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Low Template DNA

Oskar Hansson

Statistical methods in forensic genetics 7-10 October 2013, Copenhagen

(slides adapted from Peter Gill)
(Advanced DNA Interpretation workshop ISFG 2013, Melbourne)

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What is Low Copy Number?

- Let's make a list of what LCN is not
  - Its not related to an overall quantity of DNA (such as 200pg)
  - Its not restricted to 'touch DNA'
  - Its not related to any particular technique
- NY court found it to be a simple extension of an existing technique
- R. v. Reed accepted that the 34 cycle definition was not relevant to any definition of LT-DNA
- Why can't a definition be adduced?

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Stochastic variation

- In a heterozygous sample, one allele is amplified more than the other
- Leads to heterozygous imbalance or allele drop-out
  - Good quality DNA will always give heterozygous balance >60% i.e. both target alleles are amplified with similar efficiency
- Much more pronounced with low level DNA as there is less template DNA
- If one target gets amplified more in the first rounds of PCR then it becomes preferentially amplified in later rounds

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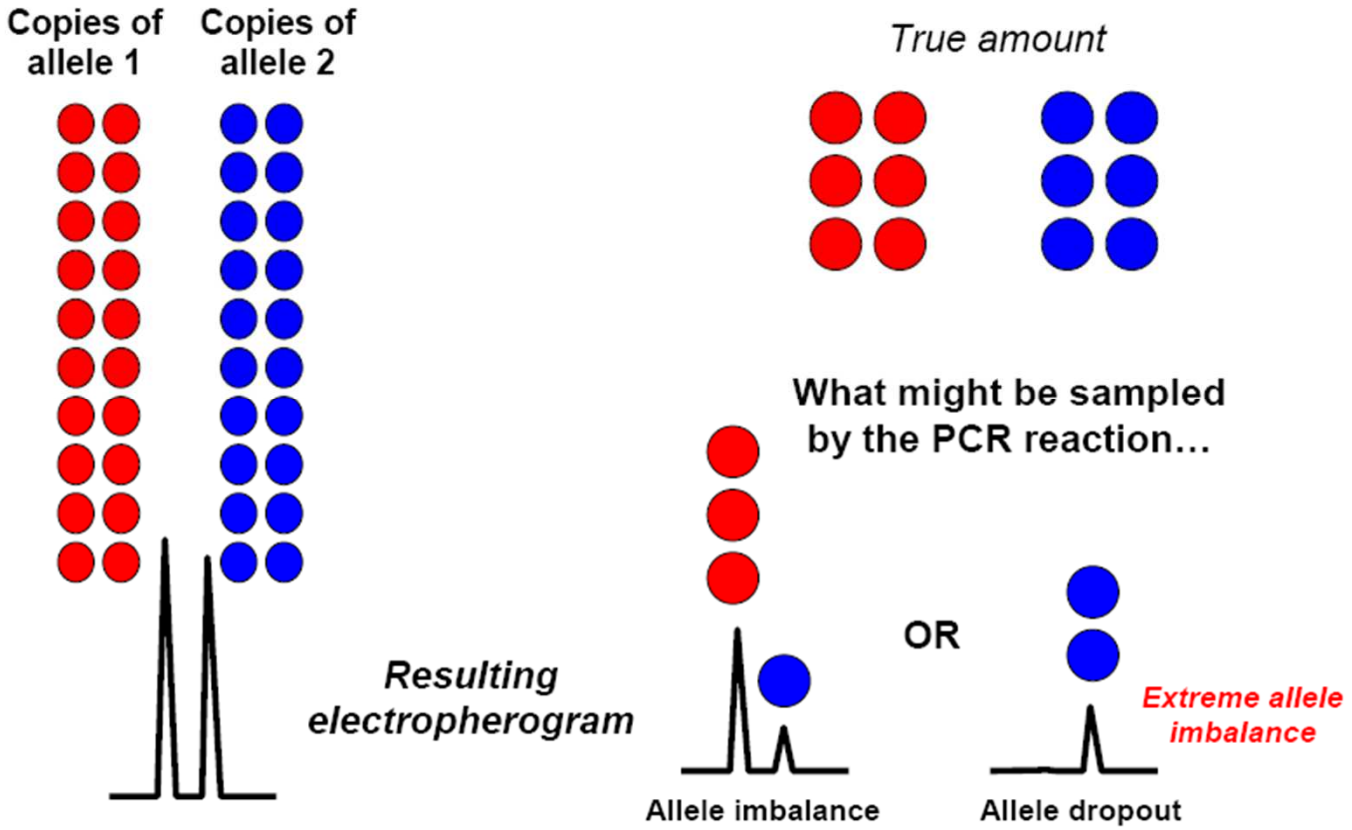
Heterozygous imbalance OR allele drop-out
(from J Butler, NIST)



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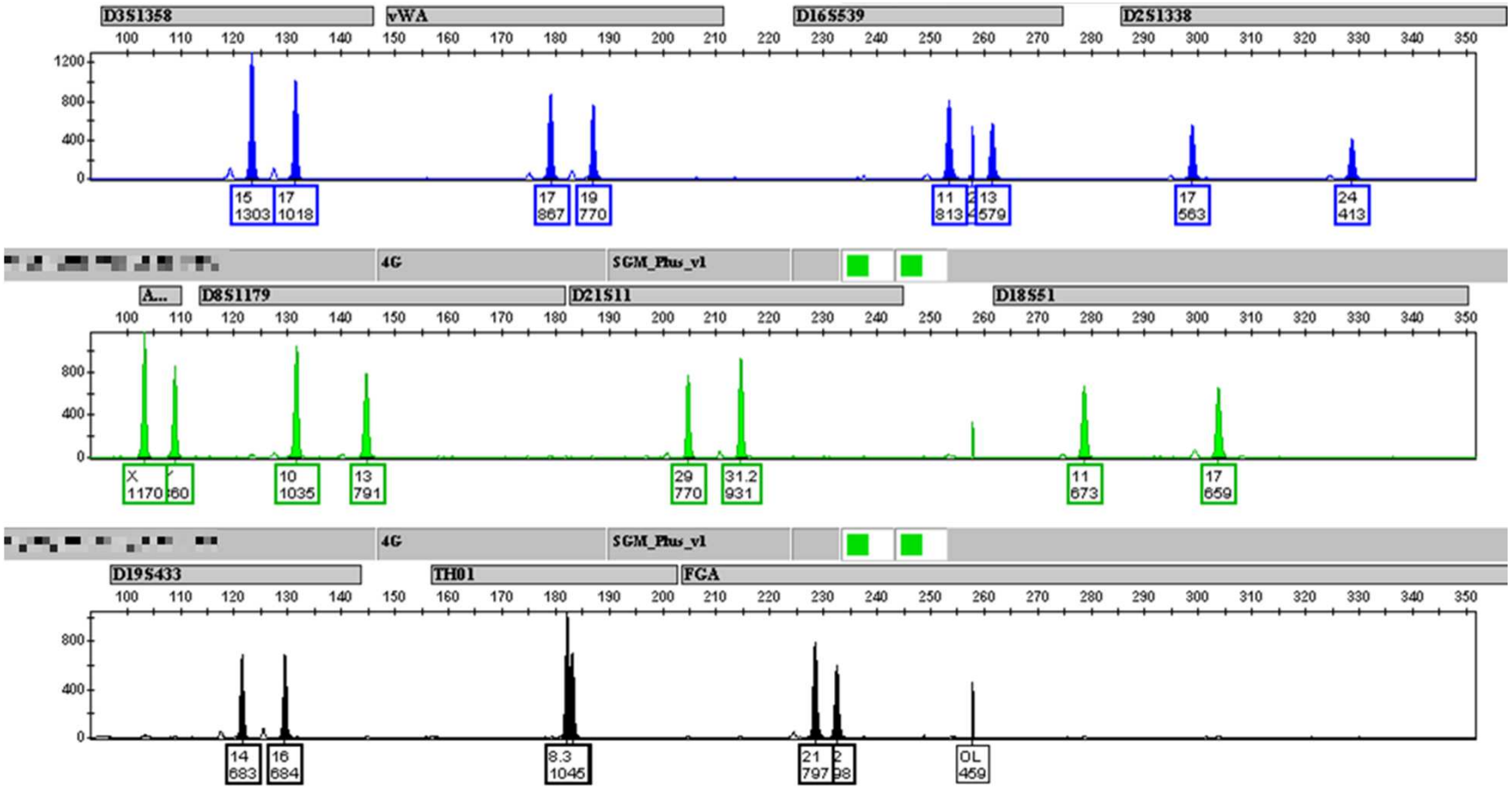
Example of good level DNA



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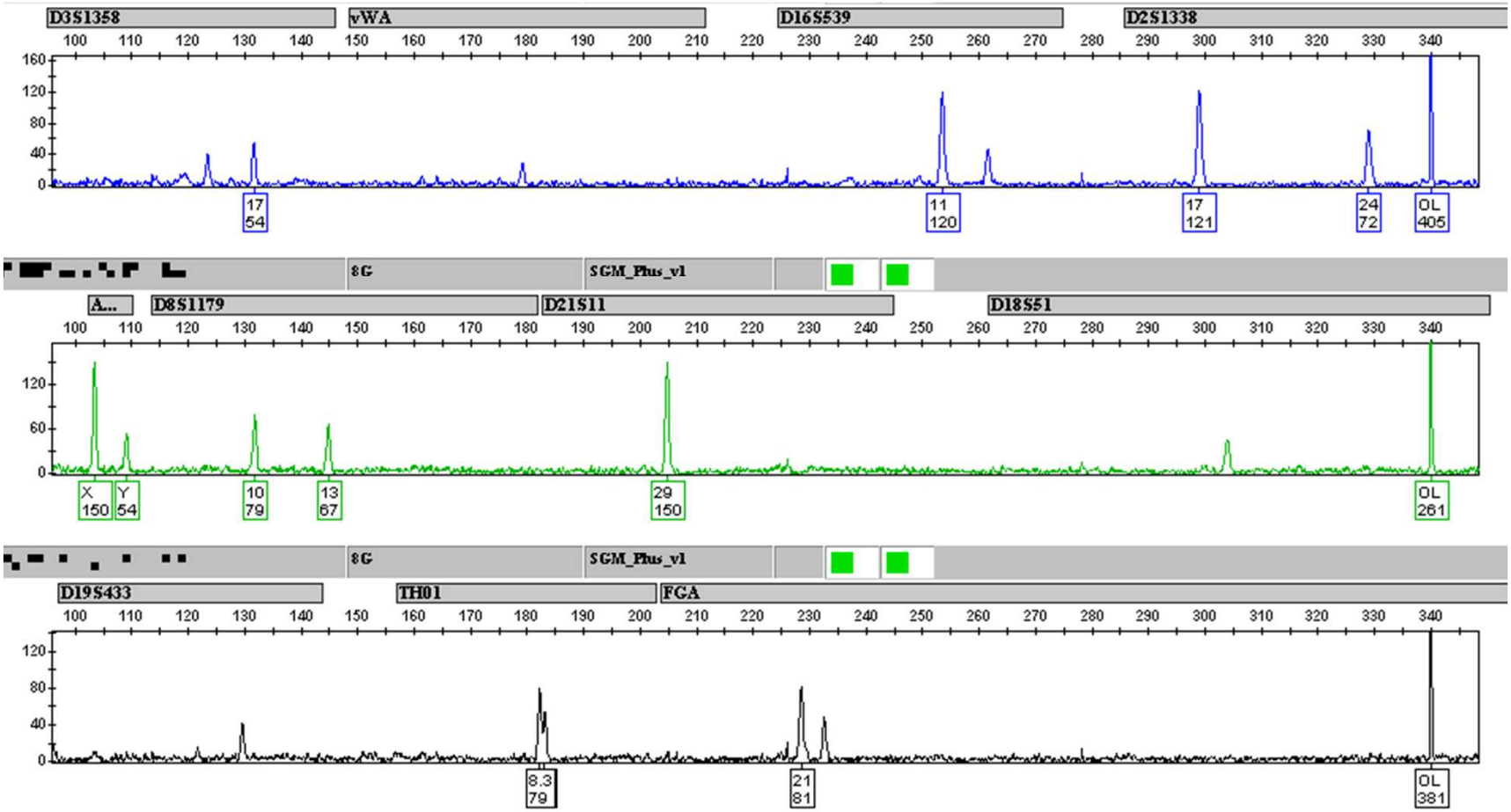
Example of low level DNA



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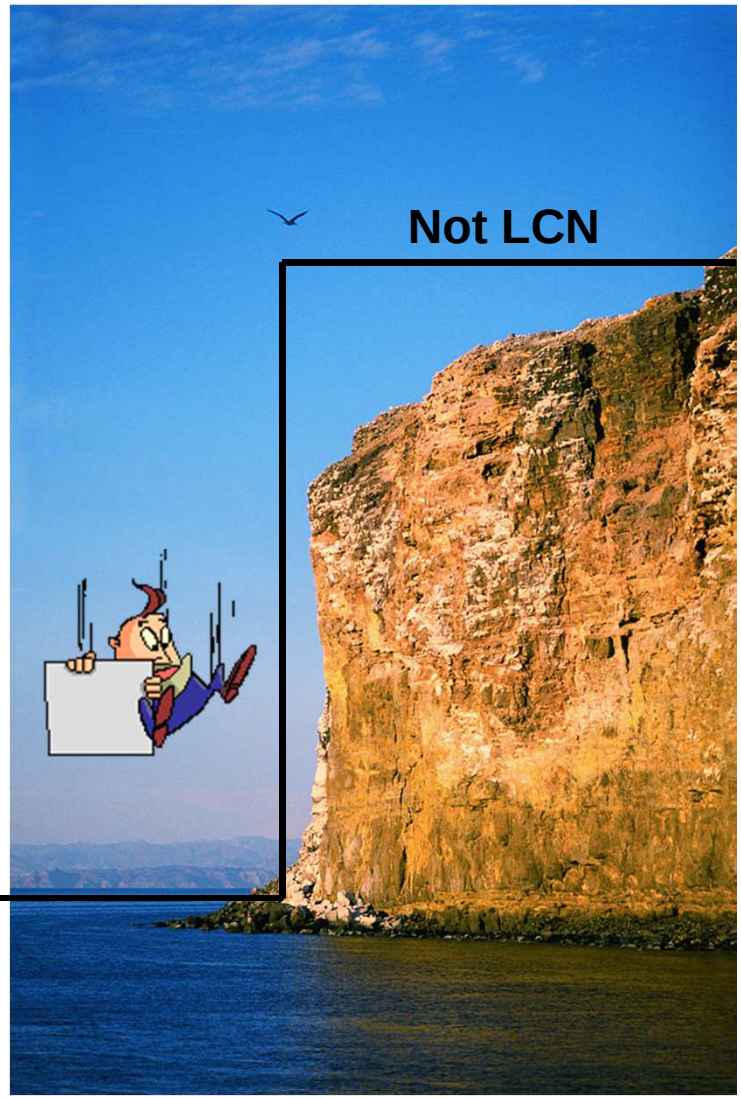
Illogical use of thresholds

- Falling off the cliff

For example if we have a rule that states:
150 RFU – This is conventional
versus 149 RFU – This is LCN

- There is nothing in between



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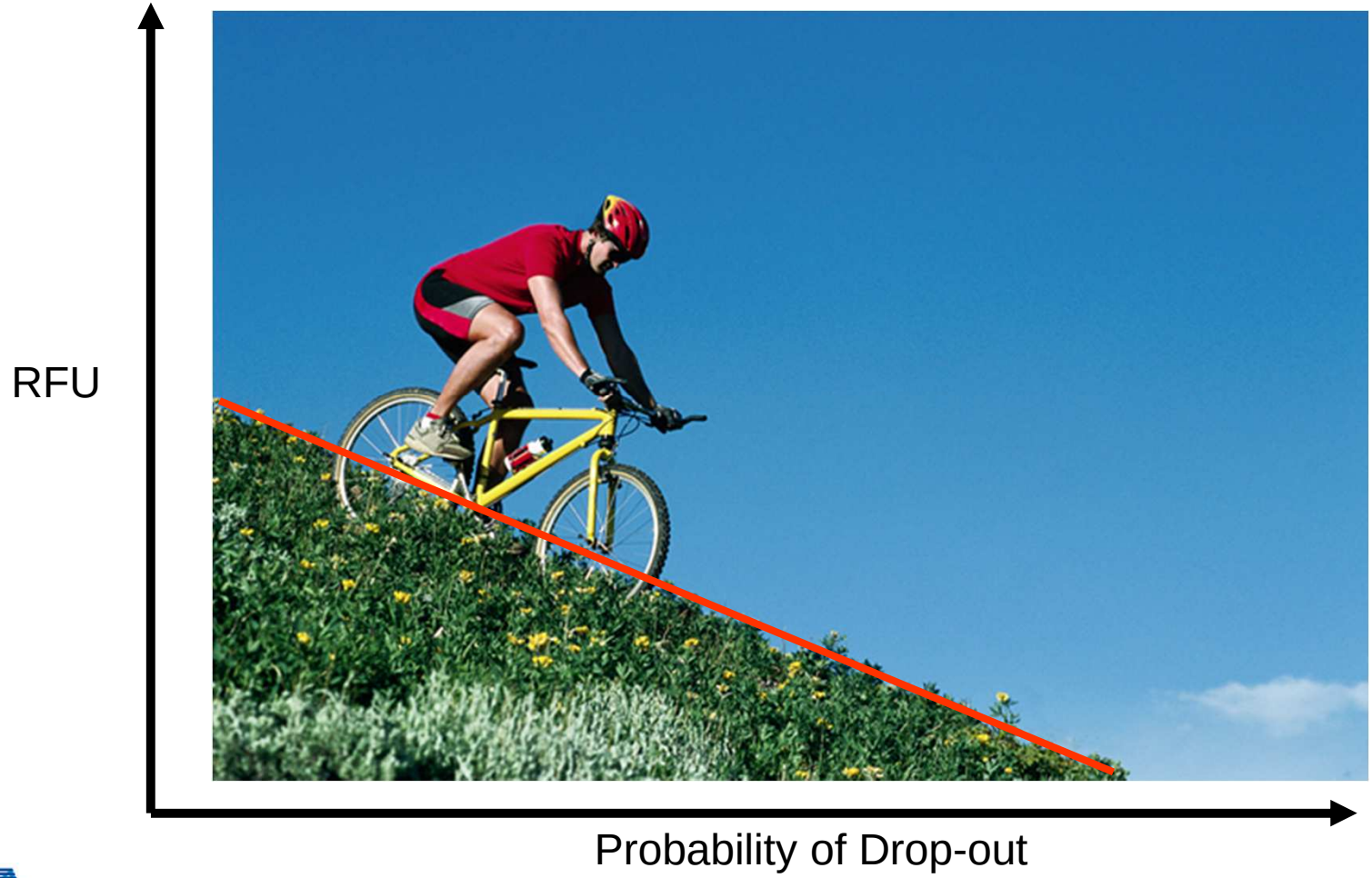
In reality it's a gentle ride downhill



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Continuum of change

- The peak height rules break down when the quantity of DNA becomes very low – in particular the Hb guideline will no longer hold true
- Allele drop-out can lead to a heterozygous locus being genotyped as a homozygous locus
  - In standard DNA profiling, a homozygous peak height of 150 RFU is often used (stochastic threshold) i.e. single peaks <150 RFU are labeled ‘F’ indicating allele drop-out may have occurred

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This is why we prefer a universal method

Forensic Science International: Genetics 4 (2010) 221–227

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

A universal strategy to interpret DNA profiles that does not require a definition of *low-copy-number*

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Papers outlining heterozygous balance

Holt CL, Buoncristiani M, Wallin JM, Nguyen T, Lazaruk KD, Walsh PS. (2002) TWGDAM validation of AmpFlSTR PCR amplification kits for forensic DNA casework. J. Forensic Sci. 47(1): 66-96.

Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. (2004) Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTR Identifiler PCR amplification kit. J. Forensic Sci. 49(6): 1265-1277.

L.A. Dixon, C.M. Murray, E.J. Archer, A.E. Dobbins, P. Koumi & P. Gill (2005) Validation of a 21-locus autosomal SNP multiplex for forensic identification purposes. For. Sci. Int. 154 (1): 62-77

Gill, P., Sparkes, R. and Kimpton, C. (1997). "Development of guidelines to designate alleles using an STR multiplex system." *Forensic Sci Int* **89**(3): 185-197

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Degradation

- Occurs with fragmented / degraded DNA as there are more of the small target molecules available for amplification
- Leads to a distinctive slope in peak heights across the profile

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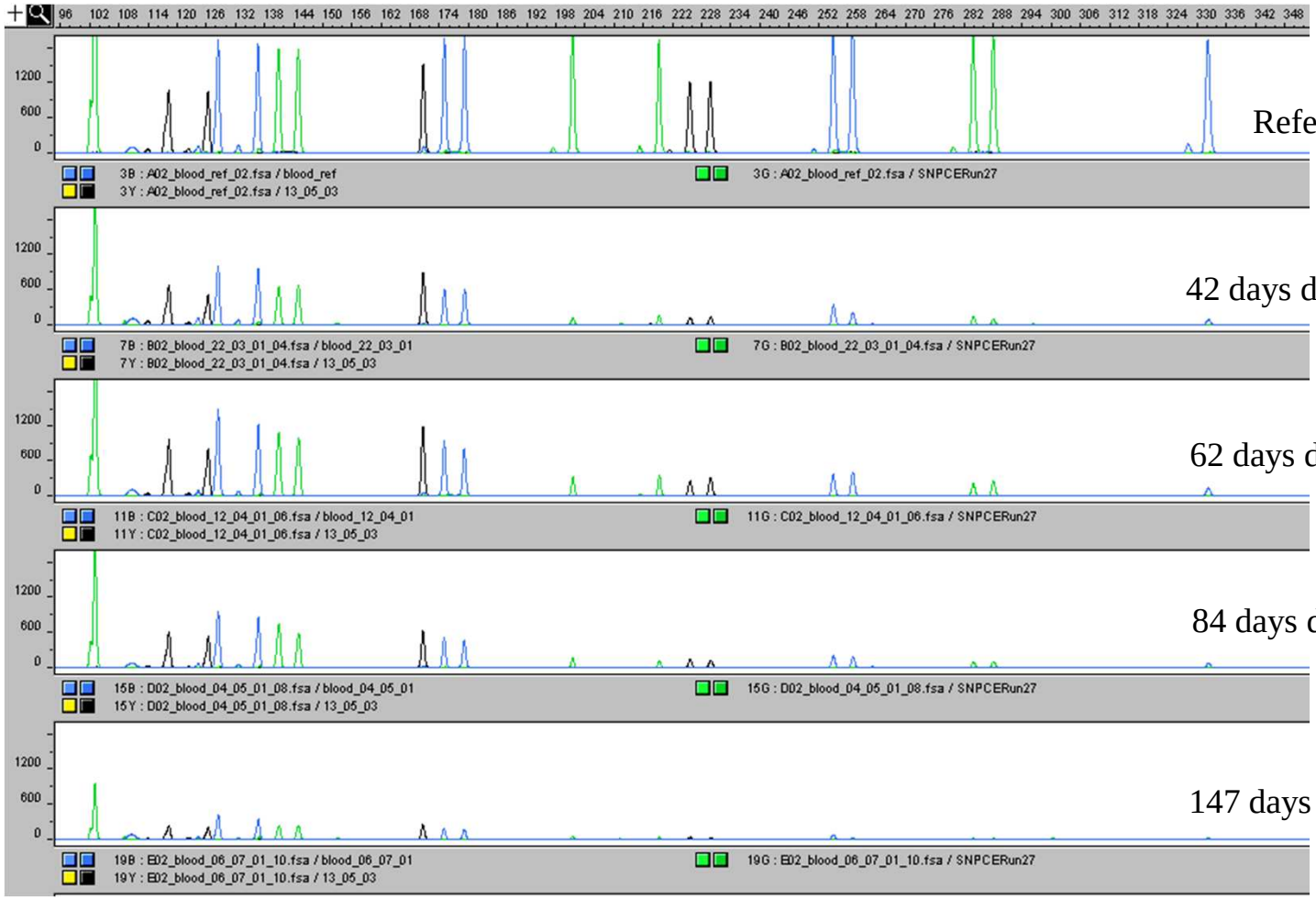
Effect of degradation



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Allele drop-in

- A contamination event resulting in only one or two foreign alleles
- Independent from gross contamination in that it comes from different sources

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Contamination

- Gross contamination is identified as being from a single contributing source
- Dependent on transfer event as to when contamination occurred
- Could be pre-incident or post-incident

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New methods

- Incorporate probability of dropout and dropin
- Uses statistical theory that is well established
- The theory can be used to evaluate complex mixtures
- No limitation on number of contributors
- No limitation on number of replicates that can be combined to form a single LR

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