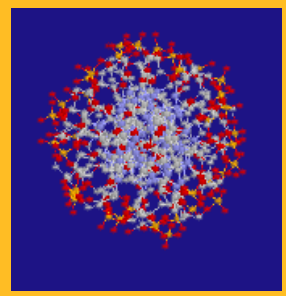
DNA PROFILING



All photos and data used in this slide show are staged; no person’s identifiable DNA is presented

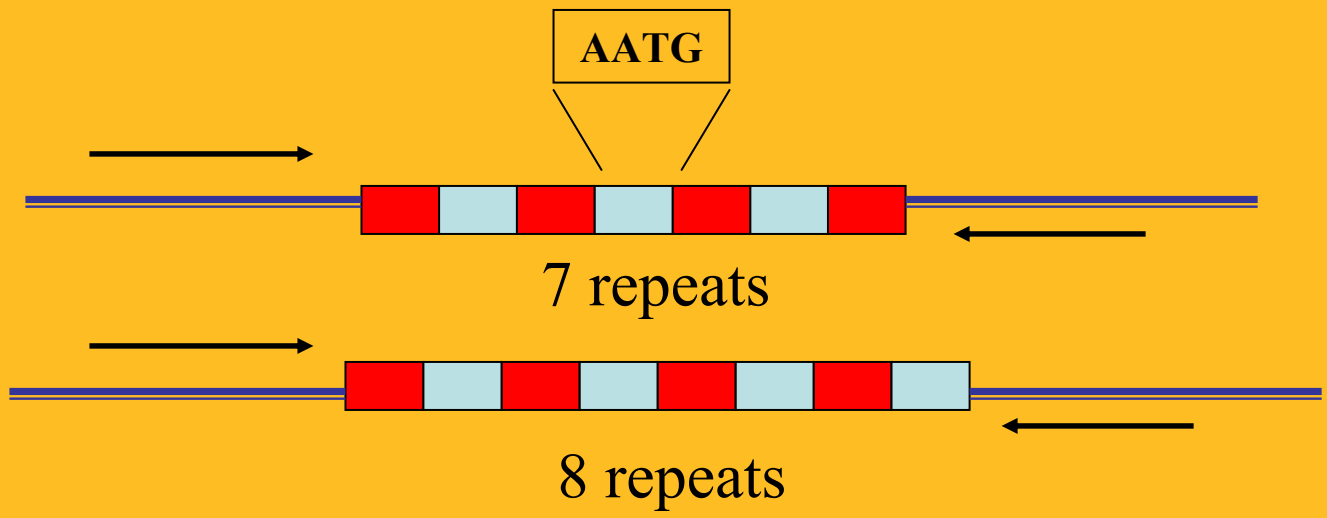
What is DNA Profiling?

- It is a method of identifying an individual by unique characteristics of that person's DNA

What is Analyzed in the DNA?

- DNA profiling depends on regions of non-coding DNA that show great variability between individuals (are polymorphic which means *many forms)*
- Modern profiling uses Short Tandem Repeats, STRs
- These are short sequences of DNA, usually 2-5 base pairs (bp) long, that repeat, or ‘stutter’ many times

Short Tandem Repeats (STRs)



the repeat region is variable between samples while the flanking regions where PCR primers bind are constant

Homozygote = both alleles are the same length

Heterozygote = alleles differ and can be resolved from one another

An Example of a STR in locus D7S280

- D7S280 is a region (locus) of human chromosome 7. Its DNA sequence, as obtained from GenBank (a public DNA database) is:

```
  1 aatttttgta ttttttttag agacggggtt tcaccatgtt ggtcaggctg actatggagt
 61 tattttaagg ttaatatata taaagggtat gatagaacac ttgtcatagt ttagaacgaa
121 ctaacgatag atagatagat agatagatag atagatagat agatagatag atagacagat
181 tgatagtttt tttttatctc actaaatagt ctatagtaaa catttaatta ccaatatttg
241 gtgcaattct gtcaatgagg ataaatgtgg aatcgttata attcttaaga atatatattc
301 cctctgagtt tttgatacct cagattttaa ggcc
```

- The STR repeat sequence is gata
- Different alleles of this locus have from 6 to 15 tandem repeats of the ‘gata’ sequence

New Technology

- STR analysis has largely replaced the original RFLP analysis (DNA Fingerprinting) developed in 1985 by Dr Alec Jeffreys
- RFLP analysis requires good amounts of non-degraded DNA but STR analysis can be done on less than one billionth of a gram (a nanogram) of DNA (as in a single flake of dandruff)

A Historical Perspective

- In the course of his research on variability in human DNA, Alec Jeffreys developed a method of forensic DNA typing.
- This method, termed 'DNA Fingerprinting', was used for the first time to solve two rape/murder cases in the UK in 1987.
- Jeffreys was knighted in 1994 for Services to Science, and has been the recipient of numerous other honours

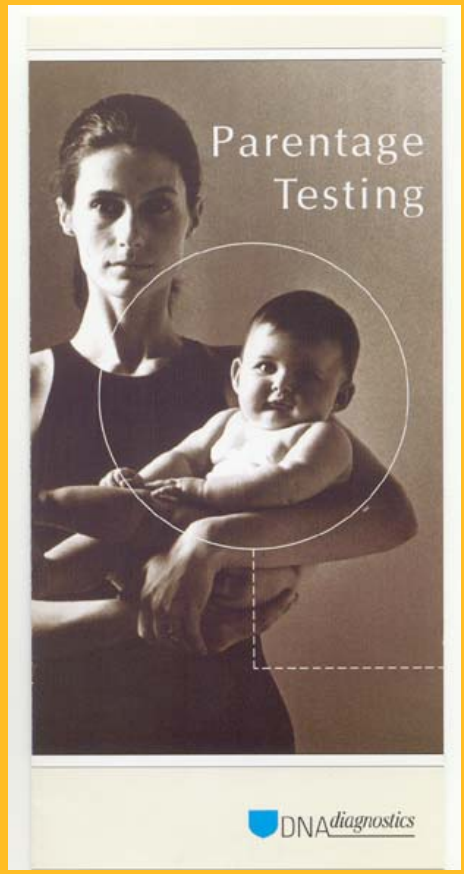
DNA Fingerprinting & DNA Profiling - same or different?

- DNA fingerprinting, as developed by Sir Alec Jeffries, targeted particular repeating sequences of DNA (9-80 bp long) found at a number of loci (multilocus). Jeffries described the pattern produced in a "fingerprint" as unique to an individual. Technology at the time (1985) required good DNA samples and took 1 - 2 weeks for a result.
- Advances in technology have led to DNA profiling, using smaller short tandem repeats (STRs) also from a number of loci. The smaller STRs are more likely to survive DNA degradation, use less DNA (because of PCR technology), and can be processed within 24 hours.

Some uses of DNA Profiling

- Forensic work on crime scenes
- Parentage testing (explored in more detail)
- Victim identification in mass disasters
- Animal identification- e.g. racehorses
- Conservation biology and evolutionary studies

Parentage Testing as conducted at DNA Diagnostics, Auckland



Why Test?

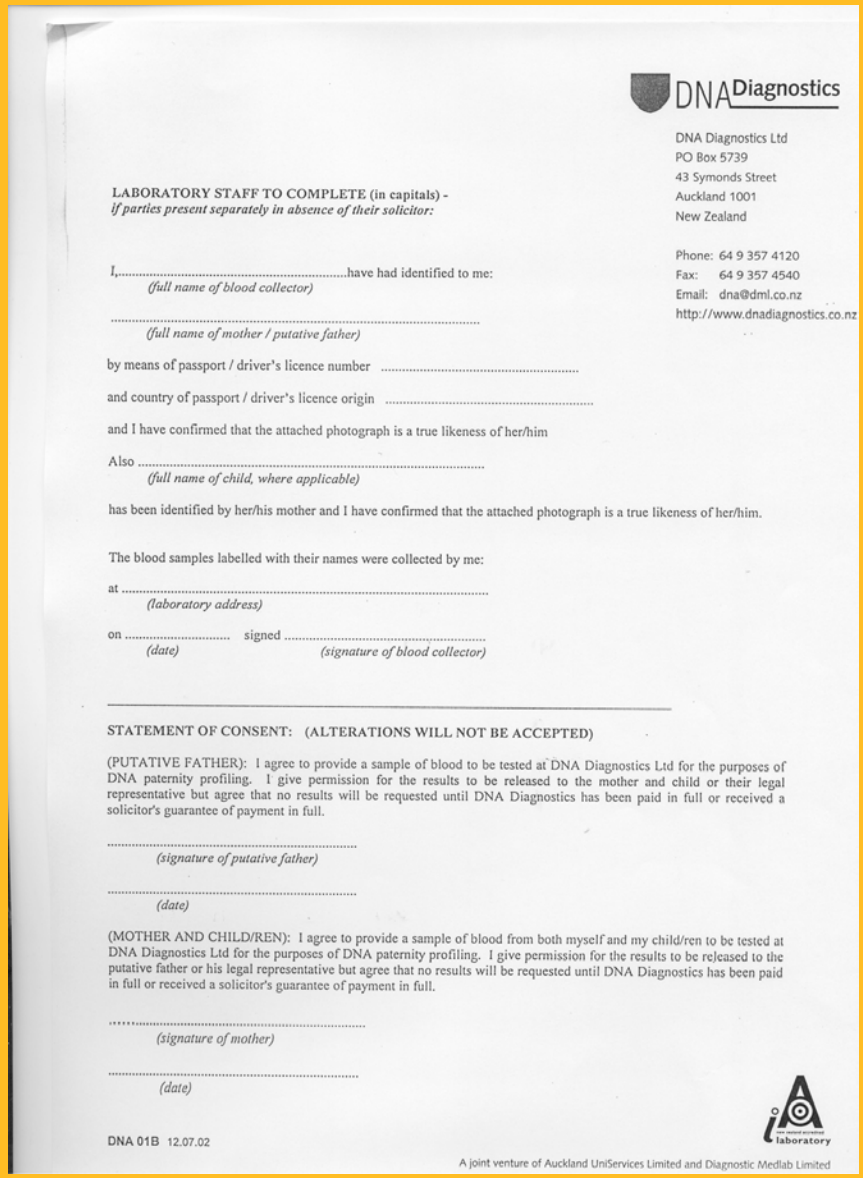
- Parentage - e.g. disputes over who is the father of a child & is thus responsible for child support
- Determining whether twins are identical or fraternal
- Estate cases (these may involve obtaining pathology samples of deceased individuals)
- Immigration - establishing that individuals are the true children/parents/siblings in cases of family reunification

Why Test? ctd

- Bone marrow transplant monitoring - to check that the transplanted marrow is still present
- Determination of maternal cell contamination in chorionic villus sampling (used to investigate the possibility that a fetus has a severe inherited disease)- is the tissue sample really fetal?
- Etc.

The Steps

- Identification is established, by photo ID or by identification by a legal representative
- A consent form is signed and witnessed
- A case number is assigned



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LABORATORY STAFF TO COMPLETE (in capitals) -
if parties present separately in absence of their solicitor:

I,.....have had identified to me:
(full name of blood collector)

.....
(full name of mother / putative father)

by means of passport / driver's licence number .....

and country of passport / driver's licence origin .....

and I have confirmed that the attached photograph is a true likeness of her/him

Also .....
(full name of child, where applicable)

has been identified by her/his mother and I have confirmed that the attached photograph is a true likeness of her/him.

The blood samples labelled with their names were collected by me:

at .....
(laboratory address)

on ..... signed .....
(date) *(signature of blood collector)*

STATEMENT OF CONSENT: (ALTERATIONS WILL NOT BE ACCEPTED)

(PUTATIVE FATHER): I agree to provide a sample of blood to be tested at DNA Diagnostics Ltd for the purposes of DNA paternity profiling. I give permission for the results to be released to the mother and child or their legal representative but agree that no results will be requested until DNA Diagnostics has been paid in full or received a solicitor's guarantee of payment in full.

.....
(signature of putative father)

.....
(date)

(MOTHER AND CHILD/REN): I agree to provide a sample of blood from both myself and my child/ren to be tested at DNA Diagnostics Ltd for the purposes of DNA paternity profiling. I give permission for the results to be released to the putative father or his legal representative but agree that no results will be requested until DNA Diagnostics has been paid in full or received a solicitor's guarantee of payment in full.

.....
(signature of mother)

.....
(date)

DNA 01B 12.07.02

laboratory

A joint venture of Auckland UniServices Limited and Diagnostic Medlab Limited

The Steps, II

- DNA samples are collected- in the case of parentage testing, from the mother, child and putative (possible) father(s)
- They are usually blood, but a buccal (cheek cell) swab is acceptable

The Steps, III

- If the samples need transport they must be sent in leak proof containers for the courier’s safety.

The Steps, IV

- The samples are processed, and DNA is extracted from each
- Primers for each locus are added. Each primer is labeled with a fluorescent marker

The Steps, IV, ctd

- DNA Diagnostics currently uses an AmpFlSTR Identifiler ™ PCR Amplification Kit which targets 15 STR regions plus a sex specific region.
- Kits allow standardization and accuracy, as DNA samples are added to a pre-made mix

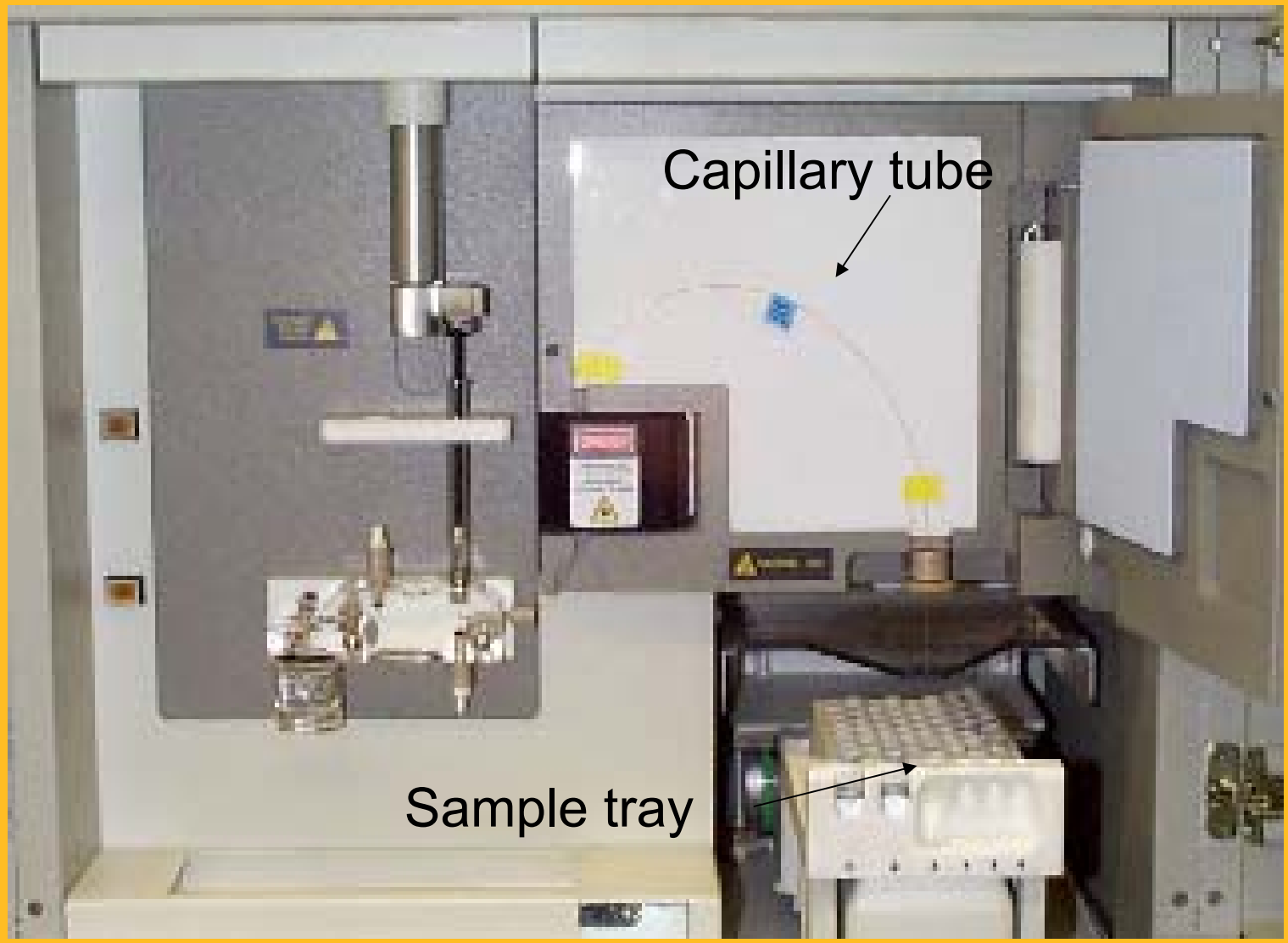
The Steps, V

- The DNA and fluorescent primers are run through the polymerase chain reaction (PCR) to amplify the targeted STR regions on the DNA
- The samples are audited continually to ensure accuracy

The Steps, VI

- The amplified DNA in a sample is separated by electrophoresis in a genetic analyzer
- The analyzer has a gel-filled capillary tube through which the DNA travels (this replaces the gel slab of earlier days)
- DNA fragments move through the gel tube by size, smallest first
- A laser reads the fluorescent marked DNA loci

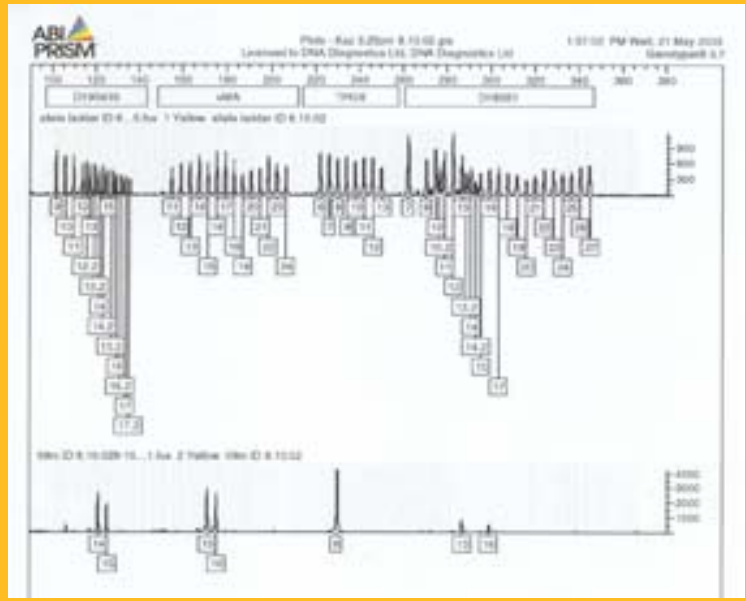
An ABI Prism 310 Genetic Analyser



Note-other models of this Analyzer have more capillary tubes and can process more samples at a time, but this model is sufficient for the demand for testing to date through DNA Diagnostics

Analyzing the Read-out

- Digital output from the Analyzer is read and interpreted by genotyping software
- Each STR region read has two peaks, for the regions (loci) on an individual's maternal and paternal chromosomes with that locus. *note - if both regions are the same length, there is one peak*
- Data is shown both graphically and numerically



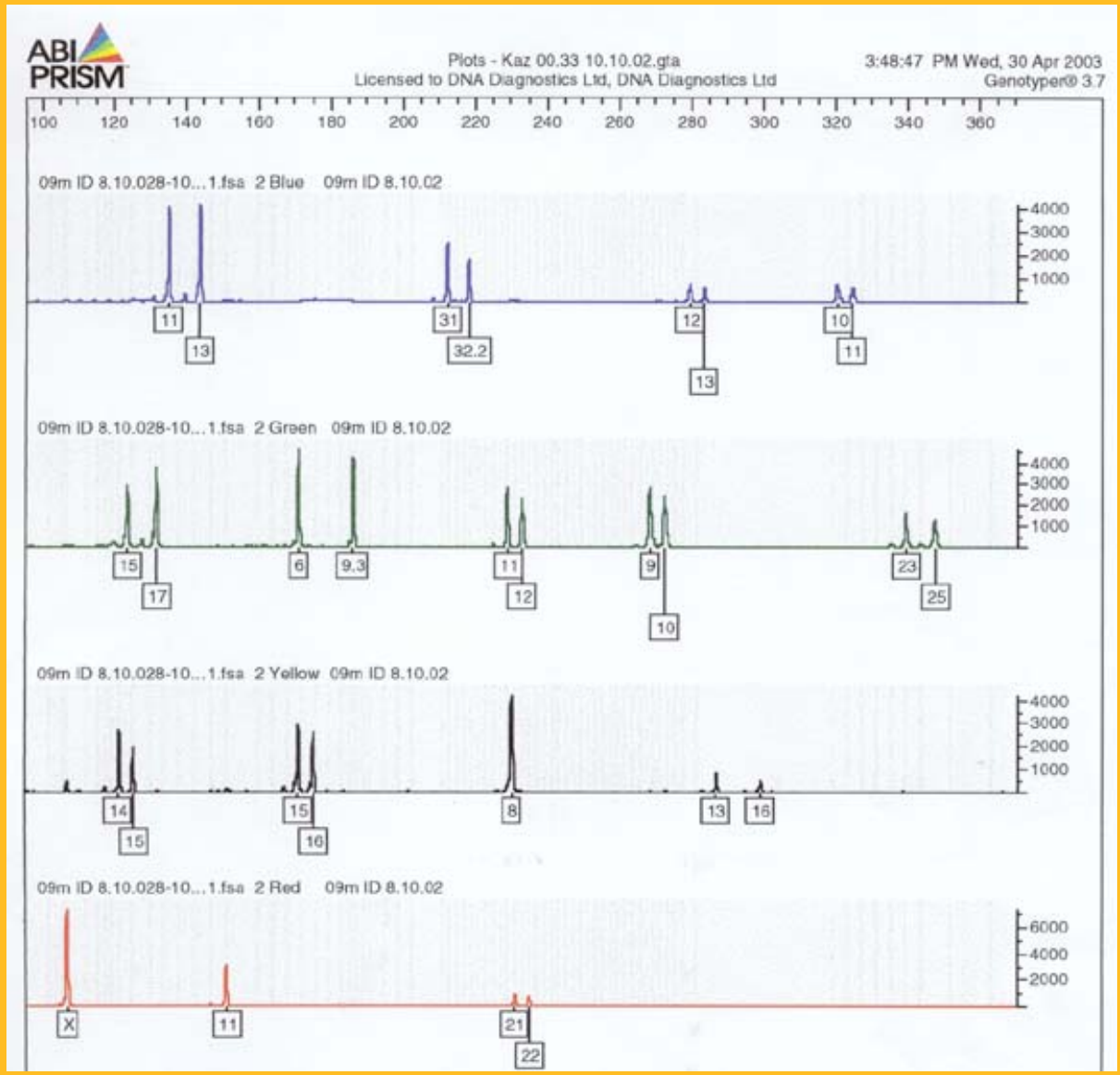
A sample showing 4 loci- The top line is a 'ladder' for comparison

Locus D19S433 = 14,15

Locus vWA = 15,16

Locus TPOX= 8,8

Locus D18S51= 13,16



A sample print -out for one person, showing all loci tested. Different colours help with interpretation

Whose STR?

- A child will inherit one of the STRs at each locus from its mother, and since usually in parentage tests these are determined, then by elimination the other STRs at each locus come from its father
- The father can donate either of his two STRs at each locus
- If a child has STRs different from those of the putative father, then that man can be eliminated as a possible father
- If a child has a particular STR that is the same as the putative father, it is necessary to examine possible matches with other STR loci and examine probability in Parentage Analysis

Parentage Analysis

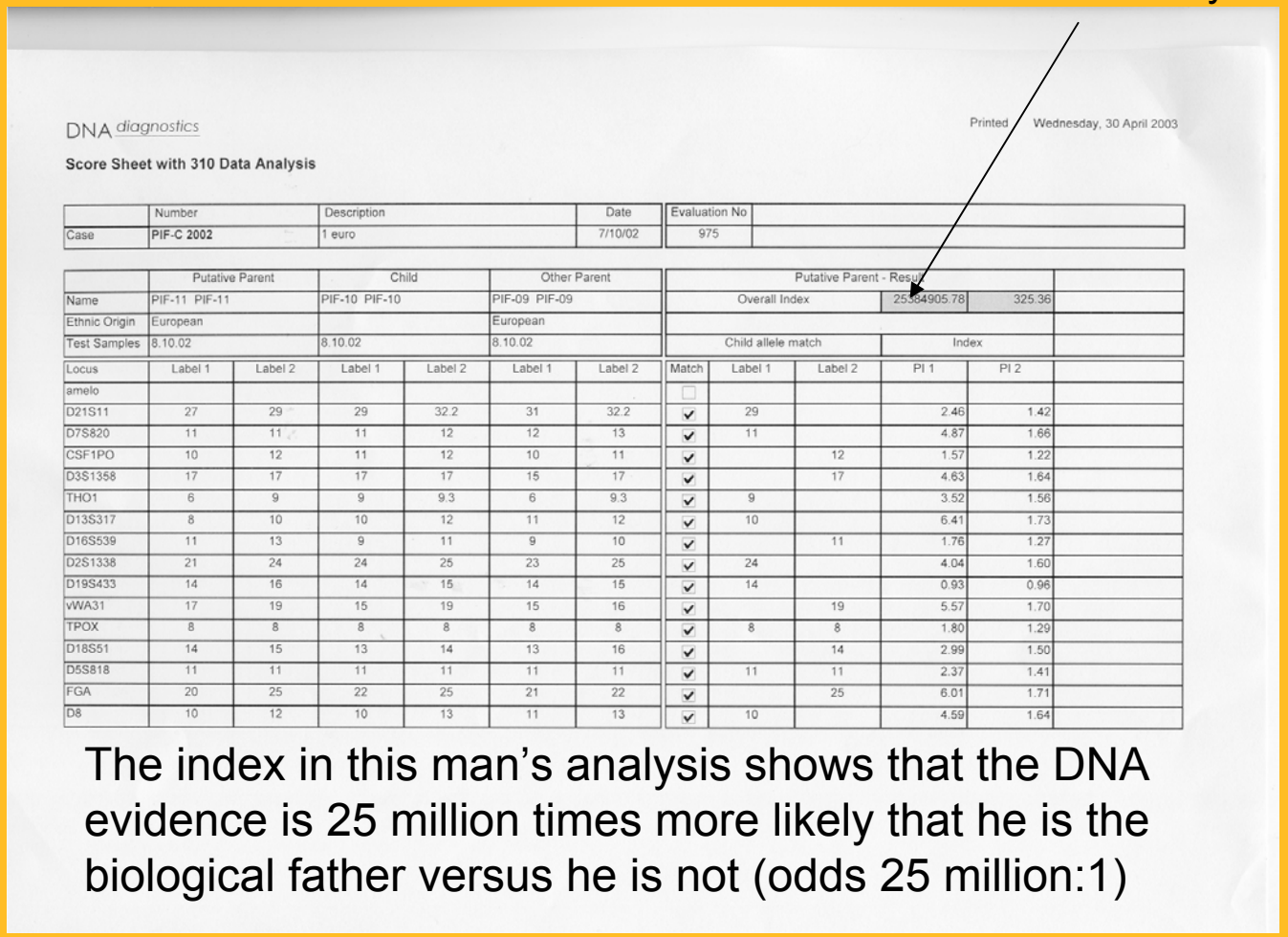
- For each STR tested, the data obtained is used to calculate a paternity index (the probability of the evidence given that a particular man is the father versus he is not the father)
- This is based on the frequency in the population of the alleles at that locus
- In New Zealand there are databases for European, Maori/Cook Islander, Asian and Tongan/Samoan. International databases are used for other ethnicities

Analysis II

- Each STR site index is an independent event, so using probability law that says "the probability that two independent events may happen together is the product of their individual probabilities", an overall paternity index is calculated by multiplying together the indices for each locus

Parentage Analysis II, ctd

Paternity index



DNA *diagnostics*

Printed Wednesday, 30 April 2003

Score Sheet with 310 Data Analysis

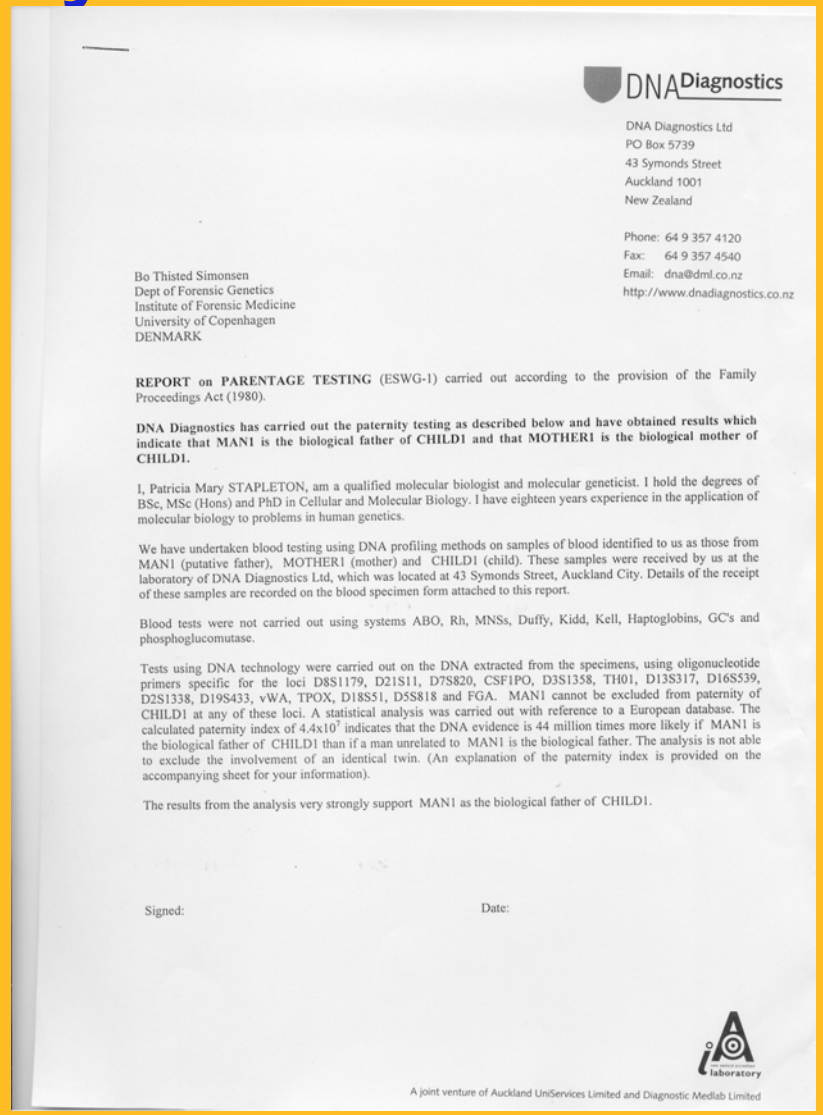
	Number	Description	Date	Evaluation No	
Case	PIF-C 2002	1 euro	7/10/02	975	

	Putative Parent		Child		Other Parent		Putative Parent - Result				
Name	PIF-11 PIF-11		PIF-10 PIF-10		PIF-09 PIF-09		Overall Index			25384905.78	325.36
Ethnic Origin	European				European						
Test Samples	8.10.02		8.10.02		8.10.02		Child allele match			Index	
Locus	Label 1	Label 2	Label 1	Label 2	Label 1	Label 2	Match	Label 1	Label 2	PI 1	PI 2
amelo							☐				
D21S11	27	29	29	32.2	31	32.2	☑	29		2.46	1.42
D7S820	11	11	11	12	12	13	☑	11		4.87	1.66
CSF1PO	10	12	11	12	10	11	☑		12	1.57	1.22
D3S1358	17	17	17	17	15	17	☑		17	4.63	1.64
THO1	6	9	9	9.3	6	9.3	☑	9		3.52	1.56
D13S317	8	10	10	12	11	12	☑	10		6.41	1.73
D16S539	11	13	9	11	9	10	☑		11	1.76	1.27
D2S1338	21	24	24	25	23	25	☑	24		4.04	1.60
D19S433	14	16	14	15	14	15	☑	14		0.93	0.96
vWA31	17	19	15	19	15	16	☑		19	5.57	1.70
TPOX	8	8	8	8	8	8	☑	8	8	1.80	1.29
D18S51	14	15	13	14	13	16	☑		14	2.99	1.50
D5S818	11	11	11	11	11	11	☑	11	11	2.37	1.41
FGA	20	25	22	25	21	22	☑		25	6.01	1.71
D8	10	12	10	13	11	13	☑	10		4.59	1.64

The index in this man's analysis shows that the DNA evidence is 25 million times more likely that he is the biological father versus he is not (odds 25 million:1)

Finally

- Further auditing and cross-checking is done to ensure accuracy
- Parentage testing results in a report that is sent to all parties tested



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REPORT on PARENTAGE TESTING (ESWG-1) carried out according to the provision of the Family Proceedings Act (1980).

DNA Diagnostics has carried out the paternity testing as described below and have obtained results which indicate that MAN1 is the biological father of CHILD1 and that MOTHER1 is the biological mother of CHILD1.

I, Patricia Mary STAPLETON, am a qualified molecular biologist and molecular geneticist. I hold the degrees of BSc, MSc (Hons) and PhD in Cellular and Molecular Biology. I have eighteen years experience in the application of molecular biology to problems in human genetics.

We have undertaken blood testing using DNA profiling methods on samples of blood identified to us as those from MAN1 (putative father), MOTHER1 (mother) and CHILD1 (child). These samples were received by us at the laboratory of DNA Diagnostics Ltd, which was located at 43 Symonds Street, Auckland City. Details of the receipt of these samples are recorded on the blood specimen form attached to this report.

Blood tests were not carried out using systems ABO, Rh, MNSs, Duffy, Kidd, Kell, Haptoglobins, GC's and phosphoglucomutase.

Tests using DNA technology were carried out on the DNA extracted from the specimens, using oligonucleotide primers specific for the loci D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA. MAN1 cannot be excluded from paternity of CHILD1 at any of these loci. A statistical analysis was carried out with reference to a European database. The calculated paternity index of 4.4×10^7 indicates that the DNA evidence is 44 million times more likely if MAN1 is the biological father of CHILD1 than if a man unrelated to MAN1 is the biological father. The analysis is not able to exclude the involvement of an identical twin. (An explanation of the paternity index is provided on the accompanying sheet for your information).

The results from the analysis very strongly support MAN1 as the biological father of CHILD1.

Signed: Date:

laboratory

A joint venture of Auckland UniServices Limited and Diagnostic Medlab Limited

Cost?

- A standard Paternity/Maternity test for two or three people costs $1125 including GST in 2003, payable in advance
- If more than three persons are tested at one time, each additional person tested costs $250 + GST.
- These costs include blood collection and transport

Quality Control

- DNA Diagnostics participates in a number of quality assurance programmes to check that their protocols and technology meet international standards
- These include running reference samples, analyzing ‘unknown’ bloodstains, and participating in paternity testing workshops run by the International Society of forensic Genetics

Further Investigation

For further work on this topic, the University of Arizona Biology Project has an excellent activity, Blackett Family DNA2, www.biology.arizona.edu/human_bio/activities/Blackett2/overview.html

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- www.scientific.org/tutorials/articles/riley/riley.html
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For NCEA Biology A.S. 3.6

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