2012 Mixture Interpretation Workshop:
Mixtures **U**sing *SOUND* **S**tatistics, **I**nterpretation, & **C**onclusions

Profile Enhancement: Pros and Cons

Does Increased Profile Information Follow Signal Optimization?

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Profile Enhancement

- What does this mean?

- Enhance: to heighten or increase; *especially* **:** to increase or improve in value, quality, desirability, or attractiveness <*enhanced* the room with crown molding>

SWGDAM Interpretation Guidelines

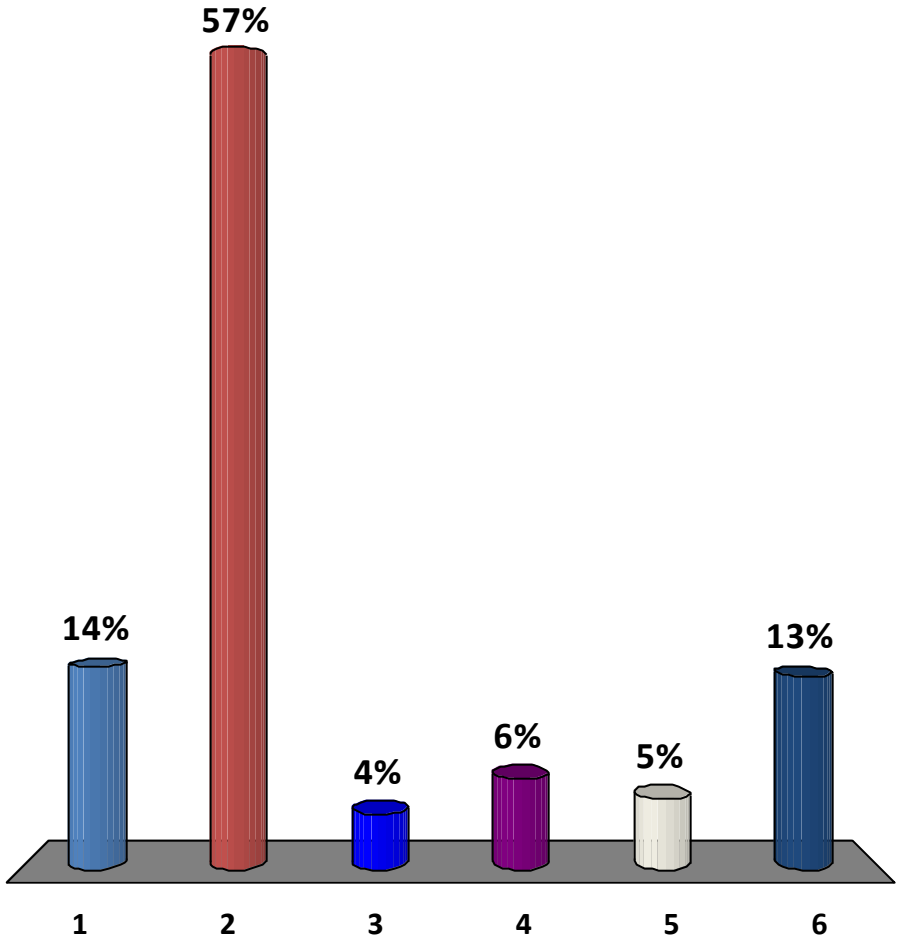
- It is anticipated that these guidelines will evolve further as future technologies emerge. Some aspects of these guidelines may be applicable to low level DNA samples. ***However, this document is not intended to address the interpretation of analytical results from enhanced low template DNA techniques.***

- 3.2.1.1. If measures are used to enhance detection sensitivity (i.e., allelic height), the laboratory should perform additional studies to establish independent criteria for application of a separate stochastic threshold(s). Such measures may include but not be limited to increased amplification cycle number, increased injection time, and post-amplification purification/concentration of amplified products.

Which of these “enhancement” procedures is allowed by your SOP? (up to 4 responses)

1. Increase in PCR cycle number
2. Increased injection times
3. Increase in injection voltage
4. Increase in both time and voltage
5. Post PCR purification, not resulting in subsequent concentration
6. Post PCR purification that both purifies and concentrates the sample

Data from 117 responses
ISHI Mixture Workshop (Oct 2012)



Which of the procedures do you prefer to use? (up to 3 responses)

1. Increase in PCR cycle number
2. Increased injection times
3. Increase in injection voltage
4. Increase in both time and voltage
5. Post PCR purification, not resulting in subsequent concentration
6. Post PCR purification that both purifies and concentrates the sample

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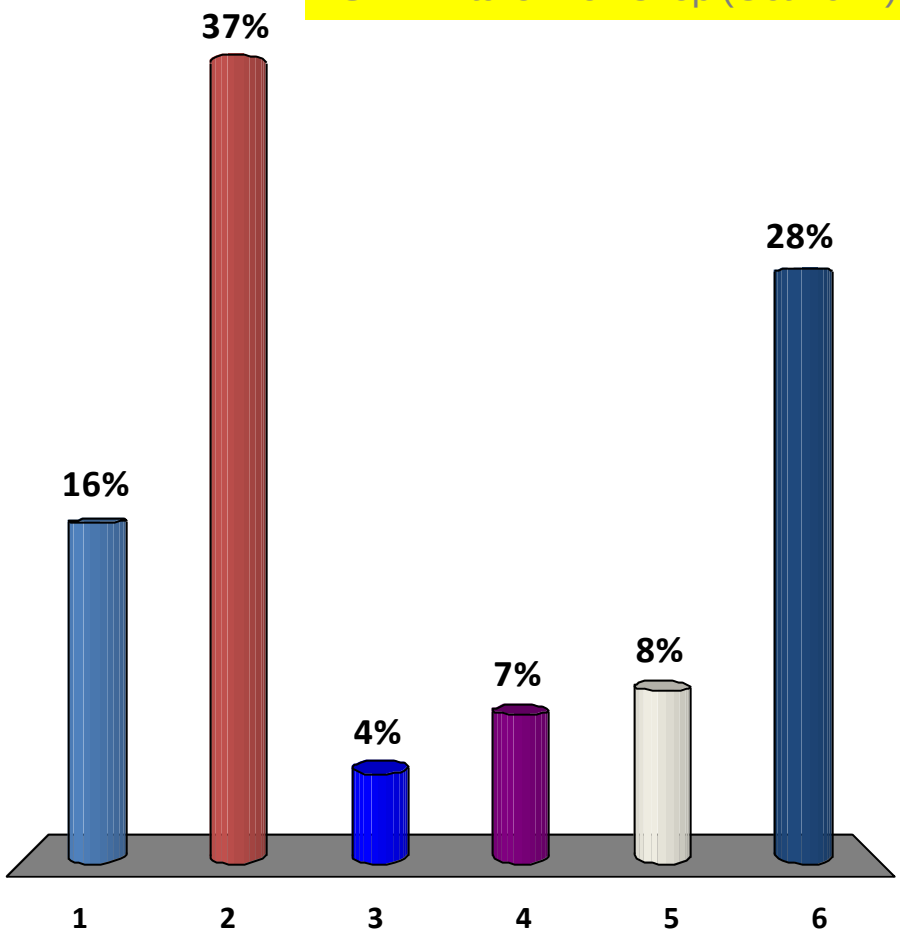
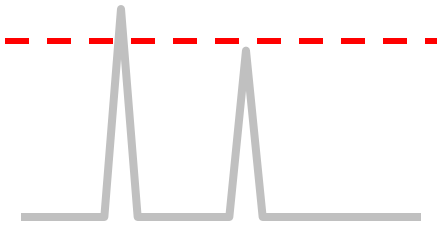


General Points to Consider:

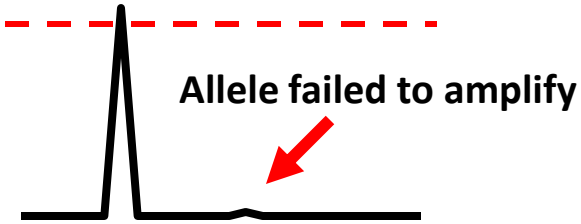
- Enhancement is relative to what you have defined your starting (SOP)parameters to be.
- For LT samples we are looking for reliable information gain.
- Consider that increased peak height alone does not constitute more information.

Stochastic Effects and Thresholds

Regular Injection

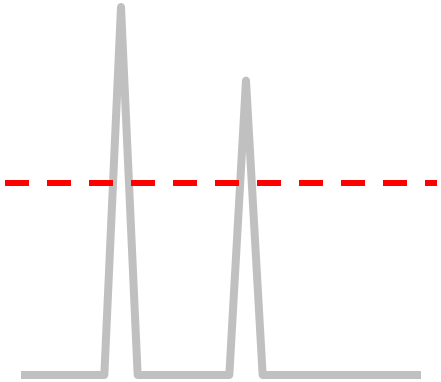


When PCR amplifying low levels of DNA, allele dropout may occur

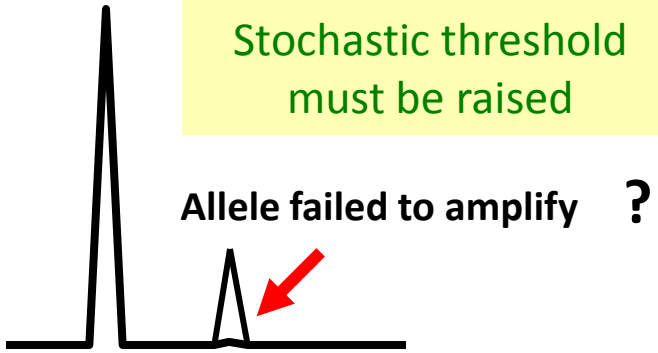


False homozygote

Injection Following Desalting (MiniElute)



Stochastic threshold must be raised



Whether there is information gain may depend on whether the sample is single source, two person or > two person mixture.

Before enhancement	After enhancement
Apparent single source partial profile; observe some complete genotypes but also possible allele or locus drop out.	Apparent single source profile, with more peaks observed, more genotypes determined, no locus drop out, may still be some allele drop out.
Apparent 2 person mixture	Increase in number of peaks observed; mixture contains 3 or more contributors.
Greater than 2 person mixture	Increase in number of peaks, may or may not be “real” information gain .

Be cautious when assessing information gain:

- Consider the possibility of allele drop in.
- Consider the possibility of elevated stutter.

Any process applied to the sample should increase the profile information with minimal affect on background noise and artifacts

Two basic approaches to enhancing signal:

- Increase in PCR cycle number: Such as using 34 cycles v. 28 cycles

- Post PCR procedures: Altering the amount of PCR product injected into the capillary
  - Increased injection time and or injection voltage
  - Purification of PCR reaction mix
    - Removes salts and some primers
    - Concentrates the template

- These two approaches are not the same
  - Increasing cycle number- affects the PCR itself
  - Post PCR purification and change in injection parameters increases the number of amplicons which can be observed post electrophoresis

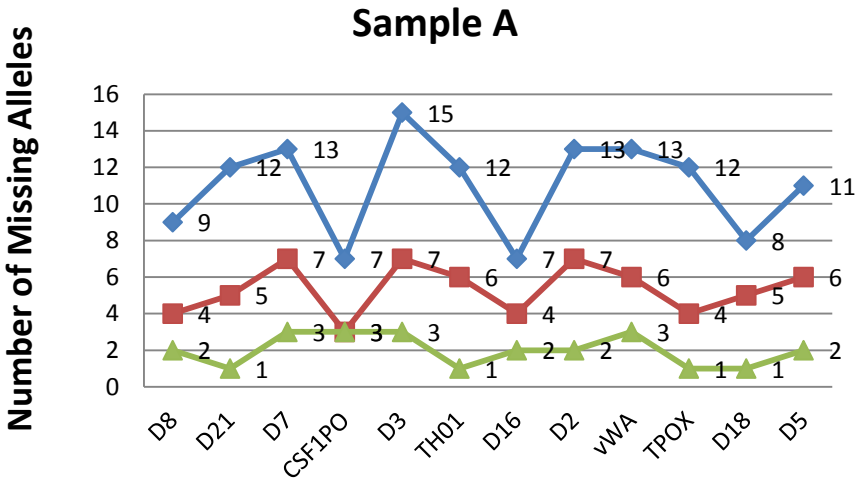
Increasing Cycle Number:

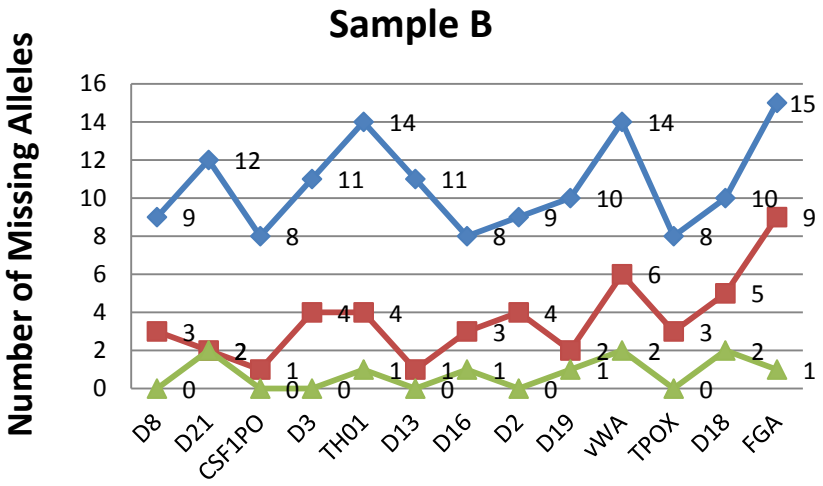
- There are numerous reports in literature of allele drop in and increased % stutter
- Many protocols involve combining information from multiple amplifications
  - In order to do multiple amplifications , each reaction uses a lower amounts of template per reaction than the total amount of template available.

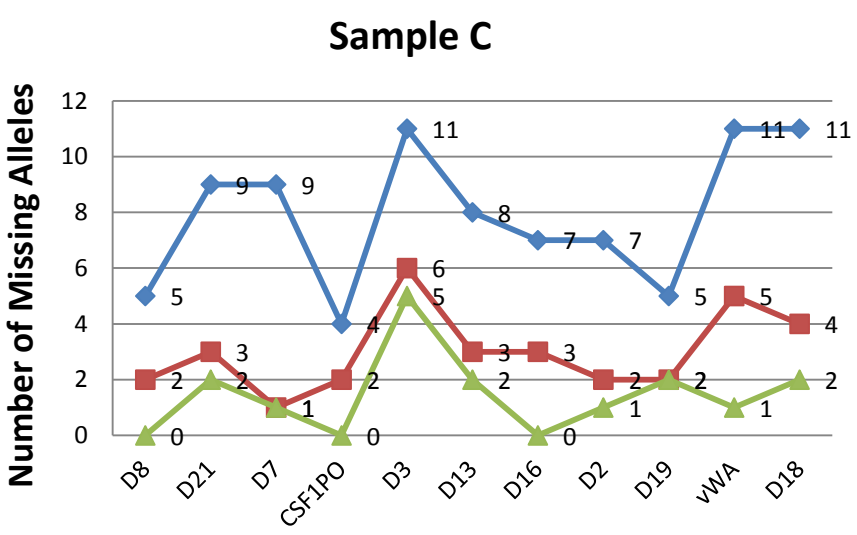
Increase Amount of Amplicon Injected:

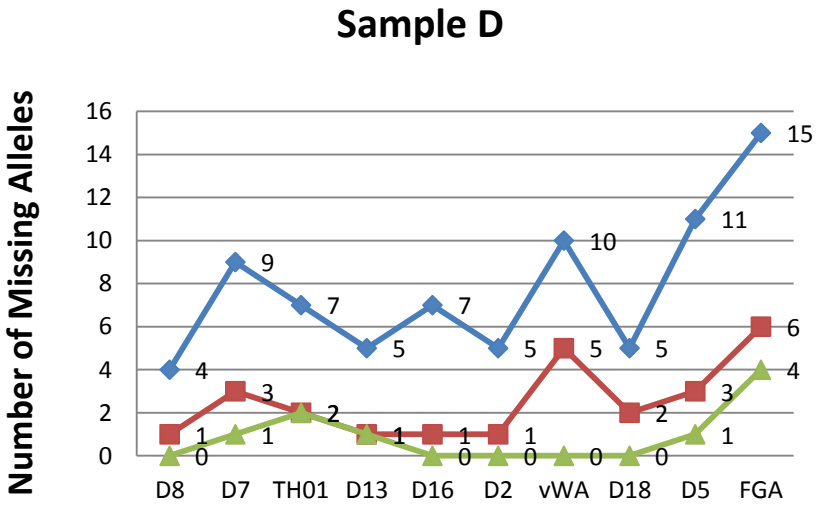
- Increase in injection time or voltage or both
  - Pros- more product enters capillary, may have information gain
  - Con- too long an injection time will cause increase in peak width
  - Con- time needed to assess validation of procedure to evaluate AT and ST (probability of drop out)

Number of missing alleles per locus per injection time for 0.0625ng & 0.125ng









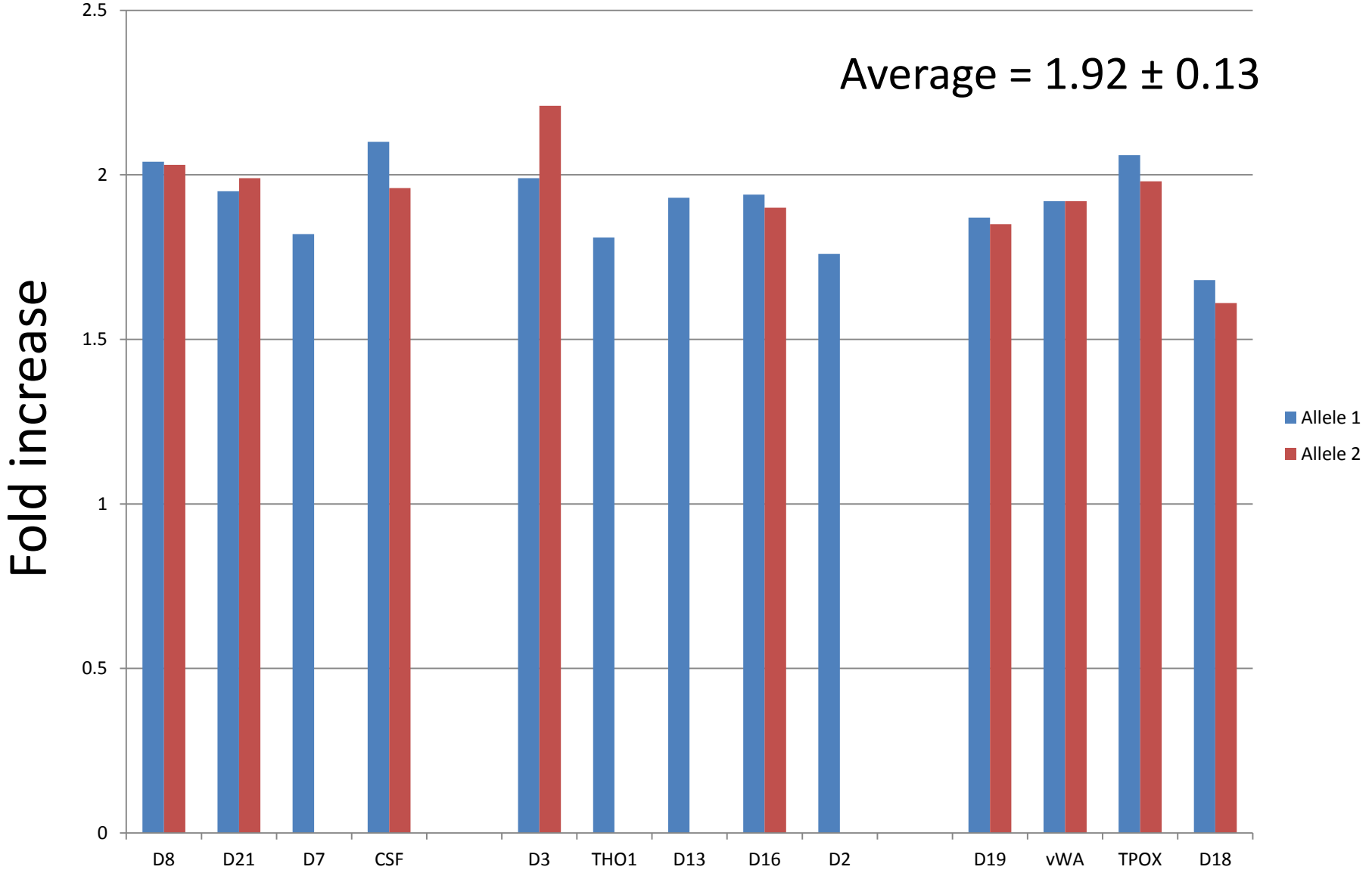
2 sec. inj. 5 sec. inj. 10 sec. inj.

Increase Amount of Amplicon *Injected*:

- Purification of amplified product to remove salts and primers
  - Salt concentration is reduced
  - Amount of primer is reduced
  - Therefore less competition of negatively charged ions during injection

- Concentration of amplified product by decreasing volume as a result of the purification procedure
  - More amplicons are added to the CE sample preparation from a more concentrated post purification sample

Fold increase in PH after purification without concentration



Fidelity of PHR

Locus	PHR-pre purification	PHR –post purification	PHR difference
D8	0.971	0.975	0.004
D21	0.648	0.634	0.014
CSF	0.575	0.615	**0.040**
D3	0.342	0.308	0.034
D16	0.748	0.733	0.015
D19	0.451	0.448	**0.003**
vWA	0.935	0.958	0.023
TPOX	0.798	0.830	0.032
D18	0.293	0.281	0.012

Additional published procedures designed produce fewer PCR artifacts and lessen the stochastic effects which contribute to allele drop out:

- Reduce drop out by extending time for primer binding.

  Extended PCR conditions to reduce drop-out frequencies in low template STR, FSI Genetics 6 (2012) p. 102

- Reduce stutter by lowering denaturation temperature

  Genotyping of simple sequence repeats –factors implicated in shadow band generation revisited, Electrophoresis 27 (2006) p. 3724

Validation of “enhancement procedures” has to be done carefully:

- Use dilution series from known samples such as known positive controls before working with evidence
  - Typical results from these samples is well understood and probably used in validation of current Standard Procedures.
- Change one variable at time
  - Do not change voltage and injection time
  - Control for concentrations and volumes
- After design of new procedure analytical thresholds and probability of drop out must be re-visited.