Assessment of GeneScan Profiles
Homopolymer assays

1. Background information

The Multiplicom MASTR assays amplify specific targets for next-generation sequencing (NGS). Since some of the NGS technologies do not accurately read homopolymer stretches, Multiplicom designed complementary Homopolymer (HP) assays. HP assays are meant to specifically analyze length differences in homopolymer stretches amplified in a MASTR assay.

HP assays are multiplex PCR-based panels that amplify fragments containing the homopolymer stretches of interest. The obtained fluorescently labeled fragments need to be analyzed on a fragment analyzer with the GeneScan module resulting in GeneScan profiles. Detailed analysis of these profiles will result in information on changes in length of the amplified fragments and the contained homopolymer stretch(es).

2. Guidelines

2.1. Check if all signal intensities are within the detection range of the fragment analyzer.

Depending on the type of fragment analyzer, we noticed that the maximum intensity is either ~8,000 or ~30,000. Signals that are too strong have a deformed top of the peak in the profile (Figure 1). When signals are too strong, the fragment analysis should be repeated with less input material. Dilute the obtained amplification products 100x in water and perform the fragment analysis again.

Figure 1: Examples of signals. Signal a and c are normal, within range. Signal b is too high: The tip is rounded, and the signal is already bleeding into the other colors. Signal d is much too high: The tip is Deformed, and there are strong signals in the other colors because of spectral bleed.

2.2. Analyze each profile in detail, looking for evidence of changes in length of any of the peaks.

Such evidence can be:

- an extra peak, accompanied by a relative decrease of another peak (evidence of an insertion or deletion of at least a few bases)
- a shoulder peak accompanied by a relative decrease of the peak showing the shoulder (evidence of an insertion or deletion of 1 or 2 bases in the fragment of the peak itself) (Figure 2)
- a shoulder peak accompanied by a relative decrease of another peak (evidence of an insertion or deletion of several bases in the fragment of the other peak)
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Analyze all peaks of each profile in detail comparing them with a reference profile of a sample known not to have insertion/deletion variants in the amplicons of the HP assay. When lacking such a sample, compare all profiles with one another and focus on the differences.

![Figure 2: Example of shoulder peak. a/ Wild type signal; b/ Variant signal. When comparing a/ with b/, it is clear that peak 1 has a shoulder in b/. Moreover, in a/ peak 1 is higher than peak 2, while in b/ they have the same height.]

2.3. Confirm the source for the changes in length observed in the GeneScan profile.

As the fragments amplify more than just the homopolymer stretch, the changes in length can be because of a change in length of the homopolymer stretch or because of insertion/deletion variants anywhere else in the fragment. Therefore, it is important to sequence the entire fragment and determine the actual source of the change in length.

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