# Validation of PCR-based forensic DNA analysis methods

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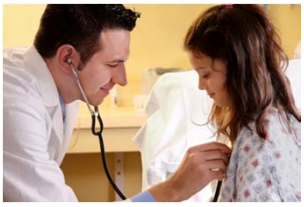
## Applications of DNA analysis

Food and feed chain

Archaeology

Bioterrorism

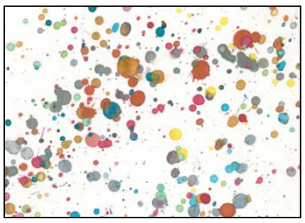


Clinical diagnostics/ personalised medicine

Environmental studies

Forensics

## Biological evidence at crime scenes



All tissue types

Foods

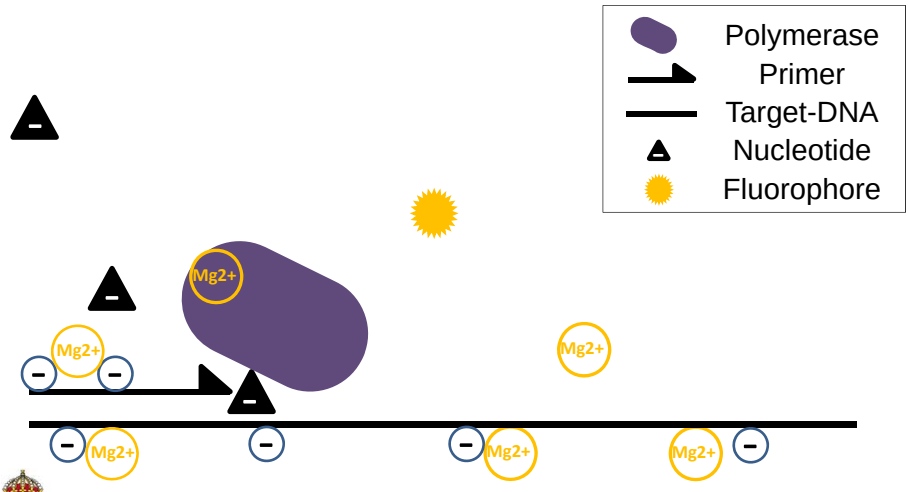
Cans and bottles

Clothes

Tobacco products

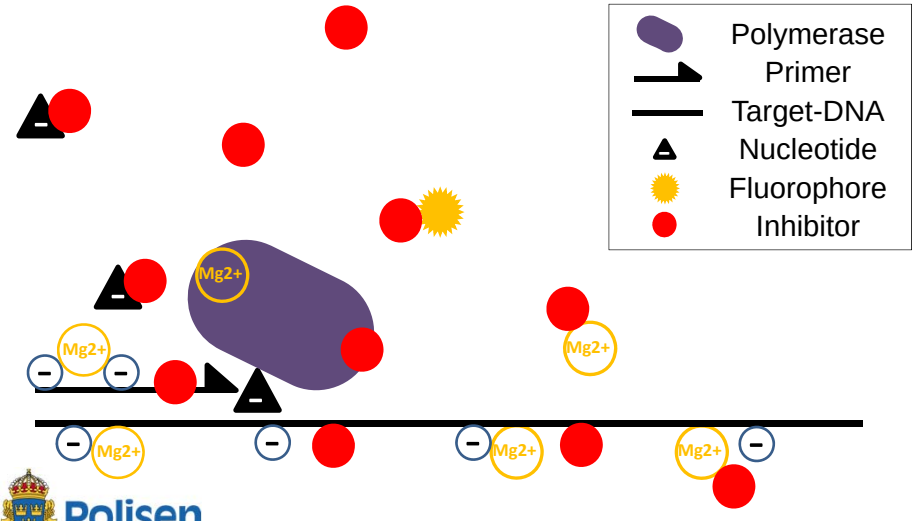
Weapons and cartridges

## Polymerase Chain Reaction – PCR




Polisen

## Polymerase Chain Reaction – PCR

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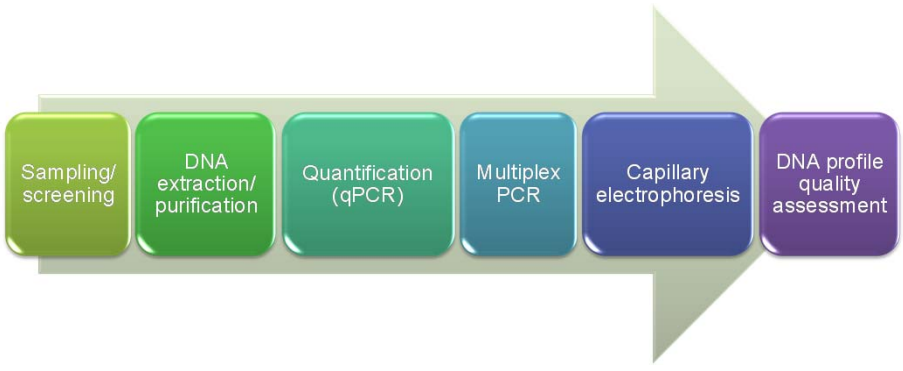
## Workflow in forensic DNA analysis

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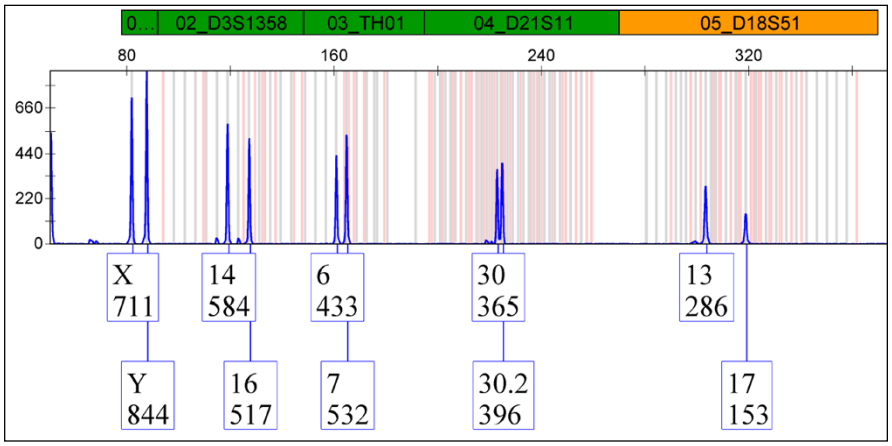
# Forensic DNA profile

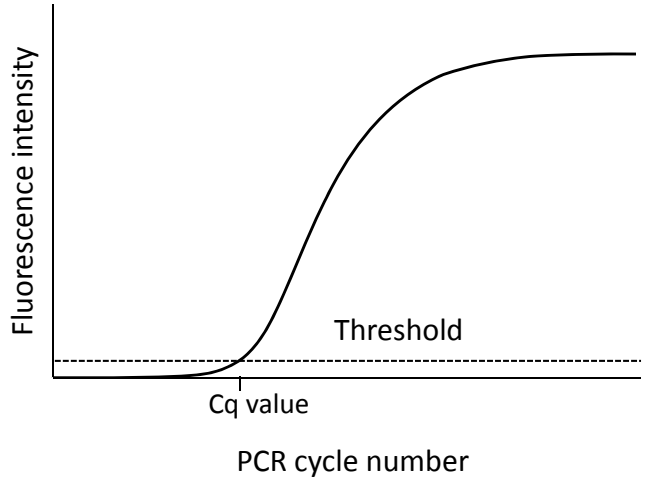
## Short tandem repeats (STR)

Amelogenin STR STR STR STR




Capillary electrophoresis – separation based on fragment size and fluorophore

# Amplification curve and quantification cycle (Cq) value from qPCR

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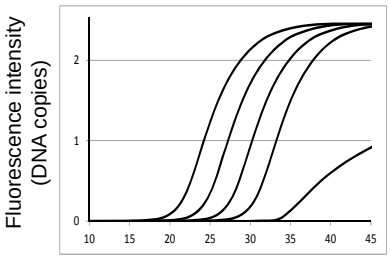
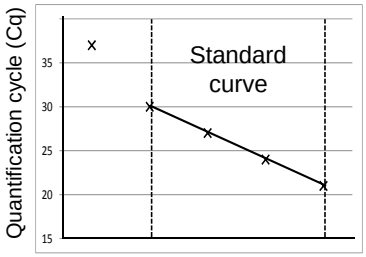
# Absolute quantification with qPCR

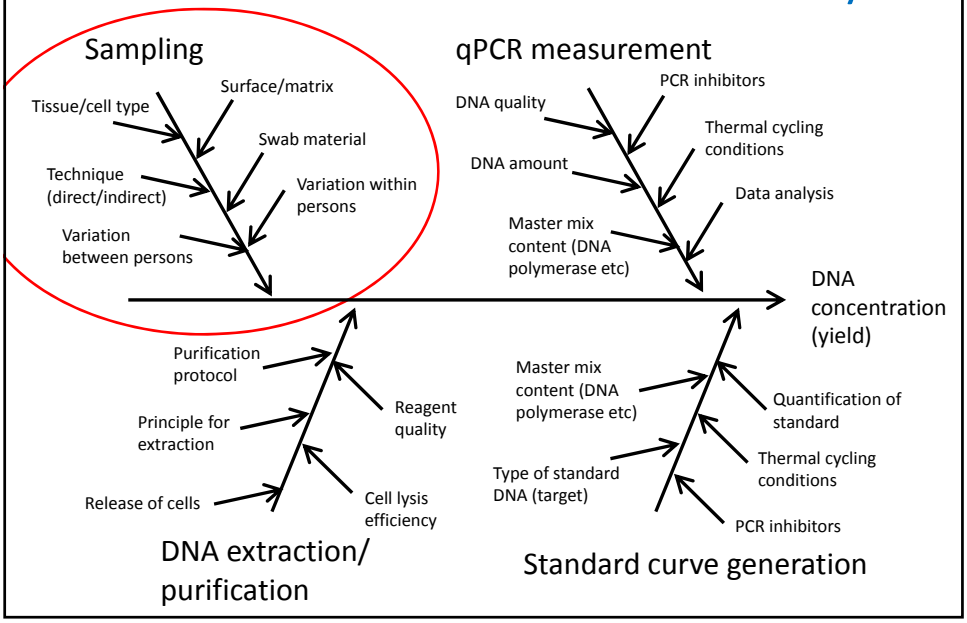



Cq=slope*Log(DNA conc.) + intercept




DNA conc. = $10^{((Cq\text{-}int.)/slope)}$

# Sources of measurement uncertainty



# Module-based validation of PCR methods

The analysis chain consists of four modules:

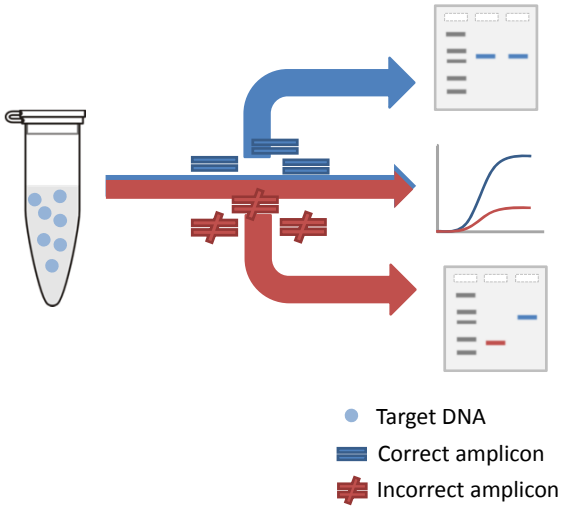
- Sampling
- Sample treatment (may be viewed as part of DNA/RNA extraction)
- DNA/RNA extraction
- PCR-based analysis

PCR-based analysis may be divided into:

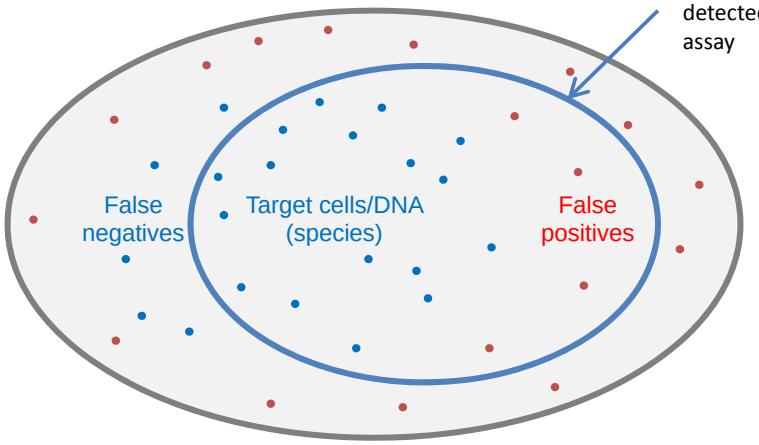
- Conventional PCR with capillary electrophoresis detection
- Quantitative PCR (qPCR)
- Reverse Transcriptase-qPCR (RNA analysis)
- Digital PCR
- Next Generation Sequencing

Each module may be validated individually

# Identity



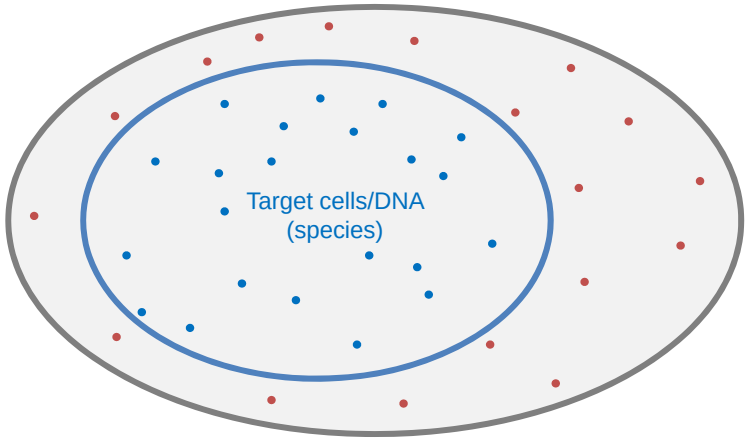
# Selectivity




- Target cell/DNA (species)
- Non-target cell/DNA (species)

Choose panel of microbial species: Some that should be detected and some that should not be detected by the assay

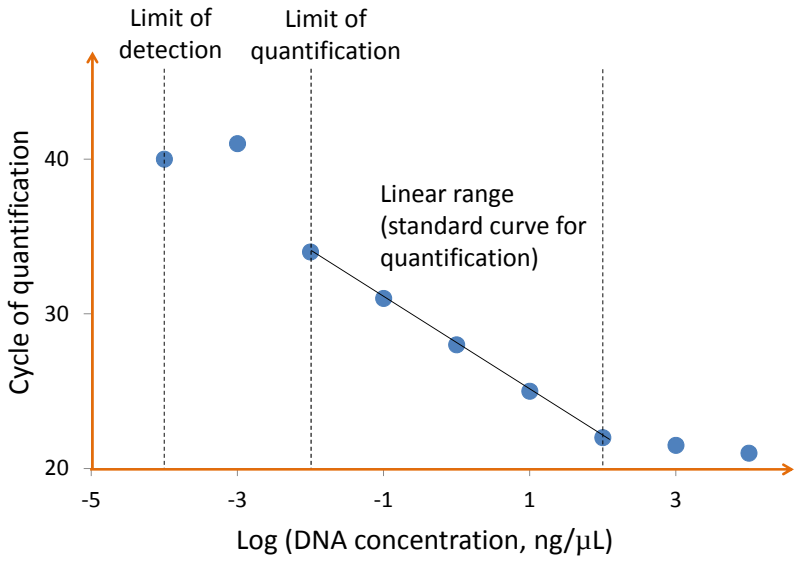
# Selectivity



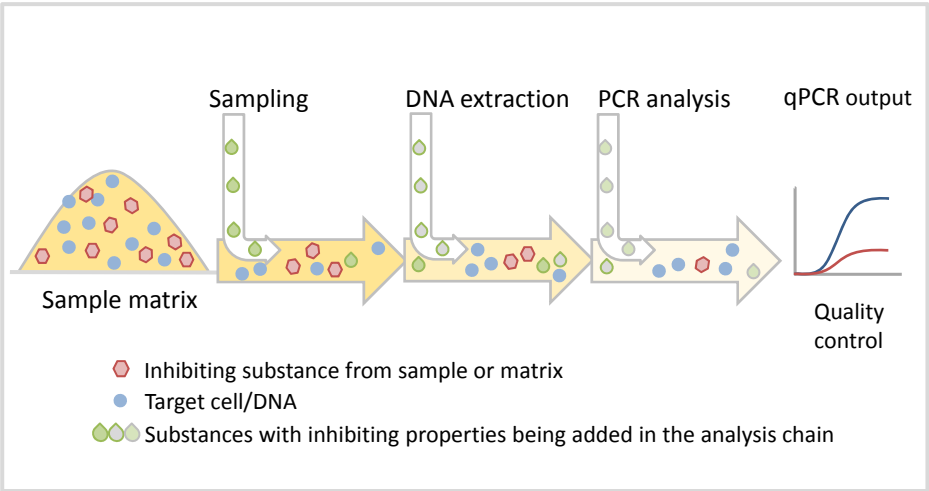

- Target cell/DNA (species)
- Non-target cell/DNA (species)

Optimise assay to improve selectivity (eg primer design)

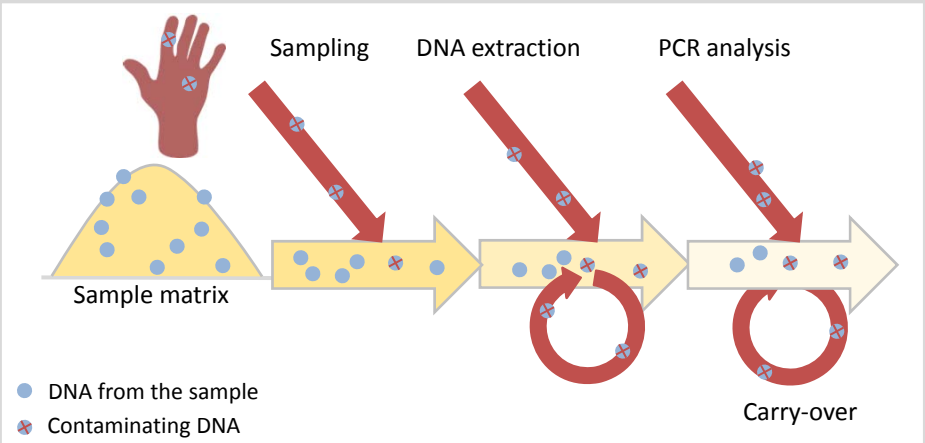
# Working range for quantitative PCR




# Matrix effects



# Risk of contamination




ISO 18385:2016 - Minimizing the risk of human DNA contamination in products used to collect, store and analyze biological material for forensic purposes

# Conclusions

- Module-based approach enables efficient validation
- Uncertainty/yield is greatly affected by sample matrix and sampling
- PCR inhibitors must be controlled in all steps of the analysis chain
- Methods with improved limits of detection increase the need for contamination control

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# Acknowledgment


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# Thanks for your attention!