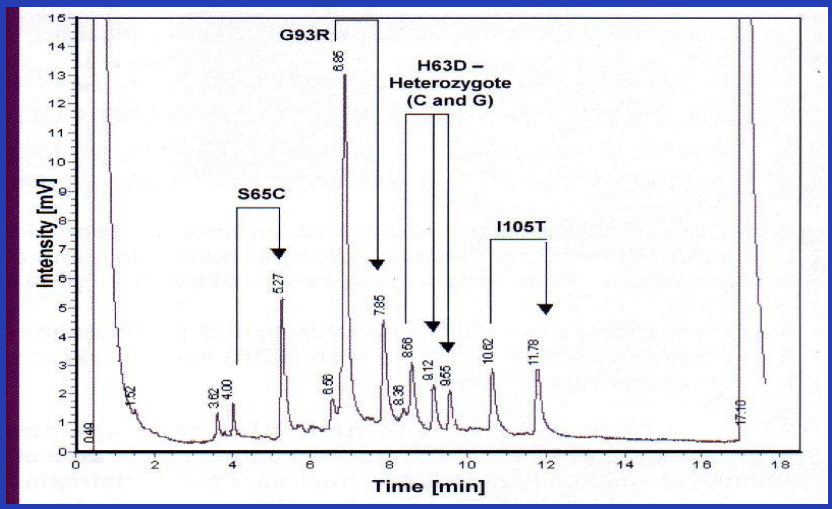
Genotyping of Mutation in the HFE Gene

Using a Single-Base Extension Assay on the Wave System

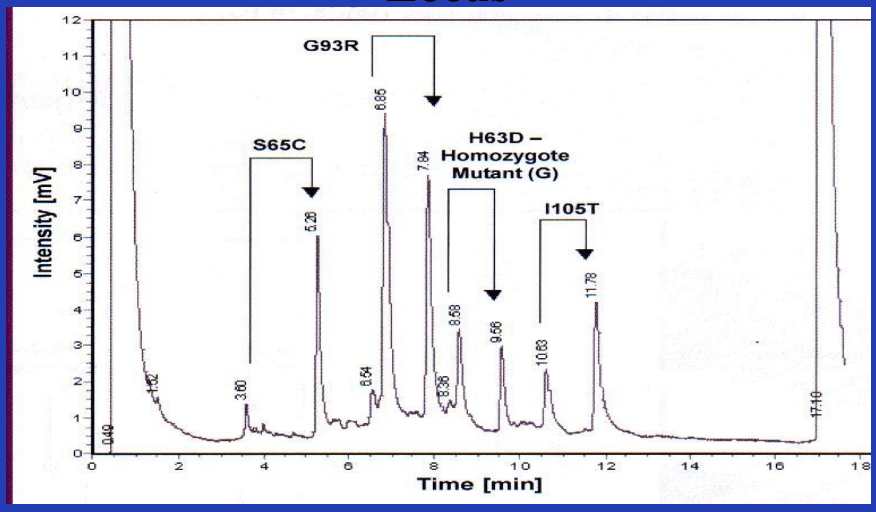
Multiplex of HFE Exon 2 Variants SBE of a Wild-type at the H63D Locus in Multiplex of Exon 2



Multiplex of HFE Exon 2 Variants SBE of a Heterozygote at the H63D Locus



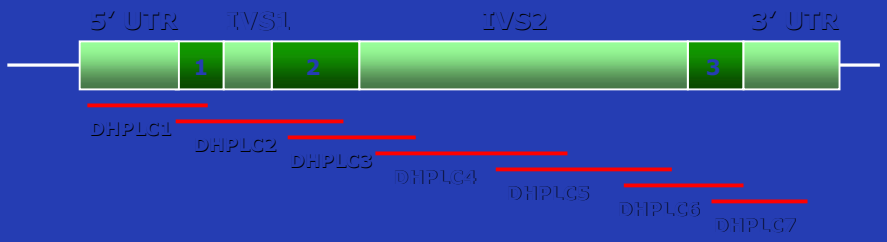
Multiplex of HFE Exon 2 Variants SBE of a Heterozygote at the H63D Locus



β-Thalassemia

- Chronic anemia
- Autosomal recessive disorder
- Carrier frequency is population-specific
- Due to a defective production of β-chain of hemoglobin A

DHPLC analysis of beta-globin gene: localization of DHPLC fragments



- Seven DHPLC fragments (DHPLC1→7)
- 300 →500bp long
- One set of PCR conditions

Human beta-globin gene project consumable kits Introduction

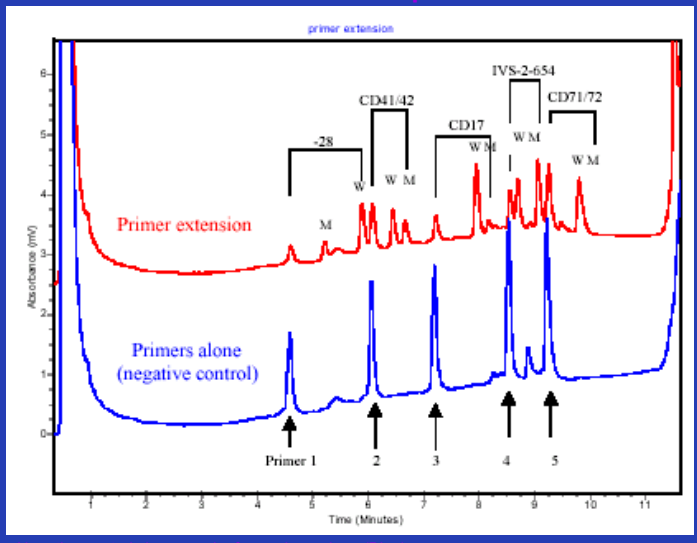
Primer extension coupled with dHPLC has been a powerful tool for genotyping. Based on this technique, we developed a consumable kit for clinical diagnosis of beta-Thalassemia disease in Chinese population. This kit genotypes five common disease causing mutations in human beta-globin gene: -28(A>G), CD17(A>T), CD41/42(-TCTT), CD71/72(+A) and IVS-2-654(C>T). The whole protocol involves four steps: one PCR reaction that amplifies all mutations to be studied; one quick column purification; one multiplex primer extension reaction, and one Transgenomic Wave DNA fragment analysis. By adding one more multiplex primer extension and a WAVE analysis, five additional mutations: -29(A>G), IVS-1-5(G>C), CD43(G>T), CD26(G>A) and CD27/28(+C) can be detected. This method is simple and easy to use. The results are very easy to read and the accuracy is equal or greater than direct sequencing.

β-globin mutations in the Chinese population

Mutations	S and SWPRC[1] (%)	Taiwan[2] (%)	Malaysia[3] (%)
28 (A→G)	19.1	10.8	11.1
17 (A→T)	19.1	10.8	2.8
41-42 (-TTCT)	44.1	28.3	52.8
71/72+A	2.1	0.0	2.8
IVS-II 654 (C→T)	7.9	45.9	19.5
Totals	92.3	95.8	89.0

[1] Br J Haematol 86:351,1994; [2] Am J Hum Genet 48:809,1991; [3] Haemoglobin 16;51,1992

Result and interpretation



Negative control shows peaks from primer alone (Blue).
For any given mutation in the primer extension (Red), there may be three possible results:

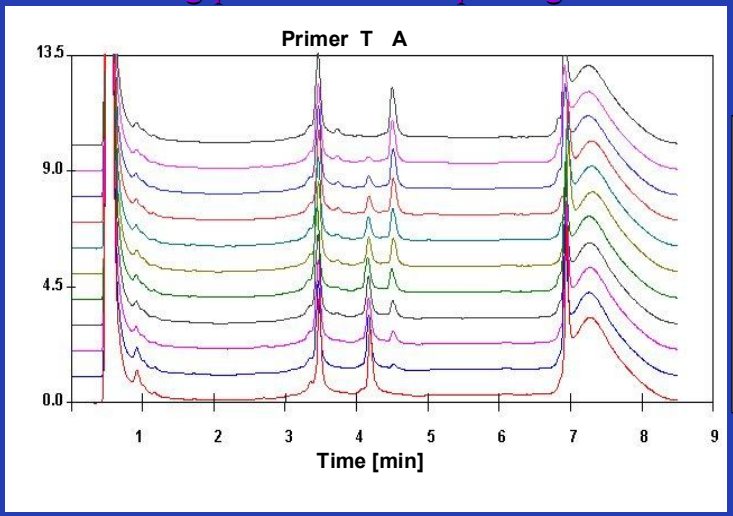
- Primer peak + wild type peak (W) indicate wild type for this site. (2 peaks)
- Primer peak + mutation peak (M) indicate homozygous mutation. (2 peaks)
- Primer peak + wild type peak + mutation peak indicate heterozygous mutation. (3 peaks)

Strategies for Increasing WAVE System Sensitivity and Throughput

- Pooling
- Multiplexing
- Fluorescence Detection

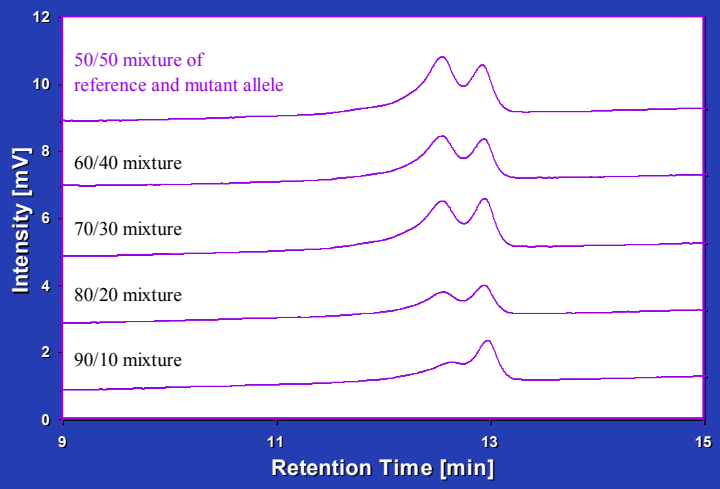
10X Pooling

Scoring-primer extension pooling for a mutation



T	A
0	10
1	9
2	8
3	7
4	6
5	5
6	4
7	3
8	2
9	1
10	0

Heteroduplex Analysis - Multiplexing (9 : 1)



DNA Fragment Sizing and Its Applications

Non-denaturing condition

Gel-like"size based separation 40-2,000 bp

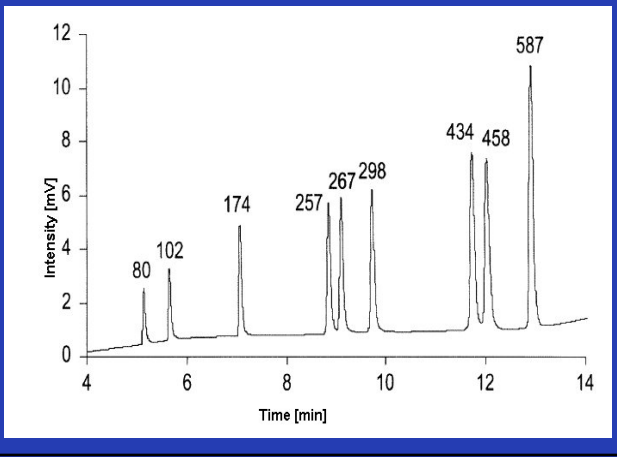
Resolution considerably better than most gel based systems (agarose and acrylamide)

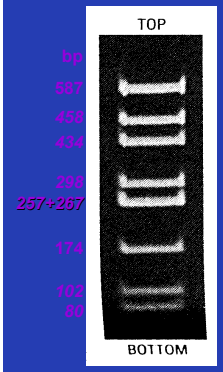
Quantitative RT-PCR (a difference from gels is exploited)

Oligonucleotide (analysis and purification

High-Resolution DNA Fragment Separations

Plasmid pUC18 was digested with the restriction endonuclease *Hae*III. Nine fragments were separated with the WAVE® System. On a 0.8% agarose gel, the 257 and 267 bp fragments co-migrated.





Characteristics of DNA Fragment Sizing

- Chromatographic separation is performed under non-denaturing conditions
- Gradient conditions for the sizing of unlabeled DNA fragments

  are predicted by WAVEMAKER™ Software
- Sizing of double-stranded DNA fragments from < 50 - about 2000 bp
- Sensitivity of UV detection: 0.5 ng/peak
- Sensitivity of fluorescence detection: low femtomole range
- Resolution: about 1% of fragment length
- Quantitation by peak integration
- Recovery of DNA fragments by peak capture

Fully Denaturing Condition

To demonstrate the separations of:

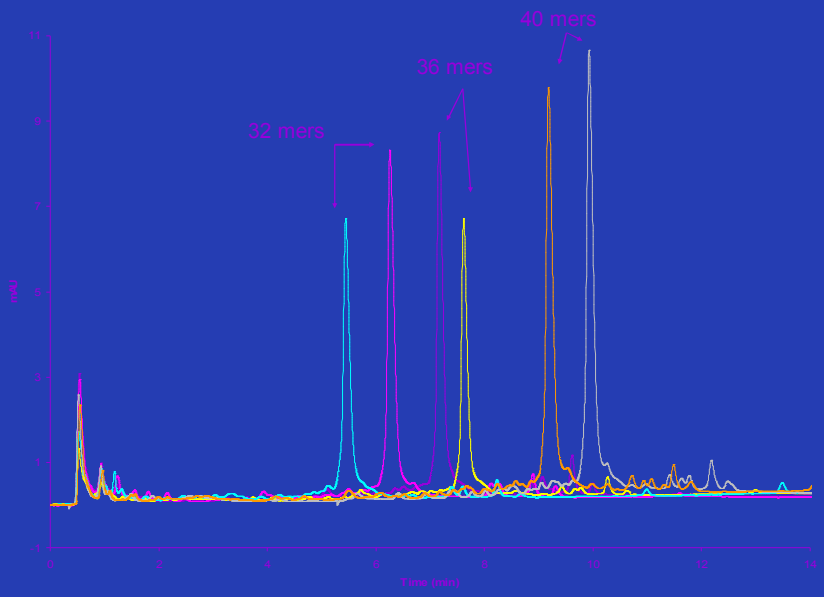
1. Two equal length oligos with one nucleotide difference (A/G)
2. NH2 labeled oligo from unlabeled oligo
3. Two NH2 labeled, equal length oligos with one nucleotide difference (A/G).
4. Cy5 fluorescent labeled oligo from unlabeled oligo
5. Two Cy5 labeled, equal length oligos with one nucleotide difference (A/G)

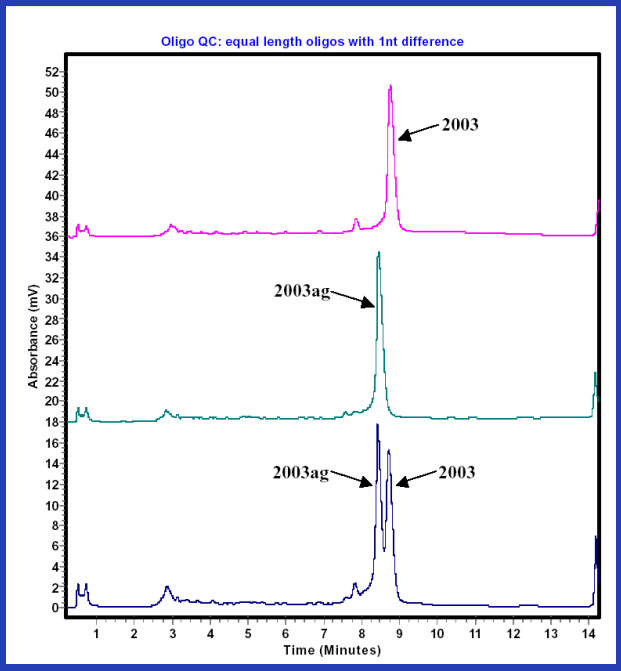
Background

Oligo sequences used for QC testing are as follows:

```
2002:      (NH2)- ttttttttttggcctgatgaggagtactgg   (30nt)

2002ag:    (NH2)- ttttttttttggcctgatgggagtactgg   (30nt)

2003:             ttttttttttggcctgatgaggagtactgg   (30nt)

2003ag:           ttttttttttggcctgatgggagtactgg   (30nt)


24nt-cy5:         cy5 - ggctctgactgtaccaccatccac   (24nt)
24nt:                   ggctctgactgtaccaccatccac   (24nt)
24nt-cy5-ag:      cy5 - ggctctggctgtaccaccatccac   (24nt)
```

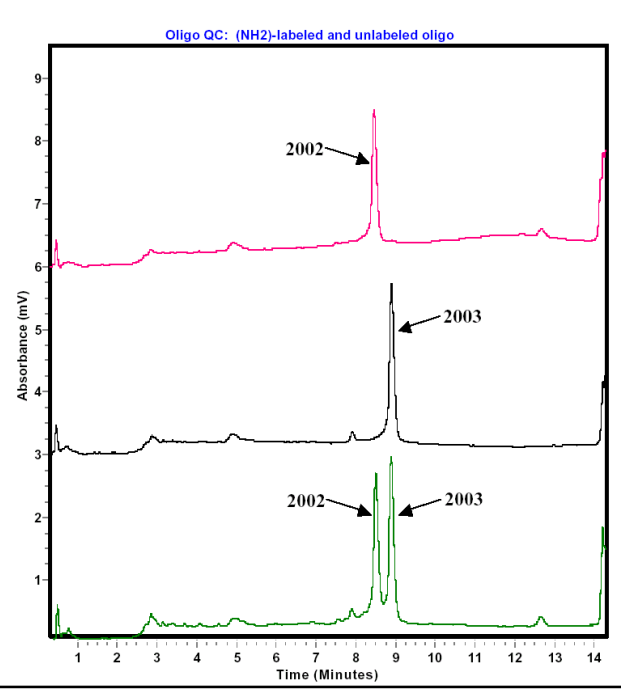
Analyze Oligonucleotides based on Sequence w/ TEAA





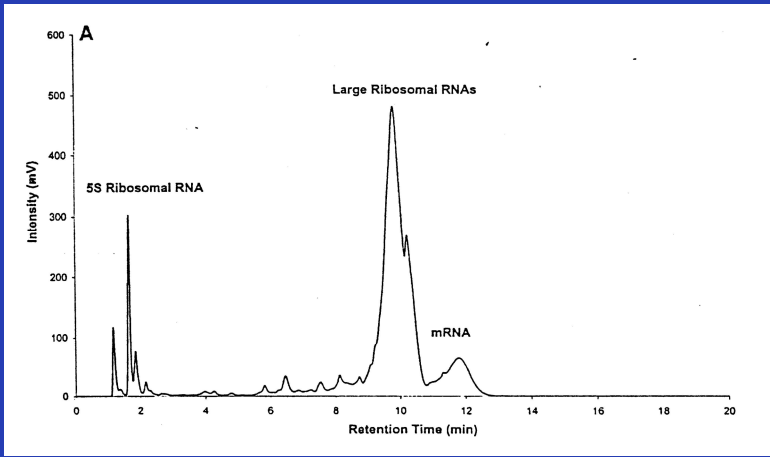
Wave Results

Analysis of equal length oligos with 1nt difference

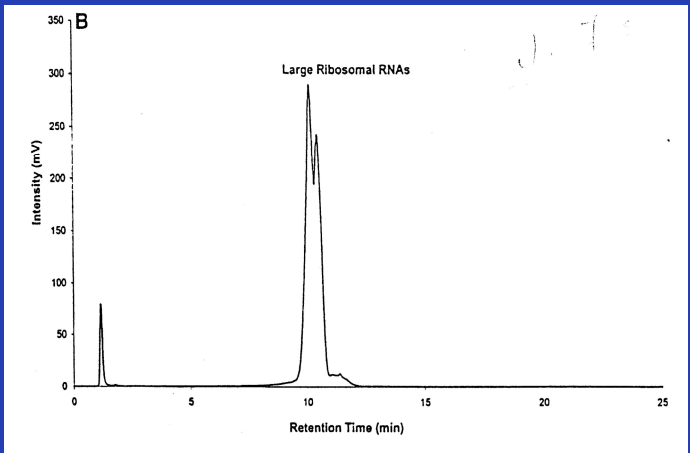


Analysis of (NH2)-labeled and unlabeled oligos

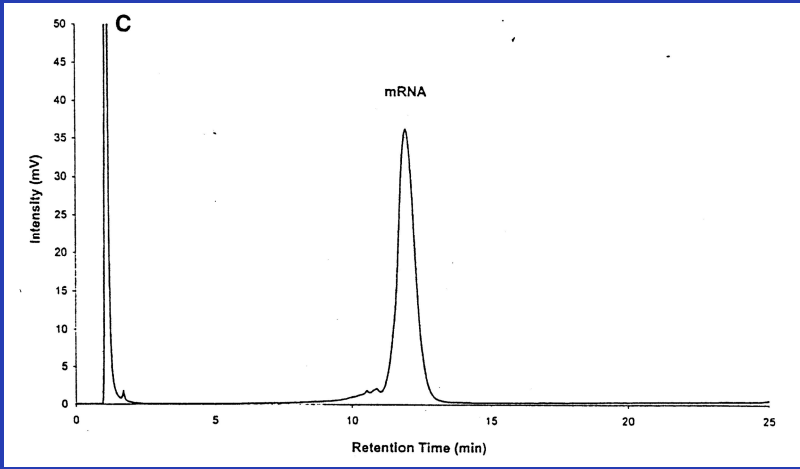
Mouse Brain Total RNA (20 μg)



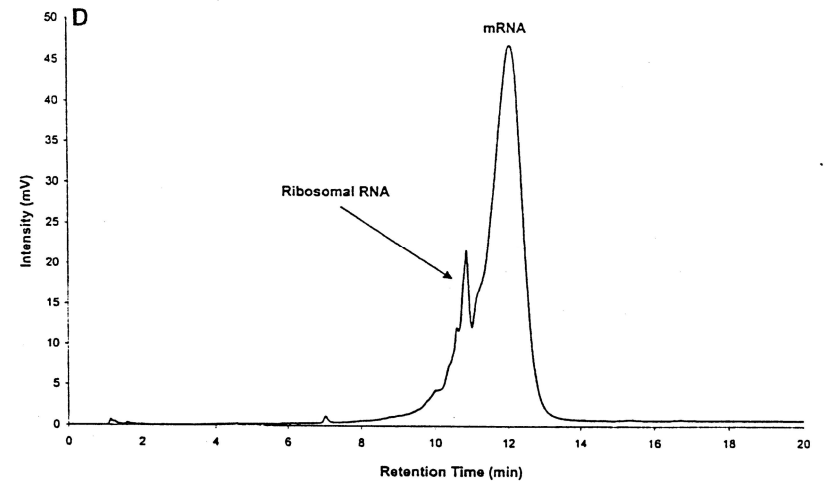
Analysis of large rRNAs



Analysis of mRNA purified by peak capture from total RNA



Analysis of mouse brain poly(A)*mRNA (5 μg) obtained after two rounds of oligo(dT)-cellulose purification

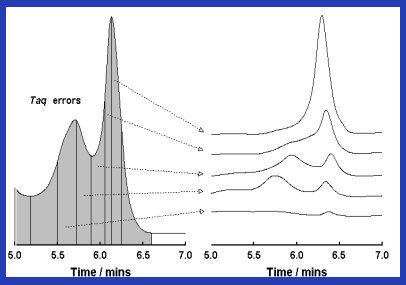


Transgenomic fragment collector for on-line purification of DNA

DNA fragment collection based on:

- peak threshold detection
- time window for a peak
- positive slope detection
- combinations of the above

True, homoduplex sequence was purified from a cocktail of PCR induced mutant species (the broad peak in front).



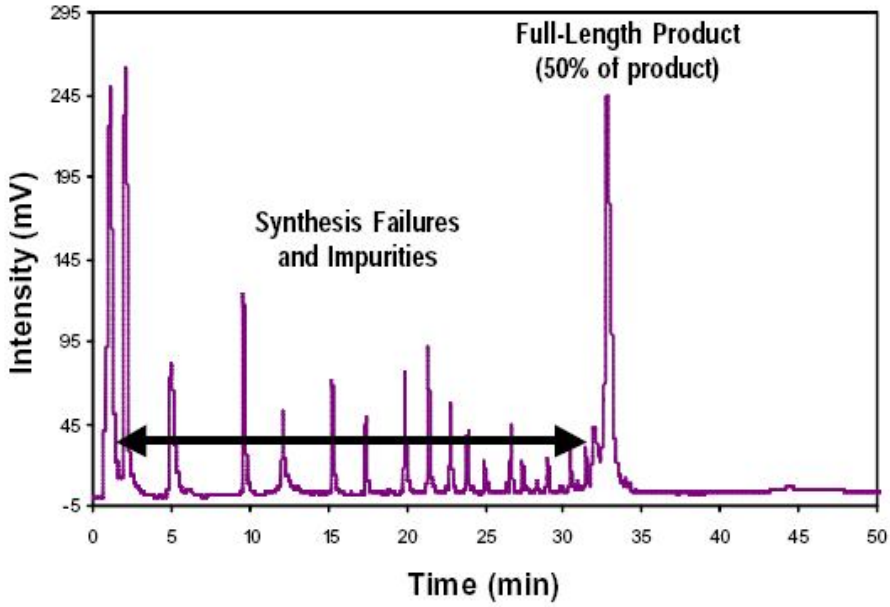


Figure 1. Ion exchange HPLC analysis of crude siRNA oligonucleotide prior to purification (siRNA oligonucleotide: Luciferase Antisense).

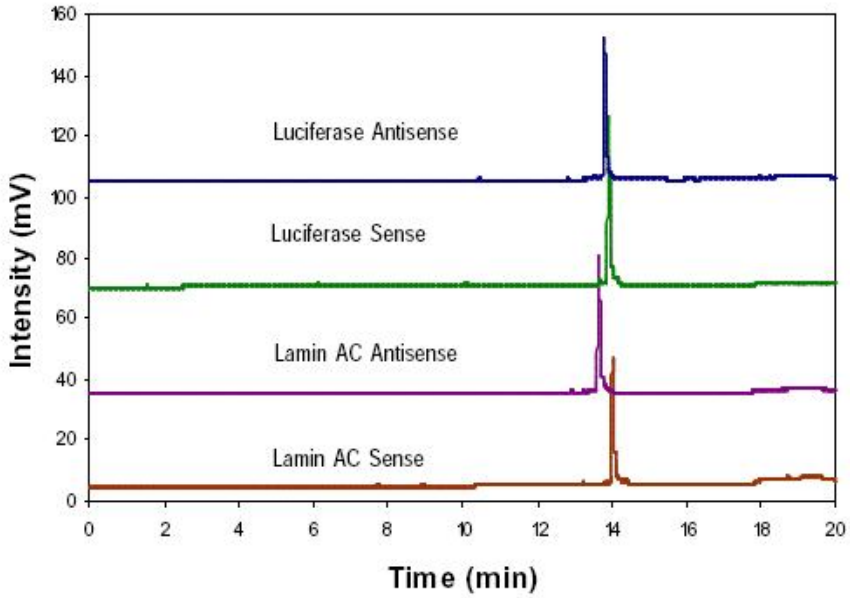


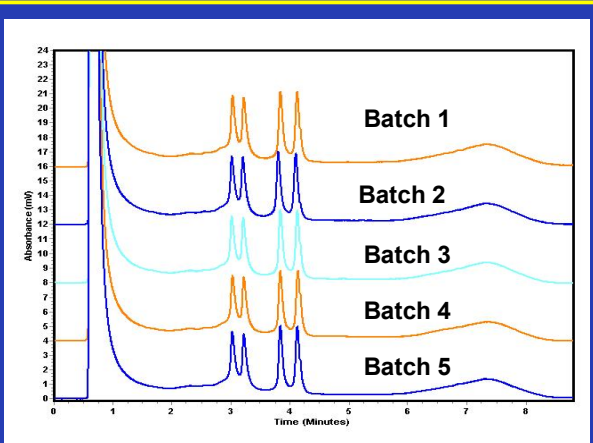
Figure 3. Analytical ion exchange HPLC analyses of siRNA oligonucleotides following purification on the WAVE Oligo System.

System Comparison

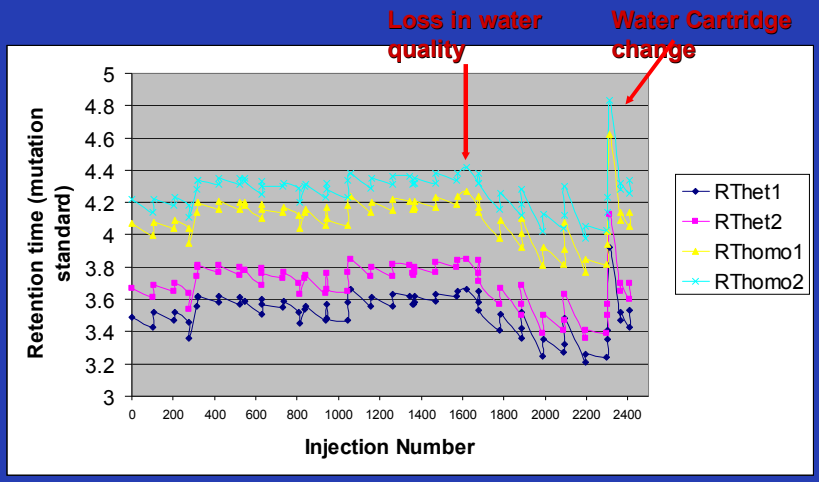
System Configuration	WAVE-MD	Model 3500	Model 3500A	Model 3500HT
WAVE® Autosampler	Model 7200 1 x 96 samples	Model 7250 2 x 96 Samples	Model 7250 2 x 96 Samples	Model 7250 2 x 96 Samples - Standard; 2 x 384 Samples - Optional
WAVE® Accelerator	No	Optional	Yes - Internal	Yes - Internal
Micro-volume Solvent Mixer	No	Optional	Optional	Yes
WAVE® running time/per injection	14min	12min	9min	4.5min

WAVE Optimized™ Buffers

WAVE Optimized Buffers Reproducibility



Use of Homemade Buffers Reproducibility Problems



Transgenomic Optimase™ Polymerase

Origins of Optimase™ Polymerase (cont)

- Phylogenetic analysis
  - closely related to *P. furiosus* (*Pfu*)
    - Stratagene
  - more distantly related to *T. litoralis* (Vent)
    - New England Biolabs.

Characteristics of Optimase polymerase

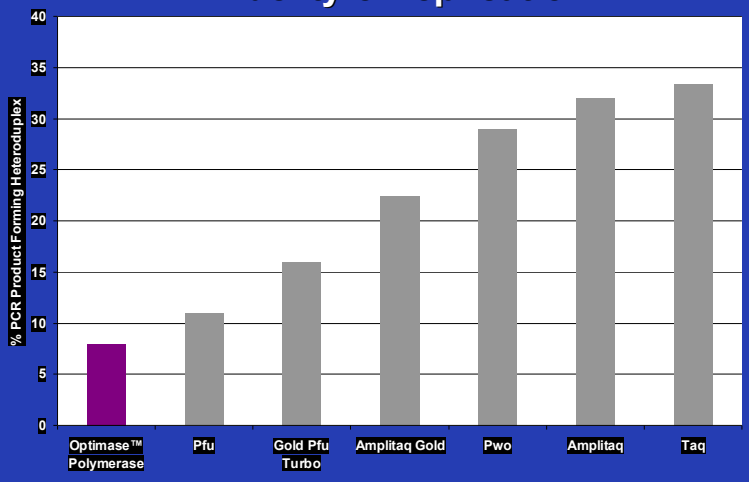
- Proofreading DNA Polymerase
  - 5' to 3' polymerase activity
  - 3' to 5' exonuclease activity
  - Highly temperature stable

Compatibility with the WAVE System

- Certain components of PCR buffers are incompatible with the DNASep Cartridge
- For example:
  - Bovine serum albumin (BSA)
  - Detergents (Triton X-100, NP40, Tween).

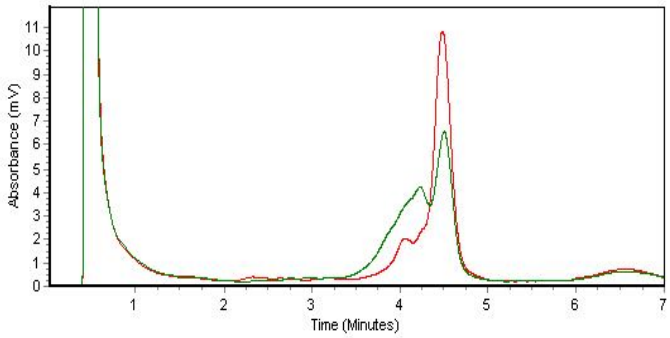
Characteristics of Optimase™ Polymerase

- Fidelity of replication



Characteristics of Optimase™ Polymerase

- Importance of high fidelity in dHPLC

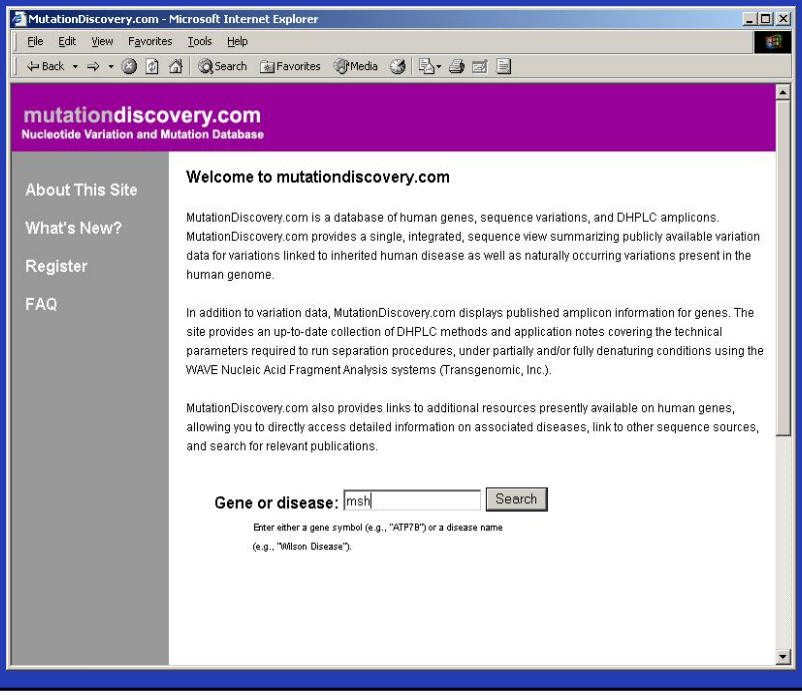


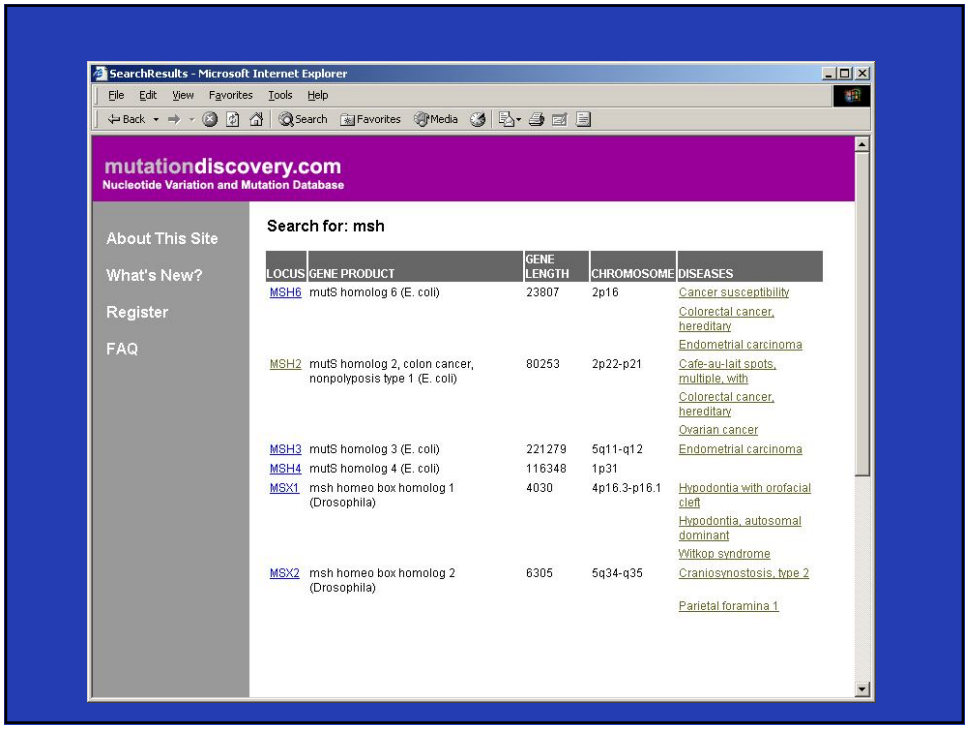
Mutation Discovery.com

Highlights

- Variation data on “all” genes, merged from multiple public sources
- DHPLC amplicon data from multiple sources
- Graphical web interface
- Private local data integration*
- Confidential data sharing*
- Integration with Navigator**
- Amplicon design support**

*August 2002; **TBD

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File Edit View Favorites Tools Help
Back Search Favorites Media
mutationdiscovery.com
Nucleotide Variation and Mutation Database
About This Site
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Register
FAQ
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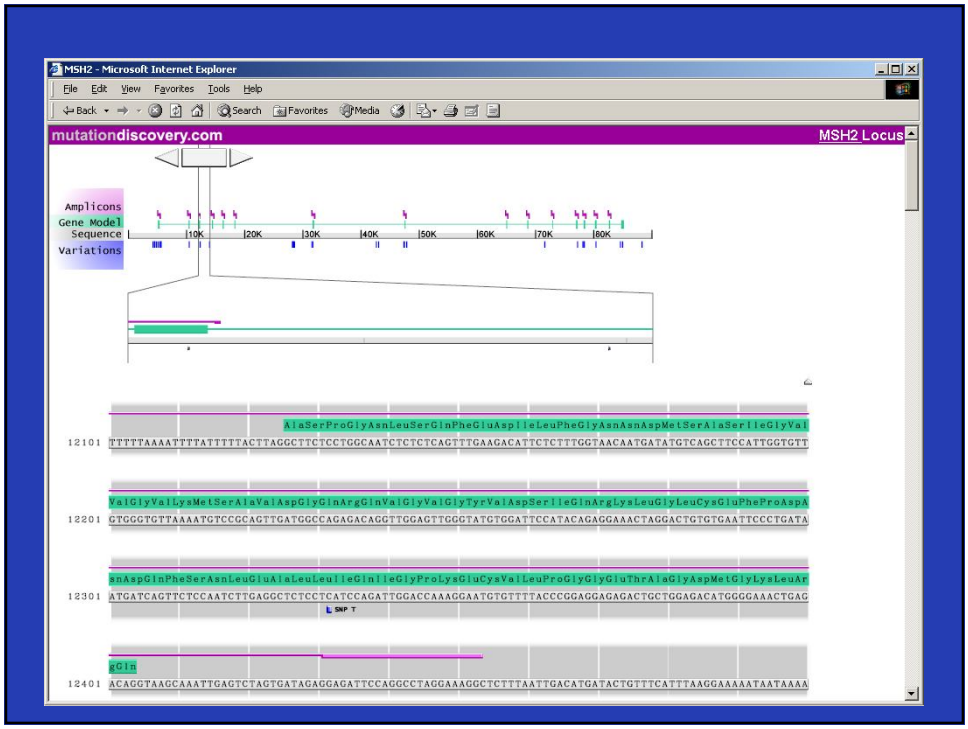
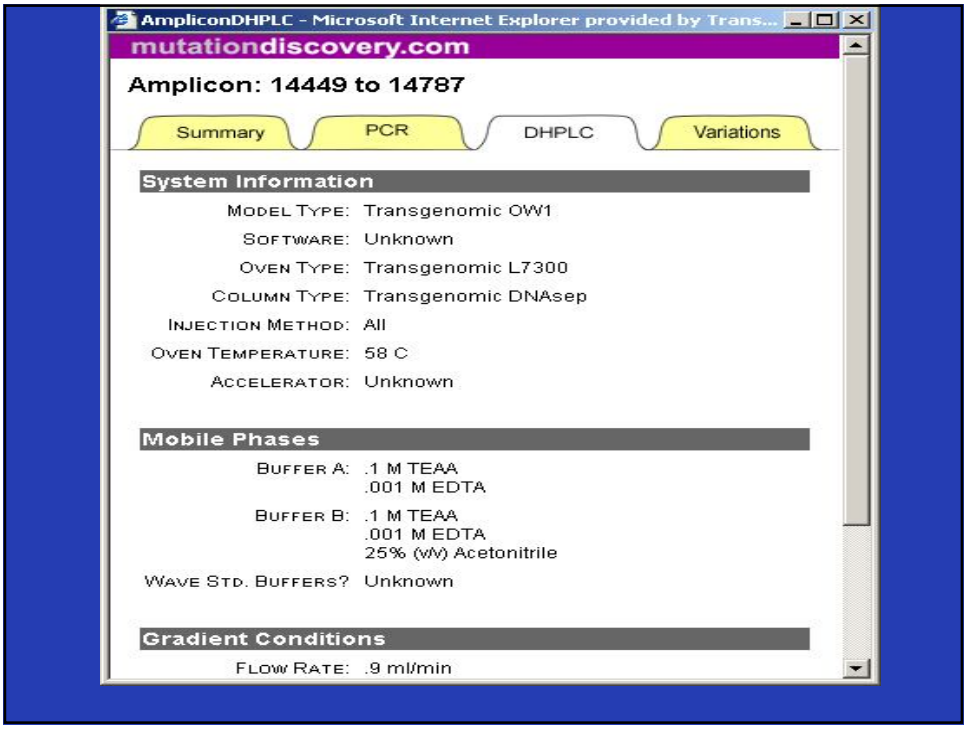
LOCUS GENE PRODUCT GENE LENGTH CHROMOSOME DISEASES
MSH6 mutS homolog 6 (E. coli) 23807 2p16 Cancer susceptibility
Colorectal cancer, hereditary
Endometrial carcinoma
MSH2 mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli) 80253 2p22-p21 Cafe-au-lait spots, multiple, with
Colorectal cancer, hereditary
Ovarian cancer
MSH3 mutS homolog 3 (E. coli) 221279 5q11-q12 Endometrial carcinoma
MSH4 mutS homolog 4 (E. coli) 116348 1p31
MSX1 msh homeo box homolog 1 (Drosophila) 4030 4p16.3-p16.1 Hypodontia with orofacial cleft
Hypodontia, autosomal dominant
Witkop syndrome
MSX2 msh homeo box homolog 2 (Drosophila) 6305 5q34-q35 Craniosynostosis, type 2
Parietal foramina 1


MSH2 - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Back Search Favorites Media
mutationdiscovery.com
MSH2 Locus
Amplicons
Gene Model
Sequence
Variations
10K 20K 30K 40K 50K 60K 70K 80K
AlaSerProGlyAsnLeuSerGlnPheGluAspIleLeuPheGlyAsnAsnAspMetSerAlaSerIleGlyVal
12101 TTTTTAAAATTTTATTTTTACTTAGGCTTCTCCTGGCAATCTCTCTCAGTTTGAAGACATTCTCTTTGGTAACAATGATATGTCAGCTTCCATTGGTGTT
ValGlyValLysMetSerAlaValAspGlyGlnArgGlnValGlyValGlyTyrValAspSerIleGlnArgLysLeuGlyLeuCysGluPheProAspA
12201 GTGGGTGTTAAAATGTCCGCAGTTGATGGCCAGAGACAGGTTGGAGTTGGGTATGTGGATTCCATACAGAGGAAACTAGGACTGTGTGAATTCCCTGATA
snAspGlnPheSerAsnLeuGluAlaLeuLeuIleGlnIleGlyProLysGluCysValLeuProGlyGlyGluThrAlaGlyAspMetGlyLysLeuAr
12301 ATGATCAGTTCTCCAATCTTGAGGCTCTCCTCATCCAGATTGGACCAAAGGAATGTGTTTTACCCGGAGGAGAGACTGCTGGAGACATGGGGAAACTGAG
SNP T
gGln
12401 ACAGGTAAGCAAATTGAGTCTAGTGATAGAGGAGATTCCAGGCCTAGGAAAGGCTCTTTAATTGACATGATACTGTTTCATTTAAGGAAAAATAATAAAA



DISCOVERY SERVICES

From the leaders in mutation detection technology…….

High-Throughput Mutation and SNP Detection

SNP Genotyping and Custom Sequencing

Bioinformatics and Statistical Genetics

Nucleic Acid Fractionation and Purification

Functional Proteomics

High-Throughput Sequence Variant Discovery
(Mutations and SNPs)

- World's largest high-throughput WAVE® DHPLC platform for mutation detection
- Capillary sequencing confirmation and identification of each variant
- Proprietary multiplexing methods improve speed of analysis
- > 99% mutation detection efficiency
- Most reliable, efficient, and cost effective approach to sequence variant discovery

Sequence Variant Discovery with the WAVE DHPLC Technology

- High Accuracy and High Sensivity
  - > 99% detection rate of unknown SNPs, no false positives
  - Detection of variants in < 2-5% of sample
  - DNASep DHPLC column chemistry
  - Precise DNA melting temperature calculations for DHPLC conditions
  - evelopment of a robust high fidelity thermostable DNA polymerase for PCR
- Transgenomic is a Leader in Mutation Detection Technology

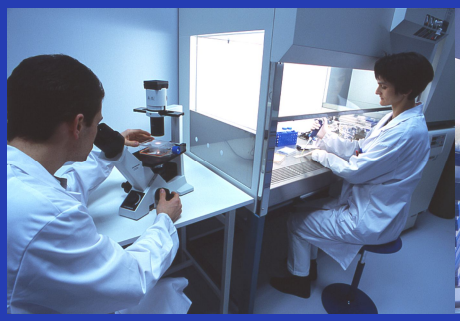
Novel and Non-detectable Human Signaling Protein Polymorphisms

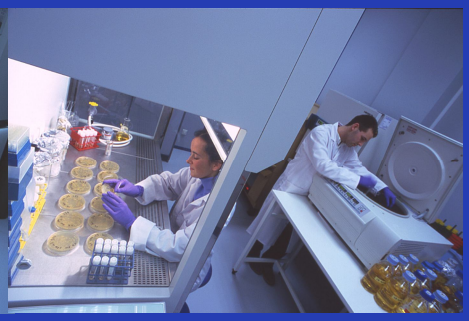
Roy A Lynch et. al., Division of Cardiology, University of Cincinnati Medical Center
Physiology Genomics, July, 2002

The frequency of single nucleotide polymorphisms in downstream signaling proteins was determined by combination heteroduplex high performance liquid chromatography and double stranded sequencing of genomic DNA from 96 to 144 congestive heart failure (CHF) patients. Analysis of fifty-six coding exons in nine signaling genes revealed seventeen novel and eight previously reported synonymous (no change in amino acid) SNPs, as well as one novel non-synonymous SNP in the Rad small G-protein. Because this initial analysis failed to detect numerous SNPs reported in the NCBI and Celera databases, double strand sequencing of relevant exons from 74 to 91 CHF patients was used to confirm the absence of ten previously reported non-synonymous SNPs. Our results show that synonymous SNPs are frequent in signaling protein genes, whereas non-synonymous SNPs are rare, suggesting a high degree of evolutionary conservation among these downstream signaling molecules.

Comparisons of our results to the NCBI and Celera databases indicates that 56% of their SNP entries are not detected in our cohort. Importantly, while 31% of database SNPs were verified, 69% of SNPs detected in our cohort are not included in these databases. These findings indicate that caution may be warranted in relying exclusively on SNP databases as catalogs for polymorphic signaling protein genes.

High throughput sequence variant discovery in candidate genes (the world's largest HTS-DHPLC platform, processing up to 6000 samples per day), using a proprietary multiplexing method.
Reliability and accuracy, along with substantial cost savings in comparison with traditional mutation/SNP discovery methods.





At Transgenomic, we believe that the identification of modifications generated by functional SNPs (mutations) in the structure (activity) of the disease-related gene products (proteins) will be a key factor for the design of new compounds correcting or enhancing the effects of those mutations in the population. Through our functional proteomic group, the client has access to the internal expertise in molecular and cellular biology including DNA recombination, protein expression systems, and protein purification necessary for this identification. In addition, appropriate cellular models and protein-protein interactions assays are developed in order to study the biological roles of functional SNPs on protein structure and activity.

多囊腎病現在可以做基因分析

台大醫院腎臟內科主治醫師暨陳萬裕教授基金會執行秘書

朱宗信 副教授

自體顯性多囊腎病（ADPKD）是一種常見的遺傳病，父母之一有病時，子女得病的機會爲50% ，估計此病的盛行率爲1/1000。目前國外研究顯示多囊腎病85% PKD1基因異常而15% 爲PKD2基因異常。國內目前接受透析（洗腎）病人已超過3萬人，其中約3% 爲多囊腎。

多囊腎病目前診斷以超音波爲主，因此無法早期發現此病。想要早期發現，則須寄望於基因分析。由於多囊腎病基因相當大，且每位病人基因突變點皆不同，因此基因診斷須分析整段基因。

在這類基因檢驗中，DNA序列分析乃是最基本且是最重要的，但昂貴之分析費用乃是最大之問題，所以如何發展出一種敏感度高，有效率且不昂貴之技術，來偵測序列中微小之變異，乃變得極爲關鍵。然而，由於多囊腎病基因之複雜性，使得基因診斷在過去變得相當困難。而目前台大醫院新進發展出之DNA突變分析儀（DHPLC）乃利用自動化之偵測來找出微小甚至是一核?酸之突變，將其合併直接序列分析，將是快速建立多囊腎病基因診斷突變基因資料庫之最有效率之利器。

此項檢驗，由於需要高科技設備及耗材，因此所需經費較高，但目前台大醫院已獲經費支持，預估可爲200位病人免費檢驗，預期在1至2年內可獲得成果。

對象：自體顯性多囊腎病的患者。

收件收集及檢查期間：即日起至92年6月30日。

報名：

病患掛號台大醫院（1）腎臟內科朱宗信醫師（星期二早上內科第2診，星期二下午腎臟內科第8診）（2）腎臟內科黃政文醫師（星期一早上內科24診）。
前述掛號可經由現場掛號、電話語音預約專線（02）23567890、本院初診電話預約專線（02）23562996、網路預約。

作法：

病患就診後確定爲多囊腎病且同意接受檢驗後於同意書上簽名並至指定地點抽血10C.C.即可，且不需禁食。

DNA突變分析儀 精準揪出乳癌變異基因

建立本土乳癌流行病學資料 台大受理民眾自費篩檢

【記者楊惠君／報導】乳癌篩檢可望提前至發病前，台大利用DNA突變分析儀(DHPLC)，快速、準確篩檢乳癌變異基因，至今已建立十一個乳癌家族資料，並找到兩個本土特異基因突變位置；即日起，台大開放一般民眾接受篩檢，每人須自費二萬元，蒐集更完整、廣泛的本土乳癌流行病學資料。

據統計，5%到10%的乳癌來自家族遺傳，是遺傳傾向最顯著的癌症之一；國外研究發現，BRCA1、BRCA2基因突變與乳癌有高度相關，帶因女性終身罹患乳癌的機率高達80%，發病年齡較一般女性提早十年，也有較高的機率罹患卵巢癌，子女同樣帶有此一突變基因機率高達50%。

包括美國、德國、英國等歐美國家，已將BRCA1、BRCA2基因突變點檢測，列爲篩檢乳癌高危險群的常規檢查。台大醫院基因部醫師蘇怡寧指出，BRCA1、BRCA2爲一種抑癌基因，一旦發生變異，即可能失去抑制不正常癌細胞增生的能力導致乳癌；DNA突變分析儀即利用自動化偵測找出微小或單一核苷酸，準確率在95%以上，爲大規模篩檢乳癌變異基因的有力工具。

國外已找到三百多個乳癌變異基因的突變點，台大利用此一儀器，也從十一個乳癌家族、十七個病人中，找到兩個本土特有的基因變異點。蘇怡寧表示，乳癌BRCA1、BRCA2基因突變爲顯體顯示遺傳，一旦此基因發生突變，家族子代中有五成機率帶因，罹患卵巢癌、子宮內膜癌的比率也相對提高。台大的個案中，曾發現一個家族、橫跨兩代，五人先後罹患乳癌的例子。

變異基因篩檢也可做爲乳癌治療的參考。台大一般外科醫師黃俊升說，保存乳房爲乳癌治療趨勢，但若爲基因變異型的乳癌患者，復發率較高，較不適合保留乳房；而基因篩檢正常者，也可放心保存乳房。

根據歐美國家統計，BRCA1、BRCA2帶因率約0.5%，台灣則無本土資料，醫師建議，若家族成員中有人罹患乳癌及卵巢癌、或家族中二個以上家族成員在五十歲前罹患乳癌、或家族中有男性罹患乳癌等高危險群，可提早接受乳癌BRCA1、BRCA2的基因檢驗。黃俊升表示，一旦發現帶有乳癌變異基因，應密切接受乳房X光和超音波篩檢，國外研究甚至發現，提早切除乳房或卵巢，可有效預防乳癌發生。

針對乳癌篩檢成本分析比較

(BRAC1 and BRAC2 gene 共有 24+27 exons 分成 84 個 amplicons 作檢測也就是 84 個 PCR products)

DHPLC(資料庫建立期)	DHPLC(已建立資料庫)
1. DHPLC 篩檢 BRAC1 和 BRCA2 gene 須 122 次 injections (因爲有些 PCR 產物須不同溫度作檢測) 2. 以 2100A 須 122x39.5=4812 元 3. 以 3500HT 須 122x43.5=5307 元 4. 若由 DHPLC 檢測出突變型須定序樣品預估爲 10-20%須定序成本爲 2400-4800 元 5. DHPLC 檢測出有突變型的圖形必先經過定序確認後若以後有相同圖形出現可歸類成相同突變所以若作相同檢測越多成本會下降	1. DHPLC 檢測出確認有相同突變型的圖形出現可歸類成相同突變所以若作相同項目檢測越多定序成本會下降 2. 84 個 PCR products 若採直接定序須 Forward and Reverse 定序也就是須由 PCR product 兩端各作一次定序,須作 84 x 2 = 168 次定序

DHPLC 和 Multi-TOF 功能性比較

	DHPLC	Multi-TOF
DNA 分析工作	1. **Non-Denature Fragment size,** LOH, STR, AFLP, RFLP, Qualitative PCR Competitive RT-PCR DNA 純化 2. **Partially Denaturing** SNP discovery And Scoring Mutation detection 3. **Fully-Denaturing Oligonucleotides,** ssDNA, RNA 分離可作 Oligonucleotide, n, n-1 mer 分離, 收集, 純化.	只能針對已知 DNA 序列變異作大量篩選

DHPLC 和 Multi-TOF 功能性比較

	DHPLC	Multi-TOF
SNP/Mutation Discovery 針對基因中未知位置序列變異探討	可利用 Hetroduplex analysis 的方式再加上溫度調控找出 Novel 突變基因	只能針對 Candidate gene 利用 Primer-extension reaction 作爲篩選工具
SNP Screening 針對已知位置序列變異探討	可利用 Hetroduplex analysis 或 Primer-extension reaction 作爲篩選工具一天可處理 280 個樣品	每天可處理上萬樣品
分析樣品前處理	直接利用 PCR 產物進樣分析	樣品純度要求較高需要經過 desalt 步驟
購置成本	380-450 萬元	須達上千萬

日期： 2003年12月7日至12月10日

地點： 陽明大學 會議中心

活動主題：

Genome 2003

	額滿人數	Title	地點
*Symposium	(600人)	Genome and Human Disease	大禮堂
*Minisymposium I	(200人)	Mini Symposium : SARS Genomics	表演廳
*Minisymposium II	(200人)	Genetic Polymorphism & Human Disease	表演廳
*Minisymposium III	(150人)	Functional Genomic Approach to Liver Diseases	第一會議室
*Workshop I	(200人)	Lecture：Integrated Platform for Functional Genomic Studies	表演廳
*Workshop II Lecture：12/9 實驗：12/10上午	(100人)	Lecture：dHPLC：Principle and Practice	第一會議室
	(10人) 需收費	Experimentation：dHPLC：Principle and Practice	

12/9 (二)	Lecture部份	Chairperson	鍾明怡 / 台北榮總
09:15~09:30	History of DHPLC Collin D'Silva (CEO of Transgenomic)		
09:30~10:30	Advances of Genetic Diagnostics taiwan Zhu Ph.D / Transgenomic		
10:40~12:00	Use of DHPLC in the diagnosis and pharmacogenomics of neurological diseases	陳彪 北京首都醫科大學 中國國家人類基因組北方研究中心	
13:30~14:30	DHPLC Application in Cancer Molecular Epidemiology 鍾翔翎 / 長庚大學公衛系		
14:30~15:30	蘇怡寧 / 台大基因醫學部		
16:00~17:00	Molecular genetic study of NMDA receptor subtype genes and schizophrenia		陳嘉祥 / 榮總大學 人類遺傳學研究所
12/10 (三)	實驗部份		
09:00~09:45	Result and discussion 1		朱萬庚
09:45~12:00	Result and discussion 2		鍾明怡 / 台北榮總

客戶名稱	科系	機型	目前研究基因	備註
		WAVE system	(DHPLC system From Transgenomic USA)	
台大醫院	第四共研	2100A	精神疾病基因...	
國家衛生研究院		3500A	Comfirm sequencing data	
長庚大學	公衛系509室	2100A	大腸直腸癌(MLH1, MSH2),乳癌基因(BRCA I, BRCA II),肝臟代謝基因	
國立陽明大學	基因體研究中心	2100A	和婦產部,陽明公衛合作	
花蓮慈濟大學	人類遺傳所	2100A	Schizophrenia Gene	
百力生物科技股			Oligo purity check and purification	
		2100A		
省立板橋醫院	檢驗科	3500A	乙型地中海貧血(B-globin Gene)	91.12.31
花蓮慈濟醫院	神經醫學	3500HT	乳癌基因(BRCA I, BRCA II),大腸直腸癌(MLH1, MSH2)	92.03.31
			Pakinson Disease	
長庚大學	醫技系	3500A	Bacteria identification	92.05.01
三軍總醫院	基因體研究中心	3500A	細胞凋亡基因FAS gene	92.06.30
台大醫院	基因醫學部	3500A	大腸直腸癌(MLH1, MSH2),腎臟癌(AKPKD gene),	
		2100A	乳癌基因(BRCA I, BRCA II),神經纖維瘤第一型(NF1)	
			乙型地中海貧血(B-globin Gene)	
		2100A		92.10.0