

User Bulletin

ABI PRISM™ Linkage Mapping Set

November 5, 1997

SUBJECT: Plus A Artifacts

Purpose of this User Bulletin

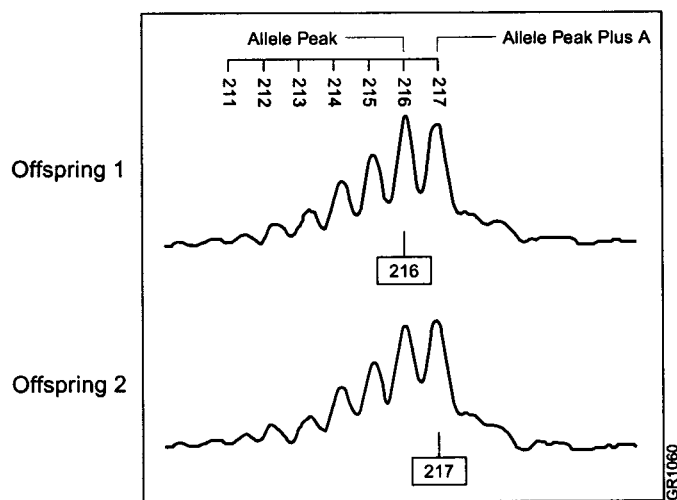
This user bulletin:

- ◆ Discusses the plus A artifact and related allele calling issues
- ◆ Describes three methods for addressing plus A artifacts

Plus A Artifacts and Automated Allele Calling

One of the main difficulties in semi-automated, microsatellite-based genotyping is the "plus A artifact"—the tendency of Taq polymerase to add a non-templated nucleotide (usually an A) to the 3' end of double-stranded DNA.¹ For a given marker, this addition is not absolute, and only a certain fraction of amplicons receive the additional nucleotide.

Ambiguity in allele calling results when the actual allele and allele plus A peaks are of near equal height. This occurs for approximately 5–10% of markers in a given laboratory. For example, the illustration below shows two individuals typed with the same dinucleotide repeat marker. ABI PRISM® Genotyper® software, however, will not correctly call the alleles even though the pattern is visible for both offspring. This error occurs because the actual allele peak (216) is the highest peak for offspring 1, while the allele plus A peak (217) is the highest peak for offspring 2. Data of this type requires manual editing to avoid missed or incorrect allele calls.



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Overview The following three methods are offered as suggestions for addressing the issues associated with plus A artifacts:

- ♦ Enzymatic Treatment of Pooled PCR Products
- ♦ Modification of Thermal Cycling Conditions
- ♦ Reverse Primer Modification

While all of these methods are useful, your research process will determine the technique you select. We recommend using the Reverse Primer Modification method with the ABI PRISM Linkage Mapping Set. This method is easier for high throughput genotyping since no significant protocol changes are required.

Enzymatic Treatment of Pooled PCR Products

Ginot et al.² describes the use of T4 DNA polymerase to remove the one base overhang from the pooled PCR products. While this method works, it requires:

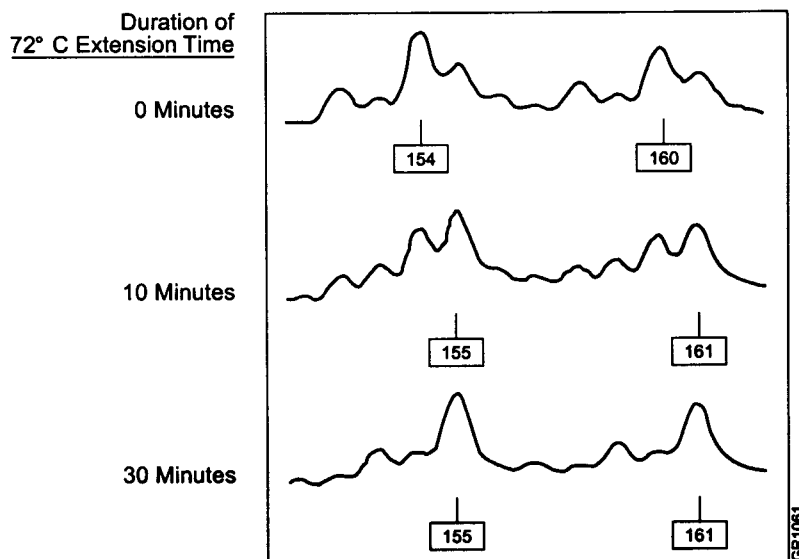
- ♦ An additional post-PCR enzymatic treatment step
- ♦ The titration of a given lot of T4 DNA polymerase to determine the optimal concentration and incubation time

Too much activity may cause degradation of the products. Too little activity may not correct the problem, and may make some markers more difficult to call.

Modification of Thermal Cycling Conditions

Smith et al.³ propose the modification of thermal cycling conditions to either promote or inhibit the plus A addition. As shown below, increasing the duration of the 72°C final extension at the end of thermal cycling promotes the addition. Decreasing the duration helps prevent it.

Note When using thermal cycling conditions to inhibit plus A artifacts, the residual activity of Taq polymerase at room temperature (or even at 4°C) can still result in enough plus A addition to create a genotyping problem.



The goal of this method is the determination of a set of thermal cycling conditions for a specific marker that result in an easily called pattern, even though the promotion or inhibition of the A addition may not be complete. It is not important whether the plus A addition is promoted or inhibited, just that one form is consistently generated for a given marker.

Disadvantages of this method are as follows:

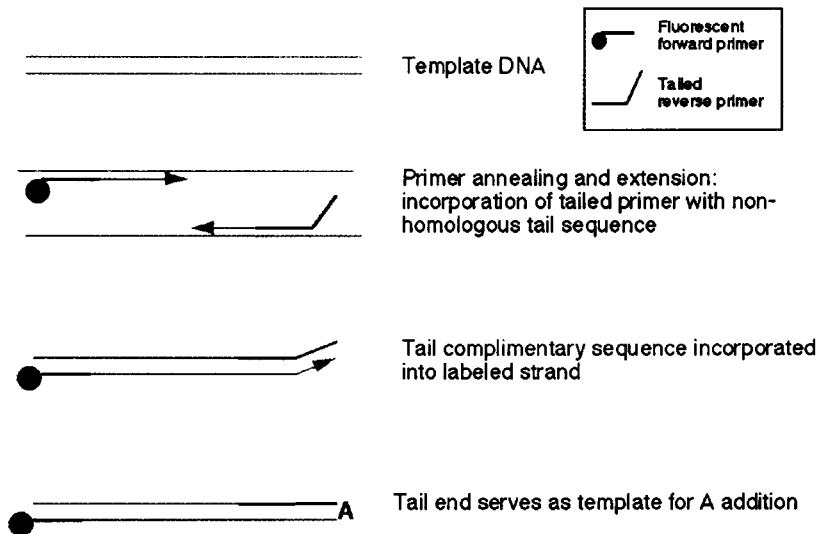
- ◆ Optimal thermal cycling conditions must be determined experimentally for each microsatellite marker
- ◆ Other variables such as the thermal cycler used, and DNA sample quality, concentration, and salt content can cause conditions to vary

Reverse Primer Modification

Using the reverse primer modification method (tailing) has the following advantages:

- ◆ It is a relatively universal fix
- ◆ The method works well
- ◆ No additional experimental steps are required

Brownstein et al.⁴ tailed the reverse PCR primer by adding various additional sequences to the 5' end (shown below). The tail either inhibited or promoted the A addition at the 3' end of the forward (labeled) strand. The most effective modification was found to be the use of a proprietary 7-base tail to promote the A addition.



Magnuson et al.⁵ studied the sequences of the 3' end of PCR templates in relation to the amount of plus A addition and noticed a trend. They found that modification of the reverse PCR primer by the addition of a single "G" to the 5' end generally resulted in enough plus A addition to generate an allele pattern that was easily called.

Our evaluation of the two tailing methods found that the 7-base tail worked more consistently than the G tail to promote the A addition.

Using tailed reverse primers greatly improves automatic allele calling. As shown below, dinucleotide repeats tend to generate complex patterns due to the combination of stutter and the plus A artifact. In this example, the 106 peak is the allele peak when using an unmodified reverse primer.

The 114 peak is the allele peak plus A when using a tailed reverse primer. It is 8 bases longer because it includes the 7-base tail and the additional A. Since Genotyper software automatically filters out stutter peaks, elimination of the plus A artifact results in easily called alleles.

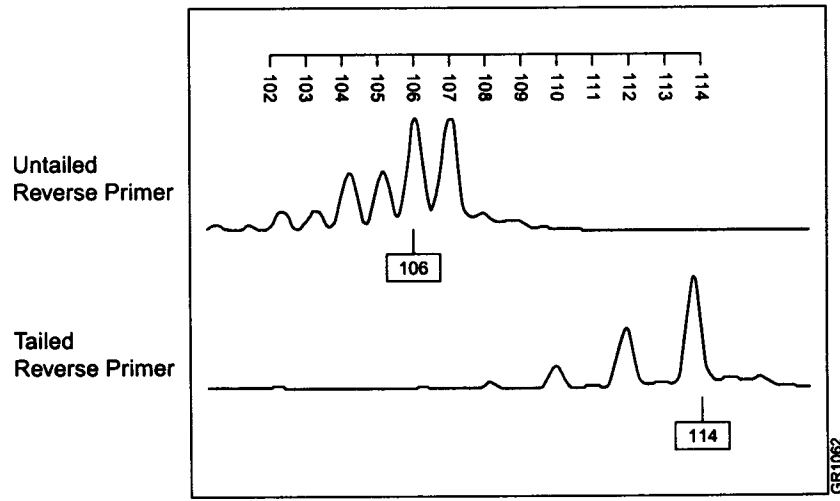


Figure 1. Unmodified versus tailed reverse primers

References

1. Clark, J.(1988) *Nucleic Acids Research*, 16:9677-9686
2. Ginot, F., et al. (1996) *Nucleic Acids Res.* 24: 540 -5412.
3. Smith, J. R., et al. (1995) *Genome Res.* 5: 312-317.
4. Brownstein, M. J., et al. (1996) *BioTechniques* 20: 1004-1009.
5. Magnuson, V.L., et al. (1996) *BioTechniques* 21: 700-7009.

Ordering Tailed Reverse Primers for the ABI PRISM Linkage Mapping Set

How To Order Panel-specific, tailed reverse primers for the ABI PRISM Linkage Mapping Set are available through PE Applied Biosystems Custom Oligonucleotide Synthesis Services. Contact PE Applied Biosystems for price and availability.

Part numbers for tailed reverse primers

Panel Number	Part Number	Panel Number	Part Number
All 28 panels	450065	—	—
1	450026	15	450040
2	450027	16	450041
3	450028	17	450042
4	450029	18	450043
5	450030	19	450044
6	450031	20	450045
7	450032	21	450046
8	450033	22	450047
9	450034	23	450048
10	450035	24	450049
11	450036	25	450050
12	450037	26	450051
13	450038	27	450052
14	450039	28	450053

Note We will be introducing version 2 of the ABI PRISM Linkage Mapping Set, which includes tailed reverse primers. Check with your local PE Applied Biosystems representative for pricing and availability.

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