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GeneMapper® *ID-X* Software and Bin Overlap

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SUBJECT: Bin overlap within the GeneMapper® *ID-X* Software

Purpose of this user bulletin

The purpose of this document is to describe overlapping bins which may occur while using GeneMapper® *ID-X* software. This user bulletin:

- Defines "overlapping bins" and describes its causes
- Describes overlapping bins in the STR Kits
- Provides options for analyzing peaks that fall into overlapping bins
- Explains how GeneMapper® *ID-X* software analyzes peaks that fall into bin-overlap regions

Definition of bins and overlapping bins

Physical and virtual bins

A bin is a region that defines an allele within a marker. There are two types of bins, physical and virtual. Physical bins are alleles physically present in the allelic ladder. Virtual bins are alleles that are not present in the allelic ladder, but have been previously reported† or discovered during developmental validation of a particular chemistry. In the GeneMapper® *ID-X* software, physical bins are shaded grey and virtual bins are shaded pink.

When creating the bin definitions, the goal is to always leave a gap of at least 0.1 nt or more between bins. However, once migration due to electrophoresis transpires and subsequent bin offsetting is applied, there are opportunities for overlapping bins to occur between bins that are only 0.1 nt apart.

Figure 1 Example of two physical bins located 1 nt apart with a gap of 0.1 nt.

Table 1 Describes alleles 11.3 and 12 in Figure 1

Bin	Bin type	Size (bp)	Bin width (nt) left of peak	Bin width (nt) right of peak	Total bin width (nt)	Gap size between bins (nt)
Allele 11.3	Physical	91.5	0.50	0.45	0.95	0.1
Allele 12	Physical	92.5	0.45	0.50	0.95	

† Previously reported in the STRbase (**www.cstl.nist.gov/div831/strbase**).

Bin offsets

Bin offsets are the size difference (in base pairs) between the reference bin locations in the panel and the actual sizes of the allelic ladder fragments. Bin offsets are automatically calculated during analysis of the allelic ladder samples. The bin offsets determine how much and in which direction (higher or lower) the reference bins shift. The offset bins are then used to genotype the other sample types.

Overlap between bins

After bin offsets are applied, bin overlap may occur between two bins, physical or virtual, that have a pre-defined separation gap of only 0.1 nt. Migrational influences may shift the bins such that the pre-defined 0.1 nt gap is eliminated, resulting in an overlap of the two bins.

Figure 2 Example of overlap between two physical bins

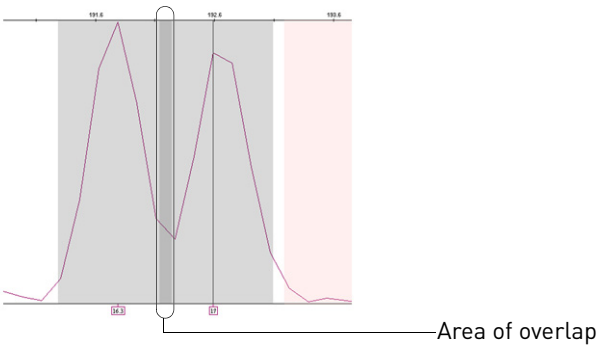
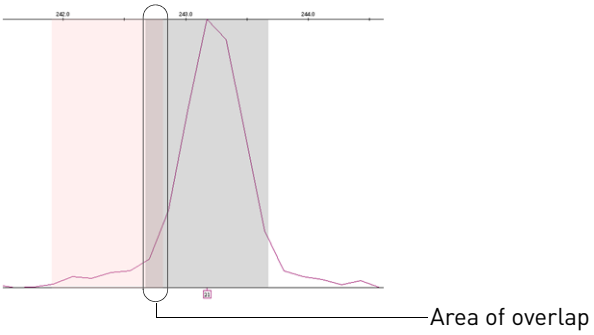


Figure 3 Example of overlap between one virtual bin and one physical bin



Possible causes of overlapping bins

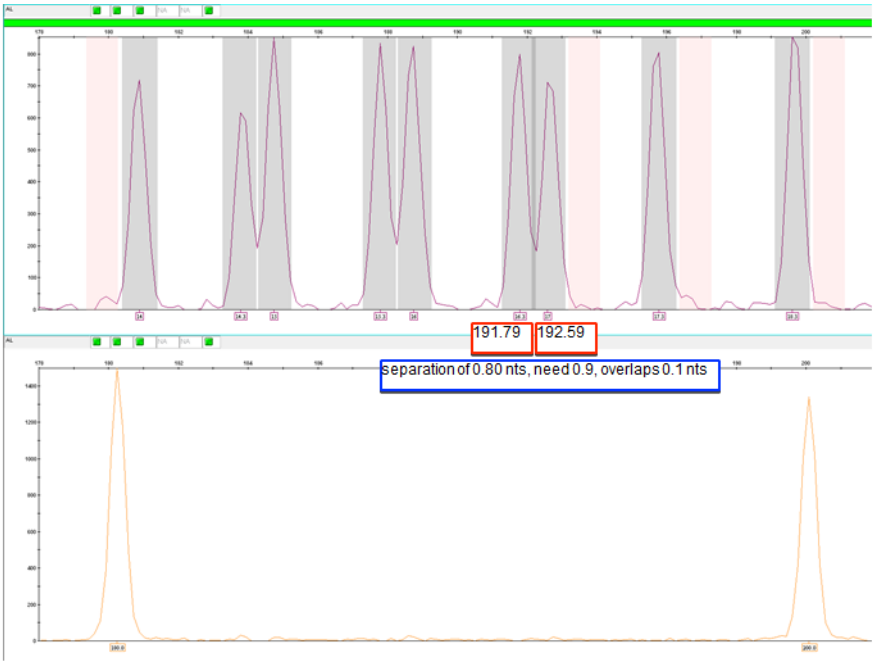
STR Kits with a high density of physical and virtual bins may be more prone to the occurrence of overlapping bins.

Due to migration conditions, bin-to-bin overlap can occur when adjacent ladder alleles within a single ladder are less the 0.9 nt apart or when the 0.1 nt gap is eliminated due to the applied bin offsets.

IMPORTANT! Reducing the bin width to correct bin overlap is not recommended. Alleles falling within the overlap region are already identified as Off Ladder (OL). Reducing the bin width causes correct allele calls to fall in the gap region. This results in more OL calls and a loss of information.

IMPORTANT! Bin overlap has been observed in data generated on both the Applied Biosystems® 3730 DNA Analyzer using POP-7™ polymer and the Applied Biosystems® 3500 Genetic Analyzer using POP-4® polymer. The frequency of bin overlap is higher in data generated on the 3730 instrument using POP-7™ polymer.

Figure 4 Example of bin width that is overlapping by 0.1 nt



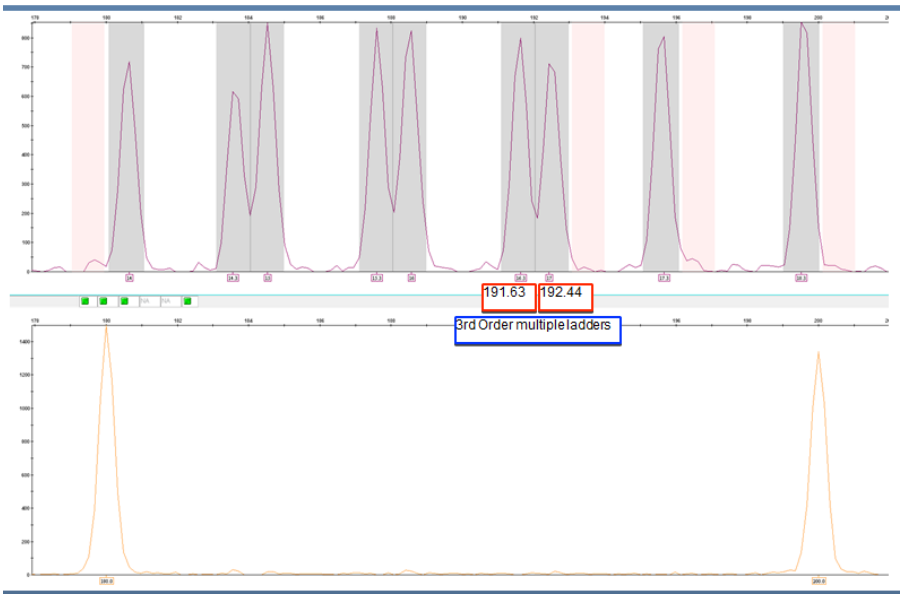
Size calling method

The Size Calling Method of the GeneMapper® *ID-X* software may affect bin overlap. The size calling algorithm impacts the peak size and changes the distance between two adjacent peaks.

IMPORTANT! The Local Southern Method sizing algorithm appears to prevent overlap more than the 3rd Order Least Squares sizing algorithm when using GeneScan™ 600 LIZ® Size Standard.

Multiple ladders

The use of multiple allelic ladders may result in different bin offsets upon comparison to analysis with a single allelic ladder. The migration rates of the allelic ladder peaks among different ladder injections may vary during capillary electrophoresis. This causes variation in the bin offset values among the multiple ladders. Therefore, when multiple allelic ladders are defined within a given GeneMapper® *ID-X* project, the bin offset values are averaged among all the defined ladders. If the averaged bin offsets eliminate the pre-defined 0.1 nt gap between bins that are 1 nt apart, then bin overlap occurs.



Minimizing overlapping bins

Here is a list of potential ways to minimize overlapping bins.

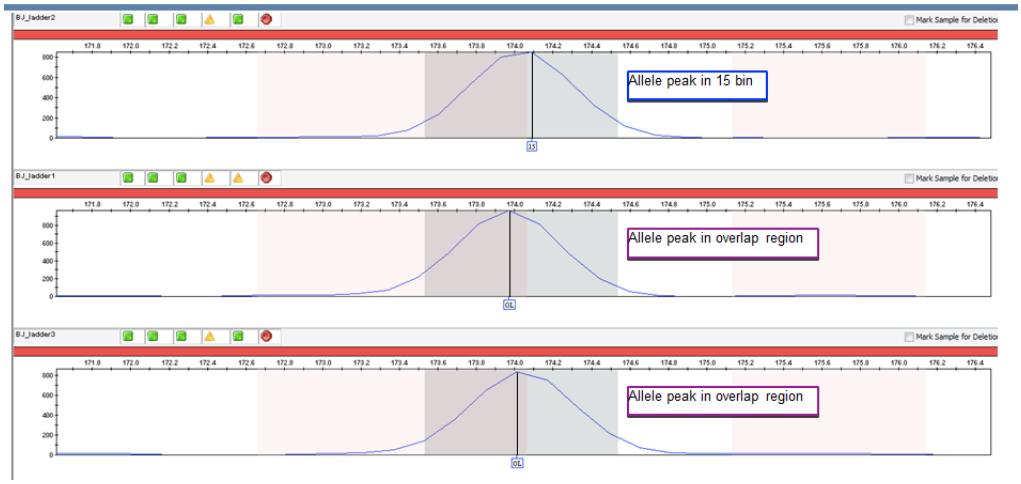
Category	Actions
Use a different ladder file	Remove the offending ladder from the data set, or replace it with another ladder that ran without overlap.
Size Calling Method	In the Peak Detector tab of the Analysis Method Editor, an option would be to choose the Local Southern Method sizing algorithm, which may reduce the affect of overlapping bins. **Note:** Using the Local Southern Method on GlobalFiler® Express data from the 3730 platform may generate sizing failure because the 60 bp size peak migrates into the primer region and may not be recognized by the software. **Note:** Conduct the appropriate internal verification studies before implementing modified analysis methods.

Recommended action

GeneMapper® *ID-X* software assigns alleles in overlapping bins as "Off Ladder" or OL. Therefore, there is no risk of incorrect genotyping. You can leave the allele call as OL, or manually assign an allele call after performing the necessary data interpretation.

IMPORTANT! Because alleles in overlapping bins are assigned OL, we recommend that you take no action to prevent overlapping bins.

Figure 5 Allele peak in a bin (top panel) and allele peak in overlapping bins called as OL (middle and bottom panels)



Documentation and support

Related documentation

For more information please refer to the following documents:

Document title	Pub. number
GeneMapper® ID-X Software Version 1.0 Getting Started Guide	4375574
GeneMapper® ID-X Software Version 1.0 Quick Reference Guide	4375670
GeneMapper® ID-X Software Version 1.0 Reference Guide	4375671
GeneMapper® ID-X Software Version 1.1 (Mixture Analysis) Getting Started Guide	4396773
GeneMapper® ID-X Software Version 1.2 Reference Guide	4426481
GeneMapper® ID-X Software Version 1.2 Quick Reference Guide	4426482
GeneMapper® ID-X Software Version 1.4 User Bulletin	4477684
GeneMapper® ID-X Software v1.4 New Features and Installation Procedures User Bulletin	4477684
GeneMapper® ID-X Software Version 1.3 User Bulletin	4470483
GeneMapper® ID-X Software Version 1.2 User Bulletin	4462639

Obtaining support

For HID support:

- In the United States and Canada – send an email to **HIDTechSupport@lifetech.com**, or call **888-821-4443** option **1**.
- Outside the United States and Canada – contact your local support office.

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the web site, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Search for user documents, Material Safety Data Sheets (MSDSs), vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents.
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- Obtain information about customer training.
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Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
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