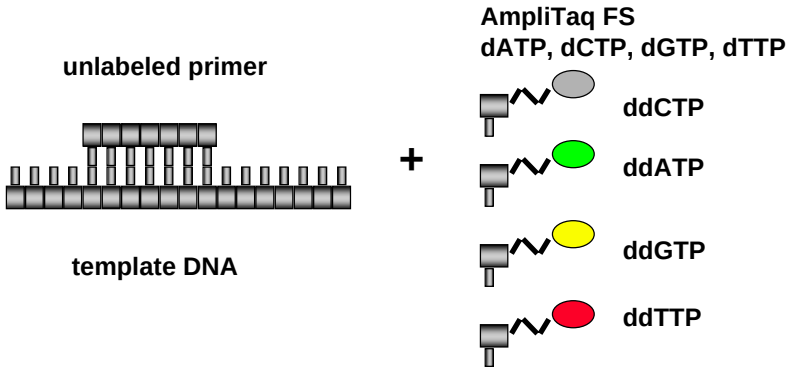
全自動毛細管DNA遺傳分析儀 - ABI PRISM 3100 的原理及應用

美商應用生命系統(股)公司
Field Application Specialist
徐英誠

Terminator Sequencing Sample Prep

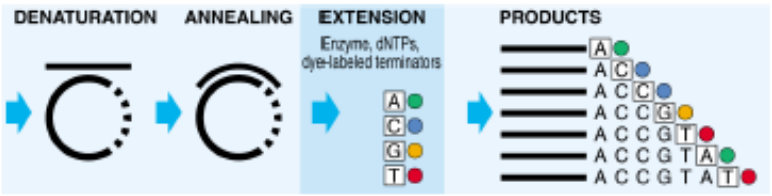
1 reaction within which each terminator is labeled with a different dye



Sample is purified by ethanol precipitation to remove unincorporated ddNTP's before loading

Sanger Dideoxy Cycle Sequencing

Performed in a thermocycler with a thermostable Taq polymerase



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Dye Terminator Cycle Sequencing Step

(1)Template +Primer +Sequencing reagents (with dye terminator)

(2) Cycle Sequencing

Annealing

Extension

Denaturation

(3) Ethanol + NaOAc precipitation

(4) denaturation

(5)loading

Advantages of Capillary Electrophoresis

- More efficient heat dissipation than slab gels
  - higher run voltages
  - faster run times
  - higher sample throughput
- Automation
  - elimination of manual operations
  - increased run to run consistency and reliability
- Sensitivity
  - less DNA per sample
  - but more strict sample requirement(ex: aware of salt contamination of the seq. product……, etc)

ABI PRISM® 3100 Overview

- **16 Capillaries**
- **Automated polymer filling, sample injection, detection & analysis**
- **Supports Sequencing & Fragment Analysis**
- **Supports capillary lengths 36 cm and 50 cm**
  - **New 22 cm for SNP genotyping and 80 cm for long sequence application (up to 1000 bp)**
- **Active temperature control**
  - Capable of subambient cooling (18℃ to 65 ℃)
- **Autosampler supports 96 & 384**
- **Up to 24 hours unattended operation**
- **PC running Windows XP**

Process of Typical Run

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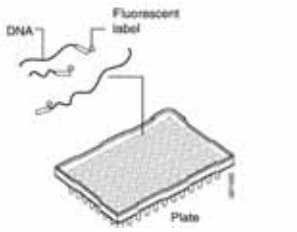
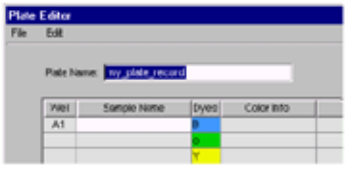
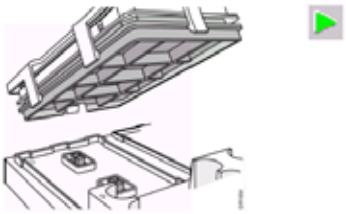
1. Sample Preparation



2. Software Setup



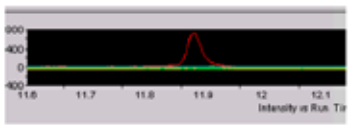
3. Beginning the Run



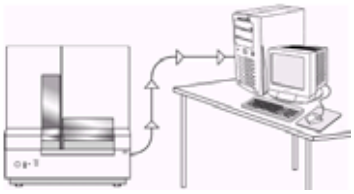
4. Electrophoresis

5. Excitation and Detection

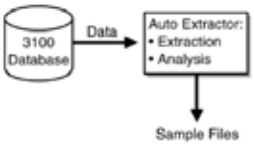
7. Data Processing: during running



6. Data Collection



8. Automatic Data Extraction and Data Analysis



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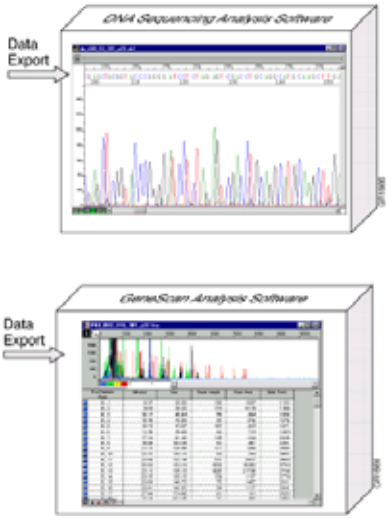
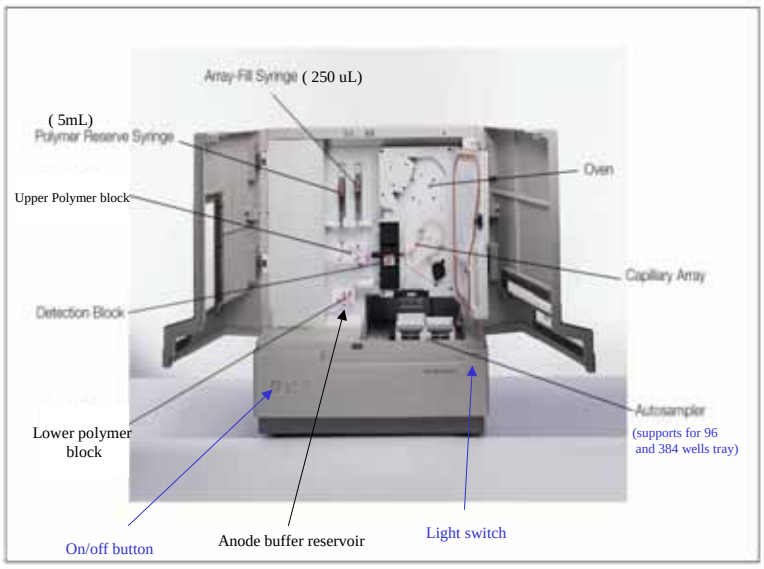
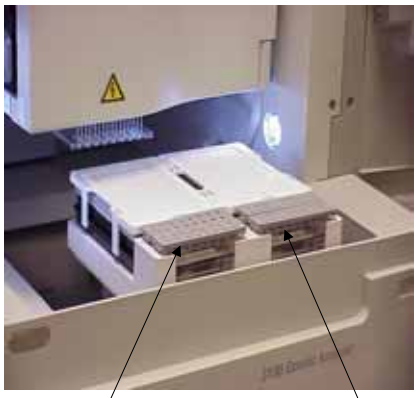
9. Viewing the Data: on the GeneMapper software or Sequencing Analysis software



ABI PRISM® 3100 : Doors Open



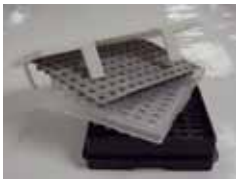
Autosampler Close-up



Site 1: 1x GA buffer Site2,3,4: H_2O

- Supports up to two 96- and or 384-well micrometer plates
- Automated sample injection
- Autosampler will stay up if power is lost

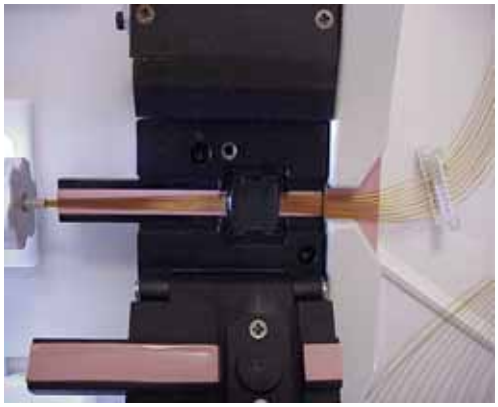
Water reservoir rinse	Water reservoir waste
Cathode reservoir 1x running buffer	Water reservoir



Instrument Open- Oven and Array

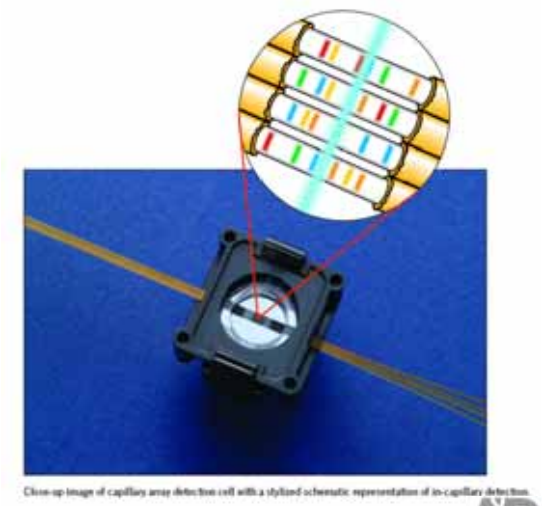
- Peltier heat pump used to heat or cool oven
  * can cool down quickly
  * facilitates subambient cooling
- Force air oven with two internal fans to provide superior thermal control and uniformity
- Array combs that clip into oven to provide reproducible array spatial alignment
- No more tape!!

3130 System Optics



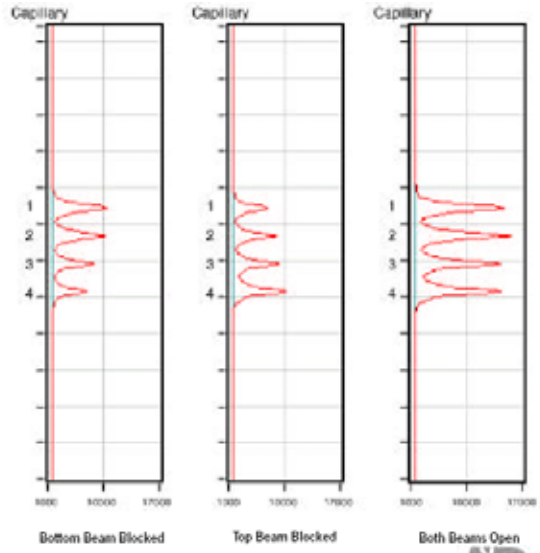
- **Laser source:**
  - *** 25 mW single argon ion laser**
  - *** running at 15 mW**
  - *** simultaneous dual side illumination**
- **Detection:**
  - *** In - capillary detection**
  - *** two- dimensional (spectral & spatial dimensions) data collected by the CCD camera**

Schematic of “In-capillary” Detection



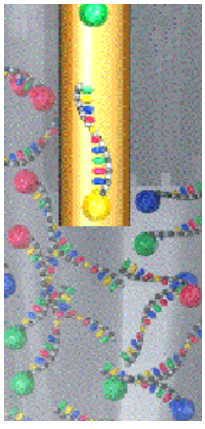
Close-up image of capillary array detection cell with a stylized schematic representation of in-capillary detection.

Dual Side Illumination



Electrokinetic Injection

- Capillary and electrode are placed into the sample
- Voltage is applied
- "-" charged DNA enters the capillary as it migrates toward the "+" electrode at the other end of the capillary



Because of the nature of electrokinetic injection, sample preparation is critical to the data quality.
Ex: salt contamination, template/primer ratio in seq. reaction

Comparison between Sequencing and Fragment Analysis Consumables

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Application	Polymer	Capillary Length	Run Time	Throughput (24 hrs)		Resolution	Performance
				3100-*Avant*	3100		
High throughput, small size fragment analysis	POP-4™	22 cm	20 min	5,760* GT	23,040* GT	400 bp	0.50 SD†
Standard fragment analysis		36 cm	45 min	2,560* GT	10,240* GT	400 bp	0.15 SD
Ultra rapid sequencing			40 min	72,000 bp	288,000 bp	500 bp	98.5% base calling accuracy
Rapid sequencing	POP-6™		60 min	48,000 bp	192,000 bp	500 bp	98.5% base calling accuracy
Standard sequencing	POP-4™	50 cm	100 min	34,500 bp	138,000 bp	600 bp	Q20 (KB™ Basecaller)
Long fragment analysis			65 min	1,760* GT	7,040* GT	500 bp	0.15 SD
Standard sequencing	POP-6™		2.5 hrs	23,400 bp	93,600 bp	650 bp	98.5% base calling accuracy
Long fragment analysis			90 min	1,200* GT	4,800* GT	500 bp	0.15 SD
Long read sequencing	POP-4™	80 cm	3.5 hrs	22,800 bp	91,200 bp	950 bp	98.5% base calling accuracy

兩大主要應用：

DNA 定序
(Sequencing)

ATGC

基因體計畫
核心實驗室
(研究機構、大學、醫院)
代客定序公司
生物醫學、農業、小型研究

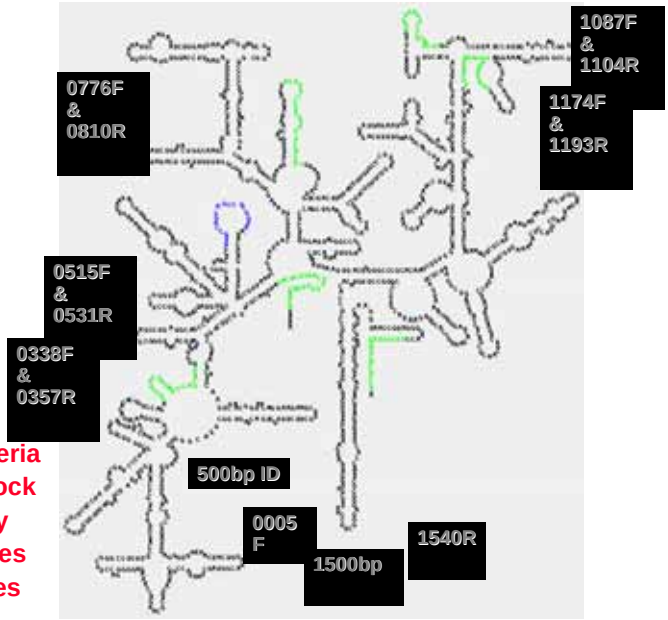
基因體計畫
核心實驗室
Gene Hunting
親子鑑定服務(檢調單位)
SSCP
AFLP等

Genotyping
(GeneScan)

Sequencing Applications 定序之應用

- De Novo Sequencing
- MicroSeq 16 S 微生物菌種鑑定
- HIV Genotyping system
- Sequencing Based HLA typing
- Mitochondrial Sequencing
- Methylation Study

16S rRNA Structure & Primer Locations

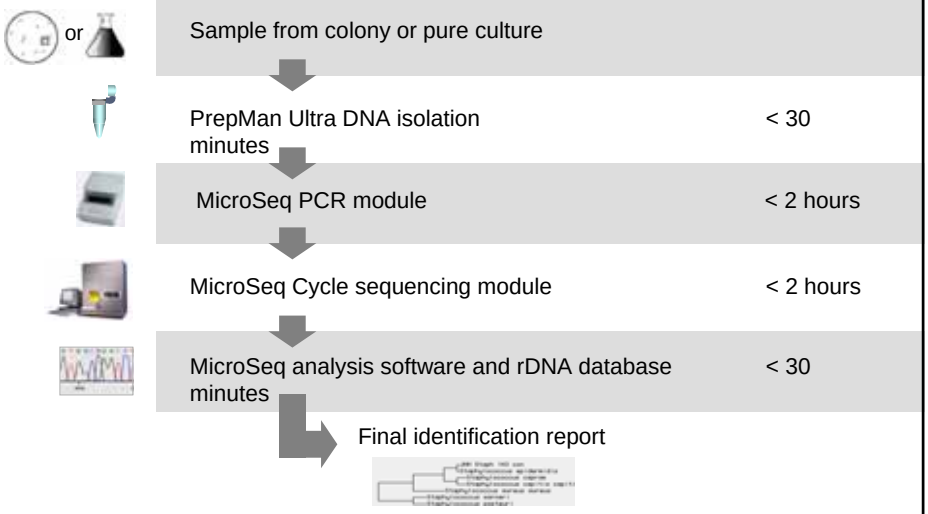


- **Present in all bacteria**
- **Slow molecular clock**
- **Used for taxonomy**
- **Publish new species**
- **In public data bases**

MicroSeq Libraries

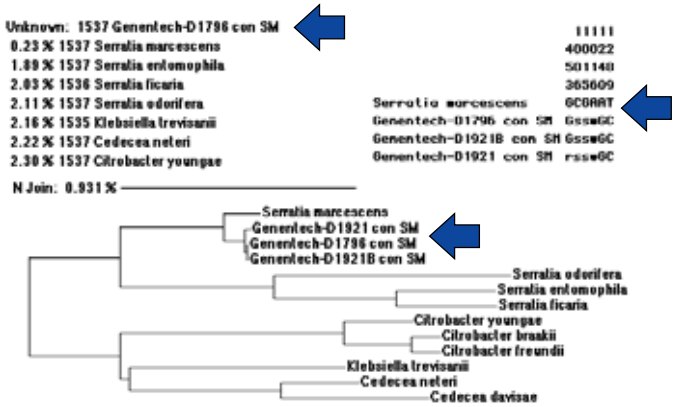
- 16S Bacteria Library Eubacteria Brand
  - Pharmaceutical / Sterility Control / Human pathogens
    - Over 1,100 entries, over 1,000 type strains.
    - Near complete Bacillus, Staphylococcus & Coryneforms (40 Corynebacteria)
    - Very strong in Gram negative nonfermenters.
    - Opportunistic pathogens, and 450-500 Aerobes.
    - Add Mycoplasmas, Ureaplasmas
- 28S Fungi Library
  - 500 Yeasts.
  - 1,000 Filamentous Fungi.
- Typing Kits
  - Methicillin Resistant Staph. aureus (MRSA) Library/ Protein A gene used for typing

MicroSeq® Workflow



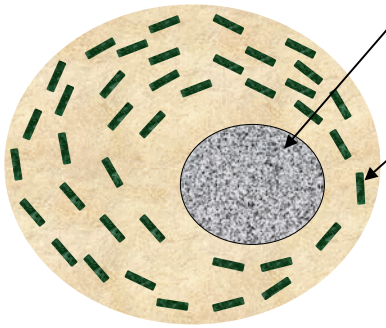
Pharma Sterility Control

Genentech Unknowns D1796, D1921 & D1921B = *Serratia marcescens*



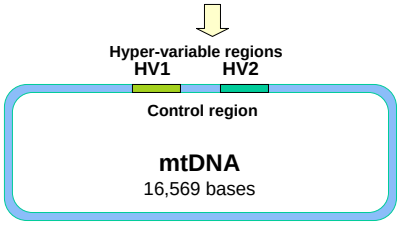
Applied Biosystems

mitoSEQr™ Resequencing System



Nuclear DNA
- 2 copies/cell
- Unique to sample
- Bi-parental inheritance
- Recombination

Mitochondrial DNA
- 100 - >1000 copies/cell
- May not be unique to sample (heteroplasmy)
- Predominantly maternal inheritance
- No recombina
- High Mutation Rate: 10-20x nuclear DNA
- Highly polymorphic in Control Region



Mitochondrial DNA Sequencing is used for

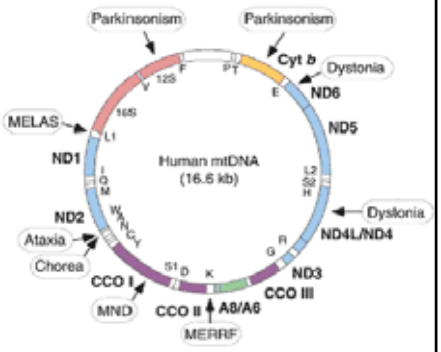
- Human Evolution & Origins
- Human mtDNA Disease Identification
- SNP and Mutation Detection
- Senescence
- Matching mtDNA sequence with maternal relatives – (even many generations apart)

Mitochondrial DNA SNP Detection

- One of the major applications is for cancer detection
  - mtDNA mutation occurs in large number and high frequency in cancers
  - Mutation not restricted by cancer type (Hum Mutat. 2006 May 2;27(6):575-582)
  - High copy number
  - Used for early cancer detection because mtDNA mutation occurs early in tumor progression (J Mol Diagn. 2005 May;7(2):258-67. Mitochondrial DNA as a Cancer Biomarker)
- Studies on other mitochondrial diseases

Mitochondrial Diseases

AD Alzheimer's Disease
ADPD Alzheimer's Disease and Parkinsons's Disease
AMDF Ataxia, Myoclonus and Deafness
CIPO Chronic Intestinal Pseudoobstruction with myopathy and Ophthalmoplegia
CPEO Chronic Progressive External Ophthalmoplegia
DEAF Maternally inherited DEAFness or aminoglycoside-induced DEAFness
DEMCHO Dementia and Chorea
DMDF Diabetes Mellitus & DeaFness
Exercise Intolerance
ESOC Epilepsy, Strokes, Optic atrophy, & Cognitive decline
FBSN Familial Bilateral Striatal Necrosis
FICP Fatal Infantile Cardiomyopathy Plus, a MELAS-associated cardiomyopathy
GER Gastrointestinal Reflux
KSS Kearns Sayre Syndrome
LDYT Leber's hereditary optic neuropathy and DYsTonia
LHON Leber Hereditary Optic Neuropathy
LIMM Lethal Infantile Mitochondrial Myopathy
MDM Myopathy and Diabetes Mellitus
MELAS Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes
MEPR Myoclonic Epilepsy and Psychomotor Regression
MERME MERRF/MELAS overlap disease
MERRF Myoclonic Epilepsy and Ragged Red Muscle Fibers
MHCM Maternally Inherited Hypertrophic CardioMyopathy
MICM Maternally Inherited Cardiomyopathy
MILS Maternally Inherited Leigh Syndrome
Mitochondrial Encephalocardiomyopathy
Mitochondrial Encephalomyopathy
MM Mitochondrial Myopathy
MMC Maternal Myopathy and Cardiomyopathy
Multisystem Mitochondrial Disorder (myopathy, encephalopathy, blindness, hearing loss, peripheral neuropathy)
NARP Neurogenic muscle weakness, Ataxia, and Retinitis Pigmentosa; alternate phenotype at this locus is reported as Leigh Disease
NIDDM Non-Insulin Dependent Diabetes Mellitus
PEM Progressive Encephalopathy
PME Progressive Myoclonus Epilepsy
RTT Rett Syndrome
SIDS Sudden Infant Death Syndrome
SNHL Sensorineural Hearing Loss
Varied Familial Presentation



Methylation Study

Epigenetic alterations of gene function are now known to contr
of cancer. Specifically, abnormal promoter region methylation i
tumor suppressor genes contributes to tightly heritable gene sil
thereby cause loss of gene function, which contributes to tumor
genesis. Re-expression of abnormally silenced suppressor genes
is being researched for potential clinical applications.

Research into gene silencing has focused on the regions of high CpG content, known as the CpG islands, and these are often located near gene transcription sites, where the transcription of DNA to RNA begins. In normal cells, most promoter-associated CpG islands at transcription sites are unmethylated. The absence of CpG methylation indicates either normal transcription activity, or the fact that a gene can be recruited to express even if it is basally silent. But in cancer cells, promoter region CpG islands are more likely to become methylated, and this can cooperate with other transcriptional silencing chro-

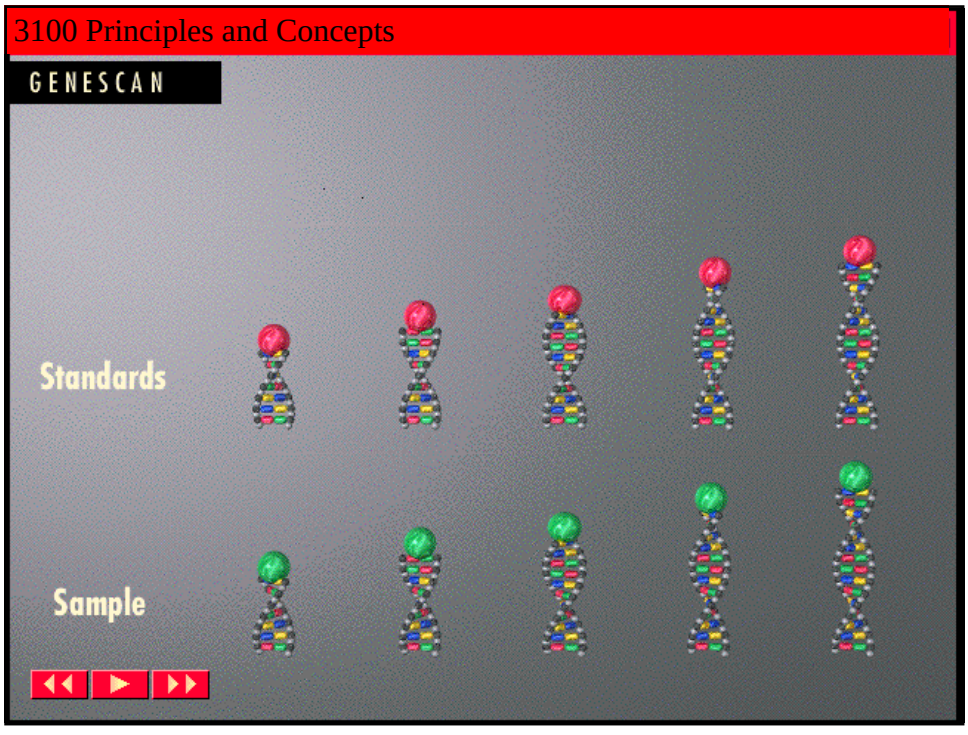
Unlike gene mutation, gene silencing associated with methylation may be reversed, reactivating the tumor-suppression activity of a gene. Two approaches to promoting re-expression of silenced genes have been studied in myelodysplastic syndrome (MDS), a hematologic disorder in which DNA methylation and gene silencing have been demonstrated. Dr. Steven Gore reports the clinical results of these two treatment approaches in "Current and Emerging Therapeutic Applications." The first approach uses methyltransferase inhibitors to stop methylation, allowing normal gene transcription to resume.

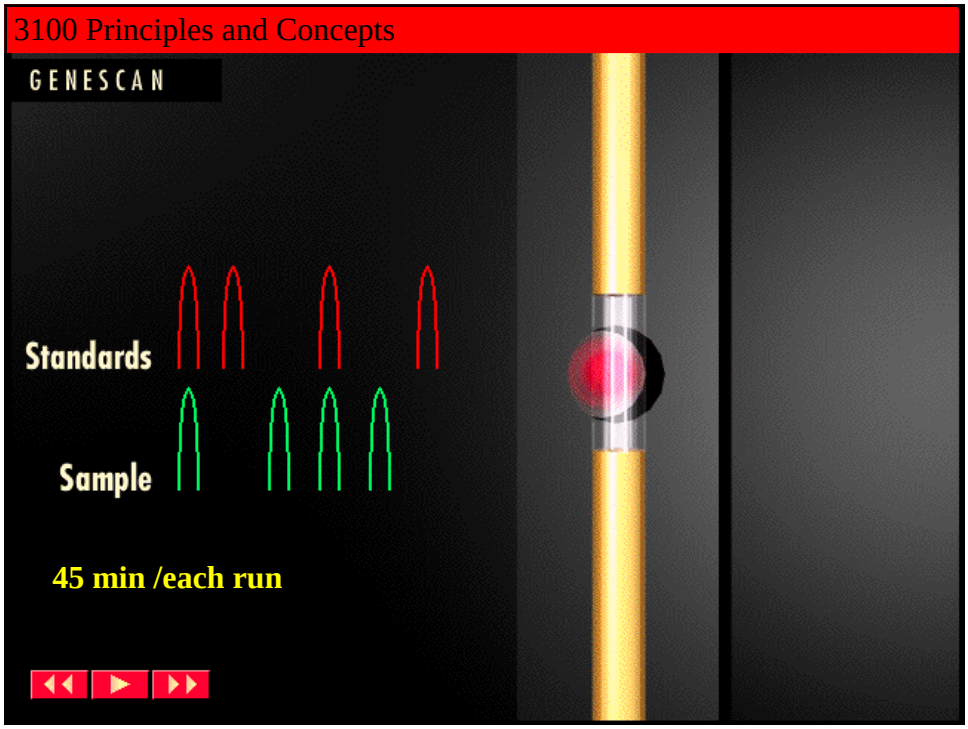
Methylation Study

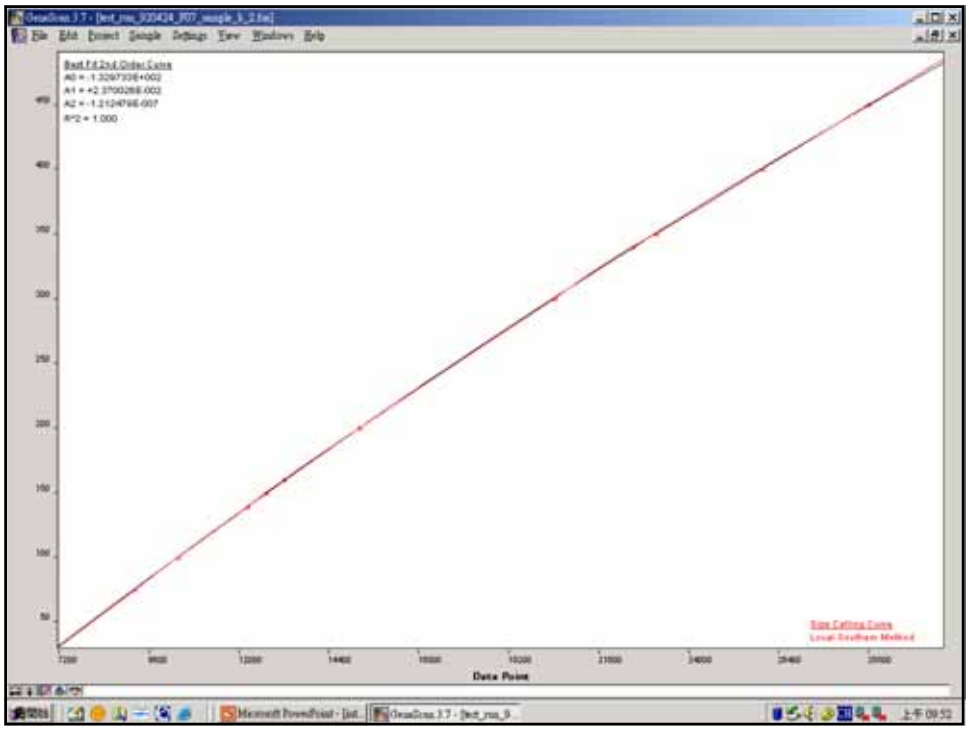
- Bisulfite treatment of gDNA converts unmethylated Cytosine to Uracil.
  - Methylated Cytosine remains a **C**.
- After PCR amplification all Uracils are converted Thymine.
- Comparing the sequences of the native gDNA to the bisulfite treated gDNA will show you the methylated bases.

	unmethylated	methylated
Target	--CG--	--C^{m}G--
Bisulfite treatment & cleanup	--UG--	--C^{m}G--
PCR	--TG--	--C G--

Date | Confidential

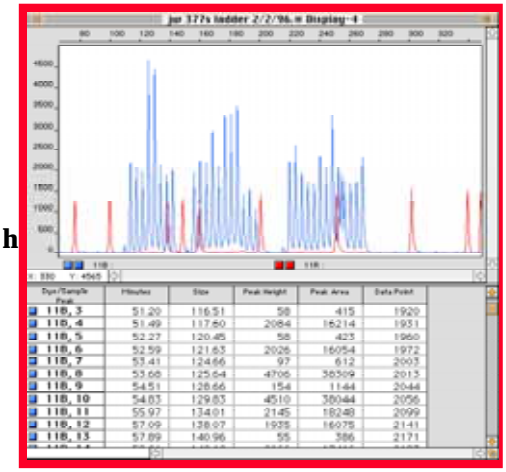



3100 Principles and Concepts
GENESCAN
Standards
Sample
45 min /each run



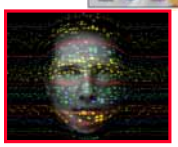
GeneMapper™ Analysis Software

- **Automatic size determination**
- **Automatic allele peak quantitation**
- **Used in conjuction with Genotyper™**

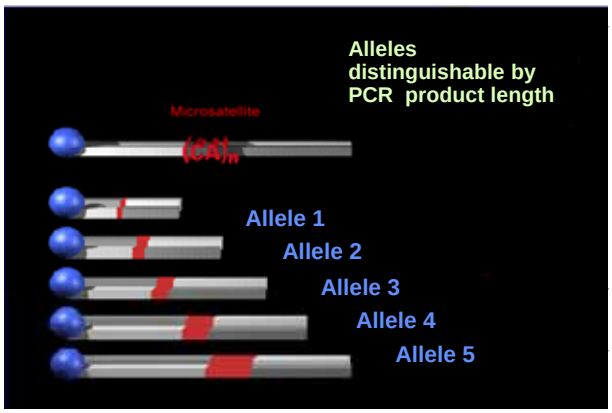


Genotyping Applications 基因比對之應用

- Human Linkage Mapping
  - microsatellite genome panel
- 人類身分鑑定
  - 16 STR Markers in one lane
- AFLP Plant Mapping Kits
- SNPs Detection
- SSCP

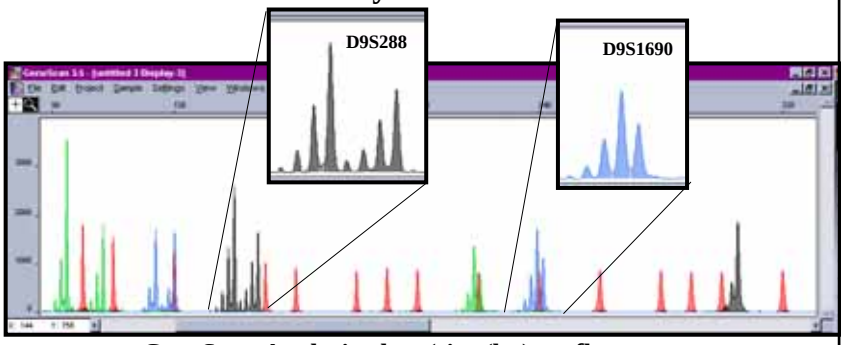


Genotype with Microsatellite Markers



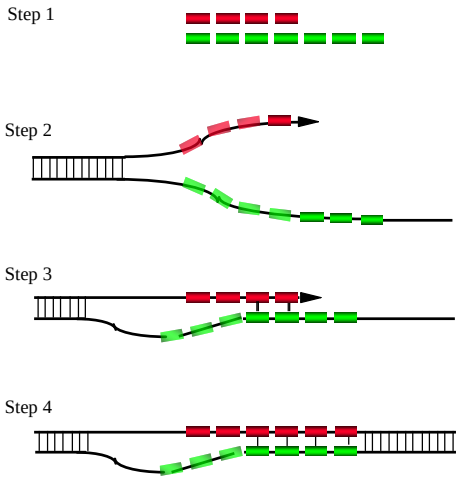
- Microsatellites are 2–7 bp repeats flanked with unique sequences constituting a 'microsatellite marker'
  - » The number of repeat units varies in the population
  - » Assumption is that each repeat unit is randomly distributed in the population
- Widely spread in the genome
- Over 7000 microsatellite markers have been mapped in the human genome

3130 GeneScan Analysis for Microsatellites



GeneScan Analysis plots (size (bp) vs. fluorescence intensity) of microsatellites on the 3100 Genetic Analyzer. GeneScan Installation Standard and the GeneScan 400-HD Size Standard run with POP4 and a 36cm capillary array are shown. The Installation Standard is a pool of six Linkage Mapping Set markers, amplified from an individual CEPH family member (1347-02).

Possible Mechanism for “Stuttering”



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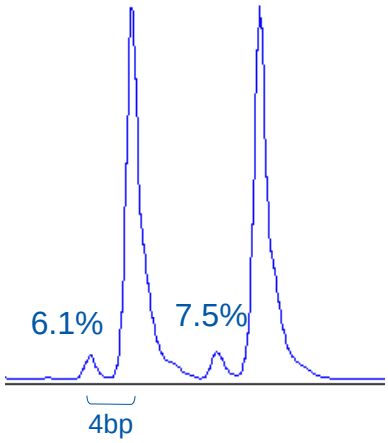
STR Stutter Peaks



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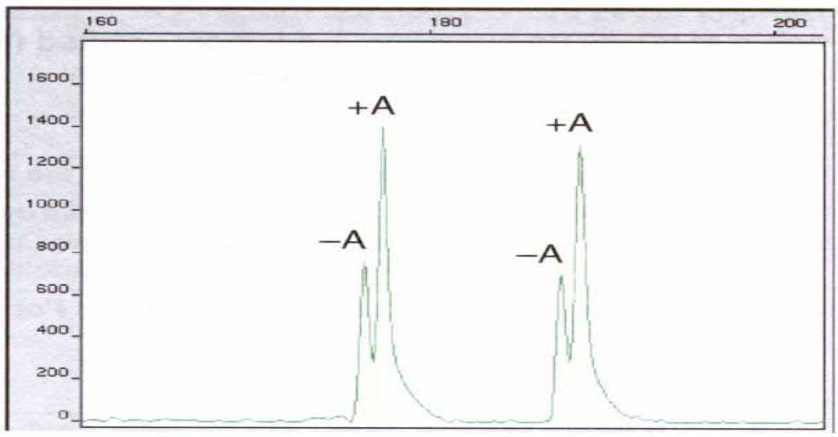
-A Peaks



人類身份鑑定系統應用範圍

- 刑事鑑定
- 親子鑑定
  - 財產繼承
  - 非婚生子女認親
  - 大陸人士來台探親
  - 子女尋親
  - 災難現場罹難者辨識

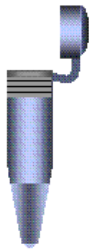
Validation on instrument, protocol, reagents!!

AmpFℓSTR® Identifiler™ Kit A Combination of Experience

親子鑑定

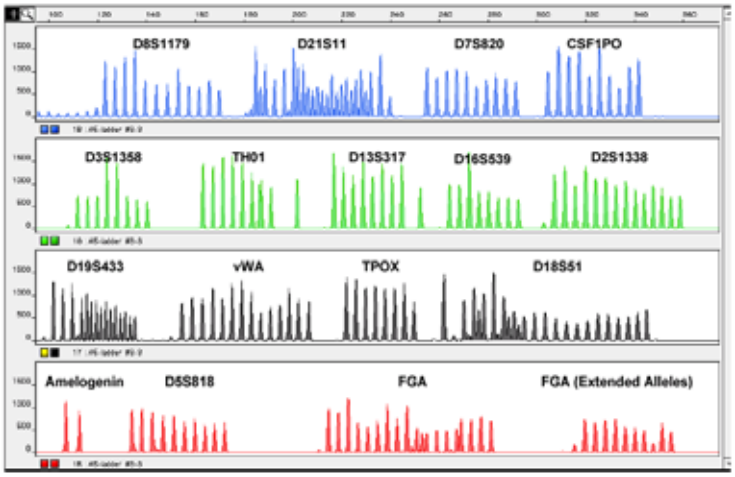
SGM Plus™ Kit	Profiler Plus™ Kit	COfiler™ Kit
D2S1338	FGA	D16S539
D16S539	vWA	D3S1358
vWA	D3S1358	
D3S1358		
D18S51	D18S51	CSF1P0
D21S11	D21S11	TPOX
D8S1179	D8S1179	TH01
FGA	D7S820	D7S820
TH01	D13S317	
D19S433	D5S818	

16 markers in one PCR reactions



Applied Biosystems

AmpFℓSTR® Identifiler™ Allelic Ladder



AmpFℓSTR® Yfiler™ Kit

DYS19
DYS385 a/b
DYS389 I/II
DYS390
DYS391
DYS392
DYS393

European Minimal haplotype

DYS438
DYS439

SWGDAM

DYS437
DYS448
DYS456
DYS458
Y GATA C4
Y GATA H4

Highly polymorphic loci

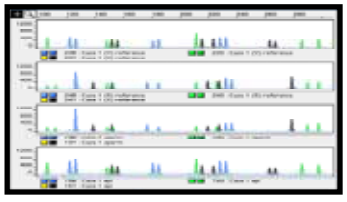
DNA片段分析實驗流程

抽取 DNA

加入Specific primers及PCR試劑

進行PCR反應

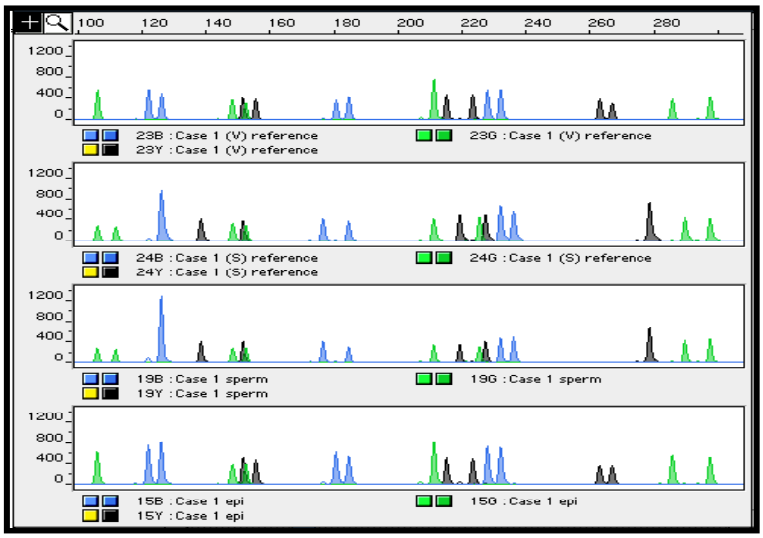
自動化電泳分離



電腦判讀結果

AmpFlSTR Profiler Plus

Nonprobative Casework



Victim Reference

Suspect Reference

Sperm Fraction

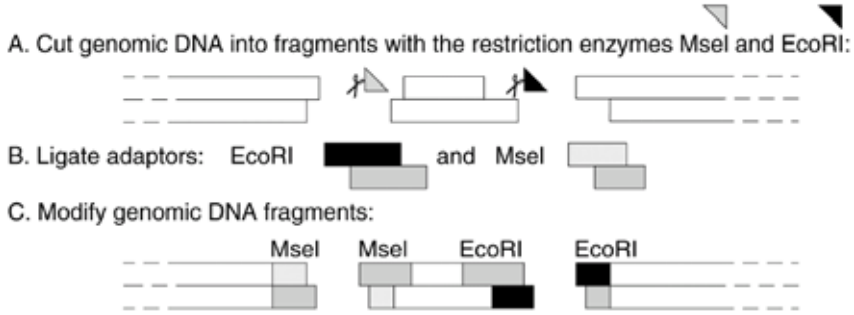
Epithelial Fraction

Advantages of AFLP

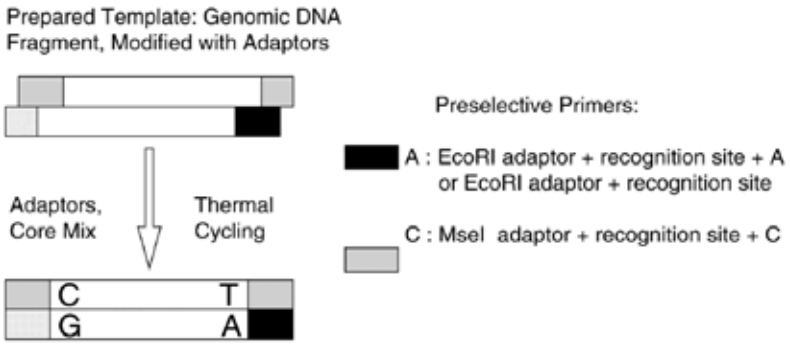
Some of the advantages of the AFLP technique are the following:

- Only small amounts of DNA are needed.
- Unlike randomly amplified polymorphic DNAs (RAPDs) that use multiple, arbitrary primers and lead to unreliable results, the AFLP technique uses only two primers and gives reproducible results.
- Many restriction fragment subsets can be amplified by changing the nucleotide extensions on the adaptor sequences. Hundreds of markers can be generated reliably.
- High resolution is obtained because of the stringent PCR conditions.
- The AFLP technique works on a variety of genomic DNA samples.
- No prior knowledge of the genomic sequence is required.

Template preparation & ligation of AFLP adaptor



Preselective amplification



Selective amplification

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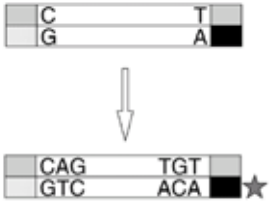
A. Choose Selective AFLP Primers: ★■ Axx ▭ Cxx ★ Fluorescent dye

★■ Axx - one of sixteen different fluorescent dye-labeled AFLP EcoRI Selective Amplification primers.

▭ Cxx - one of eight different AFLP MseI Selective Amplification primers.

B. Run Selective Amplification:



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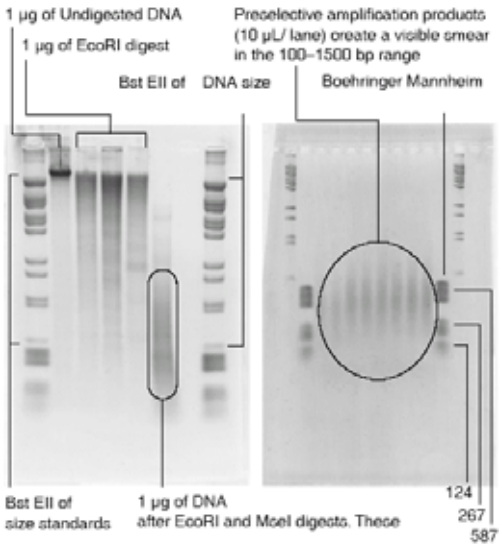

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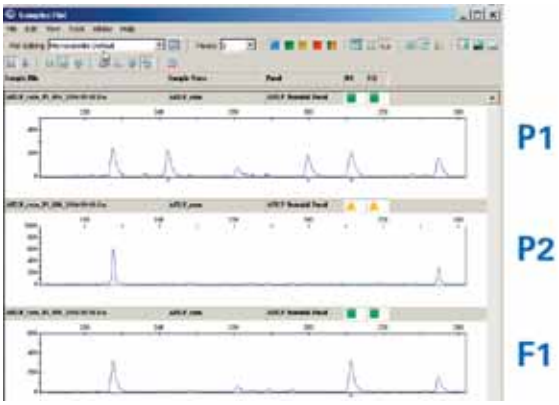
Samples | Genotypes

	Sample File	Dye	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7
1	004_B02_afip_20	B	1	0	0	1	0	1	1
2	008_D02_afip_20	B	0	0	1	0	0	1	0
3	010_E02_afip_20	B	0	1	1	1	1	0	0



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Thanks!

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