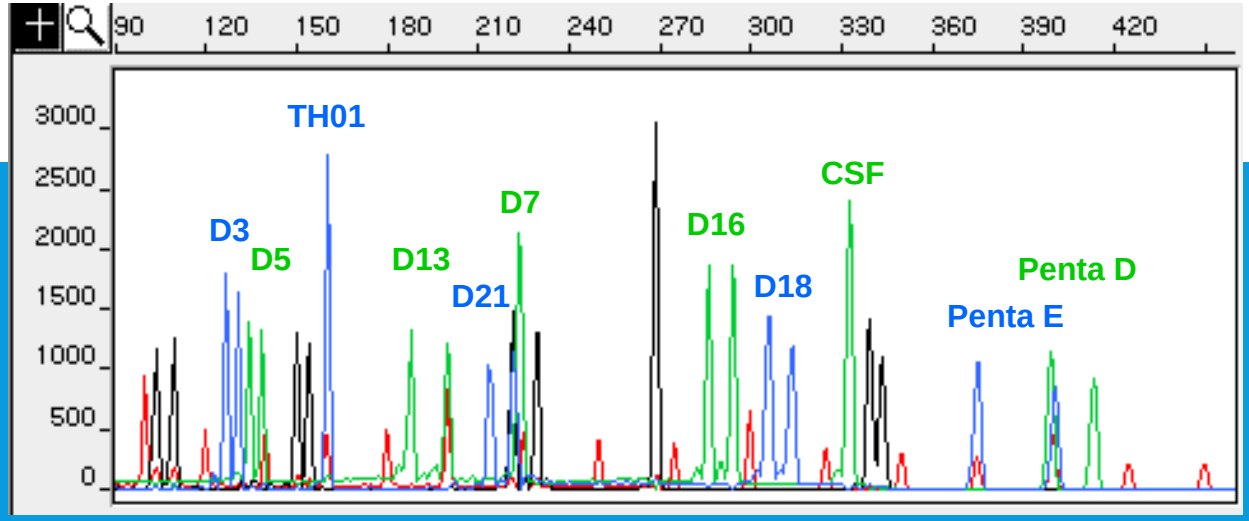
POLYMERASE CHAIN REACTION

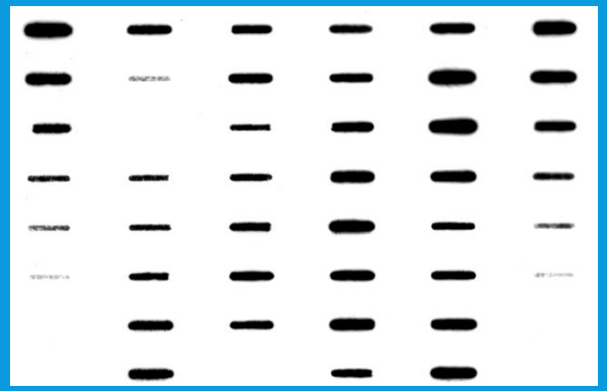
PCR for replicating DNA for improved detection

OVERVIEW OF STEPS INVOLVED IN DNA TYPING

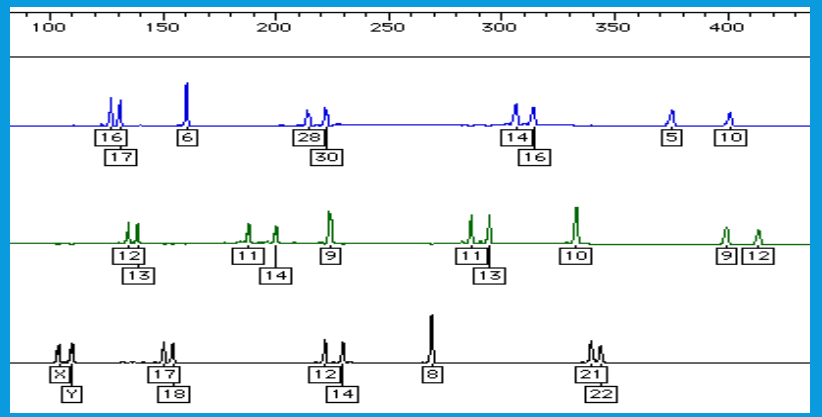
Blood Stain



PCR Amplification with Fluorescent STR Kits and Separation with Capillary Electrophoresis

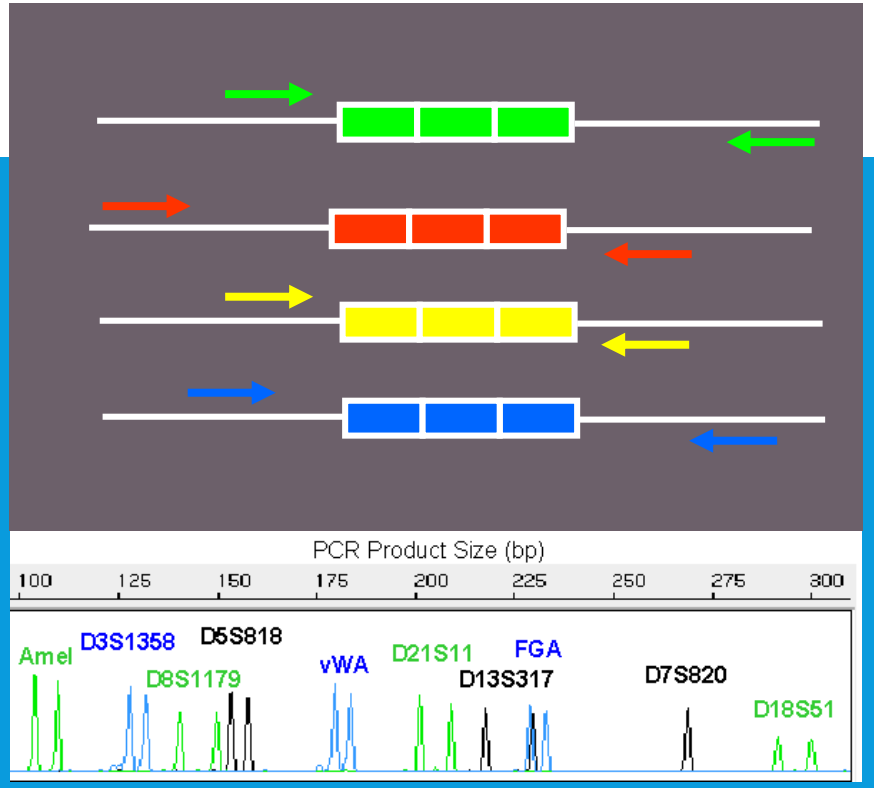


DNA Quantitation using Slot Blot



Genotyping by Comparison to Allelic Ladder

MULTIPLEX PCR (PARALLEL SAMPLE PROCESSING)



- 10 or more STR loci can be simultaneously amplified
- STR kits are commercially available

Advantages of Multiplex PCR

- –Increases information obtained per unit time (increases power of discrimination)
- –Reduces labor to obtain results
- –Reduces template required (smaller sample consumed)

ADVANTAGES OF PCR

- Minute amounts of DNA template may be used from as little as a single cell.
- DNA degraded to fragments only a few hundred base pairs in length can serve as effective templates for amplification.
- Large numbers of copies of specific DNA sequences can be amplified simultaneously with multiplex PCR reactions.
- Contaminant DNA, such as fungal and bacterial sources, will not amplify because human-specific primers are used.
- Commercial kits are now available for easy PCR reaction setup and amplification.

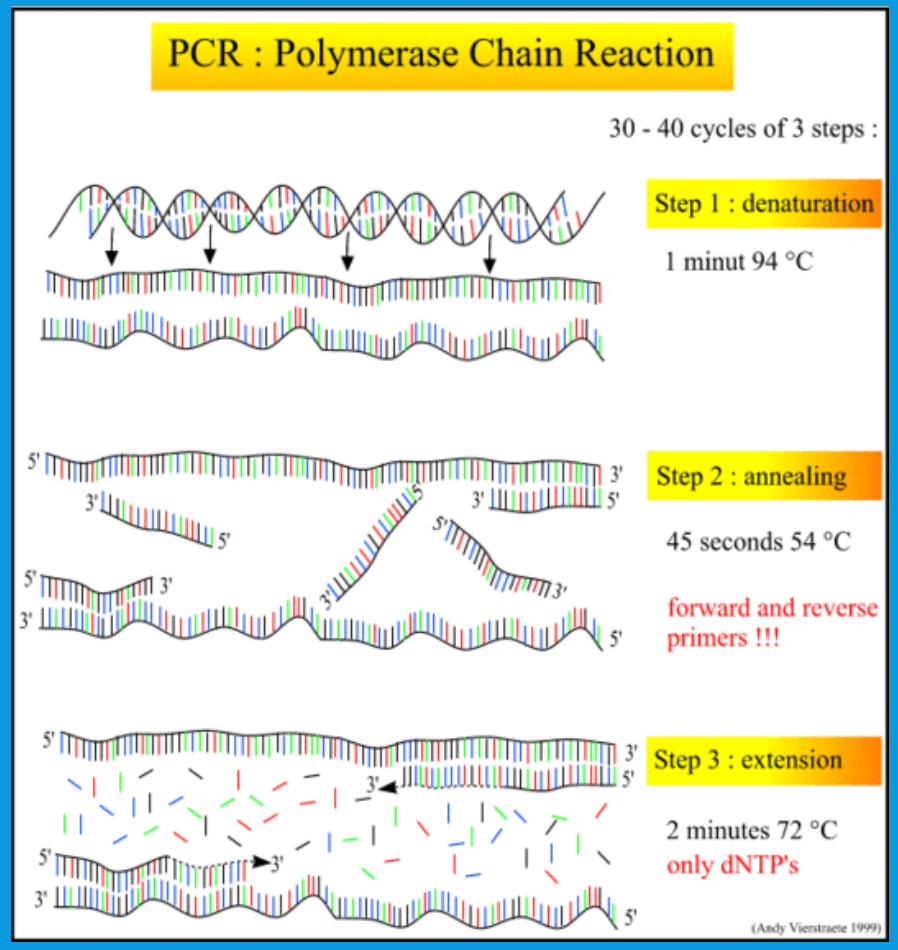
POTENTIAL PITFALLS OF PCR

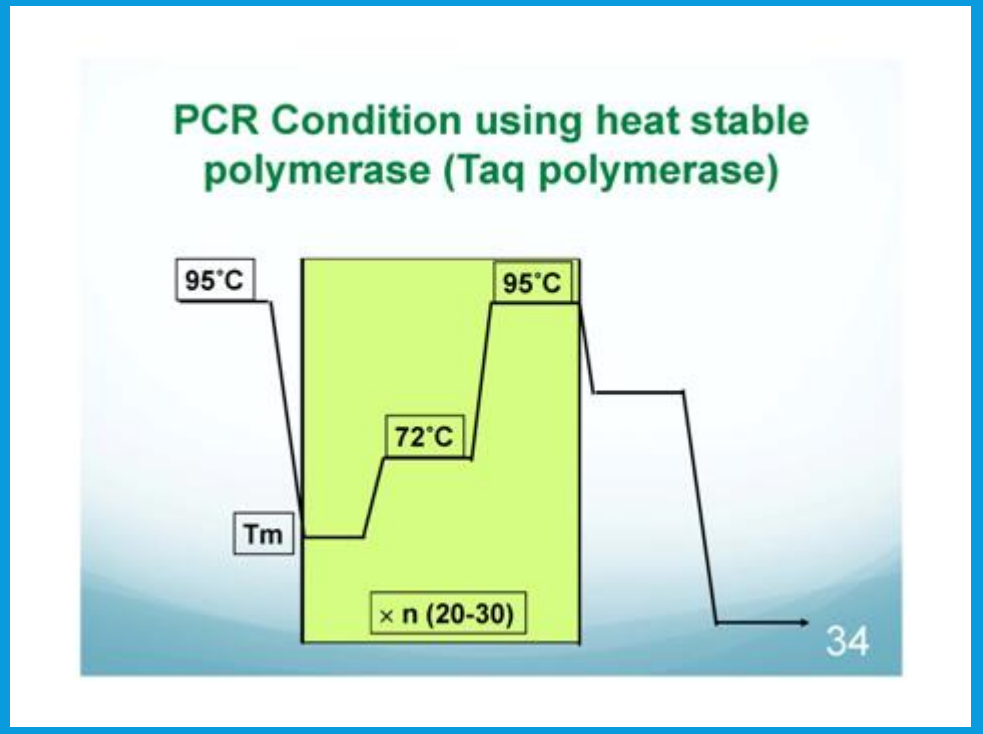
- The target DNA template may not amplify due to the presence of PCR inhibitors in the extracted DNA
- Amplification may fail due to sequence changes in the primer binding region of the genomic DNA template
- Contamination from other human DNA sources besides the forensic evidence at hand or previously amplified DNA samples is possible without careful laboratory technique and validated protocols

PCR REACTION MIX

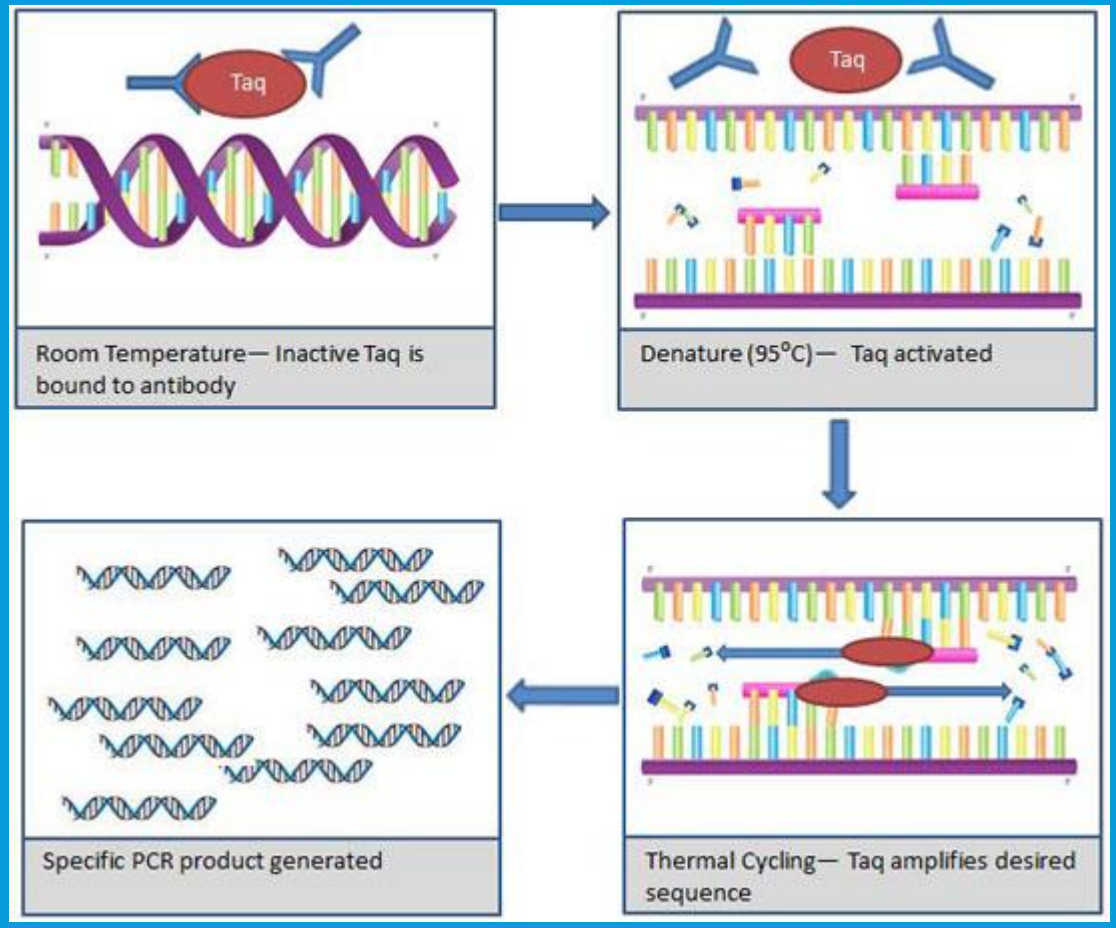
- Small microliter volumes
- Buffer
- Co-factor (e.g. $MgCl_2$)
- Taq polymerase enzyme
- Free nucleotides
- PCR primers (~20 –mers)
- DNA template

PCR AMPLIFICATION

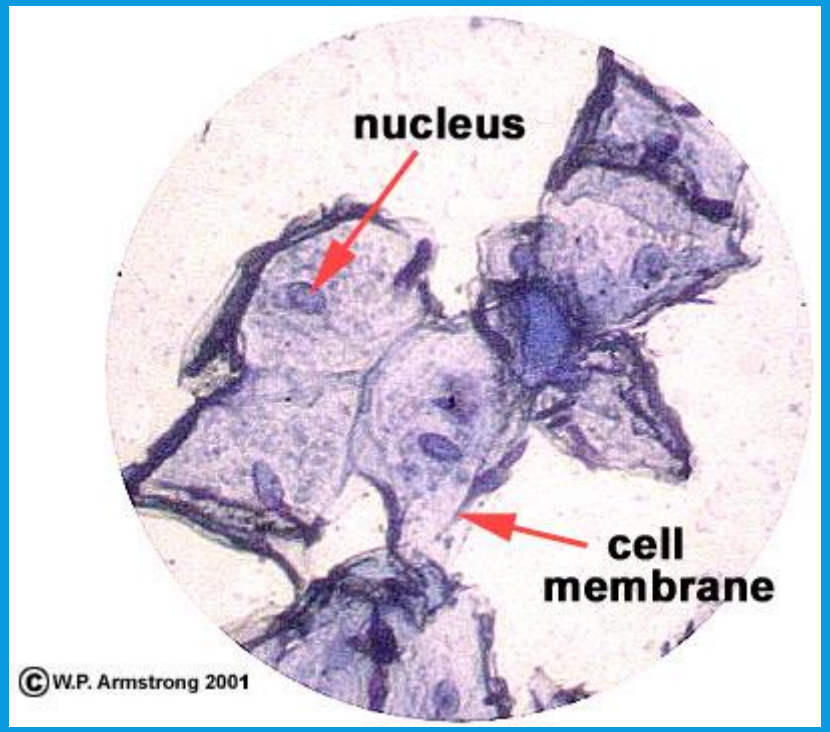


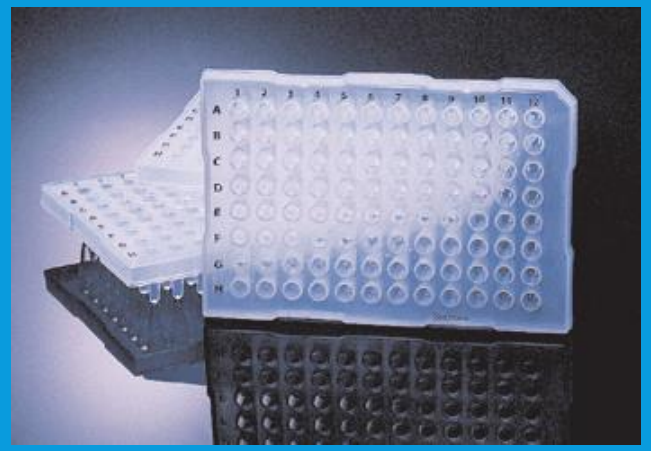


HOT START PCR: Antibody – Enzyme Complex



SINGLE CELL AMPLIFICATION TECHNOLOGY





TIPS FOR AVOIDING CONTAMINATION

- Equipment, such as pipettes, and reagents for setting up PCR should be kept separate from other lab supplies, especially those used for analysis of PCR products.
- Disposable gloves should be worn and changed frequently.
- Reactions may also be set up in a laminar flow hood, if available.
- Aerosol-resistant pipet tips should be used and changed on every new sample to prevent cross-contamination during liquid transfers.
- Reagents should be carefully prepared to avoid the presence of any contaminating DNA or nucleases.
- Ultraviolet irradiation of laboratory PCR set-up space when the area is not in use and cleaning workspaces and instruments with isopropanol and/or 10% bleach solutions help to insure that extraneous DNA molecules are destroyed prior to DNA extraction or PCR set-up