

DNA Profiling the mystery of the romanovs



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DNA PROFILING

Definition:

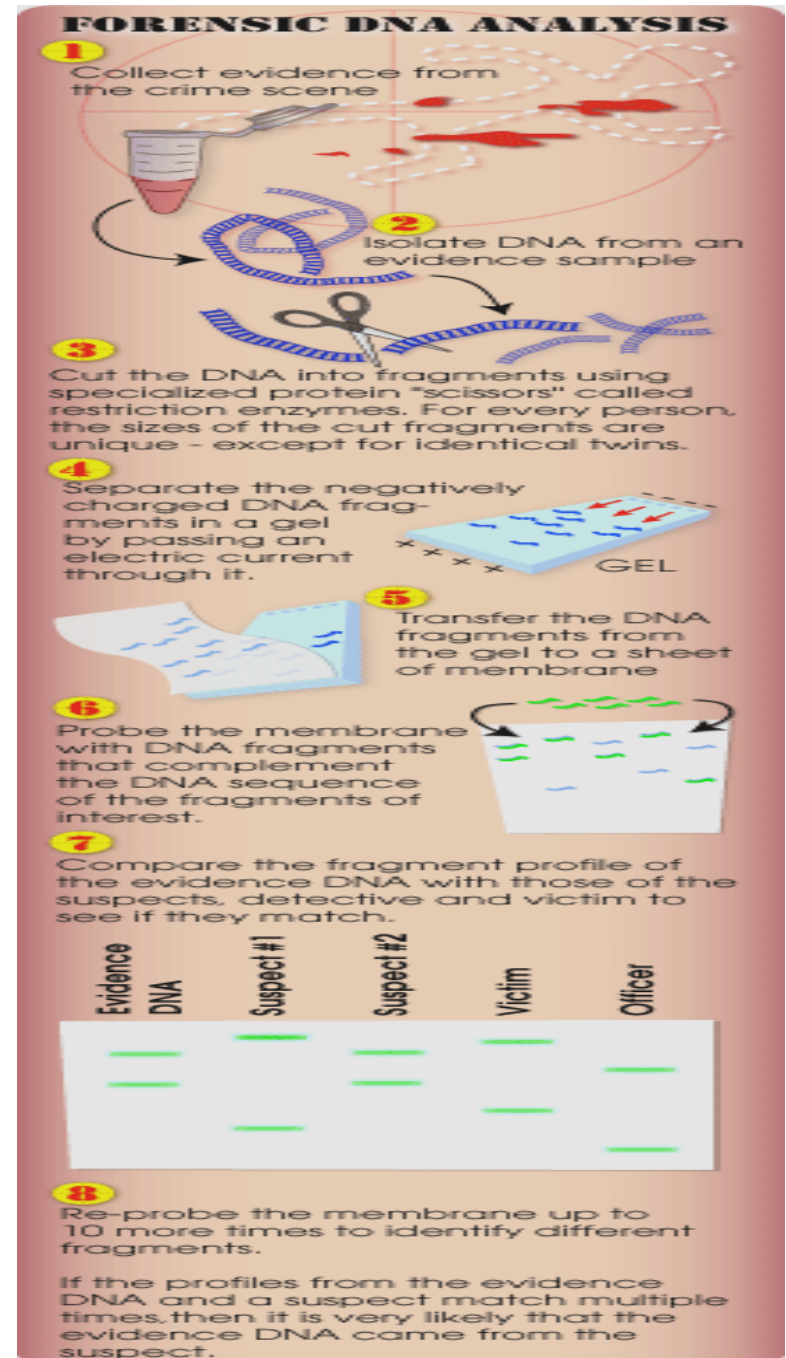
Characterization of one or more relative features of an individual's hereditary make up.

DNA Profile:

A pattern of bands usually developed on a radiograph or an X-ray film.

Principle:

- *Polymorphisms: determining which polymorphism a person has at the highly variable region.*
- *Identical DNA structure within all tissues of the same body: identical DNA in semen, saliva or hair cells.*





FORENSIC STR PROFILING



The size of the blood sample
necessary for RFLP

The size of the
blood sample
necessary
for PCR



ELECTROPHORESIS FOR STR TYPING

Capillary Electrophoresis

- DNA fragments subjected under electric field in a thin glass capillary tube.
- Detection is using fluorescent dyes.
- Size standard are assigned to determine the no. of repeats.
- Costly.

Gel Electrophoresis

- Works on same principle as CR but polyacrylamide gel is employed.
- The entire gel is scanned onto computer and image showing all bands is got.
- No need for size standards since the allelic ladder is run alongside the samples.
- Cost-effective.

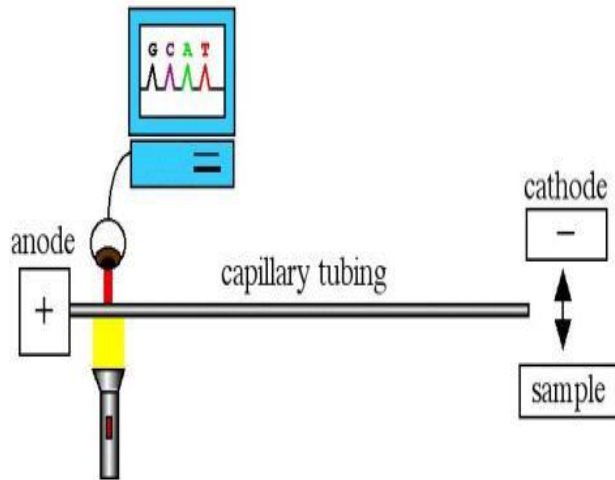
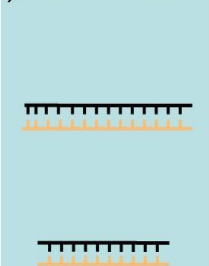


Figure 1 A schematic drawing of a capillary electrophoresis instrument. The sample is introduced on the right side and the chromatograph is detected on the left side. (Photo © Copyright 2002 Department of Biology, Davidson College).

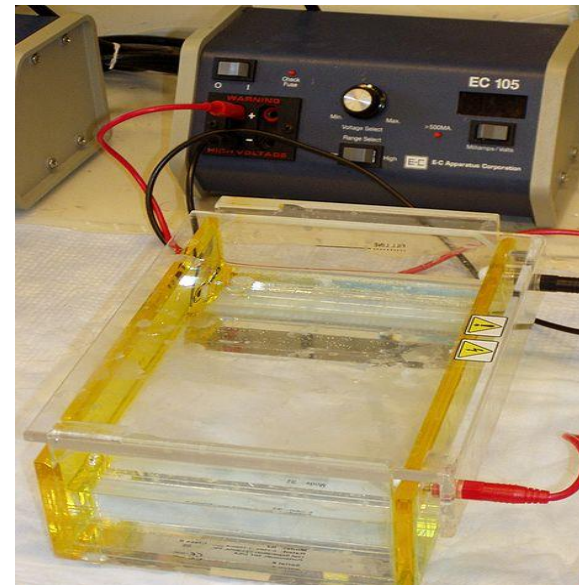
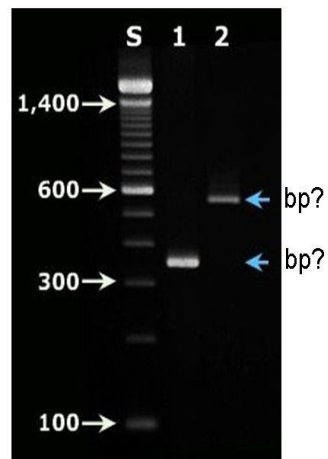


Gel Electrophoresis: Sizing DNA Fragments

(-) Negative electrode

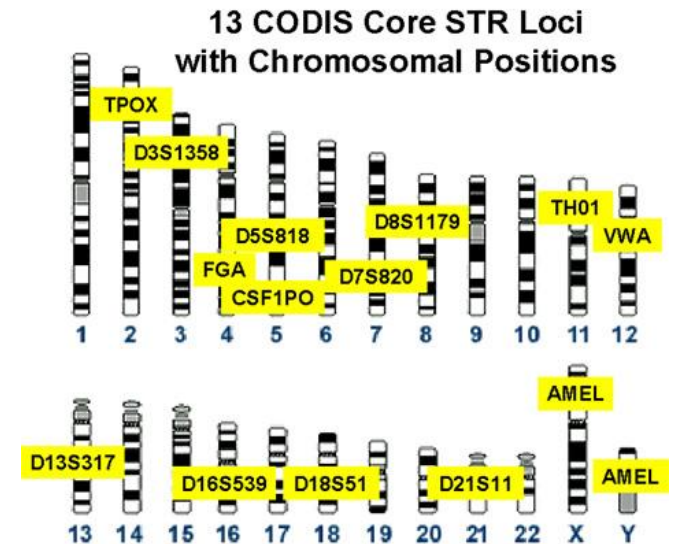


(+) Positive electrode



CODIS

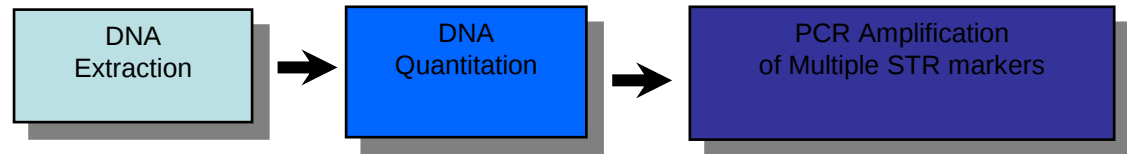
- *Combined DNA Indexing System*
- *Put forward by the FBI*
- *13 STR loci studied for reference and discrimination.*



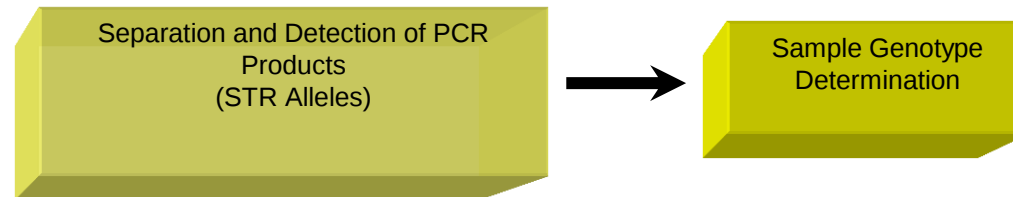
DNA samples are processed

Sample Obtained from
Crime Scene or Paternity
Investigation

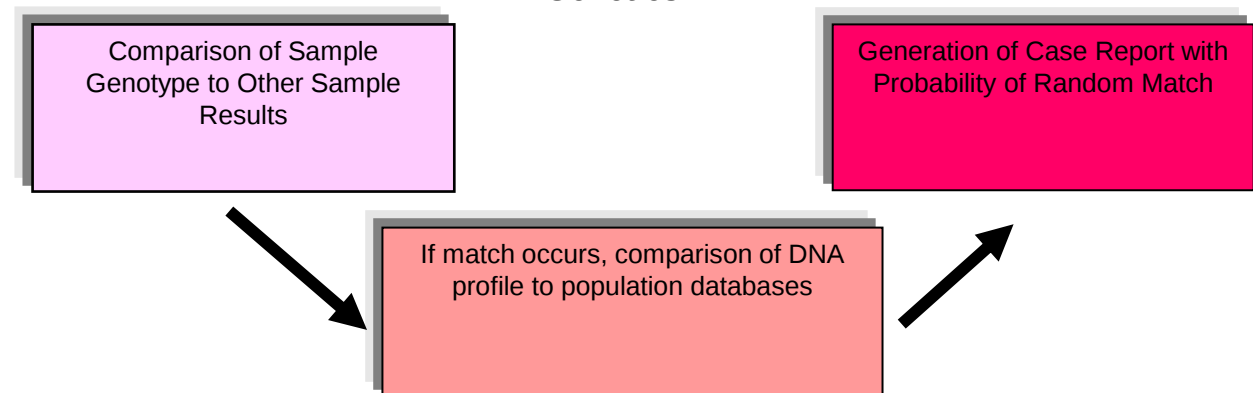
Biology



Technology



Genetics



THE ROMANOVS

- *The Romanovs were the last Russian Monarchy.*
- *Family was made up of:*
 - Tsar Nicholas II*
 - Tsarina Alexandra*
 - Daughters: Olga, Tatiana, Maria and Anastasia.*
 - Son: Alexei*



STORY IN SHORT

- *The Tsar was abdicated of his throne following which the royal family along with four loyal members was taken into exile by the enemies.*
- *On July 17, 1918 the family along with the others was executed by a firing squad.*
- *The remains were processed and abandoned in a mine shaft. Further, the bodies were transported to an open field a few kilometers from the mine.*
- *Thus the fate of the Romanovs became a mystery waiting to be solved.*

DISCOVERY AND INVESTIGATIONS OF THE FIRST MASS GRAVE

- *In late 1970s, Dr. Alexander Advonin located the mass grave containing the remains of the Romanovs.*
- *There were 950 bones obtained from the grave which were organised into 9 skeletons.*
- *Further investigations on the 9 skeletons were performed by DNA analysis exp. Conducted by Dr. Peter Gill and Dr. Pavel Ivnov.*
- *Sex and familial relationship was established by the anatomical study and STR analysis.*
- *MtDNA testing confirmed maternal relationship between Prince Philip and the Tsarina. This was also useful in relating the 3 daughters of the royal couple.*



Queen Victoria of England
b. 1819, London, England
d. 1901, Isle of Wight, England
& Prince Albert

Princess Alice Maud Mary
b. 1843, London, England
d. 1878, Darmstadt, Germany
& Grand Duke Louis IV of Hesse



Princess Victoria of Hesse
b. 1863
d. 1950
& Prince Louis of Battenberg



Princess Alice of Battenberg
b. 1885
d. 1969, London, England
& Prince Andrew of Greece



Prince Philip MOUNTBATTEN
b. 1921, Corfu, Greece
& Queen of England Elizabeth II



Tsarina Alexandra "Alix"
b. 1872, Darmstadt, Germany
d. 1918, Ekaterinburg, Russia
& Tsar Nicholas II ROMANOV

Grand Duchess Olga
b. 1895
d. 1918, Ekaterinburg, Russia



Grand Duchess Tatiana
b. 1897, Russia
d. 1918, Ekaterinburg, Russia



Grand Duchess Maria
b. 1899, Russia
d. 1918, Ekaterinburg, Russia



Grand Duchess Anastasia
b. 1901, Russia
d. 1918, Ekaterinburg, Russia

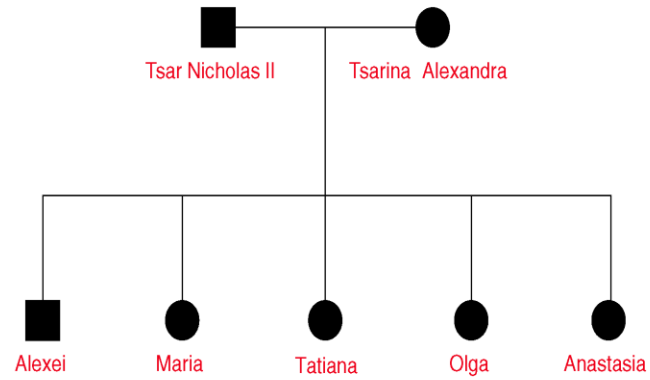


Tsarevich Alexis
b. 1904, Russia
d. 1918, Ekaterinburg, Russia



- *Maternal relatives of the Tsar, were used for reassociation of the remains of Tsar.*
- *A single point heteroplasmy at position 16169 was observed in mtDNA of the Tsar. This was seen in his relatives too.*
- *To confirm the authenticity of the heteroplasmy, the remains of the Tsar's own brother, Grand Duke Georgii Romanov were analysed.*
- *He shared the same heteroplasmy but with a different ratio.*
- *This helped in the confirmation of the presence of the remains of the Tsar and tsarina in the grave and helped in the establishing of the family tree.*

(a) The Romanov family tree



(b) The STR analysis

	STRs	
	<i>THO1</i>	<i>VWA/31</i>
Child 1	8, 10	15, 16
Child 2	7, 8	15, 16
Child 3	8, 10	15, 16
Female adult 1	8, 8	15, 16
Female adult 2	6, 6	16, 17
Male adult 1	9, 10	14, 20
Male adult 2	6, 10	17, 17
Male adult 3	7, 10	15, 16
Male adult 4	6, 9	15, 17

ANNA ANDERSON

- *Since the romanovs were missing many people claimed to be one of the missing children.*
- *The most interesting case was that of Anna Anderson, who claimed to be the youngest daughter of the Tsar, Anastasia.*
- *With the discovery of first grave and subsequent DNA analysis, Anna Anderson was exposed as an imposer.*



DISCOVERY OF THE SECOND GRAVE

- *In summer 2007, a group of archaeologists discovered few bone fragments approximately 70 mts from the first grave.*
- *A set of 44 bone fragments and teeth were recovered from the site under the guidance of Dr. Sergei Pogovelov.*

Conclusions obtained by analysis:

- a) Duplicative anatomical units : two people were present among the recovered remains.*
- b) Sciatic notch dimension: one of them was a female, 5-9 yrs of age; another was a male, 12-15 yrs age.*

CONFIRMATORY TESTS

- *mtDNA analysis can be considered conclusive. The new samples matched exactly with the mtDNA data of Tsarina Alexandra and a living relative.*
- *Autosomal STR genotypes were developed to form a family pedigree. The DNA profiles of the new samples perfectly fit into the family tree of Tsar and Tsarina.*
- *17 Y-STR haplotype remains of the Tsar, his son and a living romanov relative were perfectly in match .*

These results taken together agrees with the hypothesis that the samples recovered from the second grave was the missing children, Anastasia and Alexei of the royal couple. Thus the missing children were found back!!

THANK YOU

Acknowledgement

- ❖ The Presentation is being used for educational and non commercial purpose
- ❖ Thanks are due to all those original contributors and entities whose pictures used for making this presentation.