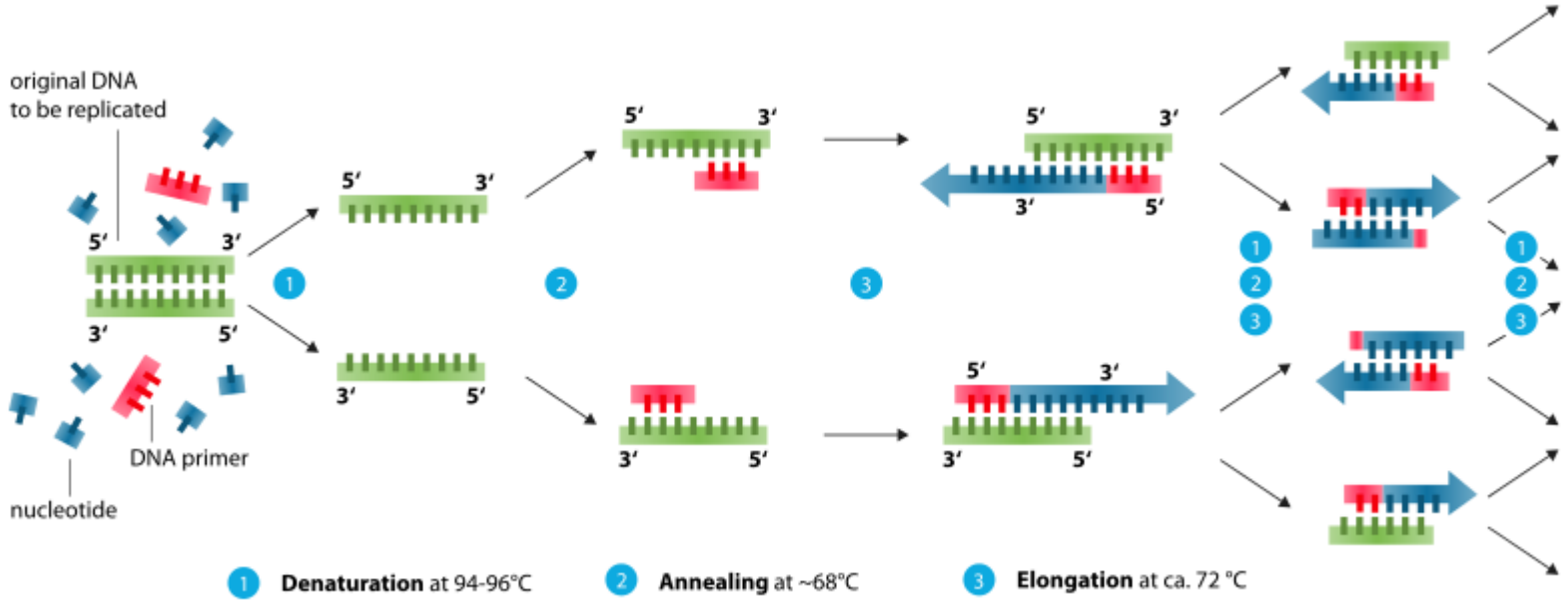
STR Typing Characteristics

Polymerase Chain Reaction

To produce an STR profile from a biological sample many identical copies of the DNA molecules within the target region (i.e. the DNA *template*) are needed. PCR (*polymerase chain reaction*) can be used to copy, or amplify, DNA through three basic steps.



Source: `https://en.wikipedia.org/wiki/Polymerase_chain_reaction`

This process can be repeated many times such that the DNA target gets amplified to millions of copies.

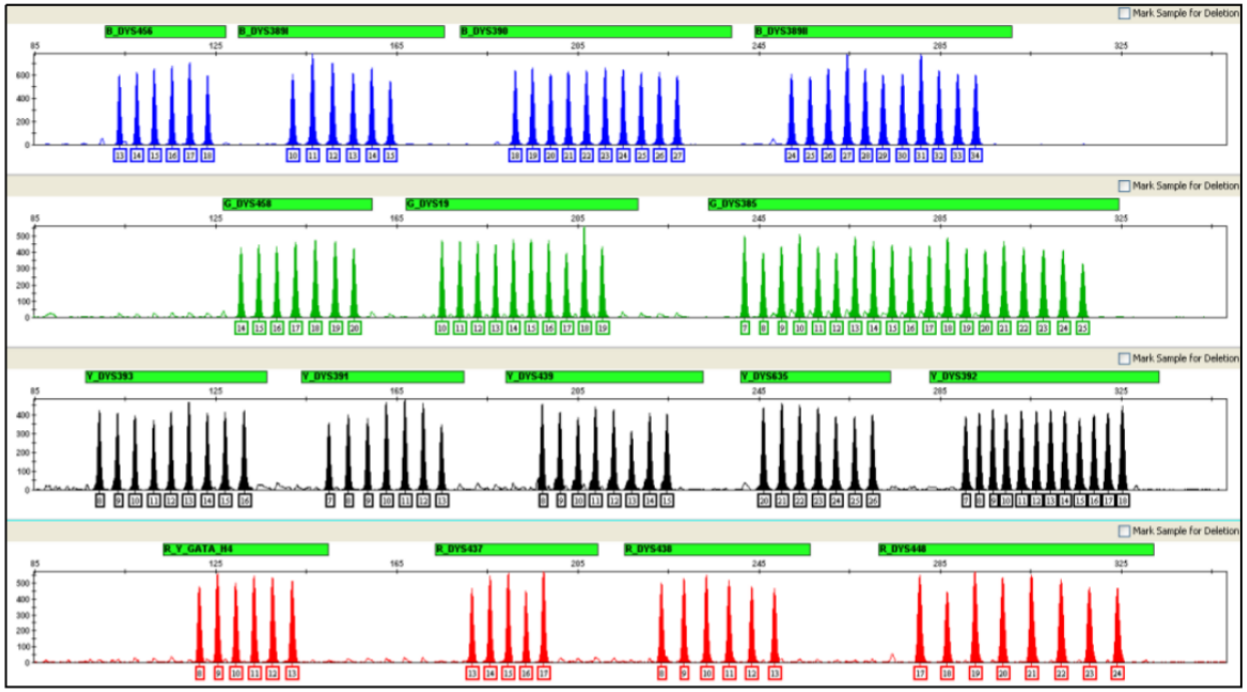
Capillary Electrophoresis

To obtain meaningful results from the PCR process, *capillary electrophoresis* (CE) has traditionally been used, allowing forensic scientists to gain access to the allele numbers contained in a DNA sample.

- DNA products are injected into the capillary where they travel in the direction of a positive charge;
- The travel time depends on the fragment size and can thus be used to infer the number of repeats;
- Primers are labeled with fluorescent dye, which will emit visible light at the detector window of the capillary.
- The fluorescence, measured in relative fluorescence units (RFU), is recorded over time and can be visualized with an *electropherogram* (epg).

Allelic Ladders

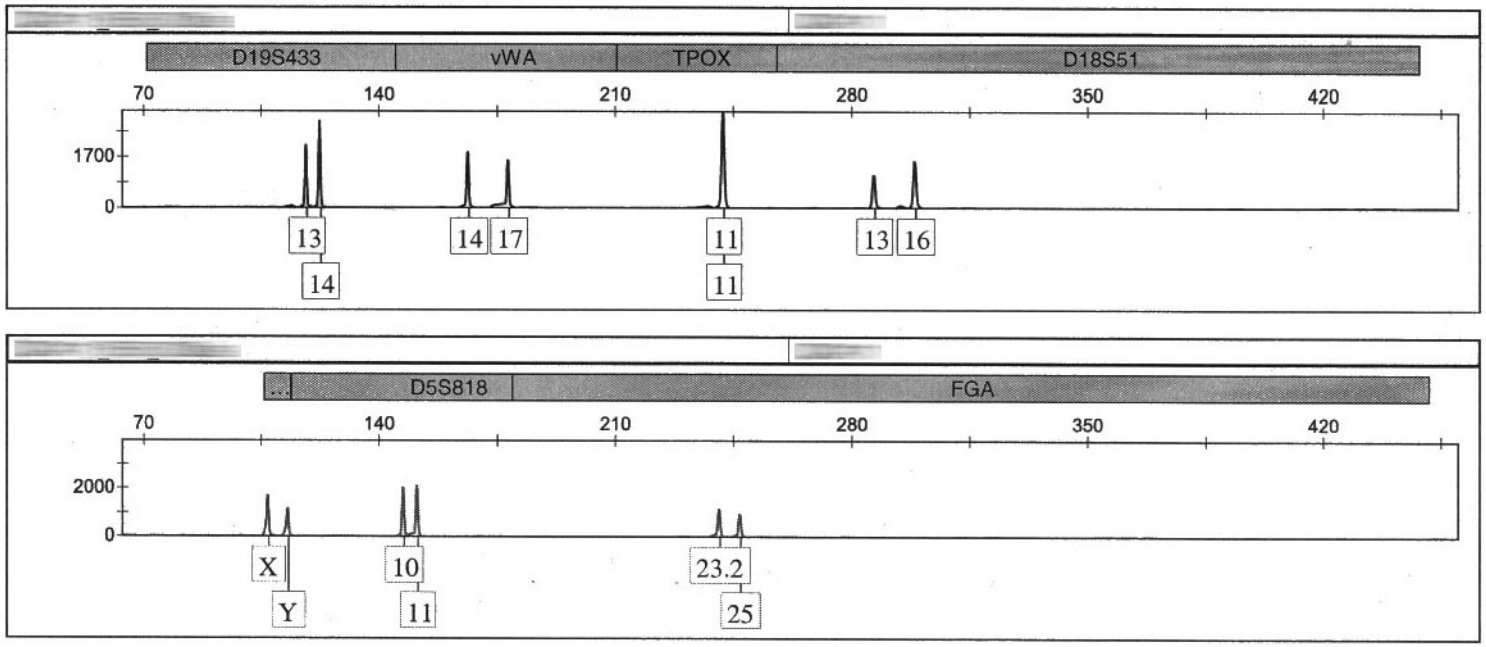
PCR-CE output can be compared to *allelic ladders* to determine allele designations.



Source: *AmpFℓSTR Yfiler PCR Amplification Kit User Guide*.

Example of an Electropherogram

An epg shows allelic designations, represented by peaks, with integer values indicating the number of complete repeat motifs and additional nucleotides separated by a decimal point.



Source: `https://en.wikipedia.org/wiki/Microsatellite`

Anomalies

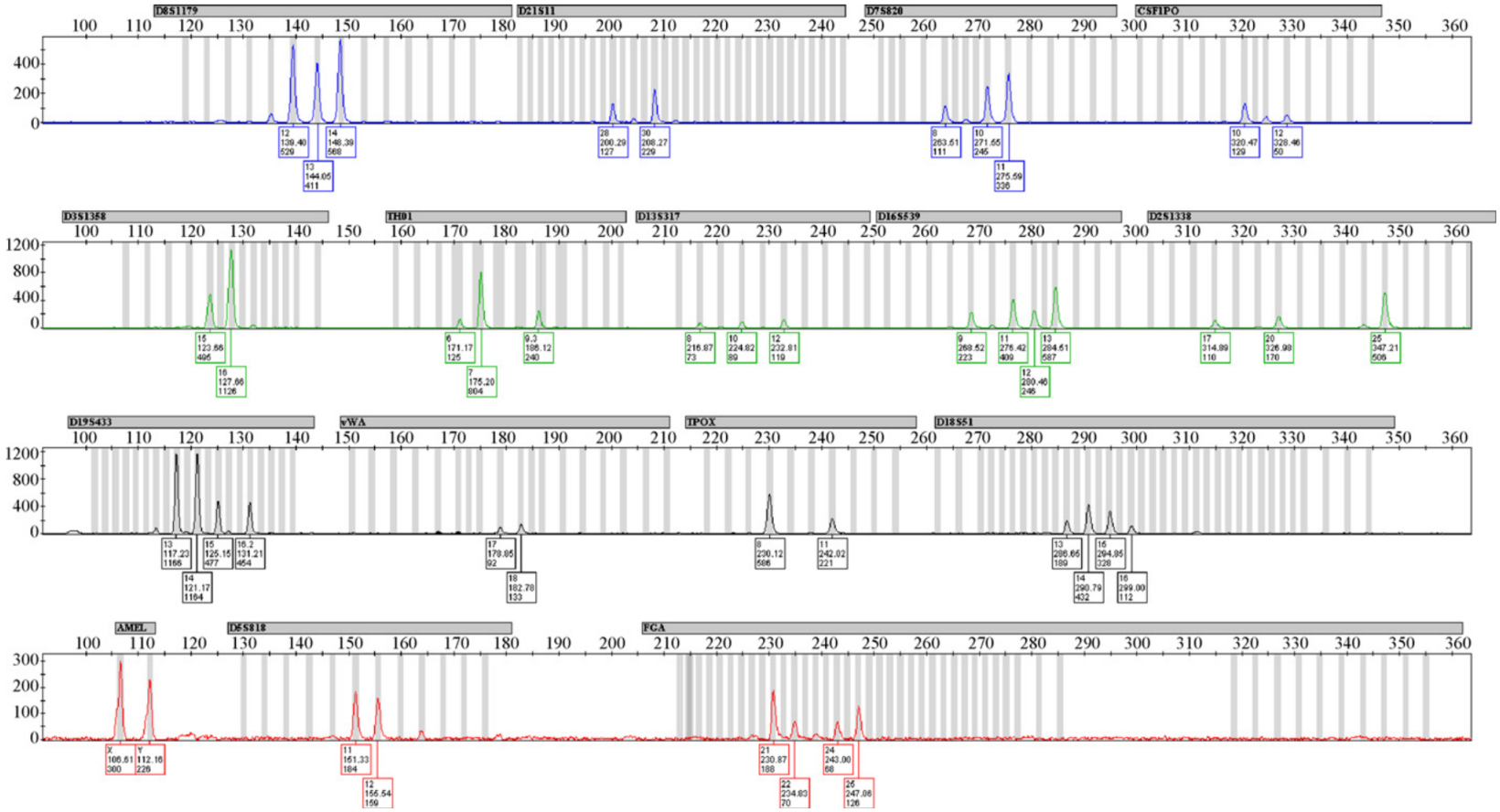
If DNA profiling technologies were flawless, and no other (human) errors have been introduced, an STR profile would provide a perfect representation.

For good-quality samples, this is a reasonable assumption and STR allele calling is usually pretty straightforward.

However, a number of anomalies may still arise. And more importantly, crime scene profiles rarely belong to this category and usually consist of low template samples that may be contaminated and/or degraded, making them even more prone to typing errors.

Peak Height Variability

Several factors play a role in observed variations within STR profiles.



Source: The interpretation of low level DNA mixtures (Kelly et al., 2012).

Template

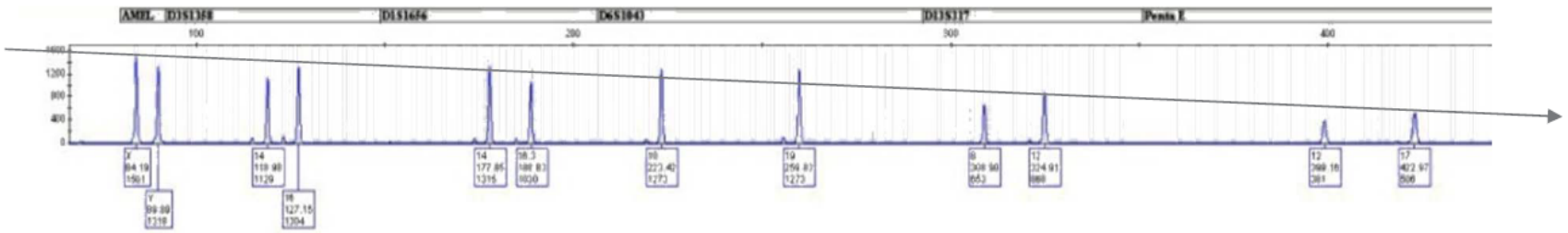
In theory, peak heights from a single contributor are expected to be approximately proportional to the amount of undegraded DNA template. The amount of DNA for each contributor to a sample will therefore directly relate to the peak height of contributors.

In practice, there exists some stochastic variation in peak height. Nowadays, only a couple of picograms of DNA is sufficient to produce results. For these *low template DNA* (LTDNA) samples, stochastic effects can play a major role and will invariably influence the analysis (and likely decrease the statistical weight of the evidence).

Degradation

DNA evidence is prone to degradation due to a variety of mechanisms and circumstances, including chemical processes and environmental conditions, causing breakage of previously intact DNA molecules.

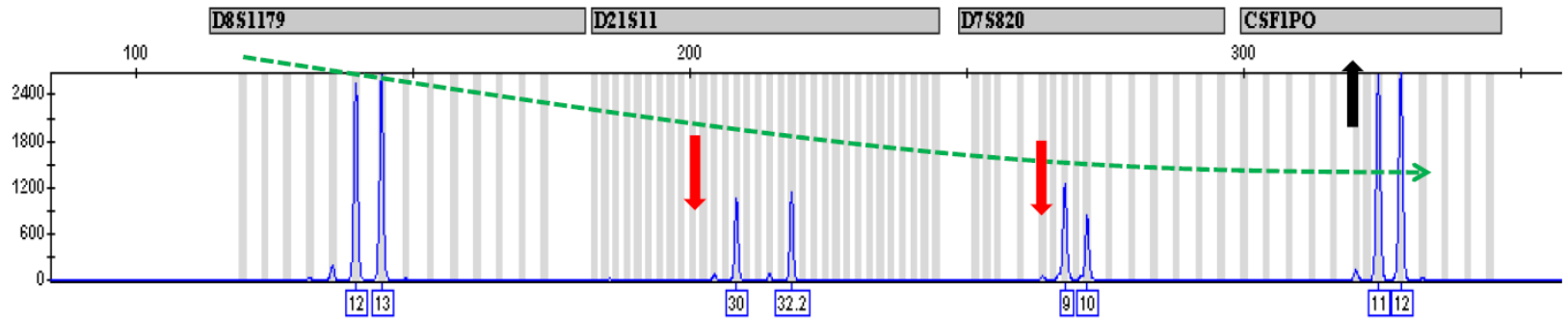
Studies suggest that degradation leads to peak heights showing a downward trend with increasing molecular weight, supposedly because smaller alleles are more resistant to degradation.



Locus Specific Amplification Efficiency

Additional variability arises from differences in amplification efficiency per locus. Observations show that some loci amplify more efficiently than others, and that these differences appear to vary over time.

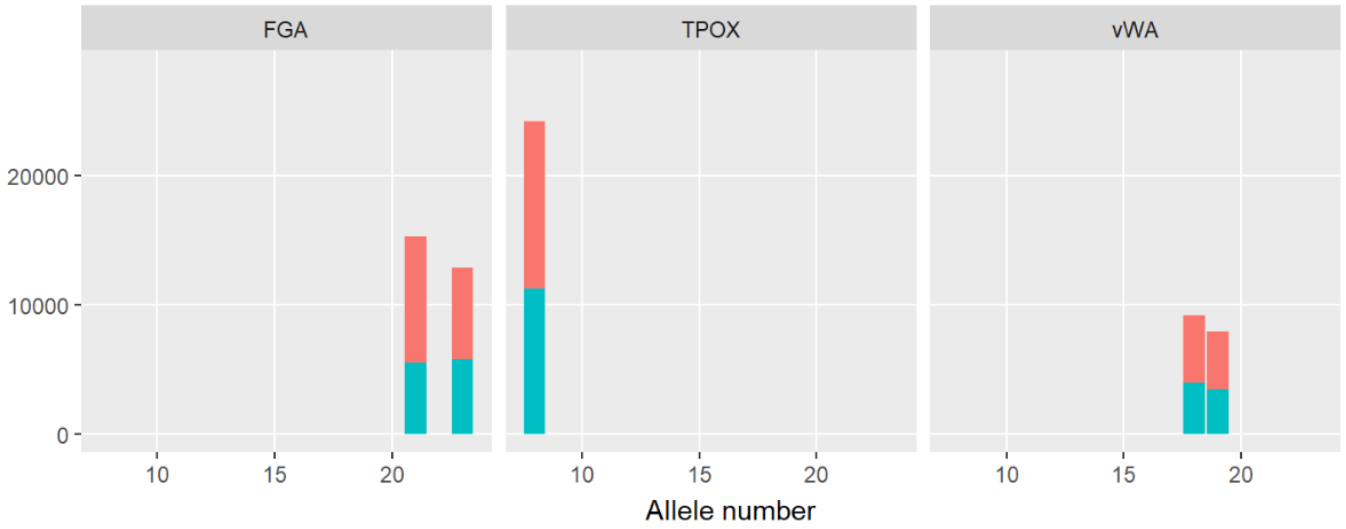
Amplification bias is thought to be a result of the large variation in target loci length.



Replicates

Replicates may show different replicate amplification efficiencies, but can be consolidated into a single analysis, even for different amounts of template DNA. As long as replicates originate from the same DNA extract, they can be used to obtain a more accurate genotype profile.

Replication is not always possible, and in case of a LTDNA sample it would probably be preferable to use as much as possible of the available DNA to give the best possible single-run profile.



Stutter

Since STR typing methods make use of the PCR process, which relies on DNA replication characteristics, replication slippage also exists during DNA amplification of STRs in vitro.