The importance of making a manual bin set in Coffalyser.Net

What is a manual bin set?

Coffalyser.Net uses "bins" (essentially just size boundaries) to determine where to look for a certain probe signal. A bin set is a collection of these bins. A manual bin set is a set of bins that you have personally adjusted and set to ensure that all probe signals in your probemix peak pattern fall within a bin. Instructions on how to create a manual bin set can be found here. Once a manual bin set has been created and saved, it can be used for all subsequent analyses by simply selecting the newly saved worksheet from the dropdown menu, and changing the probe recognition method to *manual*.

A manual bin set helps to avoid false positive results (empty bins being interpreted as homozygous deletions) and false negative results (signals of mutation-specific probes being missed due to bins being incorrectly set).

What happens if a probe falls outside of a bin?

If a probe signal falls outside of a bin, it will not be recognised. This *binning failure* results in a large red column in the binning profile, as shown here. If the missing signal belongs to a *target* or *reference probe*, this will also result in a reduced number of probes in *probe counter* shown in the *fragment analysis* window. In contrast, if the *Benchmark fragment* (92 nt) or the *Denaturation (D) fragments* (88 & 96 nt) fail to bin, this is not recognised as easily, as control fragments are not included in the probe counter.

What happens if the 92 nt *Benchmark fragment* or *D*-fragments are not binned?

If the 92 nt *Benchmark fragment* is not properly binned, the *DNA quantity* and d*enaturation scores* cannot be calculated, resulting in an incorrect calculation of the FMRS (Fragment MLPA Reaction Score) and CAS (Coffalyser Analysis Score). If quality issues are present in the data, this may lead to the incorrect assignment of a green ("good") FMRS and/or CAS. The quality of the data is therefore **overestimated.** See Figure 1 below.

Incorrectly binned *D-fragment*s can lead to a *reduced* FMRS when in fact no denaturation problem is present. The quality of the data is therefore **underestimated**.

How do I know if my 92 nt *Benchmark fragment* and *D-fragments* are binned incorrectly?

Four ways to identify a 92 nt Benchmark fragment binning problem:
In the *fragment analysis* window:
1. The scores for DNA and DD after fragment analysis are not filled in (grey) (Figure 1, 2).

In the *overview* window:
2. All samples are identified as *female* in the overview tab, even those with an identified Y probe (Figure 3).
3. The identified problems section states: "there were problems identified with the ligation fragment(s) of this sample" (Figure 3).

In the *binning profile:*
4. When the 92 nt bin is empty, it will be coloured red (Figure 4).

Three ways to identify a D-fragment binning problem:
In the *fragment analysis* window:
1. The score *DD* after fragment analysis shows a warning.

In the *overview* window:

2. The identified problems section states: "there was a problem identified with (one of) the D-fragments".

In the *binning profile*:

3. When the 88 nt or 98 nt D-fragment bin is empty, it will be coloured red

Please note: If a sample *does have* denaturation problems, you may get similar warnings. However, the binning profile will show you whether the D-fragments are indeed low (thus indicating denaturation failure), or whether there was a D-fragment binning problem.

Figure 1. A correctly binned *92 nt Benchmark fragment* will result in DNA, DD, X and Y quality scores. Note the low DNA quantity scores and resulting low FMRS score for various samples.

Figure 2. The same data shown in Figure 1 but now with a *92 nt Benchmark fragment* that failed to bin, resulting in absent quality scores for the DNA, DD, X and Y control fragments (grey).

The FMRS cannot be calculated correctly and is displayed as a *green* (i.e. a "good") score, *even for those samples affected by insufficient DNA or denaturation problems* (compare with Figure 1).

Figure 3. A *92 nt Benchmark fragment* that fails to bin will result in all samples being identified as female, even if a *Y-fragment* signal was detected. The identified problems section indicates there were problems identified with the *ligation fragment* (=*Benchmark fragment*) of the sample.
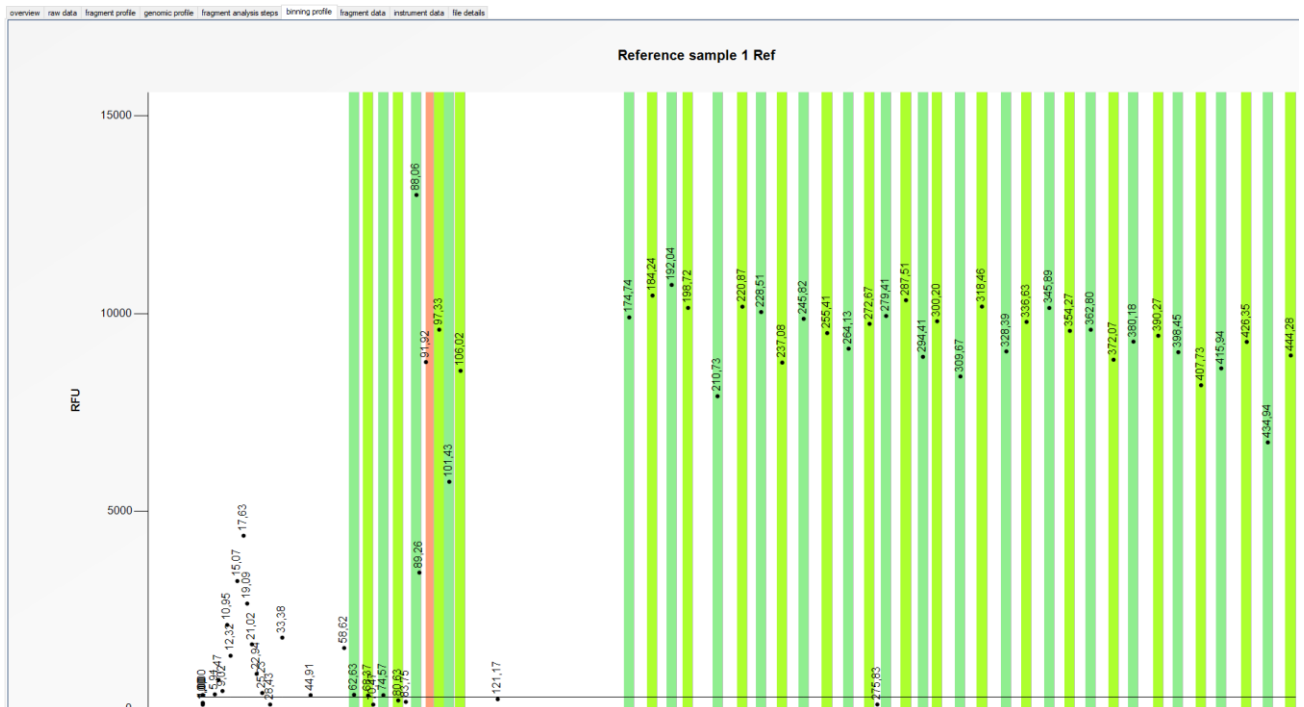

Figure 4. A red column is visible for the 92 nt fragment in the binning profile when the *92 nt Benchmark fragment* is not binned. A dot indicating the 92 nt probe signal can be seen falling just outside this column.