

12-2012

## Artifacts and Noise in DNA Profiling

Dan E. Krane

*Wright State University - Main Campus*, dan.krane@wright.edu

Follow this and additional works at: <https://corescholar.libraries.wright.edu/biology>



Part of the [Biology Commons](#), [Medical Sciences Commons](#), and the [Systems Biology Commons](#)

---

### Repository Citation

Krane, D. E. (2012). Artifacts and Noise in DNA Profiling. .  
<https://corescholar.libraries.wright.edu/biology/243>

This Presentation is brought to you for free and open access by the Biological Sciences at CORE Scholar. It has been accepted for inclusion in Biological Sciences Faculty Publications by an authorized administrator of CORE Scholar. For more information, please contact [library-corescholar@wright.edu](mailto:library-corescholar@wright.edu).

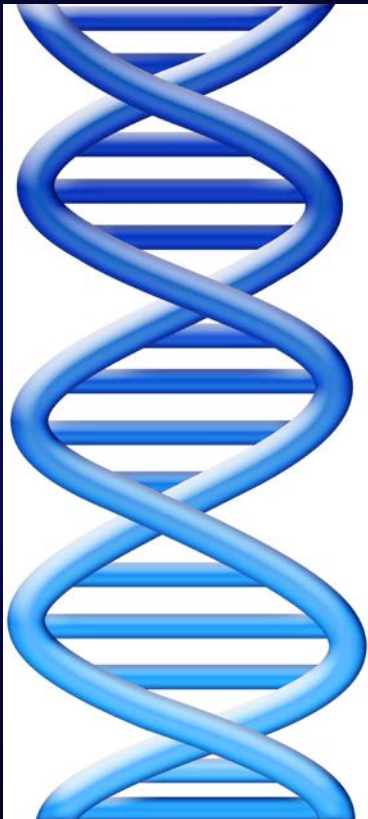
# Artifacts and noise in DNA profiling

Dan E. Krane, Wright State University, Dayton, OH

**Forensic DNA Profiling Video Series**

Forensic Bioinformatics  
([www.bioforensics.com](http://www.bioforensics.com))

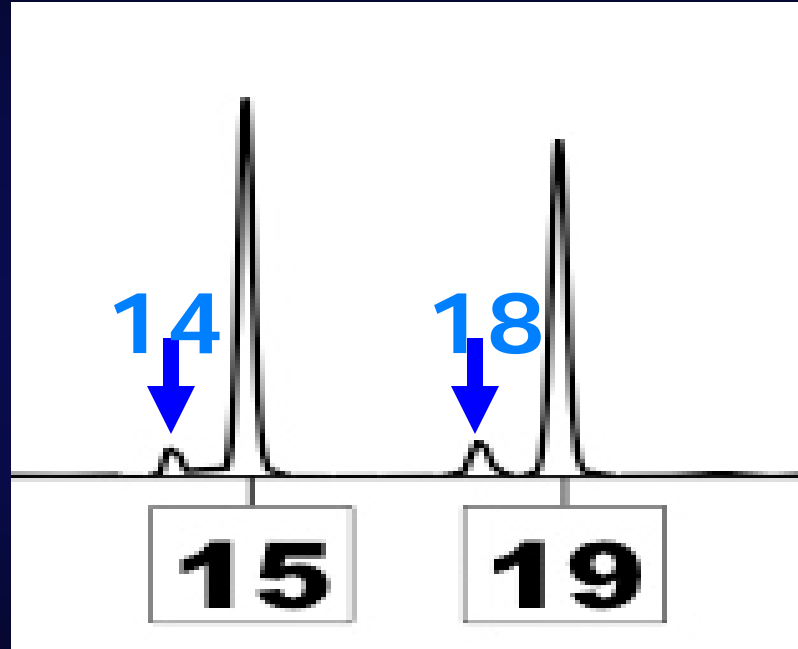
# Factors that can complicate the interpretation of DNA profiles



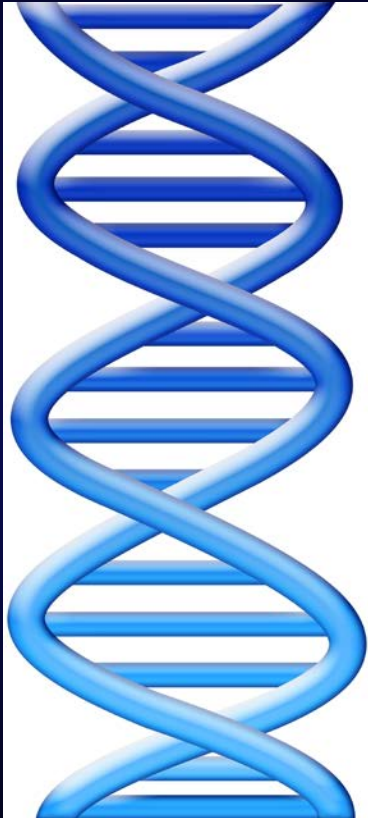
- Technical artifacts
  - Stutter
  - Spikes and blobs
  - Peak height imbalance
  - Degradation/Inhibition
- Background noise

**Stutter**  
**(little extra peaks)**

# Stutter (an artifact)

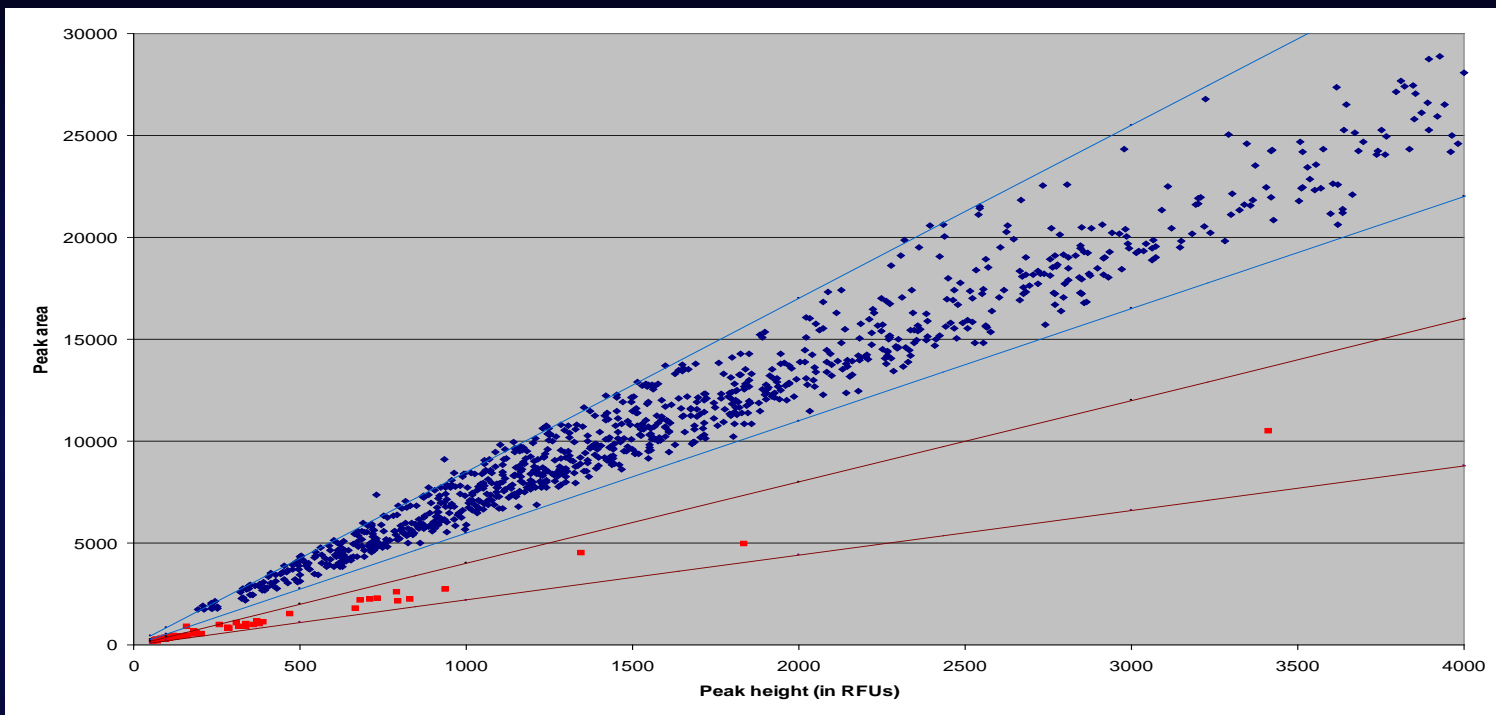


# Factors that can complicate the interpretation of DNA profiles



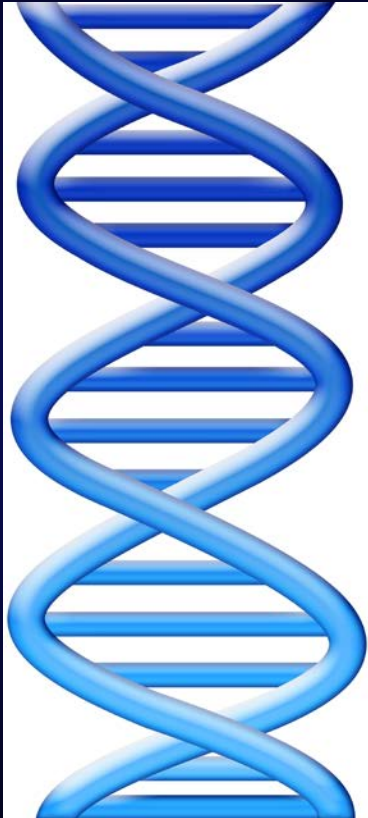
- Technical artifacts
  - Stutter
  - Spikes and blobs
  - Peak height imbalance
  - Degradation/Inhibition
- Background noise

# Spikes (and blobs)



- 89 samples (references, pos controls, neg controls)
- 1010 "good" peaks
- 55 peaks associated with 24 spike events
- 95% boundaries shown

# Factors that can complicate the interpretation of DNA profiles

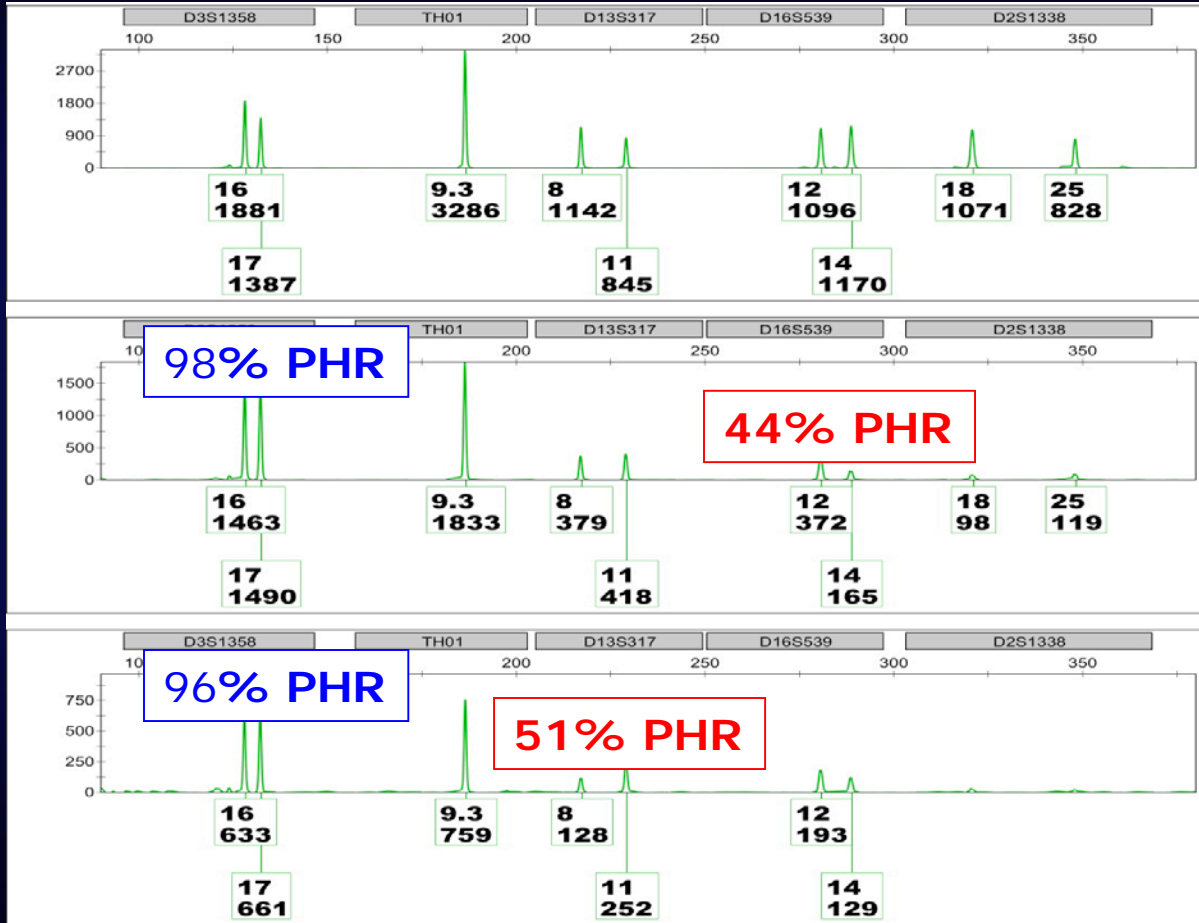


- Technical artifacts
  - Stutter
  - Spikes and blobs
  - Peak height imbalance
  - Degradation/Inhibition
- Background noise

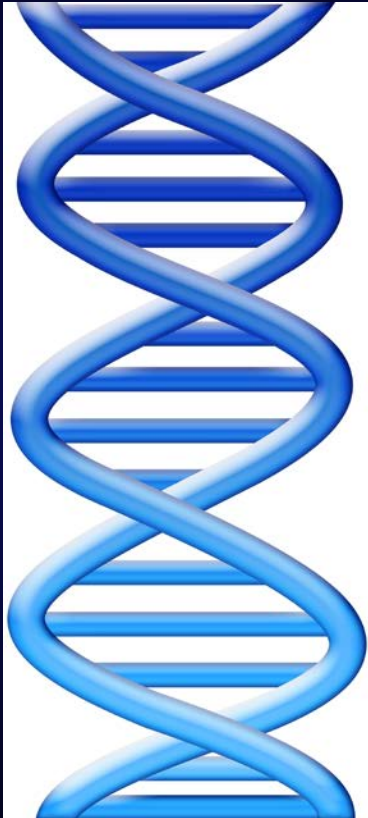


# Peak height imbalances

# Peak Height Ratios



# Factors that can complicate the interpretation of DNA profiles

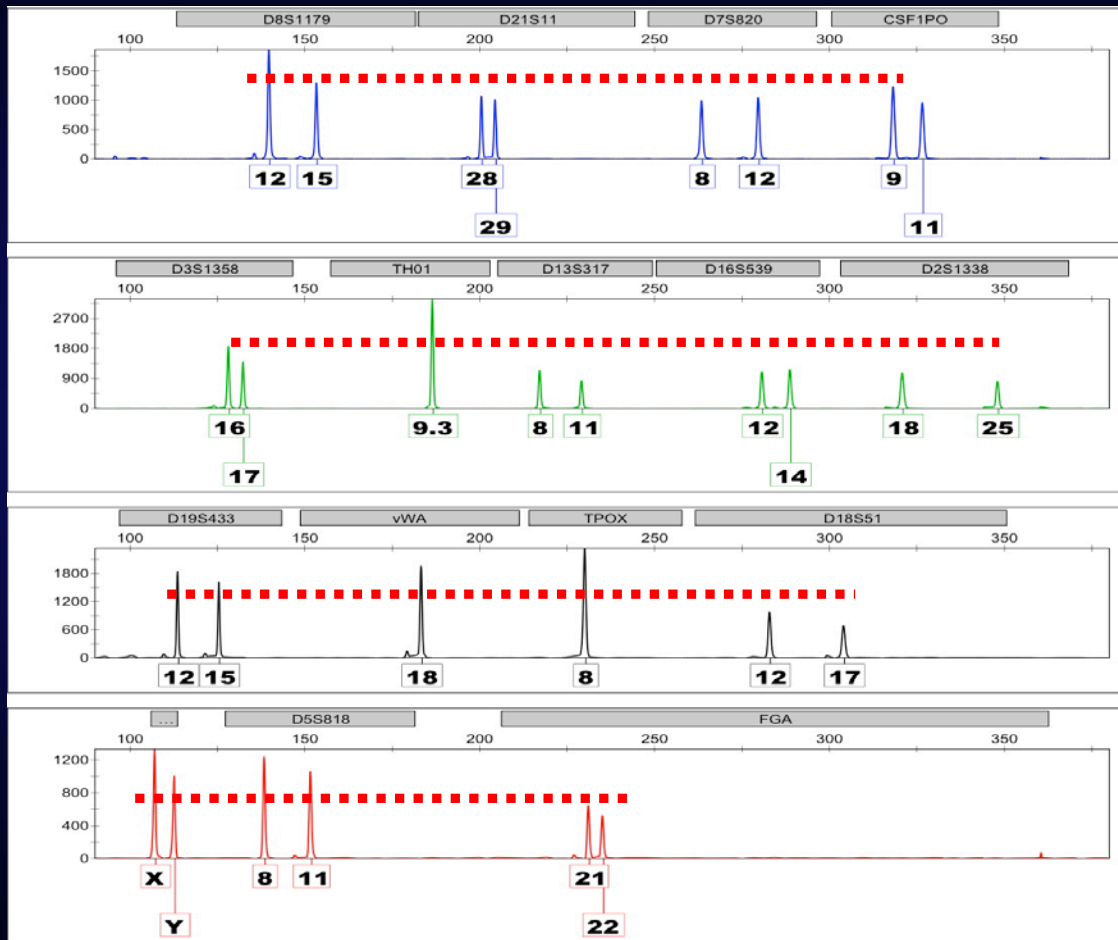


- Technical artifacts
  - Stutter
  - Spikes and blobs
  - Peak height imbalance
  - Degradation/Inhibition
- Background noise

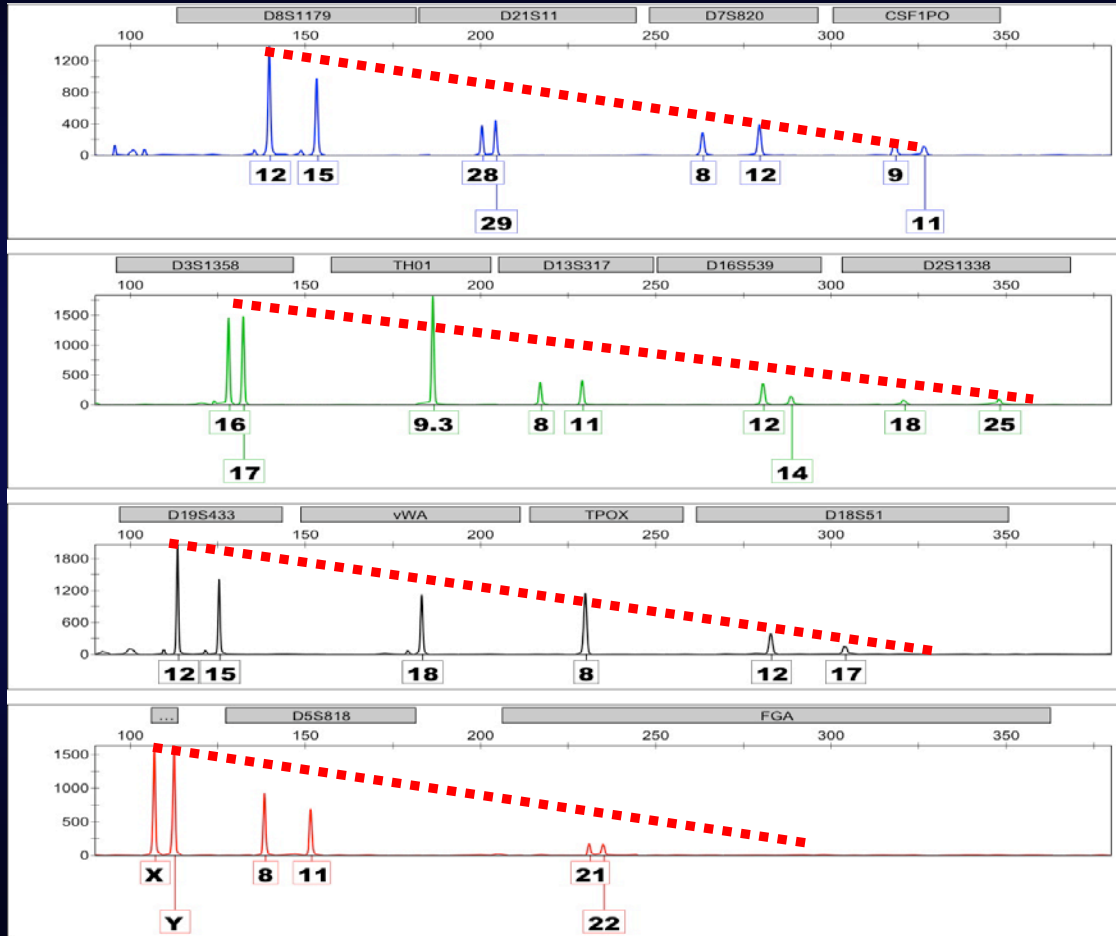
**Degradation**  
**(deterioration of DNA)**

**Inhibition**  
**(poor PCR amplification)**

# Minimal degradation/inhibition

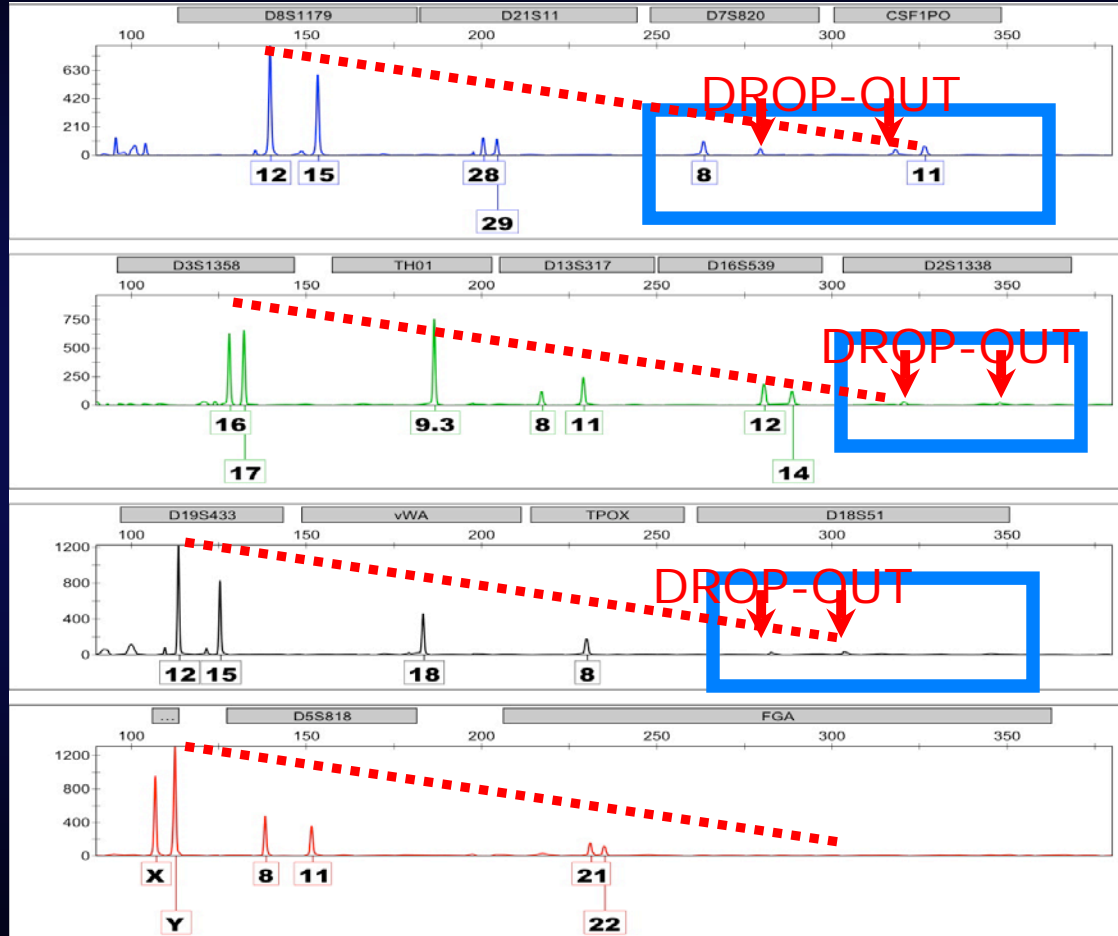


# Slightly degraded/inhibited



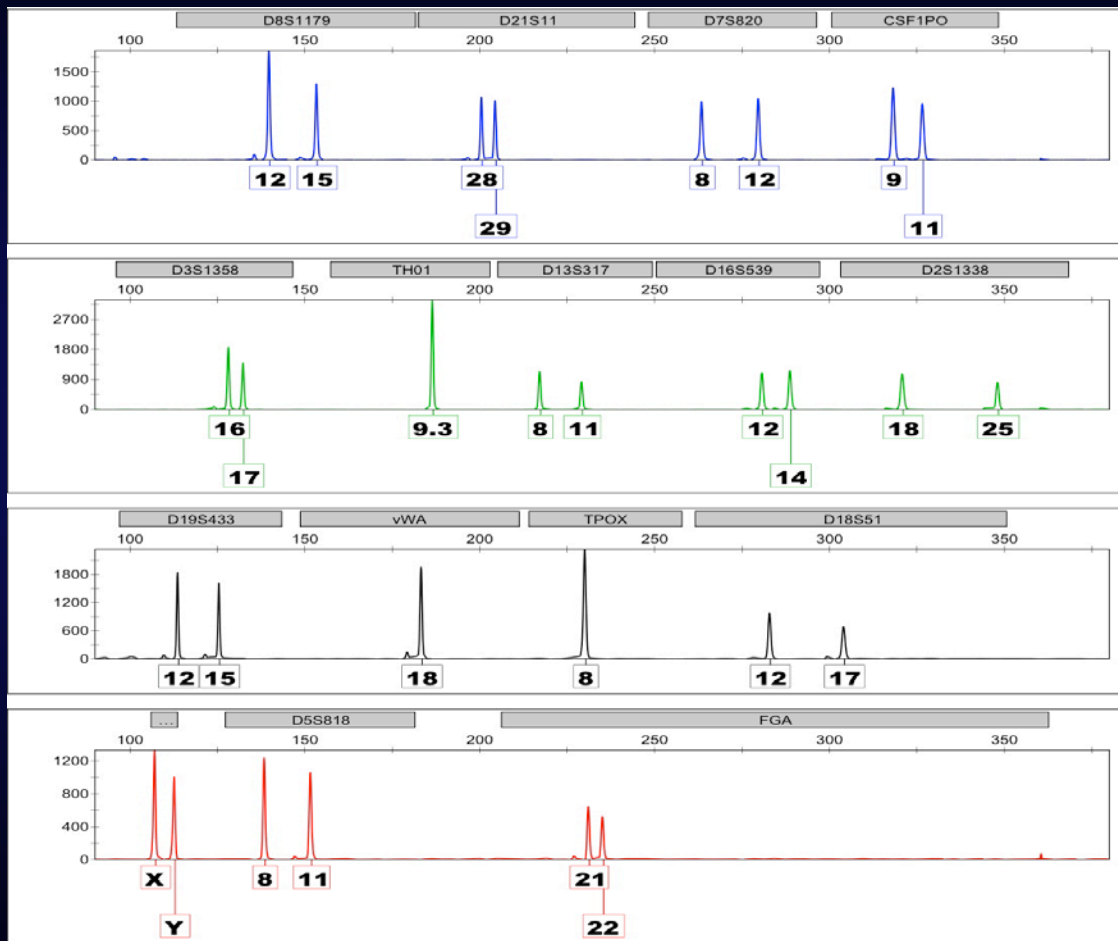
“Ski slope”

# More degraded/inhibited



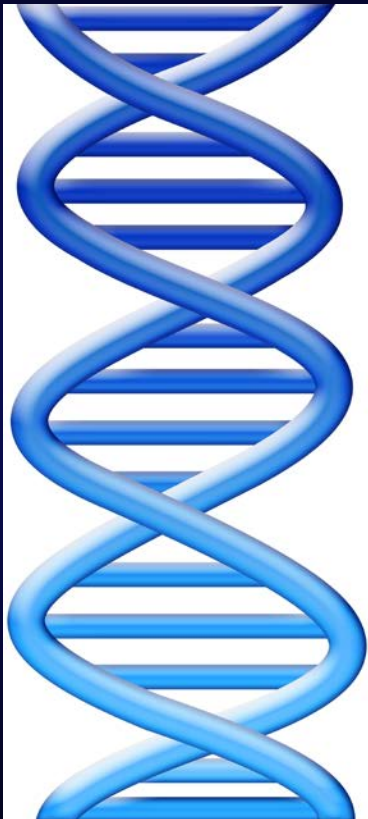
Drop out  
on right

# Minimal degradation/inhibition



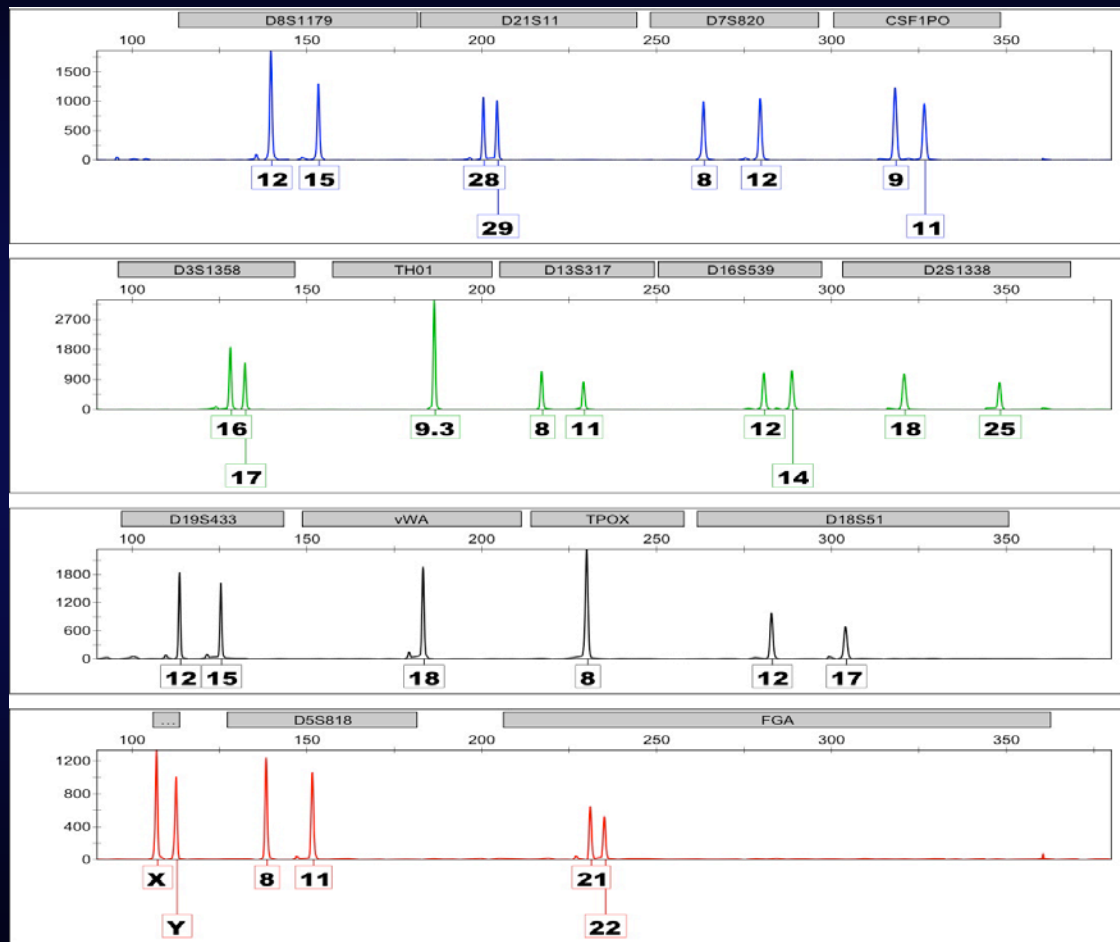


# Factors that can complicate the interpretation of DNA profiles

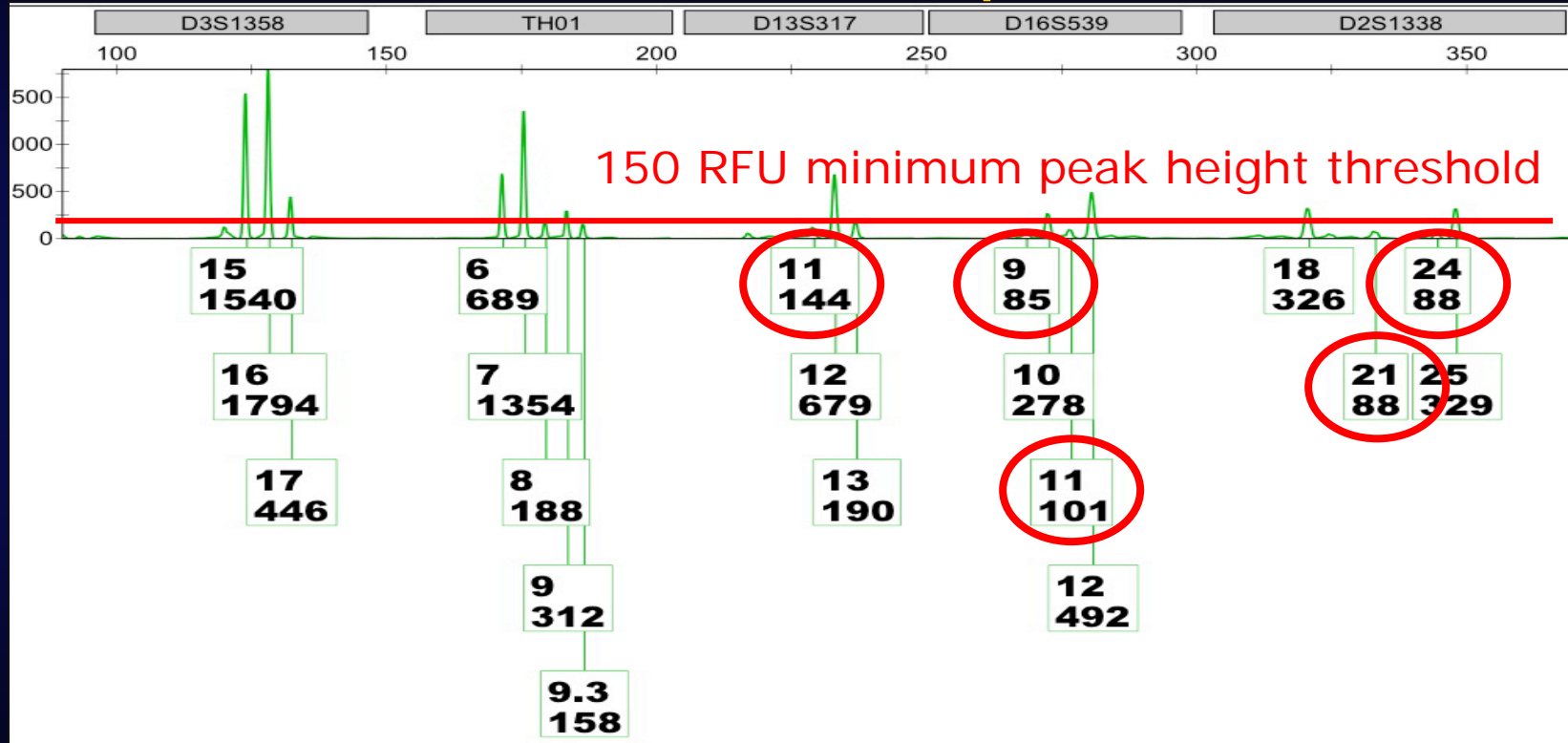


- Technical artifacts
  - Stutter
  - Spikes and blobs
  - Peak height imbalance
  - Degradation/Inhibition
- Background noise

# Sometimes signal is easy to see



# Sometimes signal and noise are hard to tell apart



# Where do minimum peak height thresholds come from (originally)?

- Applied Biosystems validation study of 1998
- Wallin et al., 1998, "TWGDAM validation of the AmpFISTR blue PCR Amplification kit for forensic casework analysis." *JFS* 43:854-870.

# Where do minimum peak height thresholds come from (originally)?

PCR products were examined on both the 377 DNA Sequencer and the 310 Genetic Analyzer. The results of 0.25 to 1.0 ng were clearly typable with peak heights of approximately 150 RFU and greater (data not shown). At 0.125 ng and less, the peak heights in both samples were not significantly above the background (< 150 RFU) or were undetectable. At 0.0313 ng specifically, peaks were extremely low or undetectable, and thus, DNA quantities as low as approximately 35 pg did not produce a typable result. Based on these results, we employed a peak height threshold of 150 RFU, below which peaks were interpreted with caution. Laboratories should determine a minimum peak height threshold for their instruments using high quality, single source genomic DNA samples which provides them with the desired sensitivity while not allowing for detection of low copy DNA. This is particularly important as the overall sensitivity of the assay may vary between laboratories.

# Where do minimum peak height thresholds come from?

- “Conservative” thresholds established during validation studies
- Eliminate noise (even at the cost of eliminating signal)
- Can arbitrarily remove legitimate signal
- Contributions to noise vary over time (e.g. polymer and capillary age/condition)
- Analytical chemists use LOD and LOQ

Measured signal (In Volts/RFUS/etc)

# Signal Measure

Saturation

$$\mu_b + 10\sigma_b$$

$$\mu_b + 3\sigma_b$$

$$\mu_b$$

0

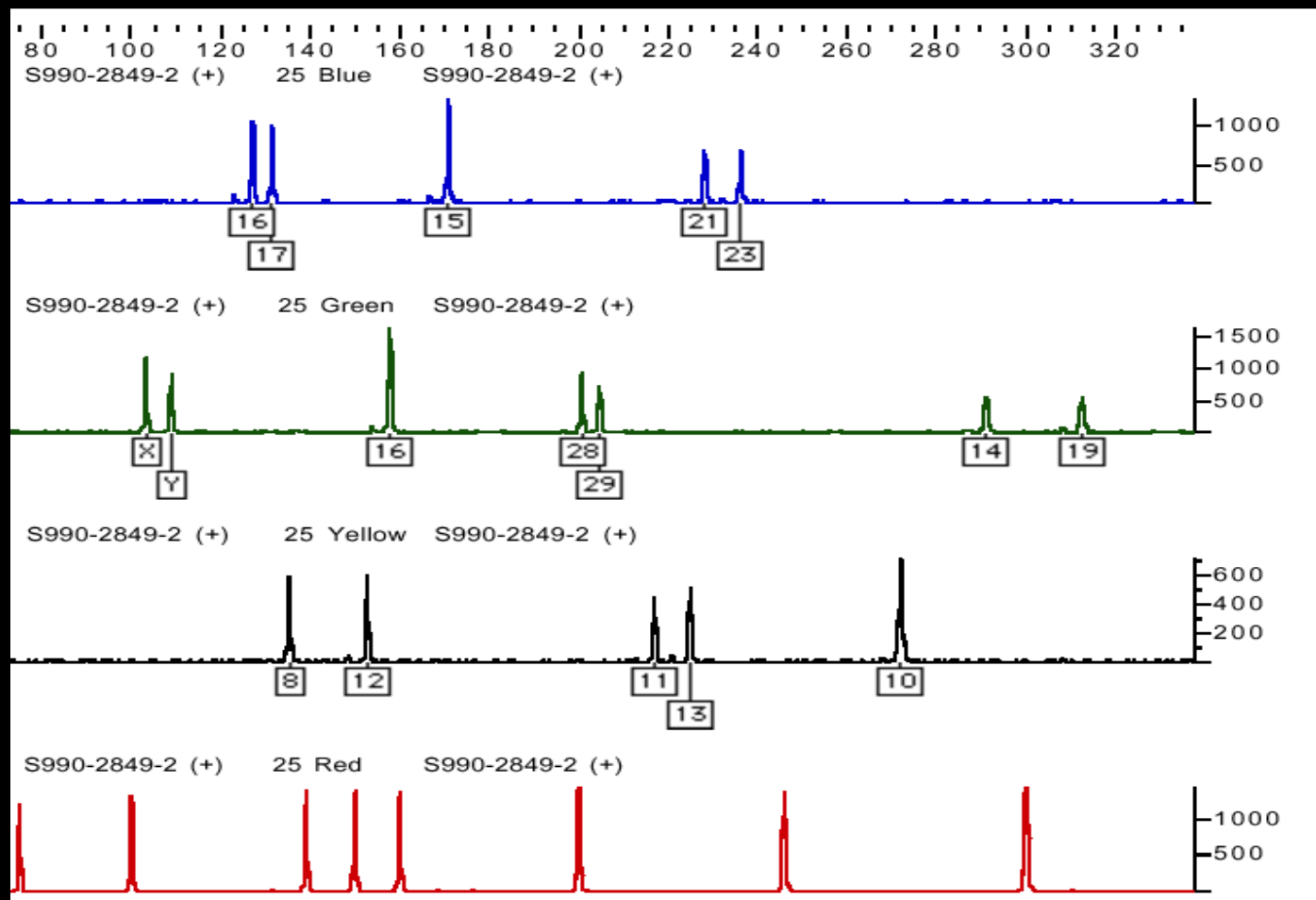


Quantification limit

Detection limit

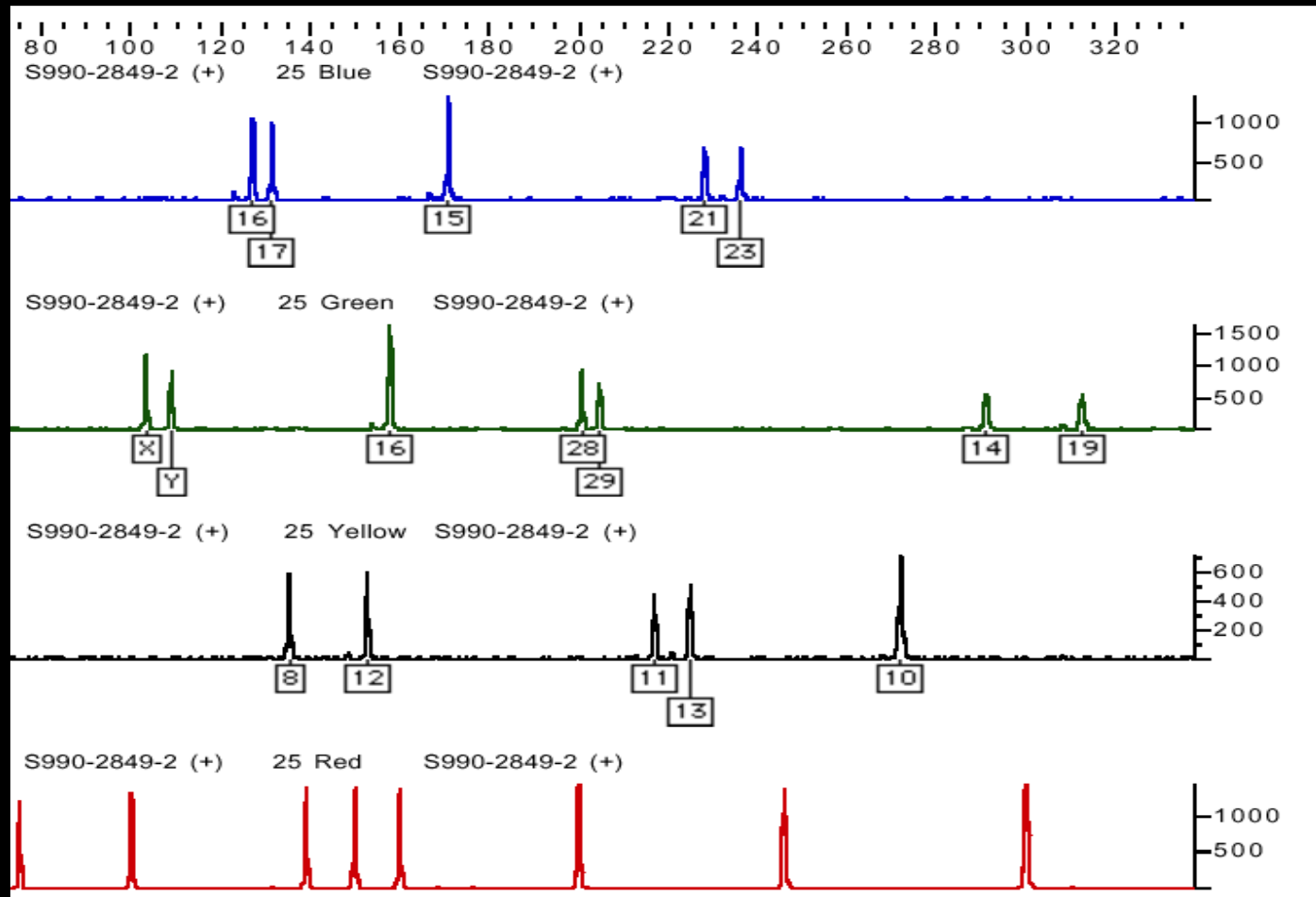
Mean background  
Signal

# Many opportunities to measure baseline

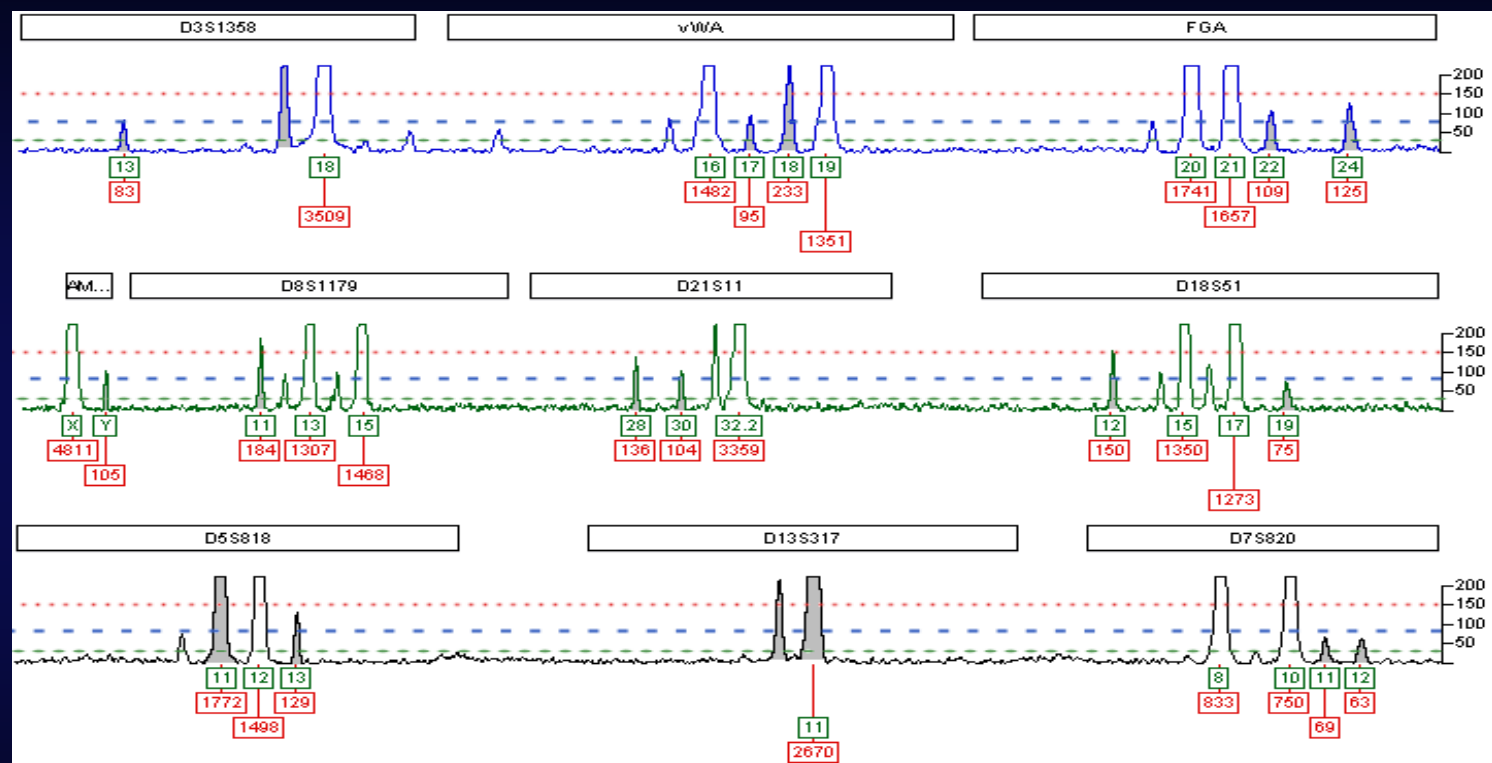




# Doesn't someone either match or not?

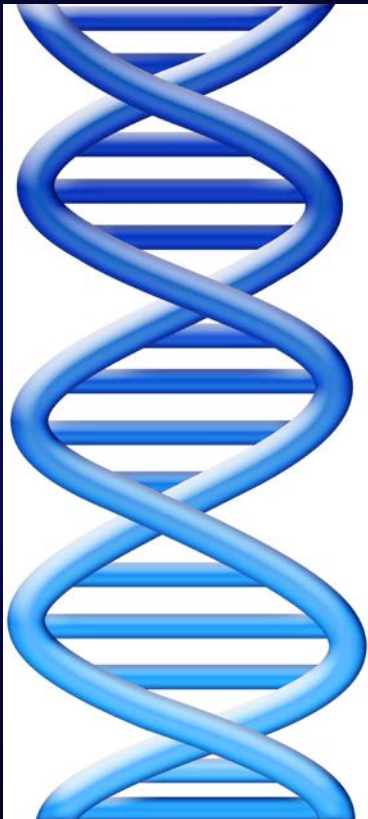


# Lines in the sand: a two-person mix?



Two reference samples in a 1:10 ratio (male:female). Three different thresholds are shown: 150 RFU (red); LOQ at 77 RFU (blue); and LOD at 29 RFU (green).

# Factors that can complicate the interpretation of DNA profiles



- Technical artifacts
  - Stutter
  - Spikes and blobs
  - Peak height imbalance
  - Degradation/Inhibition
- Background noise

# Artifacts and noise in DNA profiling

Dan E. Krane, Wright State University, Dayton, OH

**Forensic DNA Profiling Video Series**

Forensic Bioinformatics  
([www.bioforensics.com](http://www.bioforensics.com))