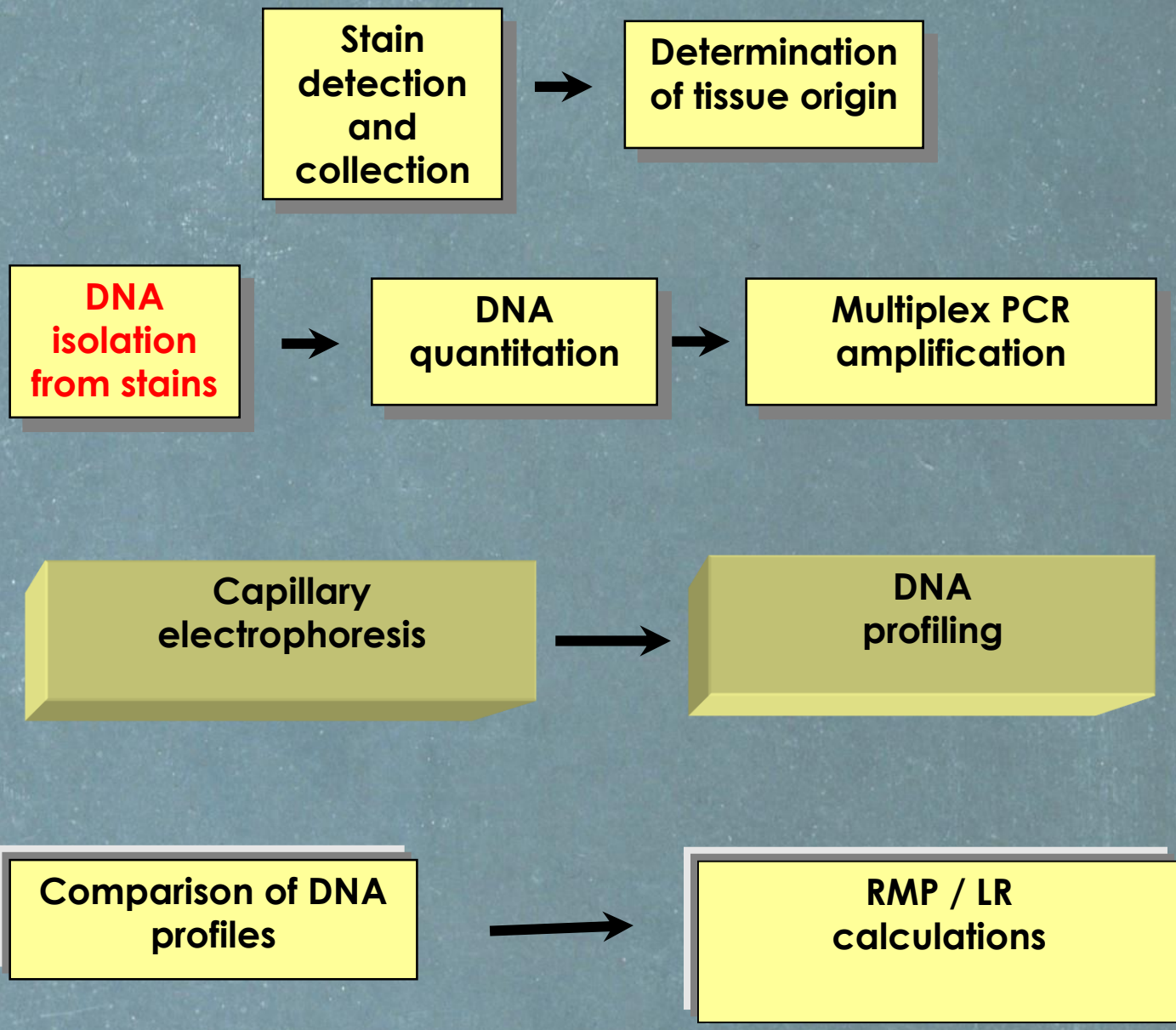
Forensic Genetics and Legal Medicine 2019-2020

18th May 2020

DNA isolation and quantitation


Stain detection and collection
Determination of tissue origin
DNA isolation from stains
DNA quantitation
Multiplex PCR amplification
Capillary electrophoresis
DNA profiling
Comparison of DNA profiles
RMP / LR calculations

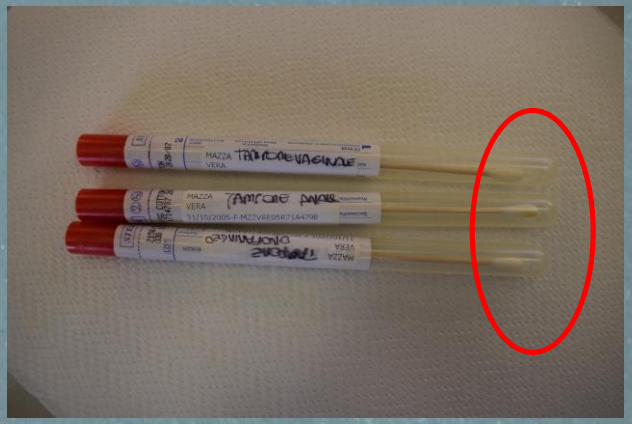
Transfer of stain to test tube

- Stain can be cut (0.5-1 cm diameter) and directly transferred to test tube. The same applies to hair evidence, that can be cut into pieces and directly transferred to test tube

- Item surface can be swabbed and swab tip (or tips, in double swabbing) transferred to test tube

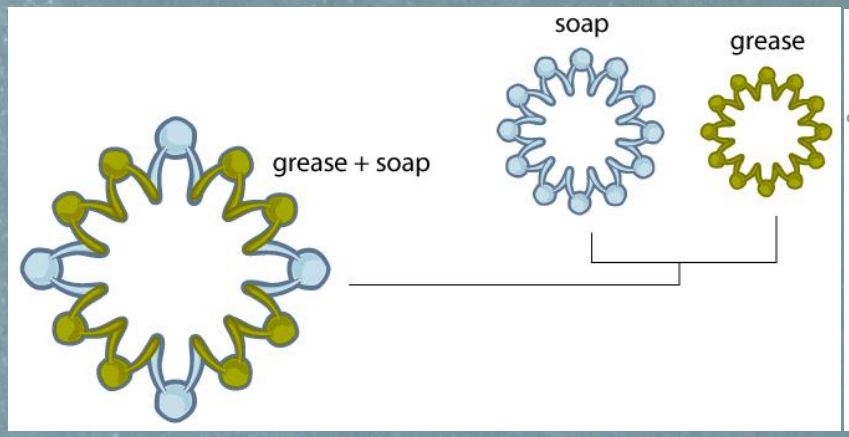
- Tip of collection swab transferred to test tube (sexual assault intimate samples)

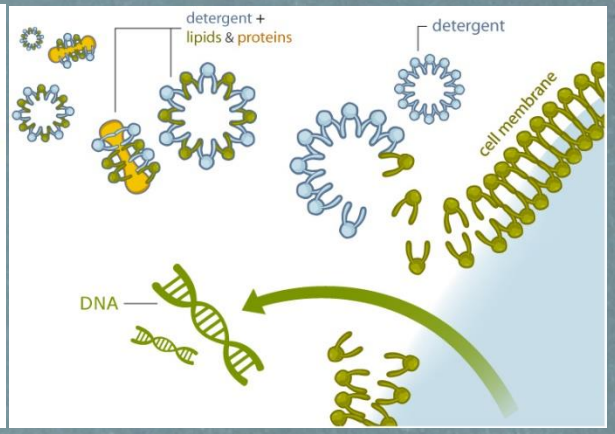
- Cellular material on large surfaces not easily swabbed (e.g. certain types of fabric) can be collected, concentrated and transferred to test tube by means of scotch tape



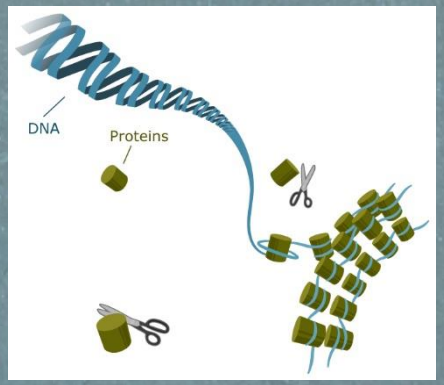
✓ **Stain** lysis in slighty basis saline buffer including:

- EDTA (prevents DNA degradation by Mg^{++} and Ca^{++})
- TRIS (interacts with cellular membrane lipids increasing its permeability)
- A detergent (e.g. SDS) that dissolves the lipid bilayer and denatures proteins



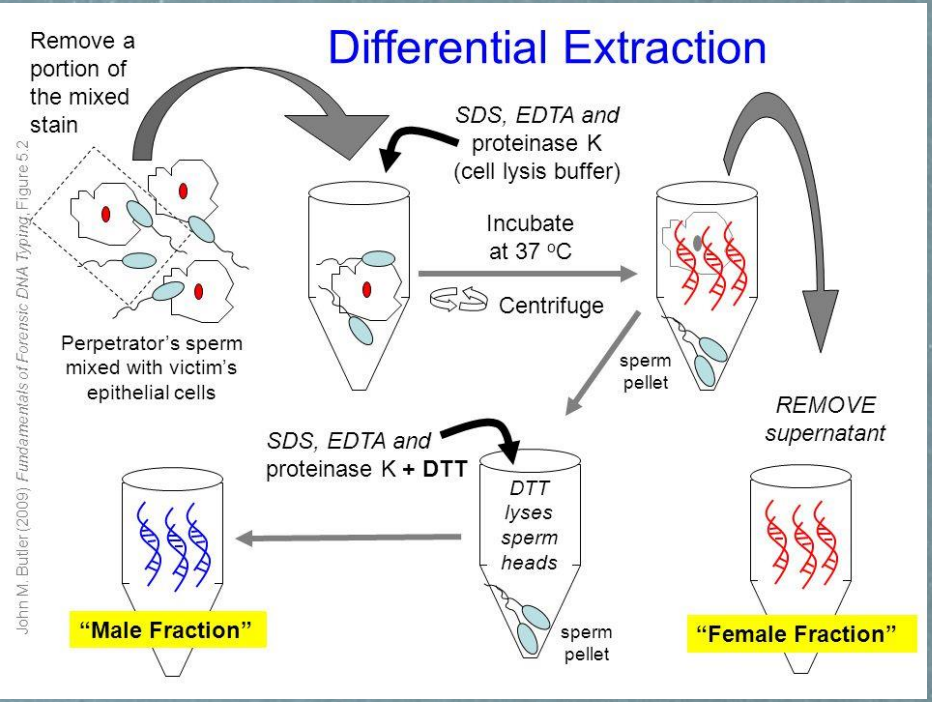


- A proteinase that inactivates cellular nucleases and digests histones



✓ Special needs:

- **Semen**: nuclear membranes of spermatozoa are rich in proteins forming disulfide bridges. Dithiothreitol (DTT) is needed to break these bonds. Differential resistence of spermatozoa / vaginal mucosa cells to standard DNA extraction methods can be used in sexual assault cases to separate male aggressor DNA



- **Hair/nails**: Addition of DTT necessary to disrupt highly cheratinized tissues

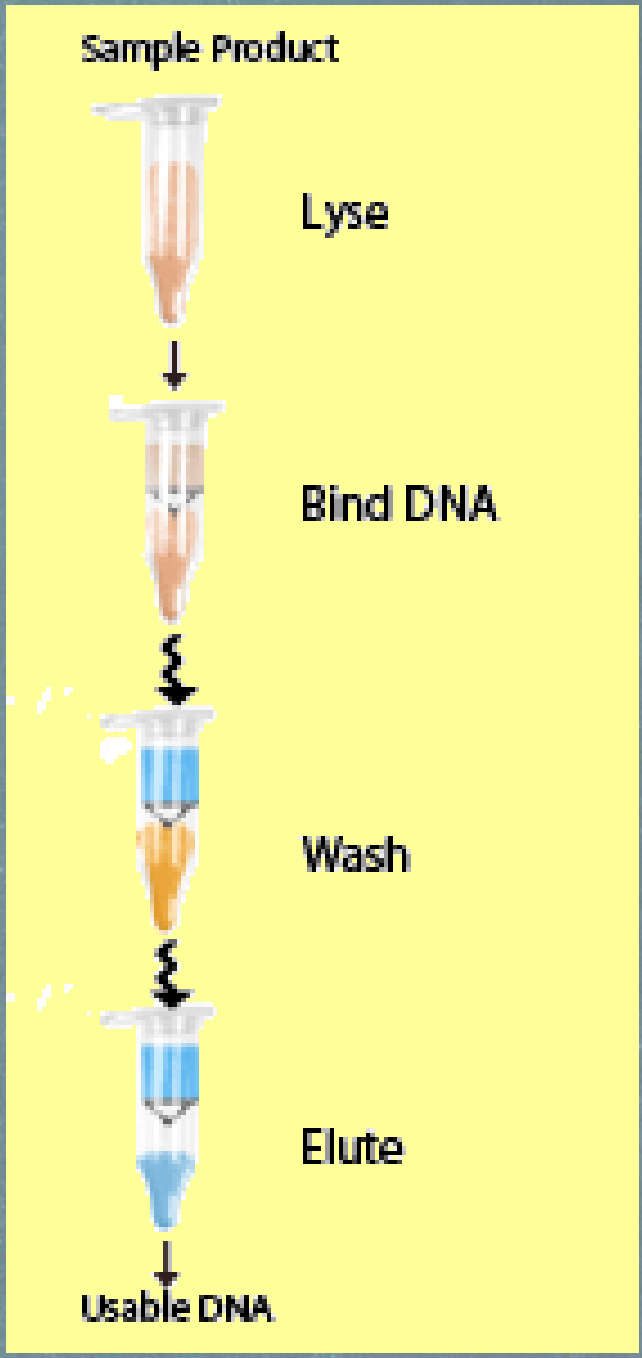
- **Bone tissue**: decalcification (EDTA wash) may be needed before lysis

✓ DNA purification from crude lysate:

- Addition to lysate of a chaotropic salt (e.g. guanidinium isothiocyanate), that disrupts the water molecules - DNA interface and favors the adsorption of DNA to a silica surface

- Silica surface is then washed with alcohol solutions to remove material different from DNA

- DNA is finally eluted in a low salt solution



Silica-based DNA purification methods are amenable to automation (e.g. by means of silica-coated magnetic beads)

Several PCR inhibitors can be co-extracted with DNA from forensic samples:

✓ Intrinsic
- Excess of heme an its oxidation products (hematin)
- Excess melanin in hair
- Calcium and collagen in skeletal samples
- Bile salts and complex polysaccharides in fecal samples

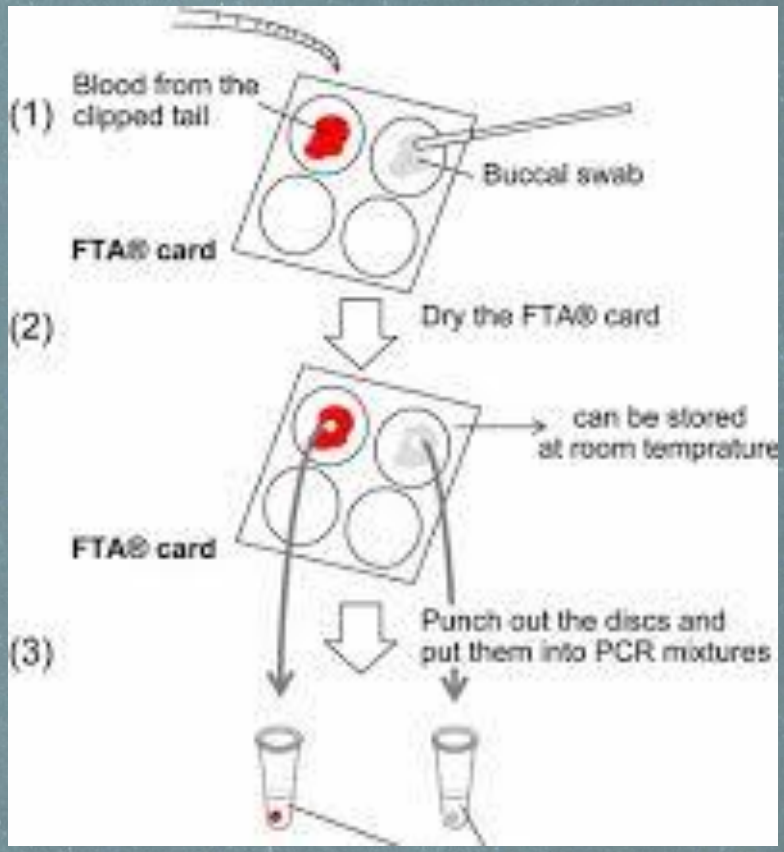
✓ Extrinsic
- Humic acids (dark colored compounds found in soils that may be encountered in samples that have been buried, particularly in skeletal remains)

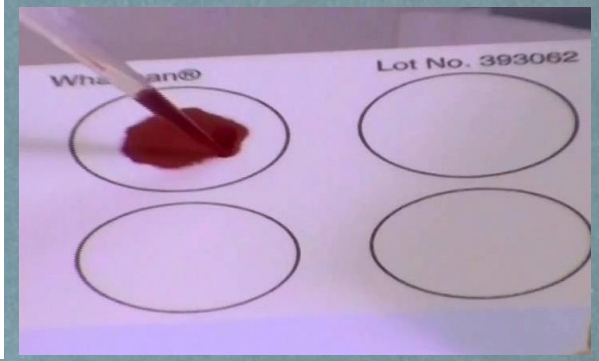
- Indigo dye (denim in fabrics)
- Tannic acid in leather

Strategies to overcome inhibition:
- Dilute sample
- Further purification
- Use of amplification facilitators (BSA to bind inhibitors, extra / alternative polymerase)

✓ **Reference sample** analysis by:

a) direct PCR of «fast technology for analysis» (FTA) cards contain





Saliva / blood cells lyse on contact with paper. Paper is treated with antimicrobial reagents preventing DNA degradation

b) Quick pre-lysis of non-FTA substrates followed by PCR with unpurified lysate

NB Trace DNA extraction experiments always carried out in presence of extraction blank

'DNA bungle' haunts German police

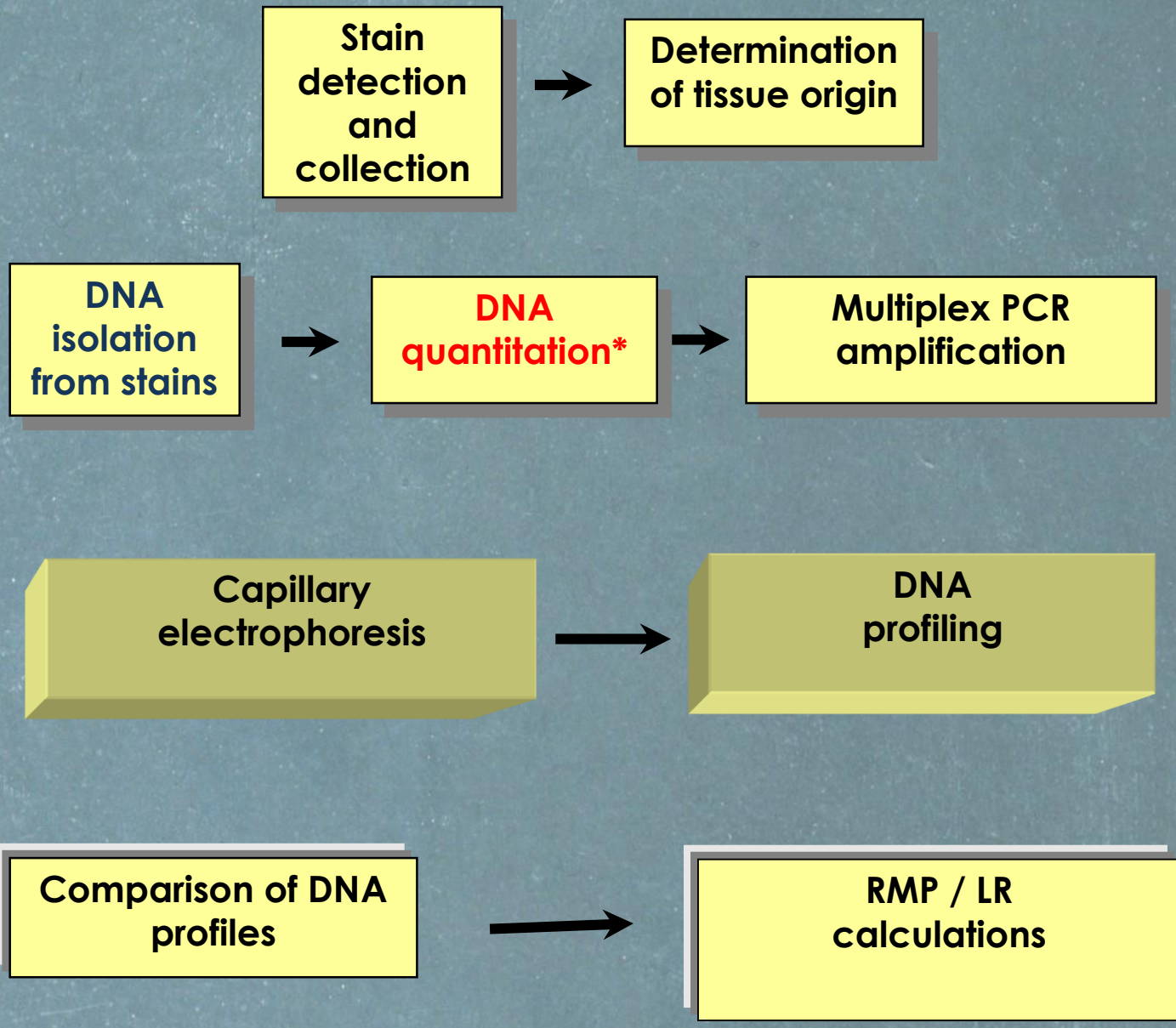
Police in Germany have admitted that a woman they have been hunting for more than 15 years never in fact existed.

This 2007 murder was believed to be the work of the phantom killer

Dubbed the "phantom of Heilbronn", the woman was described by police as the country's most dangerous woman.

Investigators had connected her to six murders and an unsolved death based on DNA traces found at the scene.

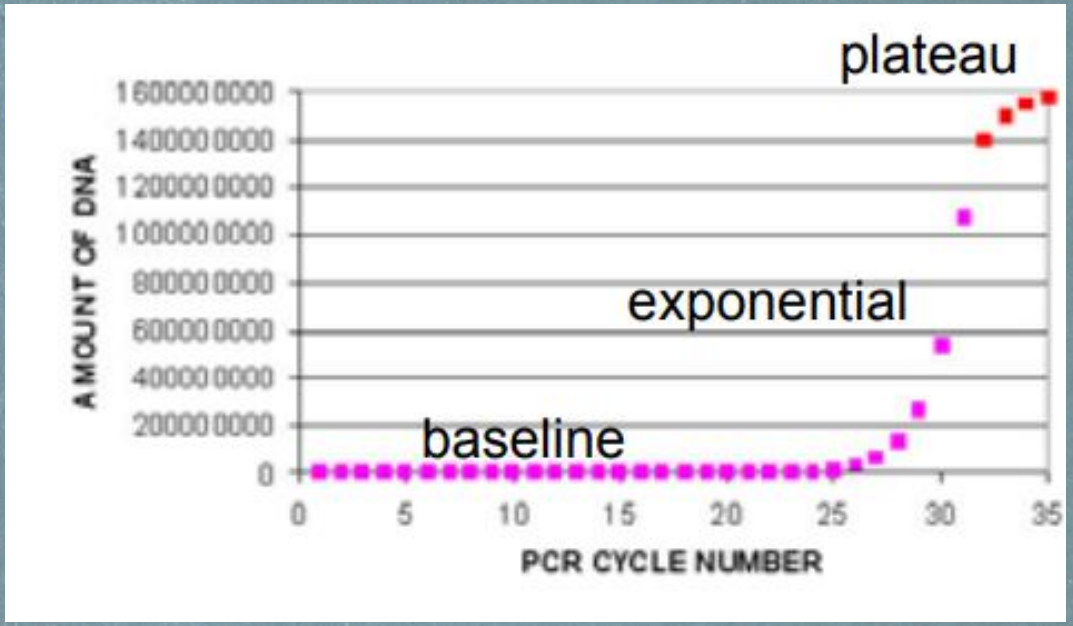
Police now acknowledge swabs used to collect DNA samples were contaminated by an innocent woman working in a factory in Bavaria.



*and PCR inhibitors and DNA degradation detection

Real time (RT-PCR) or quantitative PCR (qPCR)

- Theoretically the quantity of PCR template T doubles with each cycle.
- After N cycles the quantity of product is 2^nT
- There is a exponential relationship between the original quantity of product and the amount of template



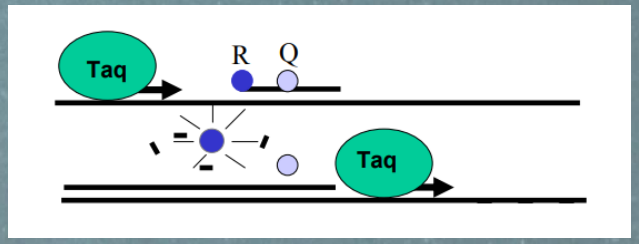
This is true for exponential phase of PCR. Loss of efficiency (limited primers, reduction in polymerase activity) will lead to a plateau phase in which product concentration is not linear. The final template amount can thus differ in PCR experiments with the same initial DNA input

- qPCR focuses on the exponential phase of amplification
- qPCR uses fluorescent detection, where fluorescence is proportional to PCR product
- real time PCR machine records fluorescence for each well at each cycle

Fluorescence detection technologies

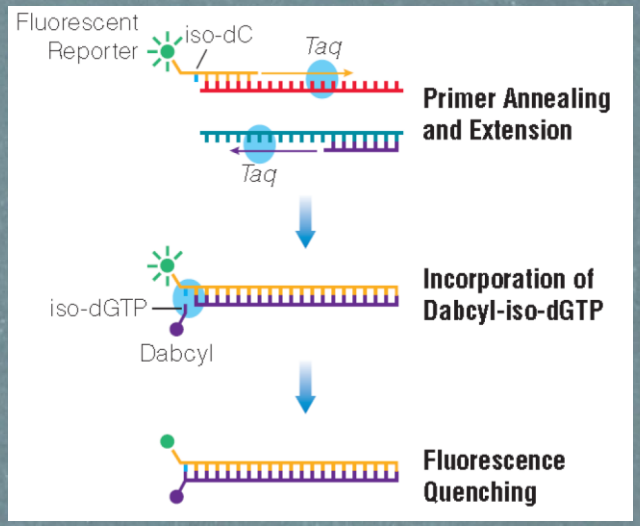
✓ TaqMan probes

- non-fluorescent probe (fluorescent reporter molecule is quenched) binds to PCR product during extension
- 5’-3’ exonuclease activity of Taq polymerase digests probe and frees reporter dye from quencher
- flourescence accumulates with PCR product



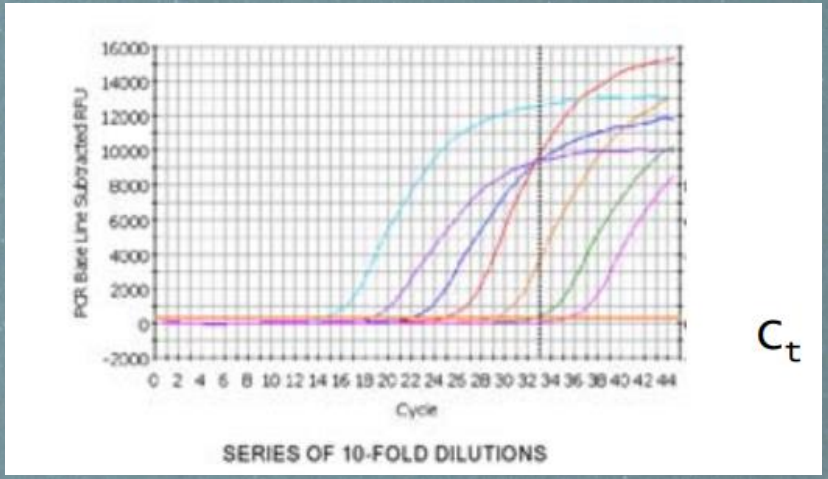
✓ Plexor technology

- One PCR primer is synthesized with an iso-dC residue and a fluorescent label at the 5′- end
- Iso-dGTP nucleotides, modified to include dabcyl as a quencher, are included in the reaction mix.
- Only dabcyl-iso-dGTP can be incorporated at the position complementary to the iso-dC residue
- Incorporation quenches the fluorescent signal
- accumulation of product is accompanied by a decrease in fluorescence

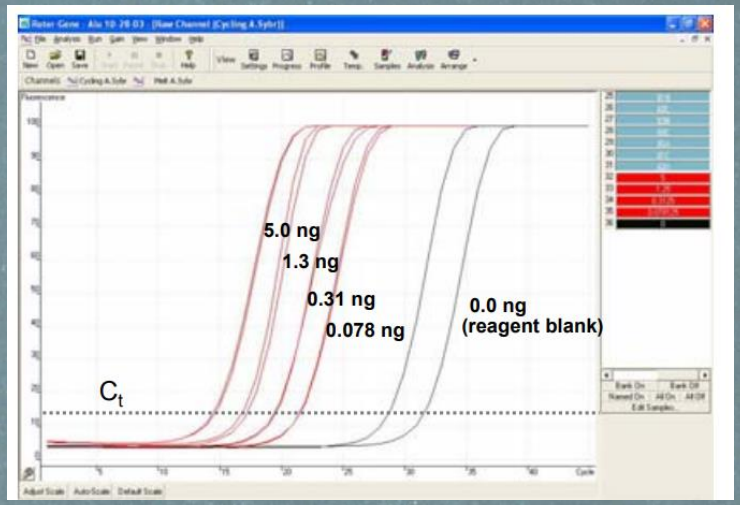


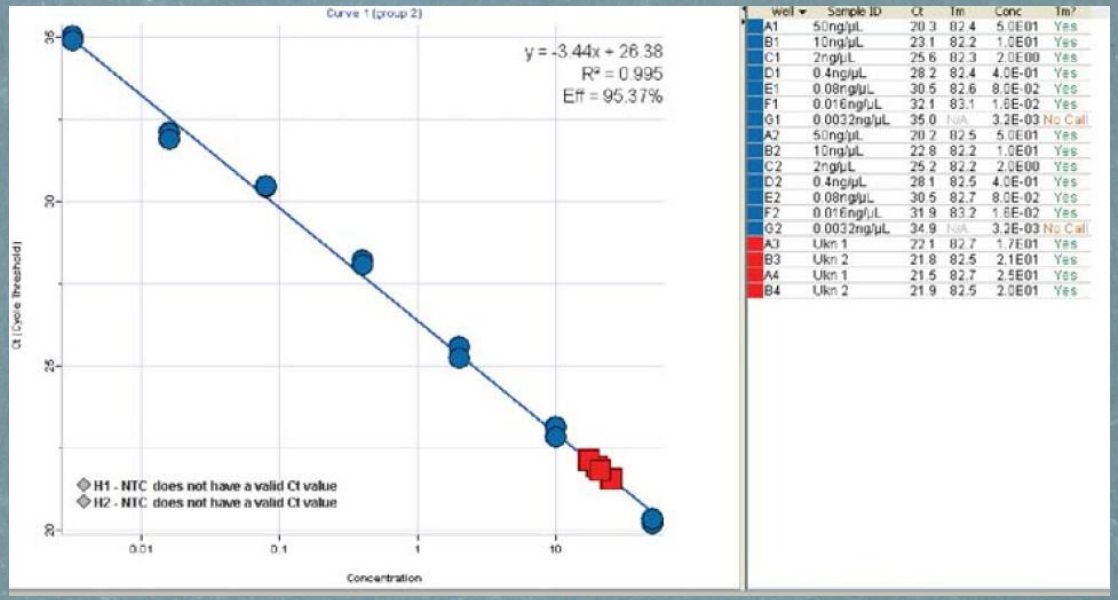
Quantitation of DNA is a based on the number of cycles required to reach a threshold intensity (Ct)

The greater the amount of starting DNA, the sooner this threshold value is reached.



The number of cycles required to reach Ct by a sample of unknown concentration is compared to the calibration curve plotted using a series of standards





Well	Sample ID	Ct	Tm	Conc	Tm?
A1	50ng/µL	20.3	82.4	5.0E01	Yes
B1	10ng/µL	23.1	82.2	1.0E01	Yes
C1	2ng/µL	25.6	82.3	2.0E00	Yes
D1	0.4ng/µL	28.2	82.4	4.0E-01	Yes
E1	0.08ng/µL	30.5	82.6	8.0E-02	Yes
F1	0.016ng/µL	32.1	83.1	1.6E-02	Yes
G1	0.0032ng/µL	35.0	N/A	3.2E-03	No Call
A2	50ng/µL	20.2	82.5	5.0E01	Yes
B2	10ng/µL	22.8	82.2	1.0E01	Yes
C2	2ng/µL	25.2	82.2	2.0E00	Yes
D2	0.4ng/µL	28.1	82.5	4.0E-01	Yes
E2	0.08ng/µL	30.5	82.7	8.0E-02	Yes
F2	0.016ng/µL	31.9	83.2	1.6E-02	Yes
G2	0.0032ng/µL	34.9	N/A	3.2E-03	No Call
A3	Ukn 1	22.1	82.7	1.7E01	Yes
B3	Ukn 2	21.8	82.5	2.1E01	Yes
A4	Ukn 1	21.5	82.7	2.5E01	Yes
B4	Ukn 2	21.9	82.5	2.0E01	Yes

→ DNA concentration of the unknown sample is derived

Commercial forensic qPCR systems include

- Probes labelled with different fluorescent dyes to separately detect total DNA (probe targeting autosomal DNA) and male DNA (probe targeting Y chromosome)

- an Internal PCR control (IPC) to detect inhibition. The IPC is the combination of an artificial DNA sequence (not found in human genome) and probes labelled with a fluorescent dye different from those used for total/male DNA. IPC is premixed to RT-PCR reagents. If the IPC Ct value of a DNA sample is several cycles higher than that of DNA standards with similar total DNA amounts, inhibition may have occurred

- A combination of small amplicon and large amplicon probes targeting autosomal DNA, each labelled with a different fluorescent dye. Comparison of small vs large amplicon Ct provides indications regarding DNA degradation in the sample