DNA FINGERPRINTING IN CRIMINAL INVESTIGATION

BY

RAJESH BISWAS,Ph.D.

HEAD OF THE DEPARTMENT, DEPARTMENT OF ZOOLOGY, GOVERNMENT HOME SCIENCE COLLEGE, SECTOR-10, CHANDIGARH

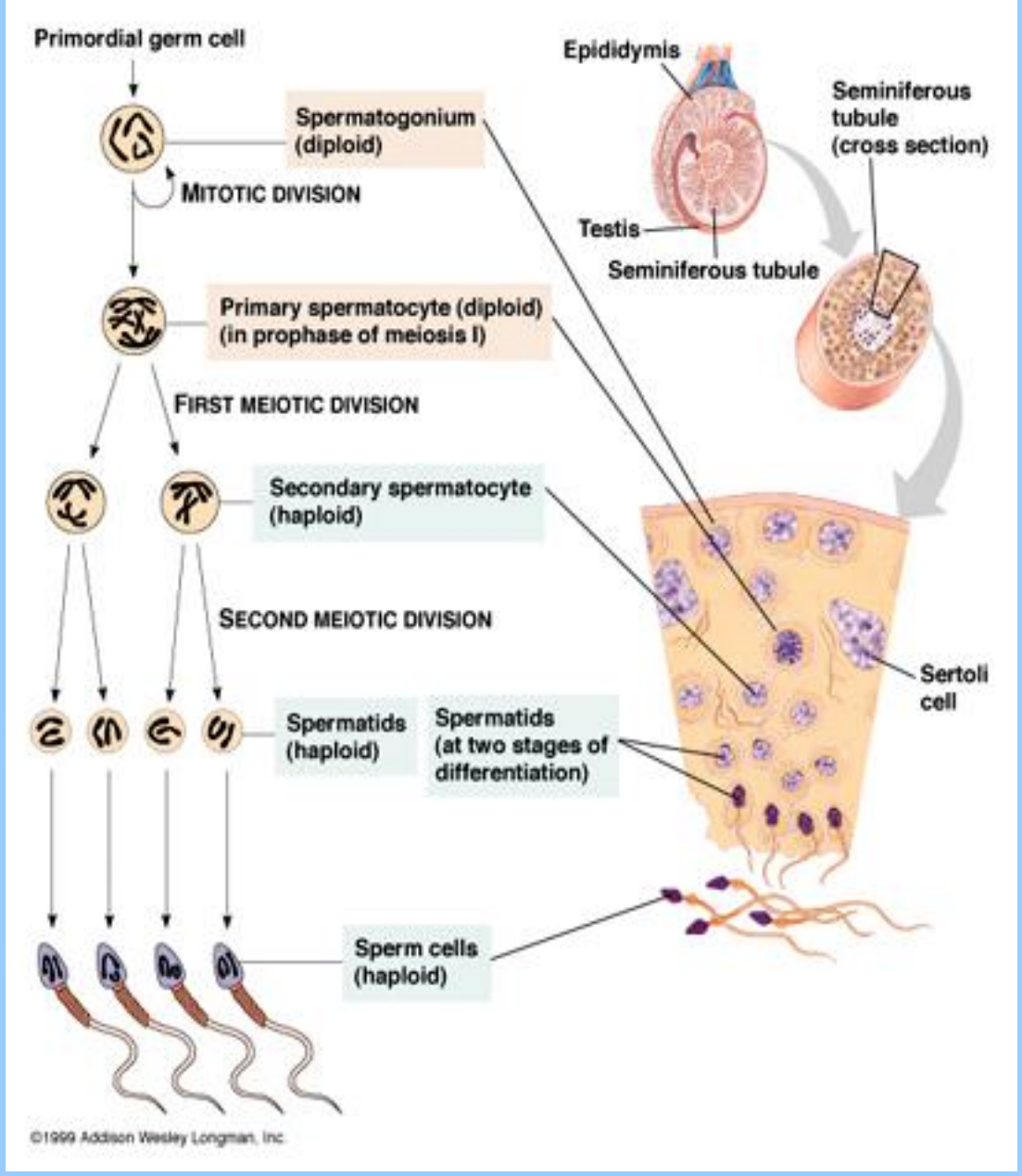
In 1985, in England, Alec Jeffreys, discovered, each human has unique DNA with the exception of identical twins.

DNA fingerprinting or DNA profiling, is a technique for analyzing and comparing DNA from separate sources, used especially in law enforcement to identify suspects from hair, blood, semen, or other biological materials found at the scene of a violent crime. It depends on the fact that no two people, save identical twins, have exactly the same DNA sequence, and that although only limited segments of a person's DNA are scrutinized in the procedure, those segments will be statistically unique.

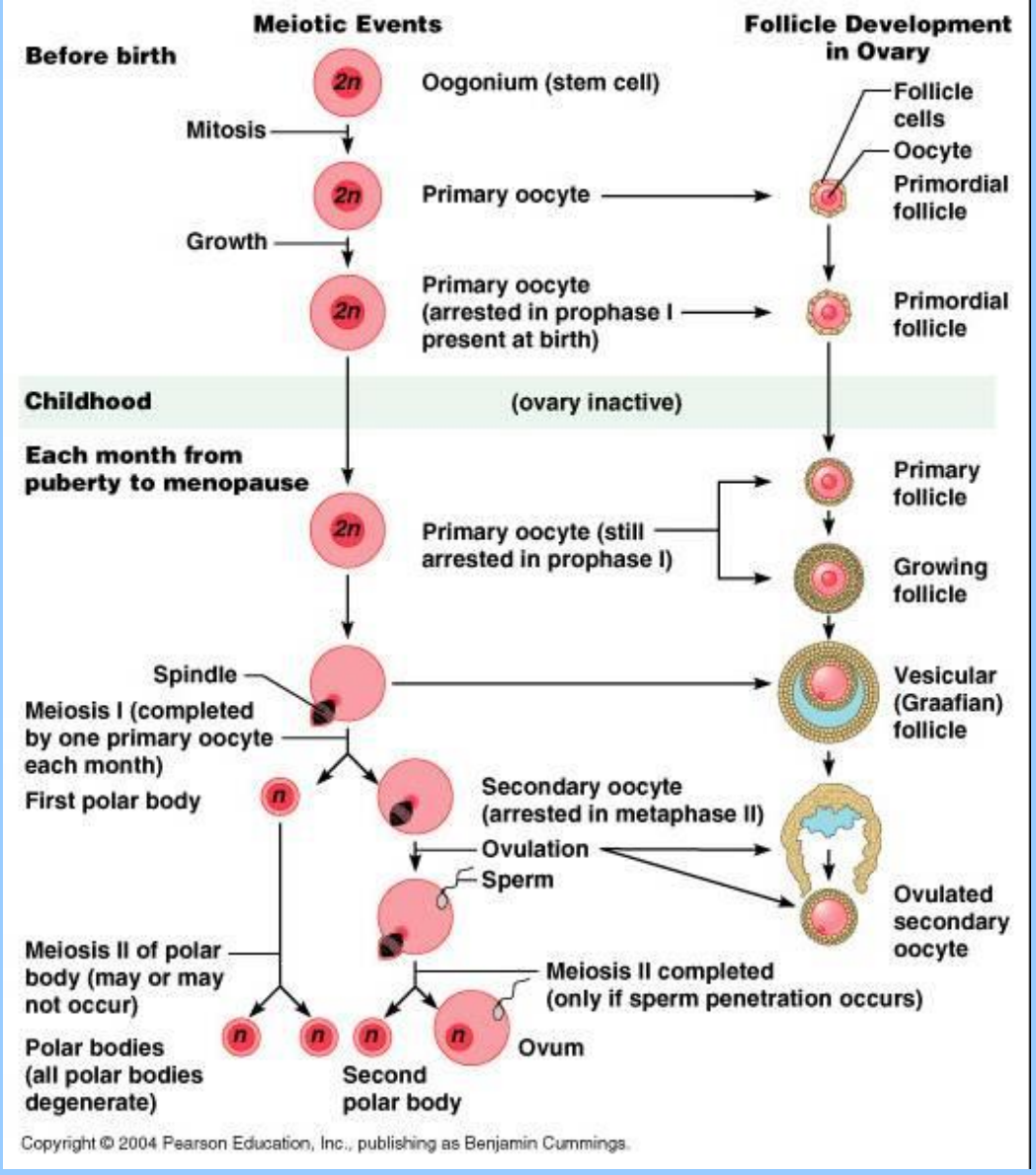
LIFE CYCLE OF HUMAN

Understand life cycle of human

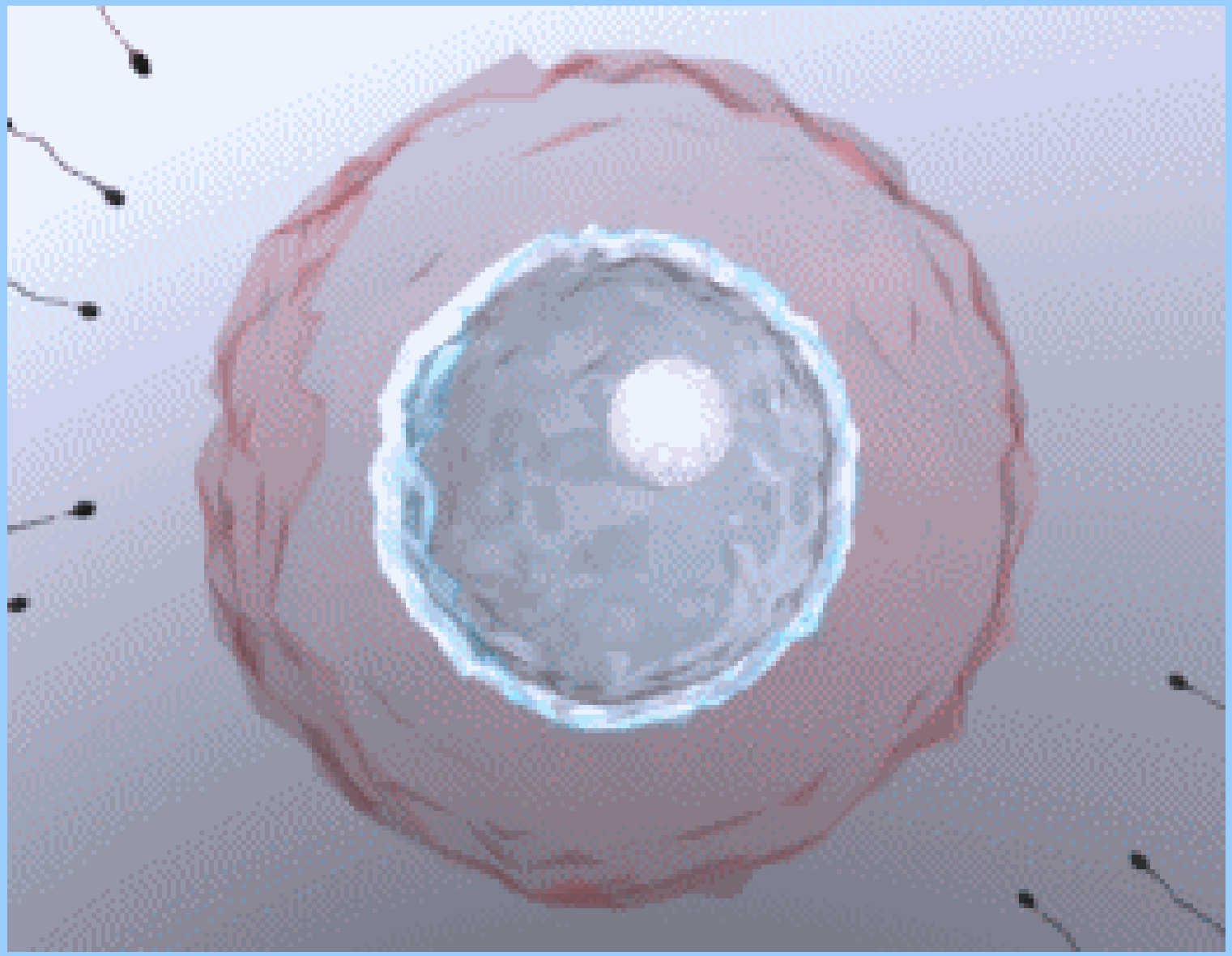
is important to use DNA fingerprinting in criminal investigation and evidence collection



Formation of Sperm, the Spermatogenesis making life ‘n’ from ‘2n’

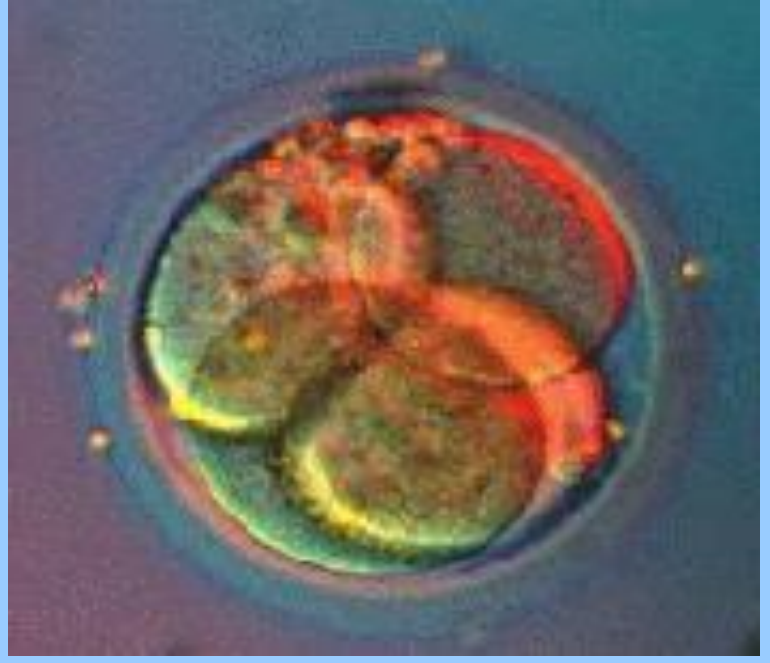


Formation of Ovum, the Oogenesis making life ‘n’ from ‘2n’



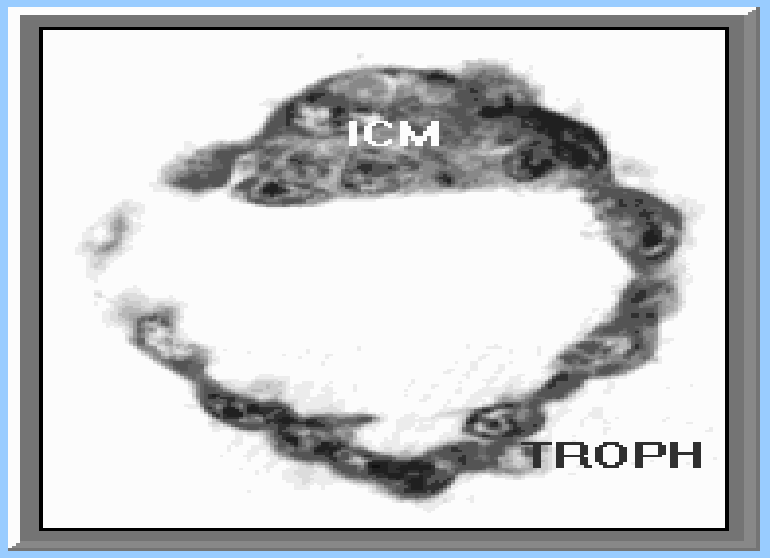
Fertilization, the formation of Zygote make life ‘2n’ again

2 Cell Stage

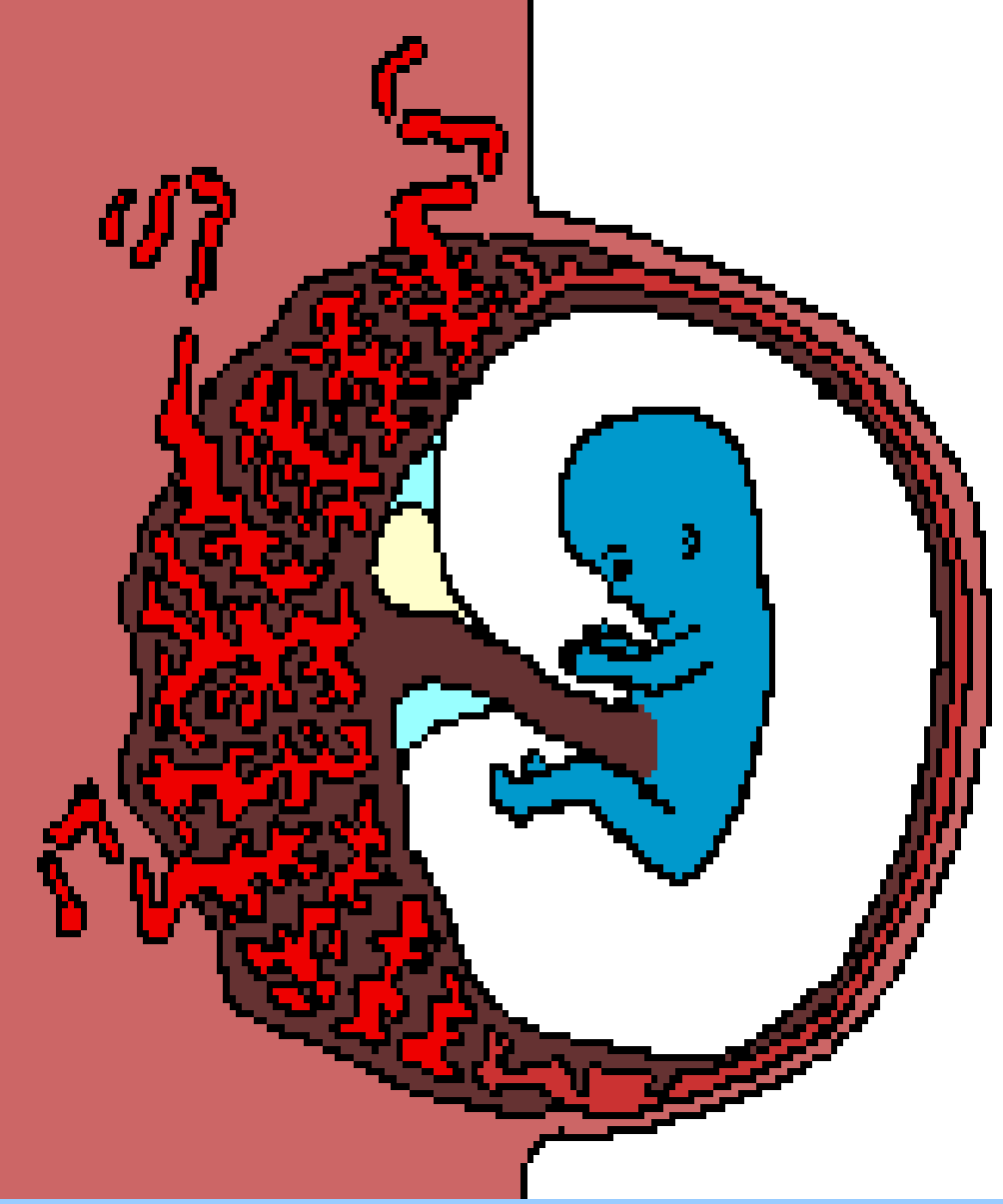


4 Cell Stage

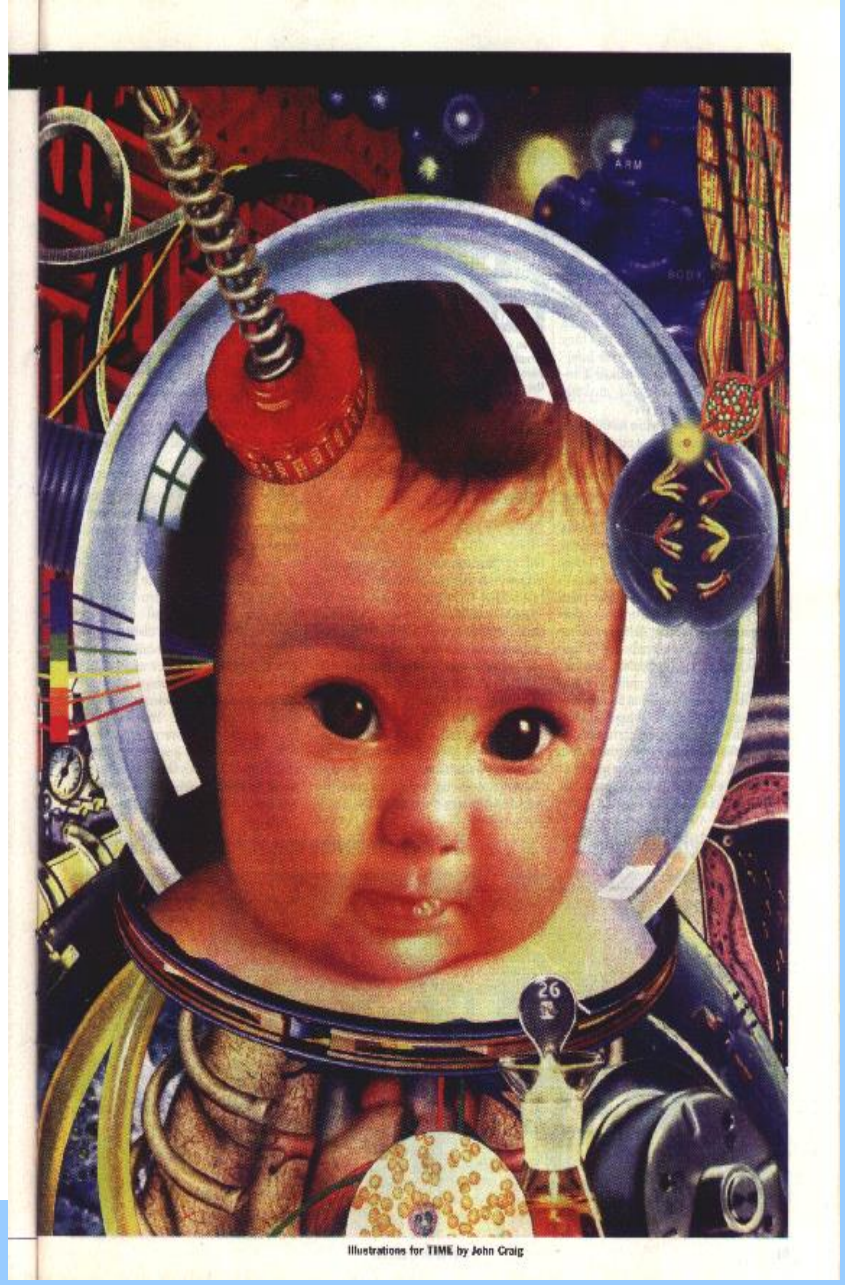
16 Cell Stage



Blastula



Week- 8

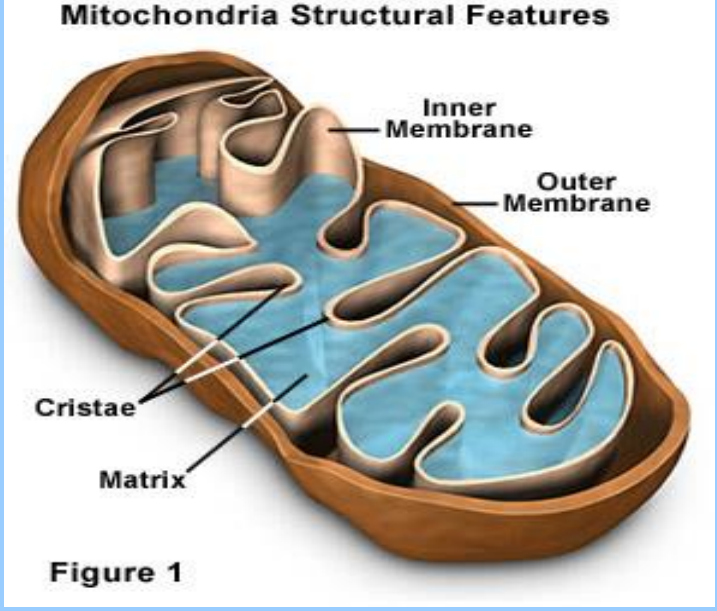


Baby

Where we can Get DNA

?

Inside Nucleus And in



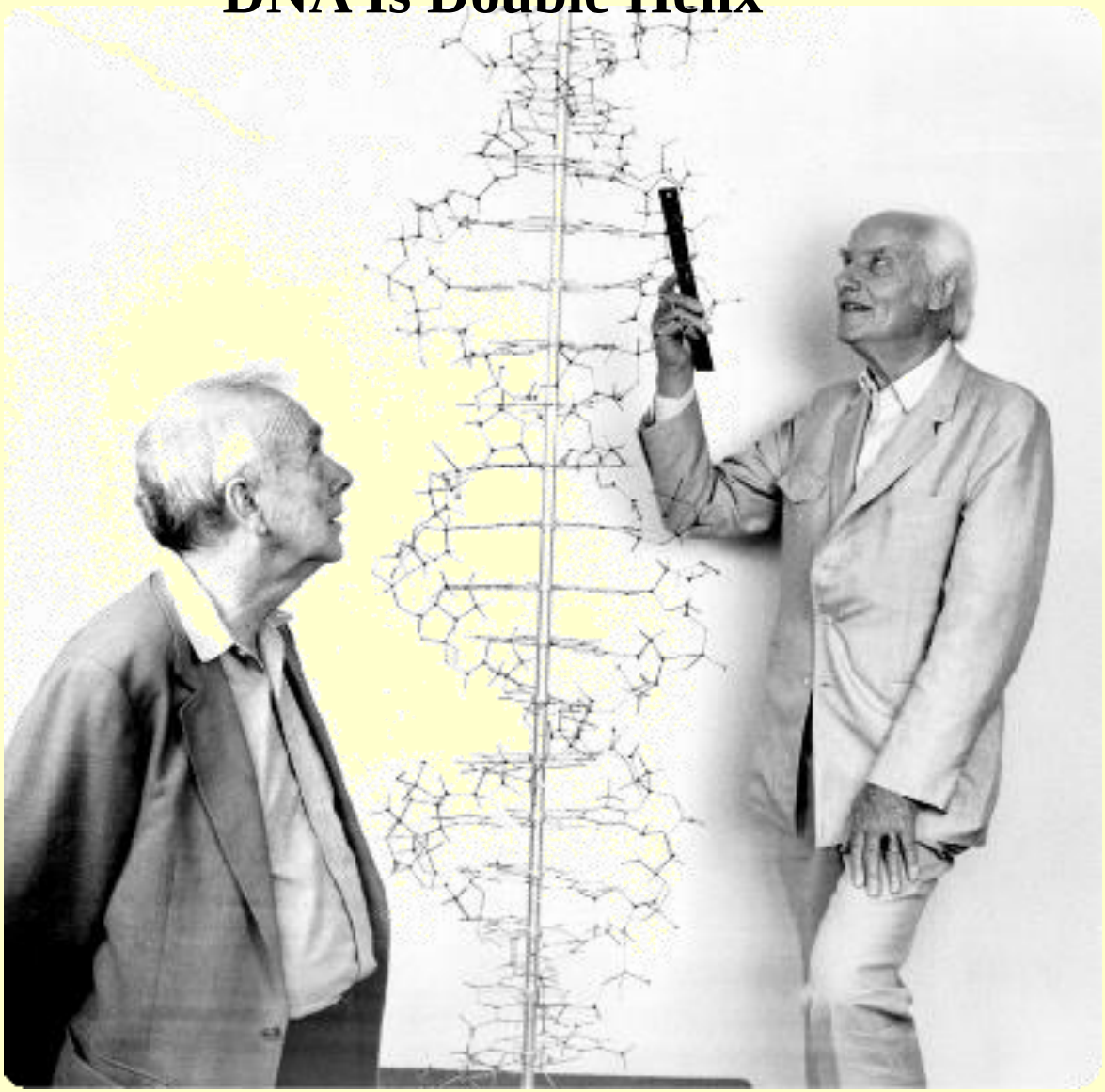
DNA is useful in forensic because it is Present in all cells and same throughout the body irrespective of its source from which it is obtained, and does not change in the course of a person's life

How much DNA present in different Cells ?

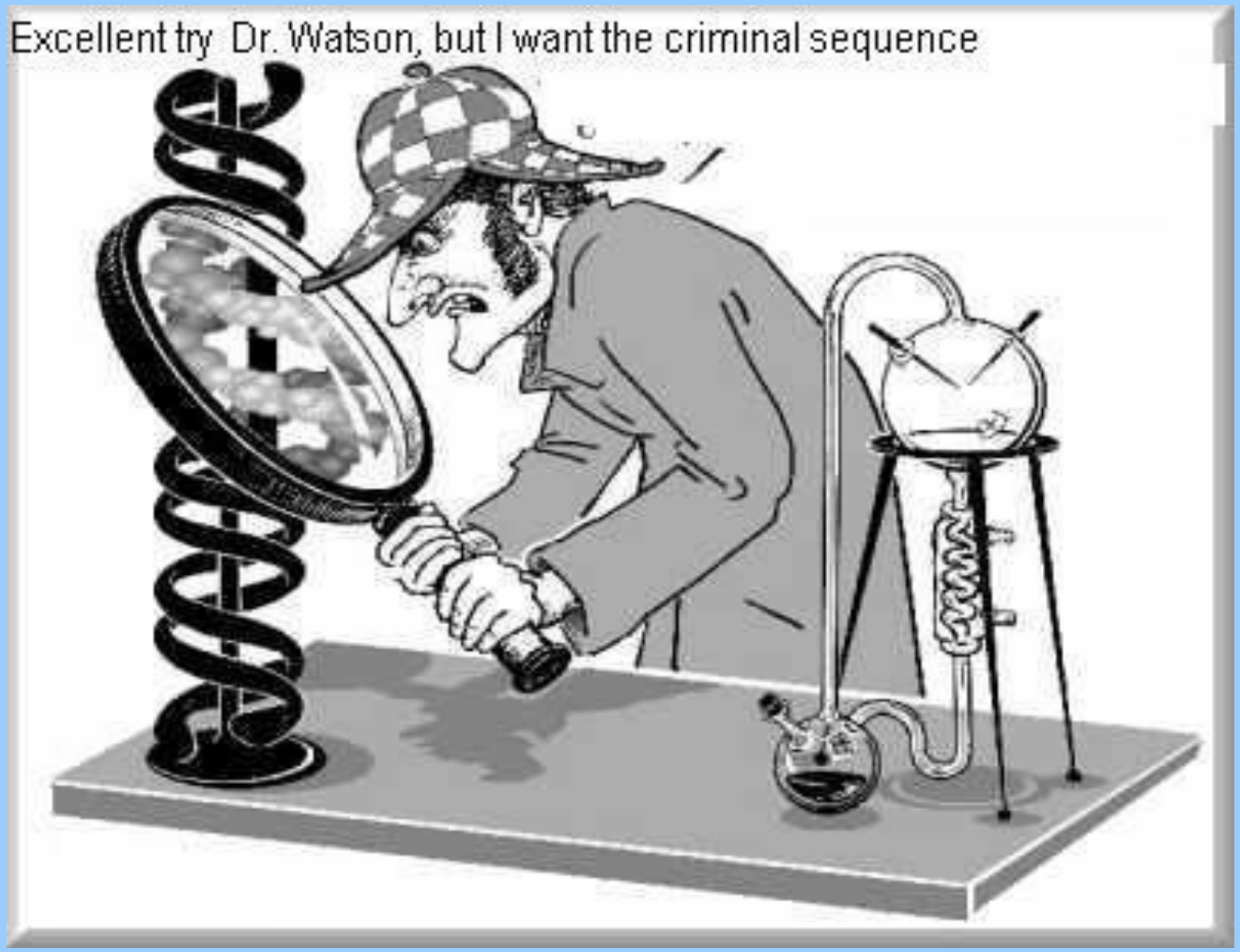
DNA contents of various tissue are as follows:

1 Sperm	- 3 pg
1 Cell	- 6pg
1 Shed hair	- 1 ng
1 Plucked hair	- 300 ng
1 drop of blood	-1500 ng

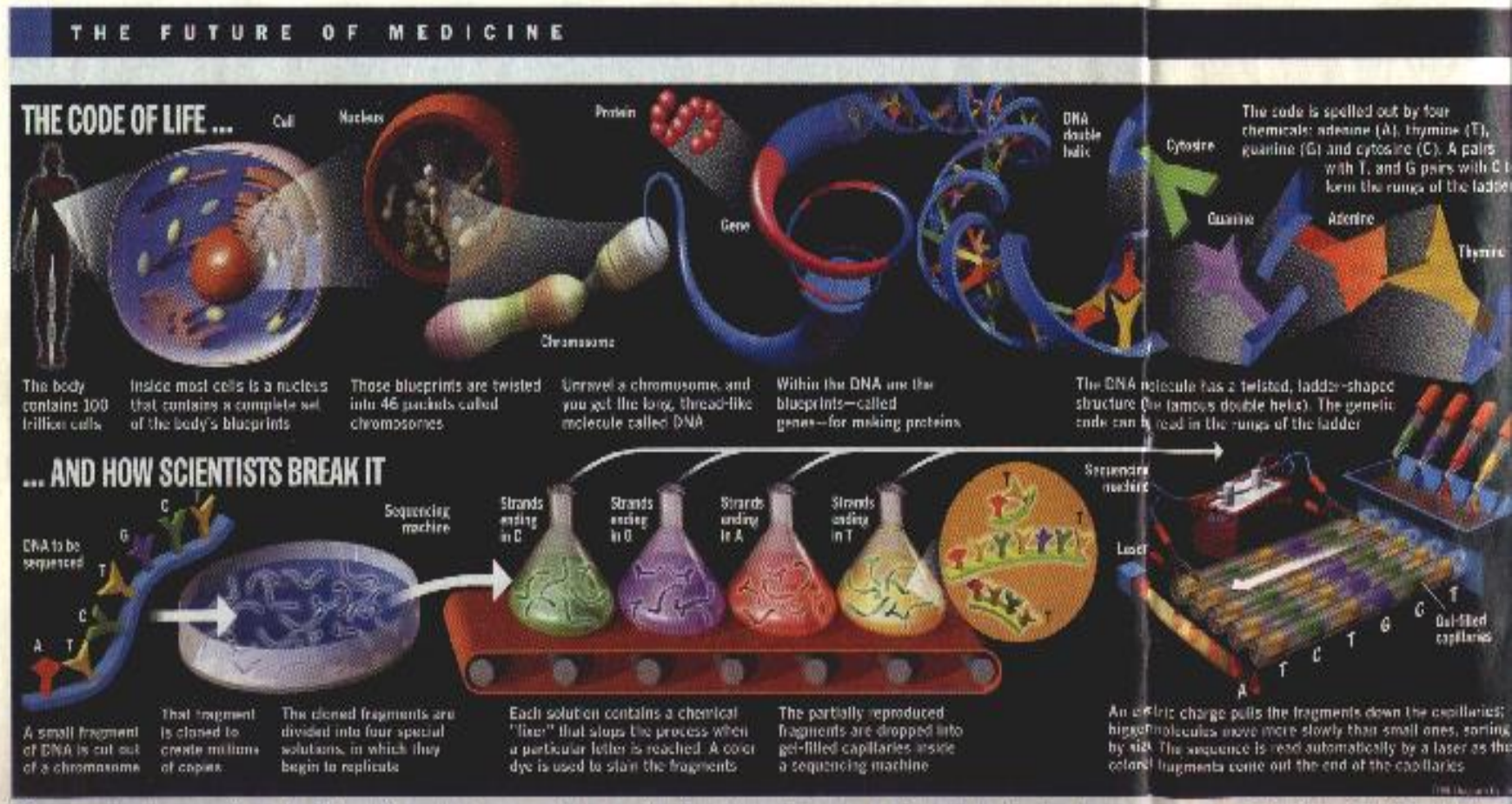
DNA Is Double Helix



Watson and Crick With original DNA model



DNA does not have any Criminal Gene



The code of life is in DNA Sequence

What is DNA sequence ?

A sequence of bases in a strand of DNA, e.g., ATTTAGGCAT etc.

IS THE SEQUENCE UNIQUE ?

By no means !

Humans differ in respect of 0.1 percent of their genomes, or about 1.42 million base pairs,or about 1 base in every 500-1000 bases.

Alternate Characters in DNA are Alleles. The Alleles are present in different locus in the DNA

Forensic Science uses the polymorphism in DNA.

What is Polymorphism ?

Polymorphism refers to different forms of same basic structure. A good example of polymorphism in human being is "ABO Blood Group System". If modification of gene exists at specific locus in a population , the locus is polymorphic.

Variable Number of Tandem Repeat (VNTR) is one group of such DNA loci that are extensively used in forensic science for human identification.

A typical VNTR segments consists of many tandemly repeated units, each unit being some 15 to 35 base pair long

The length of VNTR usually ranges from 500 to 10,000 base pair

Some of the example of forensically used RFLP based VNTR is

- **D1S7**
- **D2S44**
- **D4S139**
- **D5S110**
- **D10S28**
- **D14S13 locus**

Example of of PCR based VNTR is D1S80. D1S80 locus variably contain between 14 and 41 copies of 16 base pair repeat

Short Tandem Repeat(STRs) is another group of polymorphism DNA loci consist of repetitive sequence elements of 3 to 7 pairs in length are well distributed throughout the human genome

Some of the example of forensically used PCR based STRs is

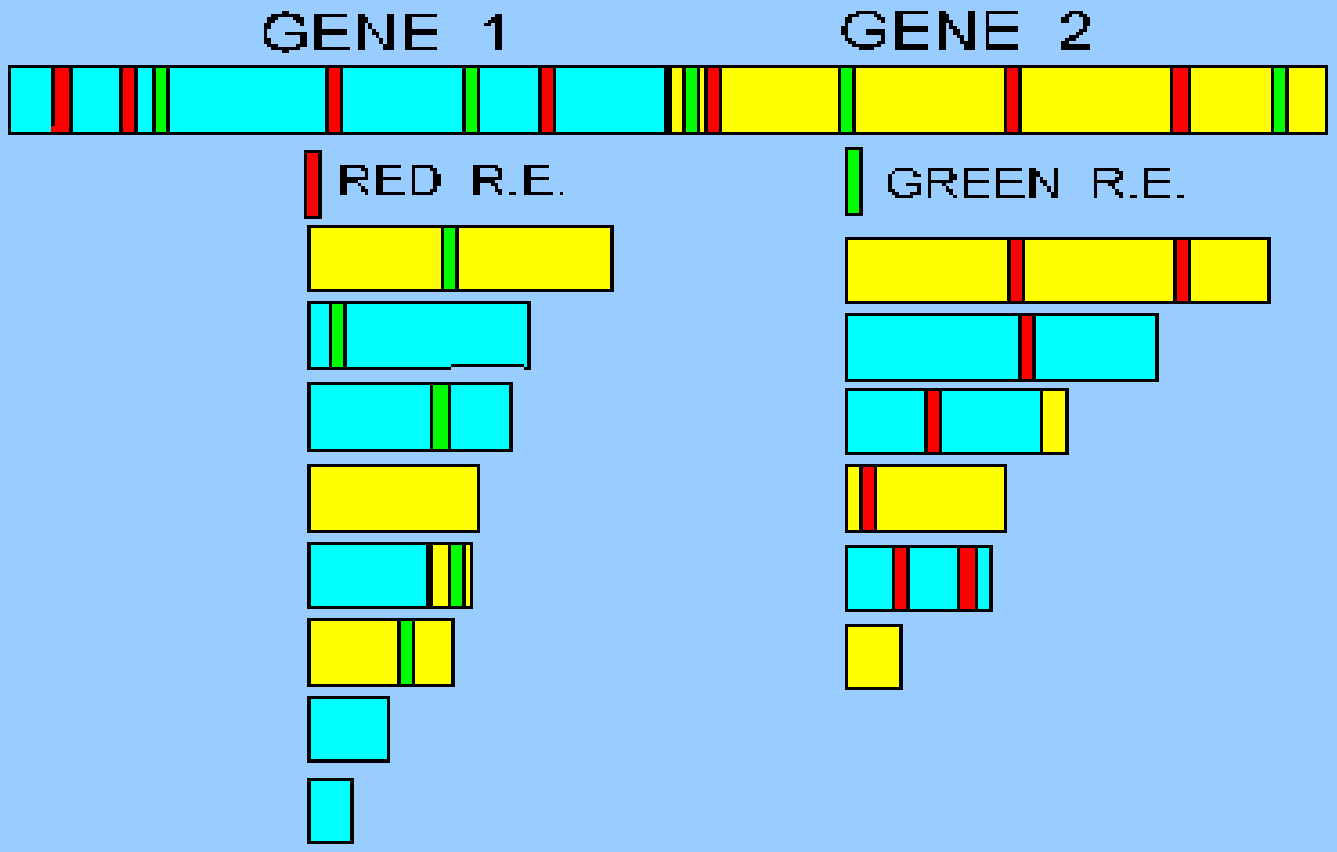
Name of locus	Chromosome location	Allele(other known allele)	Ladder size.
CSF1P0	5q, 33.3q-34	7 to 15 (6)	295-327
TP0X	2p, 25.1p ter	6 to 13 (6)	224-252
TH01	11p, 15.5	5 to 11 (9.3)	179-203
FESFPS	15q 25-qter	7-14	222-250
F13A01	6p24.3-p25.1	4-16(3.2,10)	283-331
VWA	12p 12pter	13-20(11,21)	139-167
D16S539	16q – 24qter	5, 8-15	264-305
D7S820	7sq11.21 – q22	6-14	215-247
D13S317	13q22 - 31	7-15	165-197

DNA Fingerprinting : Basic Technique

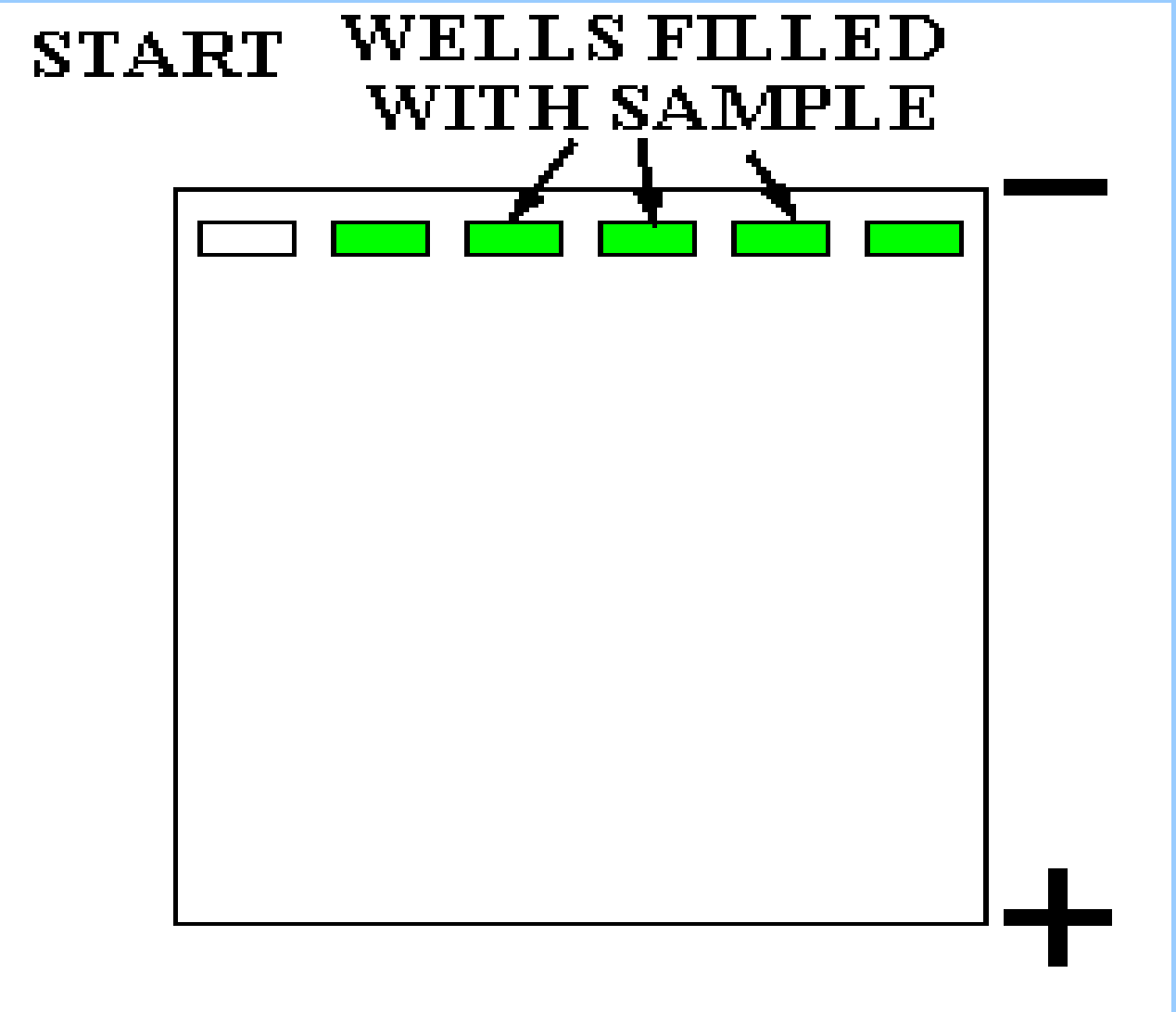
The First type of forensic DNA test to be widely used by crime laboratories was Restriction Fragment Length Polymorphism(RFLP), based on variation in the length of the DNA fragment among individual

Briefly RFLP process involves

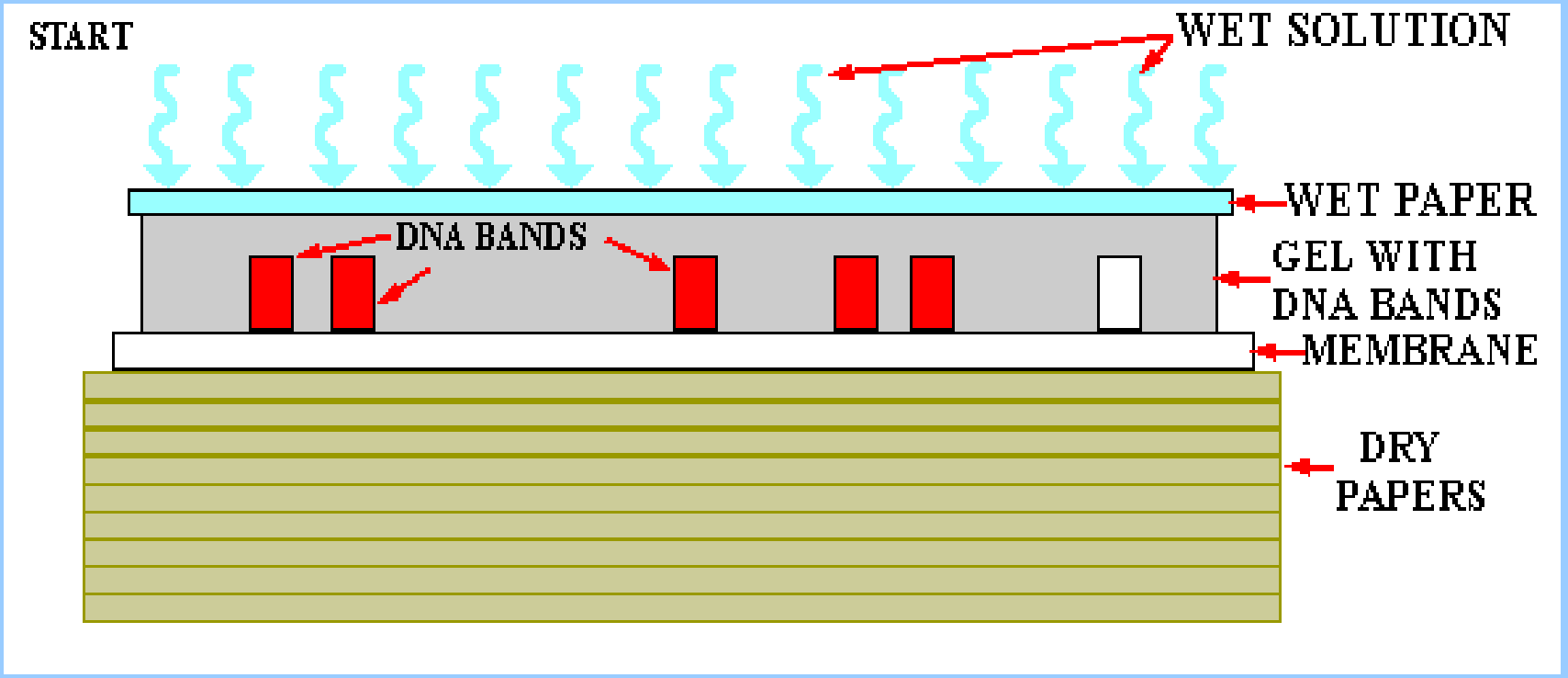
- **Digestion of human genomic DNA with restriction enzyme for creation of fragments**



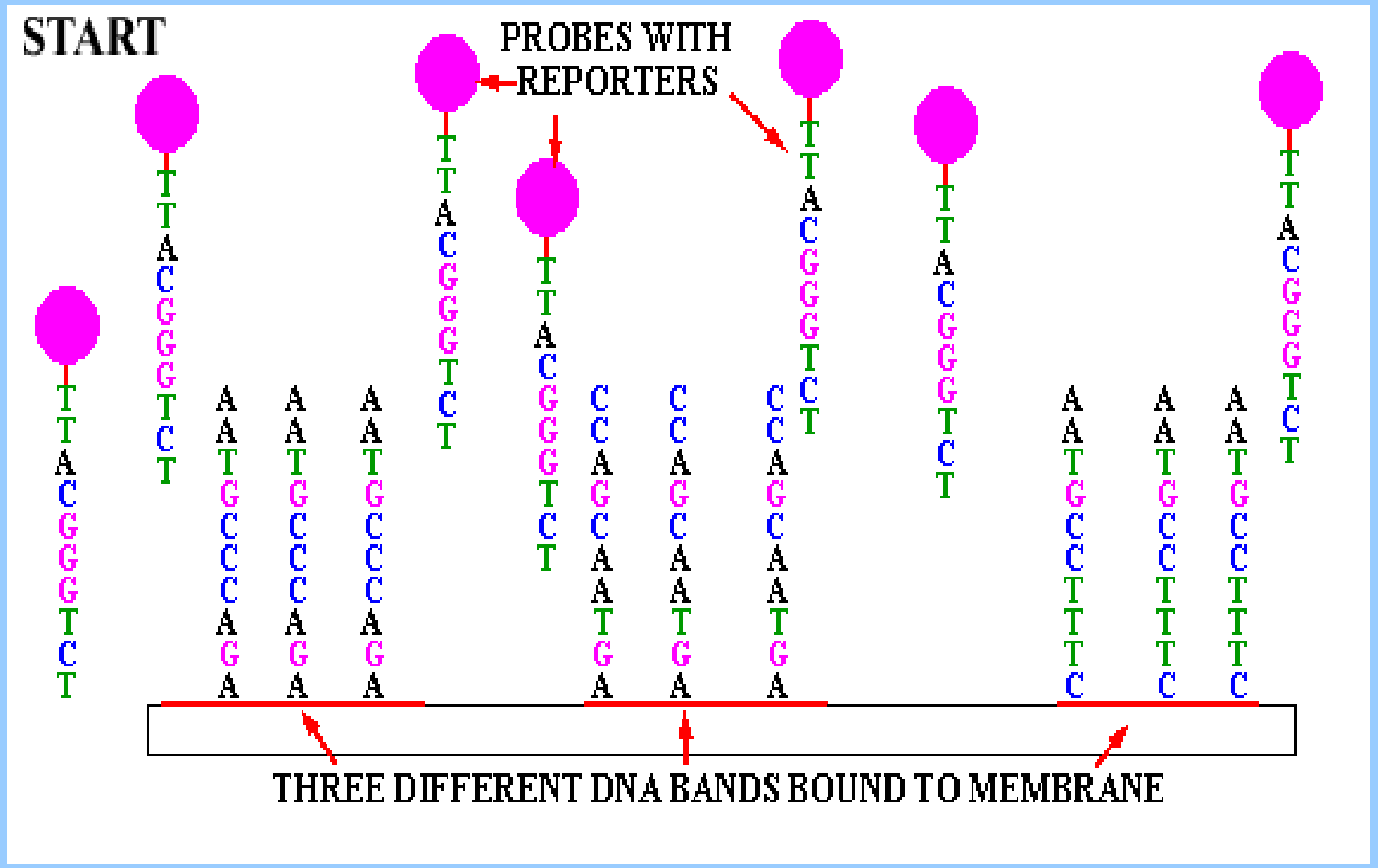
➢**Separation of Restrict fragments on an agarose gel by electrophoresis**



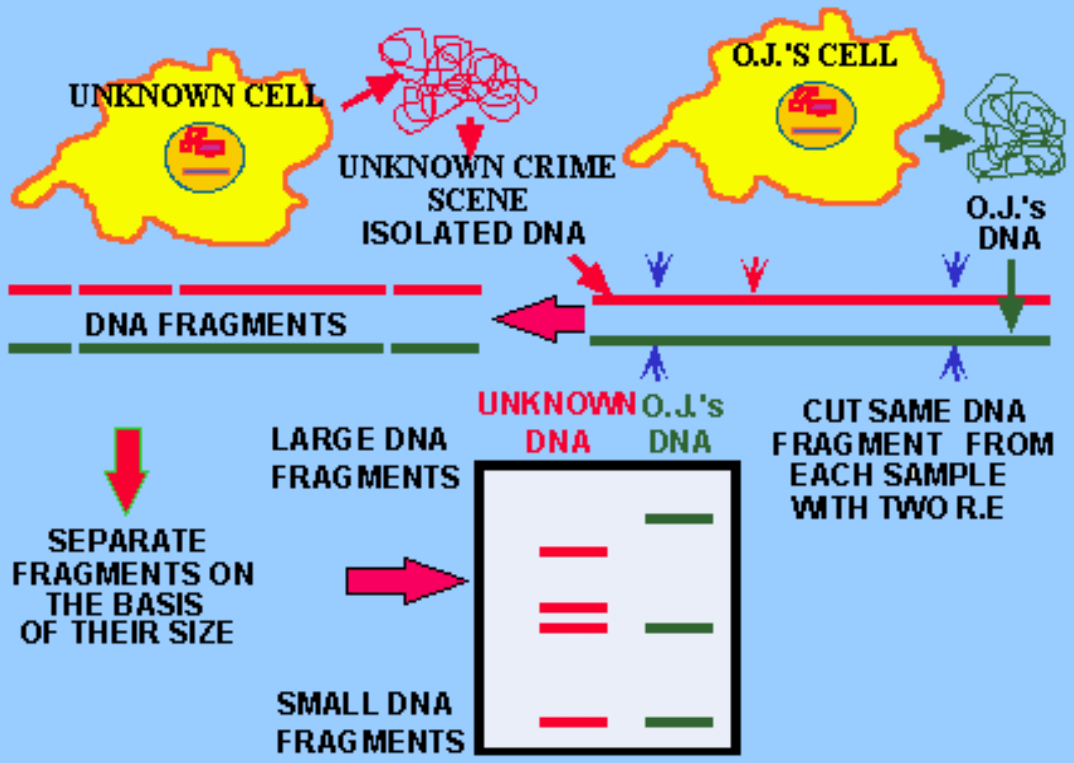
➢**Transfer of the Fragments to a nylon membrane by southern blotting**

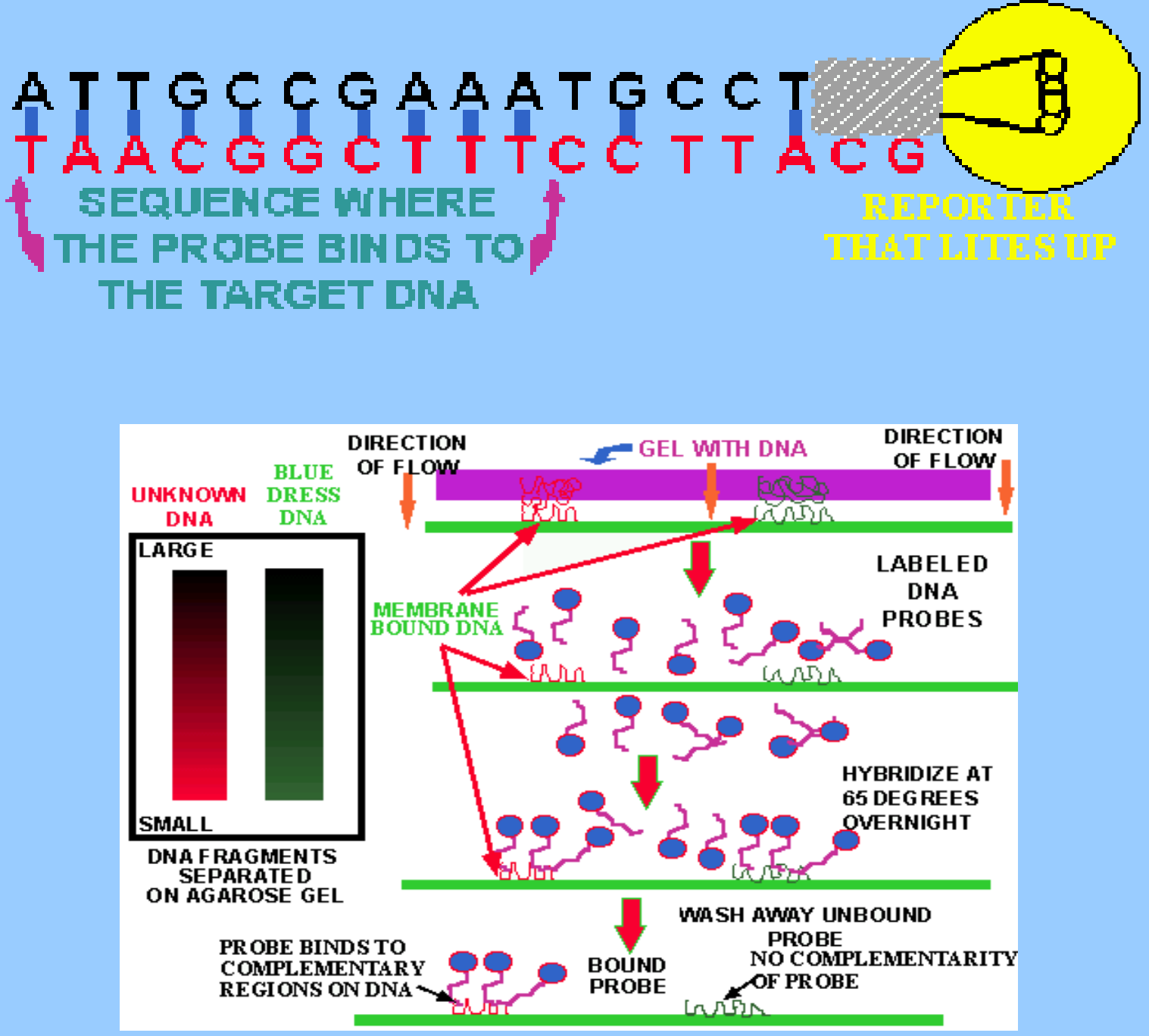


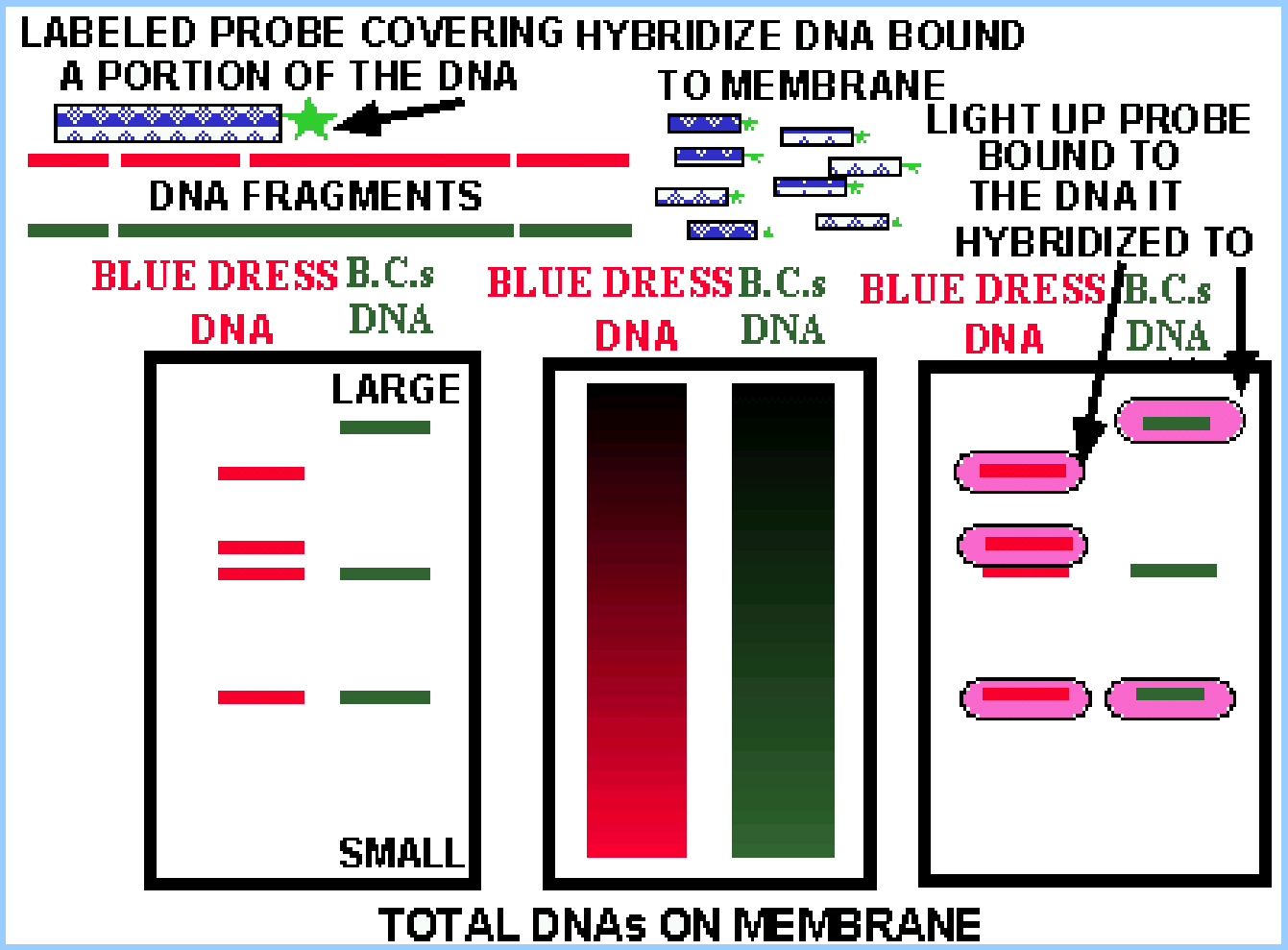
Incubation of the membrane with a chemiluminescent or radioactive probe for hybridization and develop Lumograph or Autoradiograph

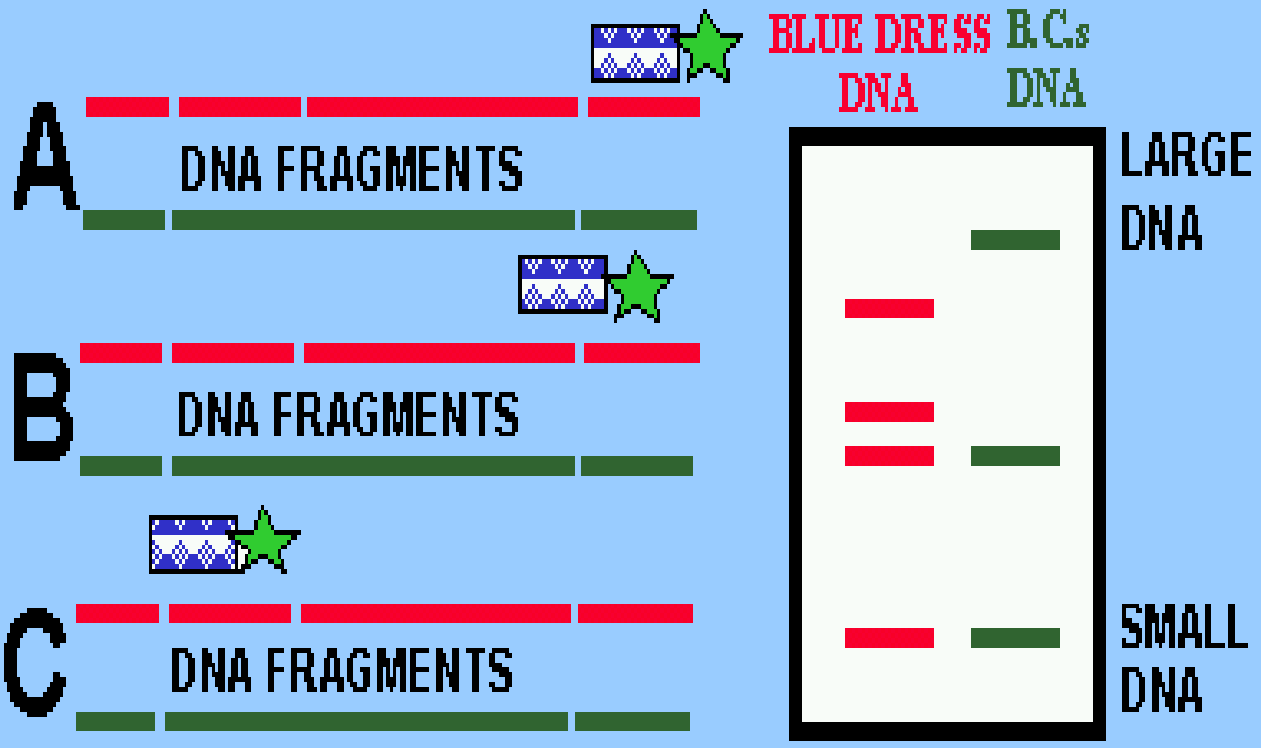


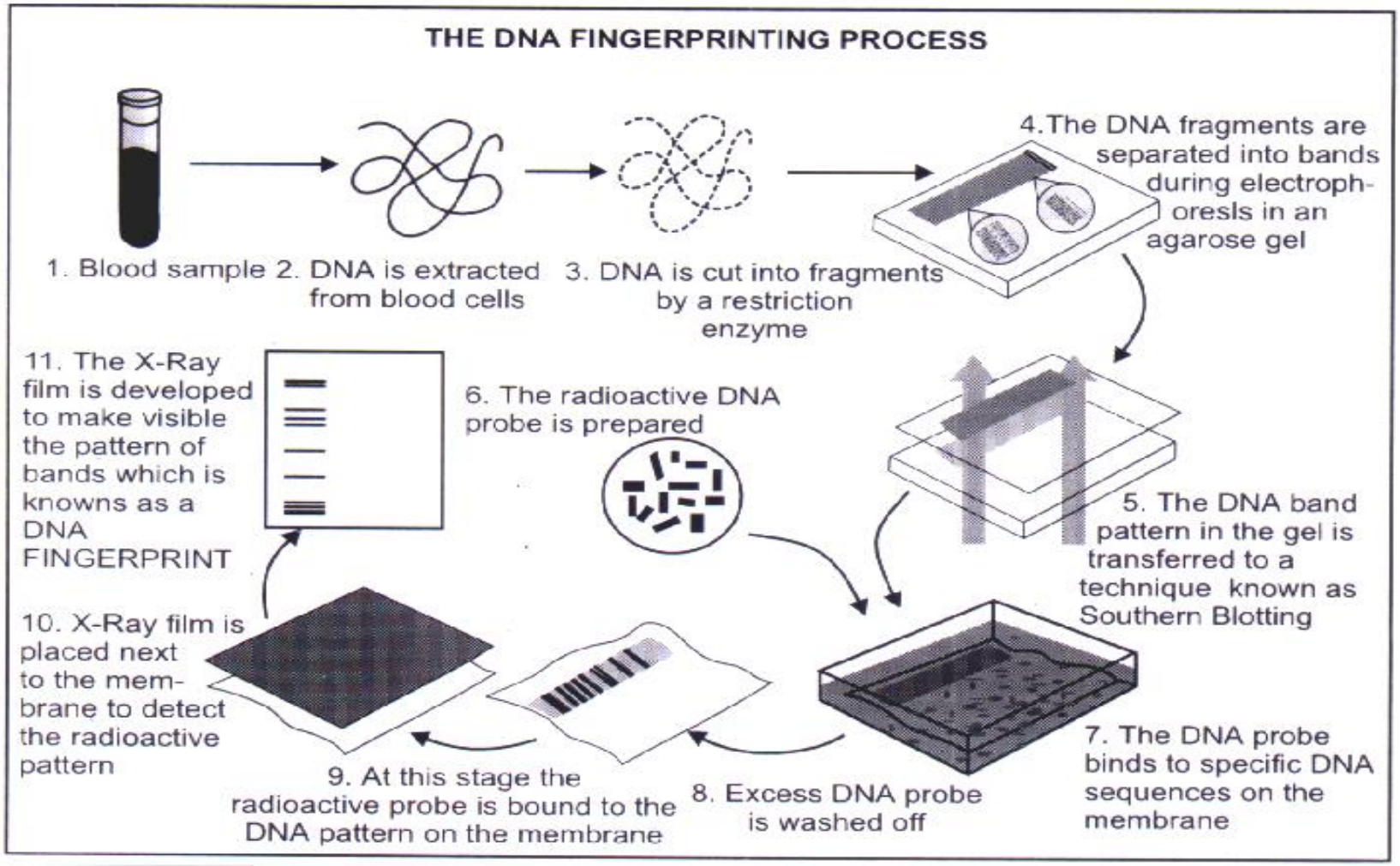
The Sizes of these Fragments are determined and compared between the evidence and reference samples




ATTGCCGAAATGCCT
TAACGGCTTTcCTTACG
SEQUENCE WHERE THE PROBE BINDS TO THE TARGET DNA
REPORTER THAT LITES UP
UNKNOWN DNA
BLUE DRESS DNA
LARGE
SMALL
DNA FRAGMENTS SEPARATED ON AGAROSE GEL
DIRECTION OF FLOW
GEL WITH DNA
DIRECTION OF FLOW
MEMBRANE BOUND DNA
LABELED DNA PROBES
HYBRIDIZE AT 65 DEGREES OVERNIGHT
WASH AWAY UNBOUND PROBE
PROBE BINDS TO COMPLEMENTARY REGIONS ON DNA
BOUND PROBE
NO COMPLEMENTARITY OF PROBE

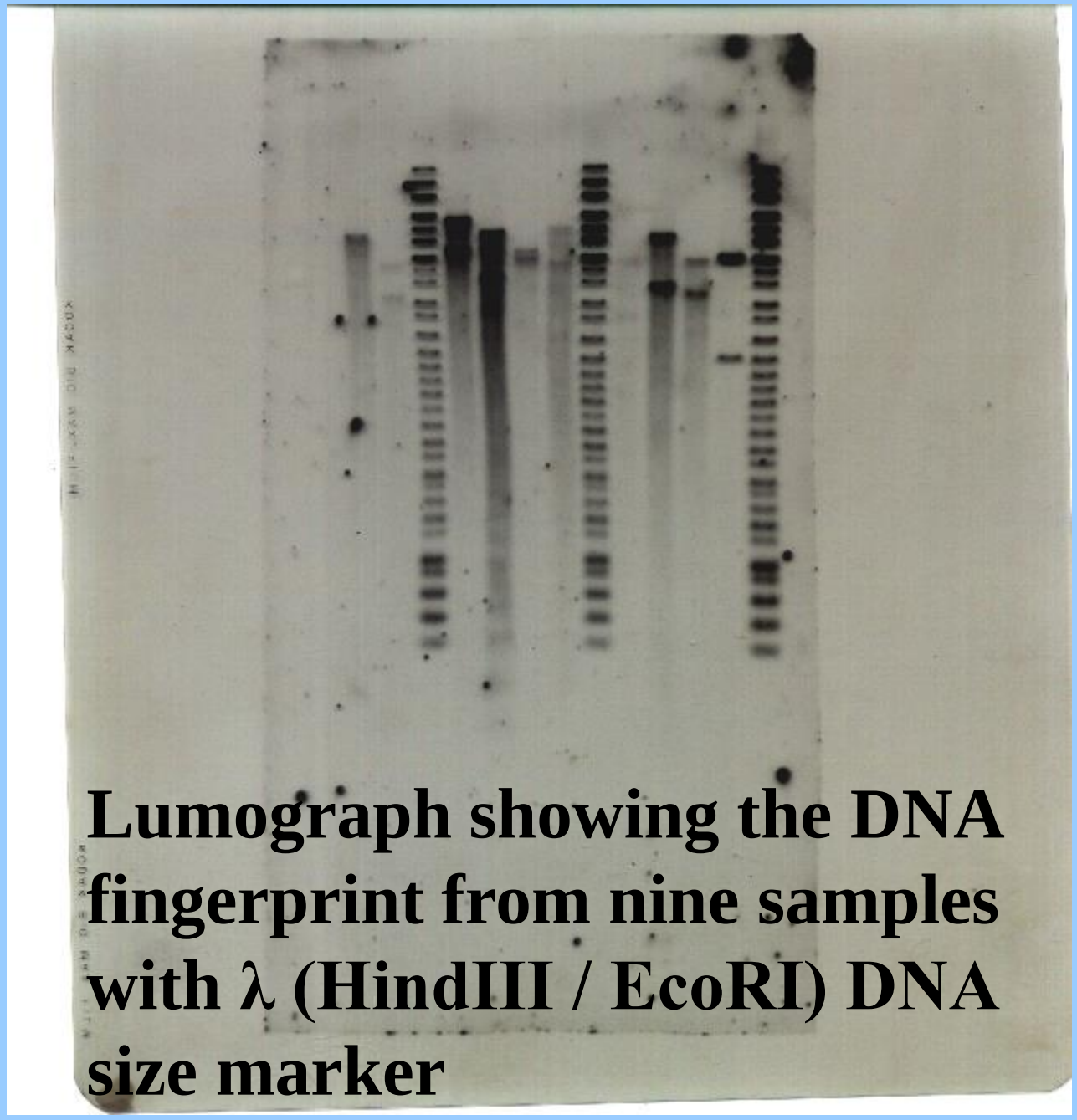

LABELED PROBE COVERING A PORTION OF THE DNA
DNA FRAGMENTS
HYBRIDIZE DNA BOUND TO MEMBRANE
LIGHT UP PROBE BOUND TO THE DNA IT HYBRIDIZED TO
BLUE DRESS DNA
B.C.s DNA
LARGE
SMALL
TOTAL DNAs ON MEMBRANE


BLUE DRESS DNA
B.C.s DNA
A
DNA FRAGMENTS
B
DNA FRAGMENTS
C
DNA FRAGMENTS
LARGE DNA
SMALL DNA


THE DNA FINGERPRINTING PROCESS
1. Blood sample
2. DNA is extracted from blood cells
3. DNA is cut into fragments by a restriction enzyme
4.The DNA fragments are separated into bands during electrophoresis in an agarose gel
5. The DNA band pattern in the gel is transferred to a technique known as Southern Blotting
6. The radioactive DNA probe is prepared
7. The DNA probe binds to specific DNA sequences on the membrane
8. Excess DNA probe is washed off
9. At this stage the radioactive probe is bound to the DNA pattern on the membrane
10. X-Ray film is placed next to the membrane to detect the radioactive pattern
11. The X-Ray film is developed to make visible the pattern of bands which is knowns as a DNA FINGERPRINT

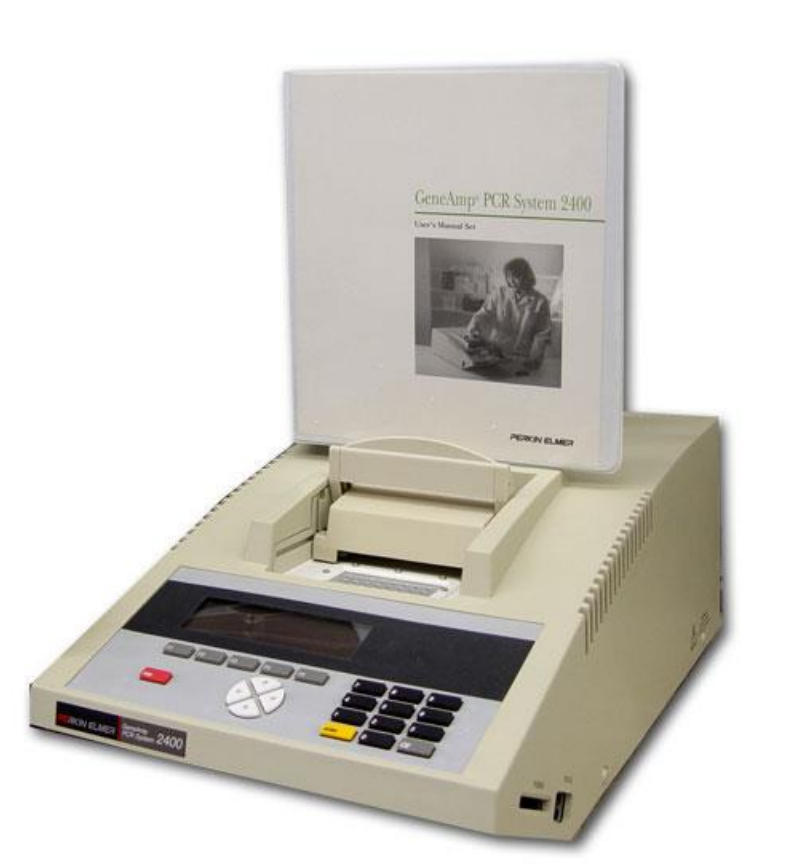
Limitations:

RFLP is a powerful but relatively insensitive technique, cannot be applied to degraded specimens, and is tedious and time consuming, taking about 6 weeks. More recently, to avoid the precautions needed to handle radioactive samples and to speed processing time, other labeling systems have been adopted, including chemiluminescent and fluorescent methods.



Lumograph showing the DNA fingerprint from nine samples with λ (HindIII / EcoRI) DNA size marker

Second type of forensic DNA test uses PCR based DNA analysis

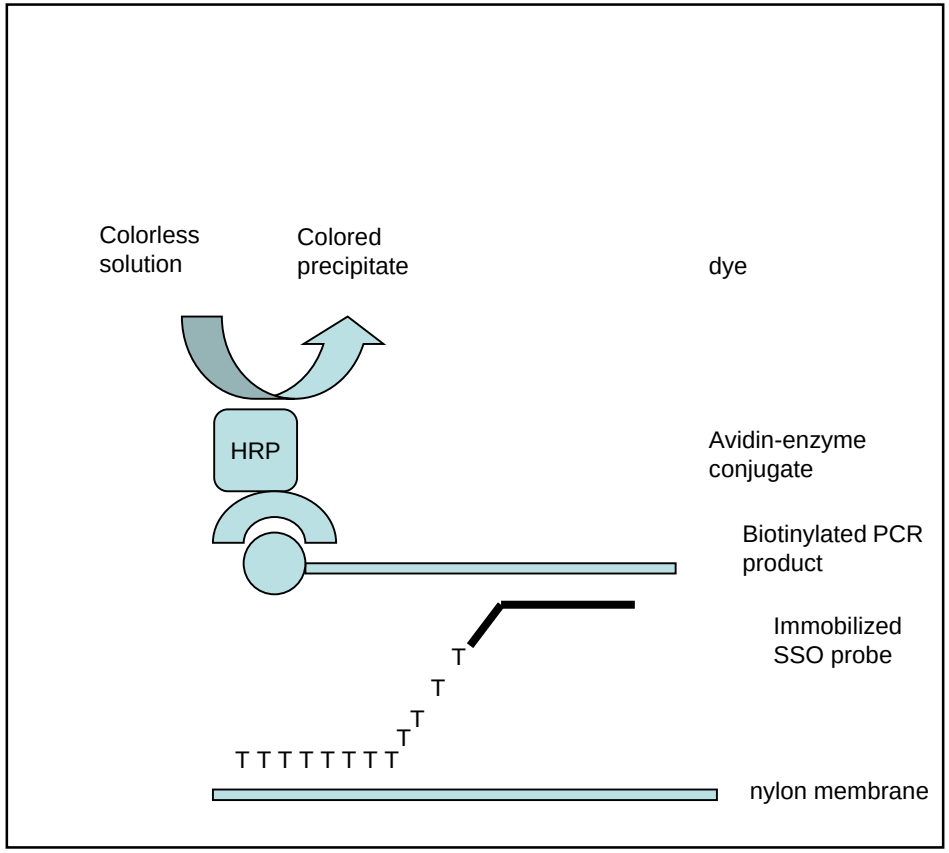


PCR Machine Gene AMP-2400
Applied Biosystem

Second type of forensic DNA test to be widely used by crime laboratories was PCR based Reverse Dot blot of HLADQA1 + PM

Characteristics of HLA-DQA1 And PM loci

	HLA-DQA1	LDLR	GYPA	HBGG	D7S8	GC
Chromosomal location	6p21.3	19p13.1-13.3	4q28-31	11p15.5	7q22-31.1	4q11-13
PCR Product Size (bp)	239/242	214	190	172	151	138
Number of allele	7	2	2	3	2	3


Colorless solution
Colored precipitate
dye
HRP
Avidin-enzyme conjugate
Biotinylated PCR product
Immobilized SSO probe
T
T
T
T
T T T T T T T T
nylon membrane

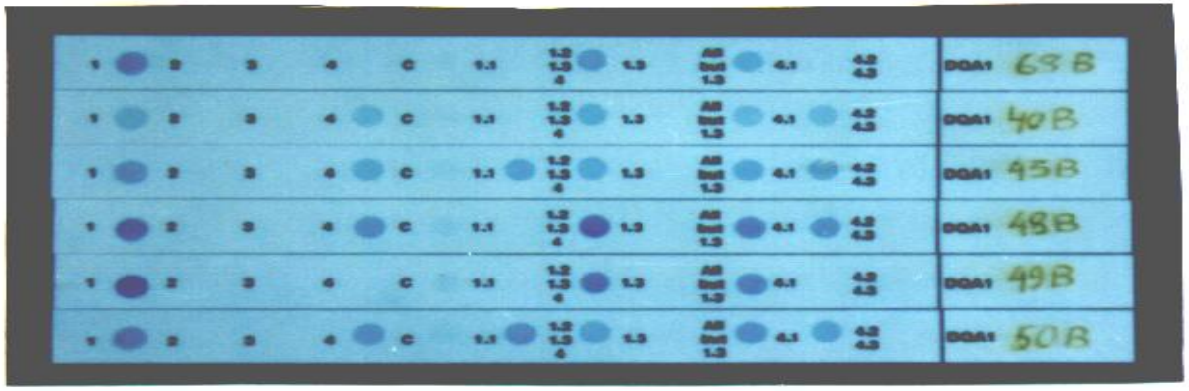


Fig.9.8 Showing Photograph of HLA-DQA1 loci of six different samples

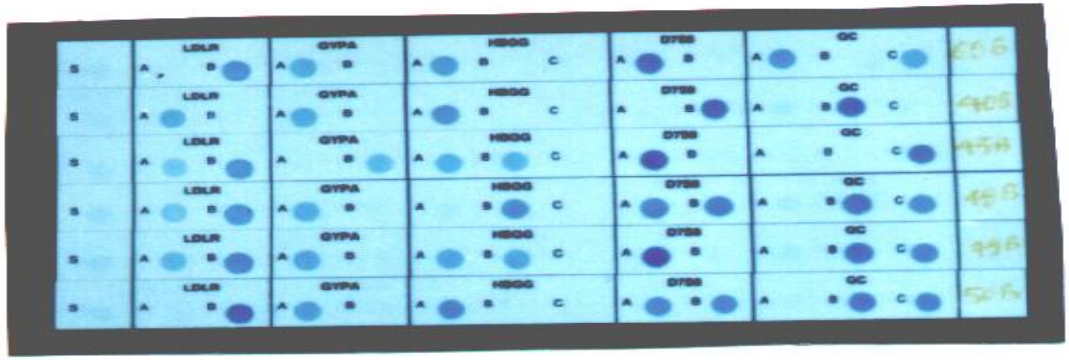


Fig.9.9 Showing Photograph of Poly Markers(LDLR,GYPA,HBGG,D7S* and GC) loci of six different samples

At present PCR based STRs a highly polymorphism loci is used for Human Identification

For Example :

In Manual sequencer

- **CSF1PO,TPOX,THO1**
- **F13A01,FESFPS,vWA**
- **D16S535,D7S820,D13S317**

Briefly PCR Base STRs process involves

- **Amplification simultaneously using multiplex PCR, using different primers simultaneously in same reaction mixture**
- **Separate the PCR Product in Denaturing PAGE**
- **Detection of DNA profile by Silver staining**

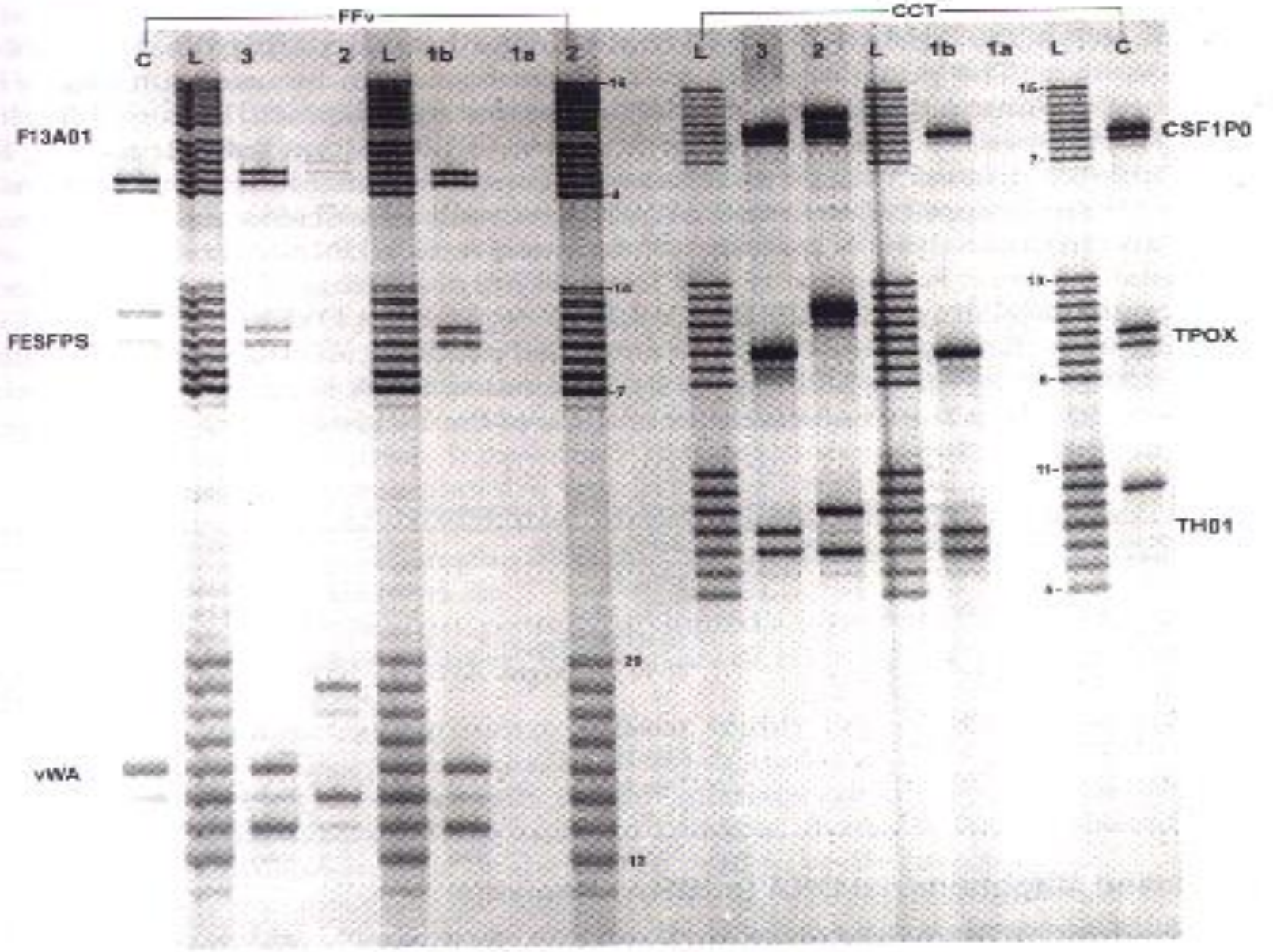


Fig. 10.10: Showing photograph of FFv (F13A01, FESFPS, vWA) and CTT (CSF1P0, TP0X, TH01) multiplex STRs separated on 4% polyacrylamide denaturing gel.
C = Control, L = Ladder, 2 = Victim blood, 3 = suspect blood, and 1a &1b = blood stain on victims cloths.

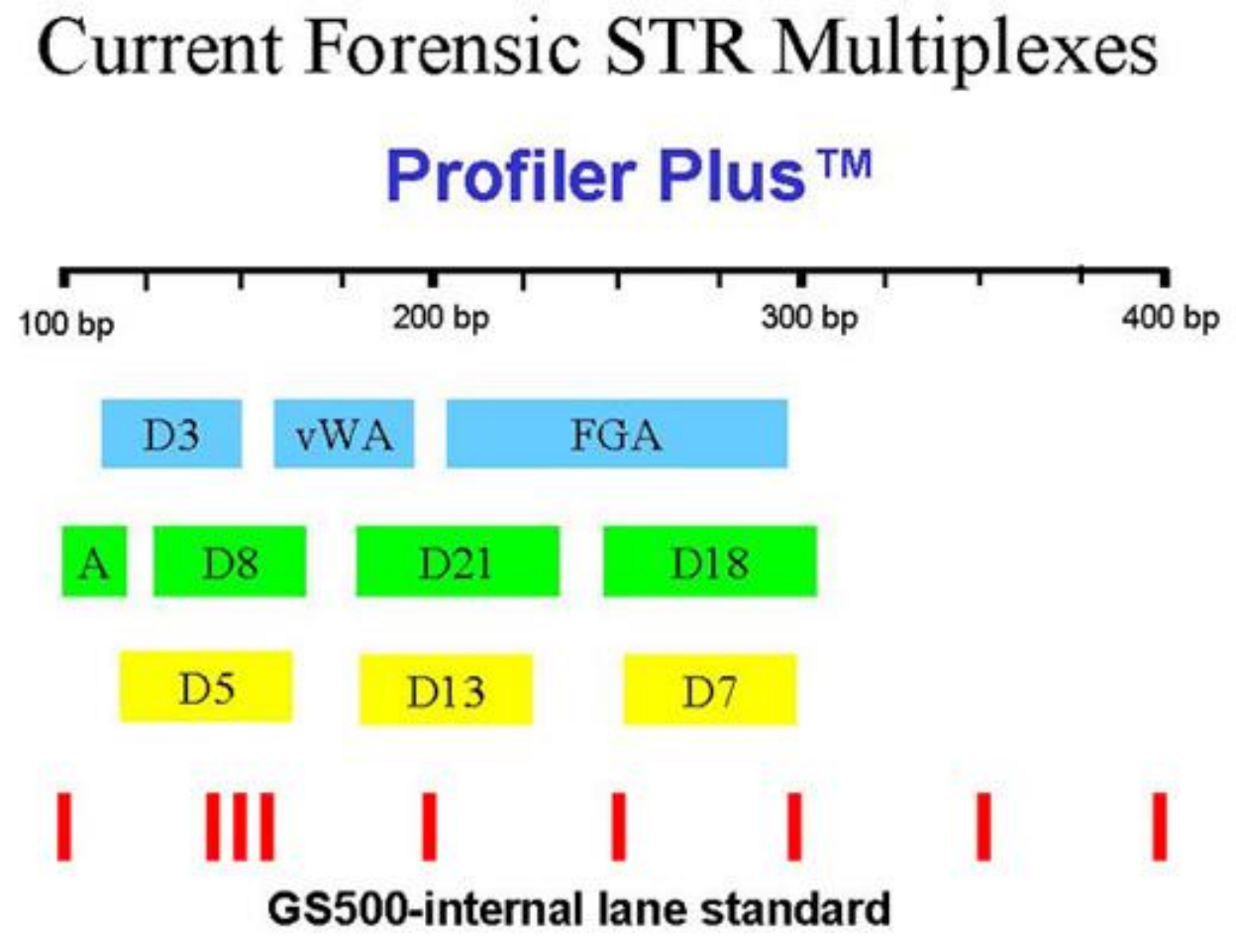
The Forensic Community has found that smaller sets of fragments, STRs, are preferable for several technical reasons.

- **The technique of using STR is easier**
- **Faster than RFLP**
- **The Small size of STR loci improves the chance of obtaining a result, particularly for samples containing minute amount of DNA and/ or Degraded DNA.**
- **The small size range of STR loci makes them ideal candidates for co-amplification while keeping all amplified alleles smaller than 350 base pairs. Many STR loci can therefore be typed from a single PCR**

- **STR alleles have discrete sizes, allowing for simplified interpretation of results.**
- **PCR based tests are rapid, giving results in 24 hours or less**
- **PCR-based tests are easy to standardize and automate, ensuring reproducible results**
- **At present fully Automated DNA Sequencer, Applied Biosystems 3130xl, is a validated for forensic use.**
- **AmpFLSTR® Identifiler is a 15 STR loci and the amelogenin gender determining marker valideted for Forensic use.**

ABI-310 automated DNA Sequencer

ABI-310 Automated DNA Sequencer



The schematic diagram illustrates the fluorescent dye label color and relative PCR product size ranges for the 9 STR loci and Amelogenin present in this particular kit.

AmpFℓ STR Systems for DNA Databases

Thirteen STR Loci Amplified in Two PCR Reactions

Forensic laboratories in North America recently established the 13 core

STR loci that will comprise the Combined DNA Index System (CODIS)

convicted offender database. The AmpFℓ STR COfiler™ PCR

Amplification Kit is designed to be used in conjunction with the

AmpFℓ STR Profiler Plus™ PCR Amplification Kit to amplify the selected

13 STR loci in two PCR reactions. The AmpFℓ STR Profiler Plus kit

amplifies nine of the selected STR loci. The AmpFℓ STR COfiler kit

amplifies the four remaining STR loci as well as two loci (D3S1358,

D7S820) also found in the AmpFℓ STR Profiler Plus kit. The overlap of

the two STR loci and the amelogenin locus provides laboratories with

the confidence that both reactions have amplified the same sample.

Table 1-1 Loci amplified by the AmpFℓSTR™ kits

AmpFℓSTR Loci	AmpFℓSTR Profiler Plus Kit	AmpFℓSTR COfiler Kit
D3S1358	X	X
vWA	X	
FGA	X	
Amelogenin	X	X
D8S1179	X	
D21S11	X	
D18S51	X	
D5S818	X	
D13S317	X	
D7S820	X	X
TH01		X
TPOX		X
CSF1PO		X
D16S539		X

TABLE 1. THE AmpFℓSTR® IDENTIFILER® KIT LOCI

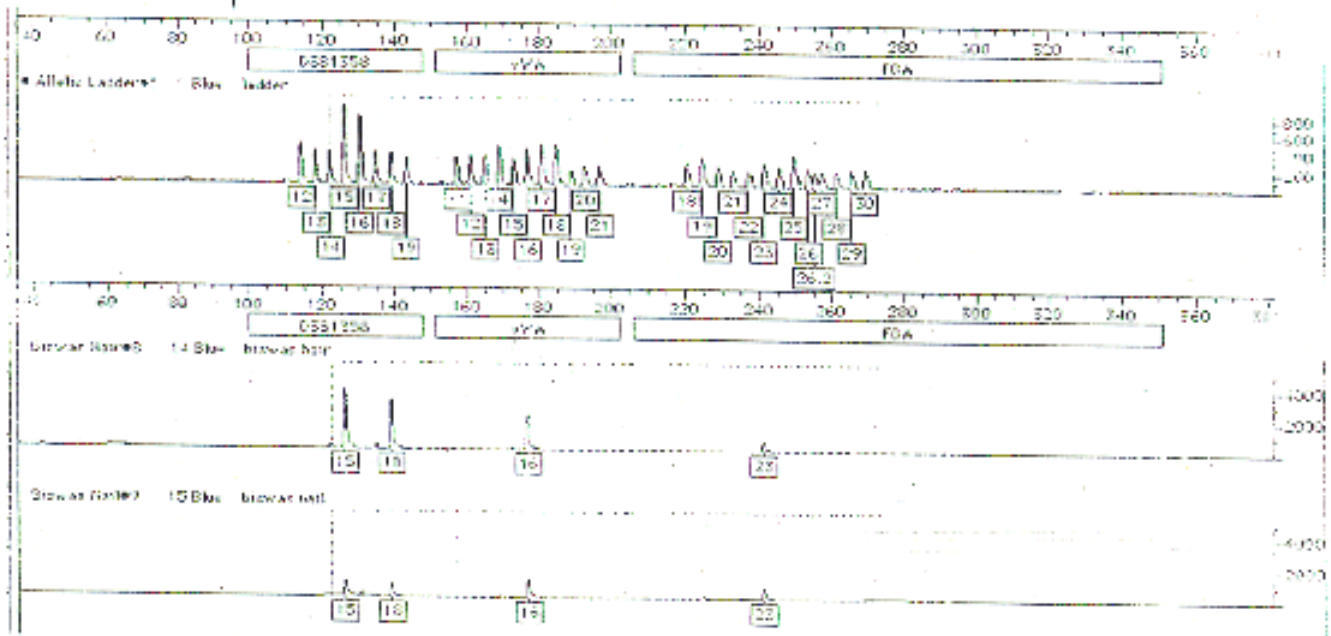
Locus Designation	Chromosome Location	Alleles Included in Identifiler® Allelic Ladder	Dye Label
D8S1179	8	8-19	6-FAM™
D21S11	21q11.2-q21	24, 24.2, 25-28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36-38	
D7S820	7q11.21-22	6-15	
CSF1PO	5q33.3-34	6-15	
D3S1358	3p	12-19	VIC®
TH01	11p15.5	4-9, 9.3, 10, 11, 13.3	
D13S317	13q22-31	8-15	
D16S539	16q24-qter	5, 8-15	
D2S1338	2q35-37.1	15-28	
D19S433	19q12-13.1	9-12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2	NED™
vWA	12p12-pter	11-24	
TPOX	2p23-2per	6-13	
D18S51	18q21.3	7, 9, 10, 10.2, 11-13, 13.2, 14, 14.2, 15-27	
Amelogenin	X: p22.1-22.3 Y: p11.2	X, Y	PET®
D5S818	5q21-31	7-18	
FGA	4q28	17-26, 26.2, 27-30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2	

* All STR loci included in the AmpFℓSTR® Identifiler® PCR Amplification Kit are co-amplified in a single PCR and analyzed simultaneously in a single gel-lane or capillary electrophoresis injection with ABI PRISM® systems.

TABLE 2. POPULATION GENETICS OF THE AmpFlSTR® IDENTIFILER® KIT LOCI

Population	Average Probability of Identity	Power of Discrimination	Average Probability of Paternity Exclusion
African American n = 367	1.31 x 10^{-19}	1 in 7.64 x 10^{18}	0.9999996
US Caucasian n = 349	5.01 x 10^{-20}	1 in 2.00 x 10^{19}	0.9999992
US Hispanic n = 290	7.65 x 10^{-21}	1 in 1.31 x 10^{20}	0.9999990
Native American n = 191	3.62 x 10^{-21}	1 in 2.76 x 10^{20}	0.9999627

Profiler Plus Loci

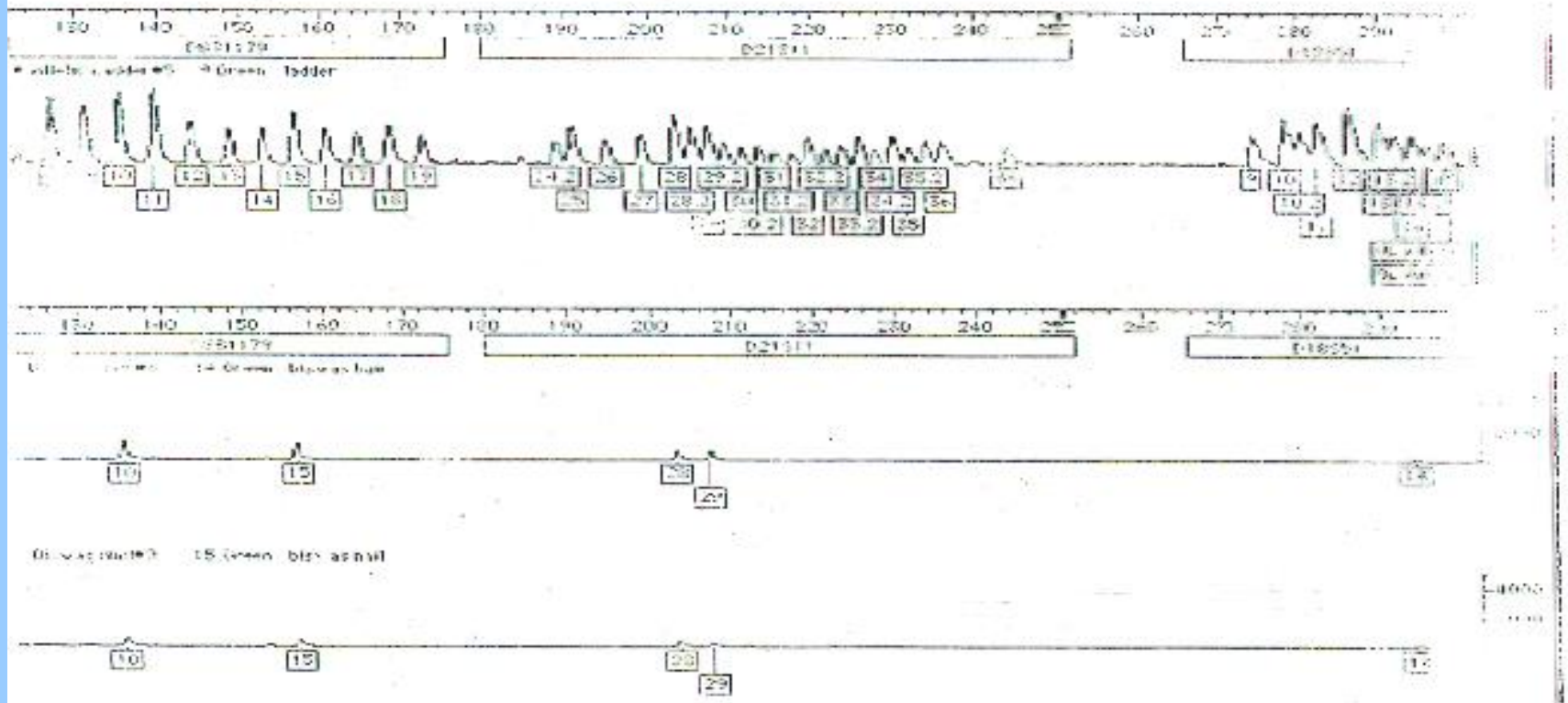


Biswas Genetic ID for Blue

D3S1358	vWA	FGA
15,18	16	23

Bioystems

Profiler Plus Loci



Biswas Genetic ID for Green

D8S1179	D21S11	D16S51
13	28,29	11

Biosystems

Table 9.3: Characteristic of some Y-chromosome STRs Loci.

Name of locus	Chromosome location	Allele	size.
DYS19	Y	10 to19	174 to 210
DYS388	Y	11 to 17	125 to 143
DYS389	Y	10 to 16(Fragm. C+D)	239 to 263
		26 to 34(Fragm. A+D)	355to 387 (with F1 & R1) 288 to 320 (with F2 & R2)
DYS390	Y	18 to 27	191 to 227
DYS391	Y	8 to 13	275 to 295
DYS392	Y	7 to 16	236 to 263
DYS393	Y	11 to 15	115 to 131

Primers for mtDNA amplification

Primer for Hypervariable Region 1		
A1	(L 15997)	5'-CAC CAT TAG CAC CCA AAG CT- 3'
B2	(H 6236)	5'-CTT TGG AGT TGC AGT TGA TG- 3'
A2	(L 16159)	5'-TAC TTG ACC ACC TGT AGT AC- 3'
B1	(H16391	5'-GAG GAT GGT GGT CAA GGG AC-3'
Primer for Hypervariable Region 2		
C1	(L048)	5'-CTT ACG GGA GCT CTC CAT GC- 3'
D2	(H285)	5'-GGG GTT TGG TGG AAA TTT TTT TG- 3'
C2	(L172)	5'-ATT ATT TAT CGC ACC TAC GT- 3'
D1	(H408)	5'- CTG TTA AAA GTG CAT ACC GCC A-3'

Primer sets used for amplification of different mtDNA

Region	Primer Set
HV1	A1,B1
HV2	C1,D1
HV1A	A1,B2
HV1B	A2,B1
HV2A	C1,D2
HV2B	C2,D1

General GUIDELINES TO minimize contamination

Do:

- Always wear clean, disposable gloves, disposable surgical mask, disposable surgical head cover and laboratory coat while handling DNA evidence at all stage.
- Handle evidence carefully.

DON'T:

- Do not eat, drink, spit and avoid sneezing while handling with DNA evidence at any stage.
- Do not put your finger inside nostril.
- Do not itch.

THANKS