MRC Holland Support

How do I adjust a bin set (create a manual bin set) in Coffalyser.Net?

You can create a manual bin set to adjust the bin set created by Coffalyser.Net. This can be done as follows:

1. Open the experiment.
2. In the fragment analysis tab, right-click somewhere in the screen and select Edit Manual Bin Set Default Channel.
3. When applicable, click Yes to replace the manual bin set with the autobin results of the last analysis.
4. Select a sample on the left to see a chart with the bin set at the top of the dialog. All identified signals are depicted as dots. A bin appears as a green bar when a signal was detected near the centre of the bin. When no signal was detected (or when the signal was too low), the bin will appear as a red bar. A purple bar indicates that a signal was detected in the bin, but more than 0.7 nt away from the centre.
5. Select a sample where all signals are present in the raw data (e.g. a reference sample or a SALSA Binning DNA). For each fragment that falls outside of the current bin settings or that is more than 0.7 nt away from the centre of its bin, do the following:
    - Find the fragment in the table with probes.
    - In the column manual binset lower bound, change the current value to a value below that of the actual length of the fragment.
    - In the column manual binset upper bound, change the current value to a value above that of the actual length of the fragment.
    - Make sure that the new bins do not overlap with other bins and that the probe fragments are in the centre of the bins. We recommend bins with a width of 4 nt.
    - The chart will show the adapted bin set.
6. Click OK at the bottom of the open window and then Yes in the pop-up screen that appears. The manual bin set will be used the next time the fragment analysis is performed.
7. Start (or repeat) the fragment analysis.

You can usually use the same manual bin set as long as your electrophoresis conditions do not change significantly. The manual bin set is stored in the analysis sheet, and can be re-

used in another experiment by selecting manual as probe recognition method in the Fragment Analysis Settings that are displayed when you start the fragment analysis.

This procedure is explained in more detail in the video below.

In case you are unable to view the video on YouTube you can also watch it here.

Background

Specific electrophoresis conditions may sometimes result in fragment lengths that deviate too much from the expected lengths. These signals will not always be properly identified by the automatic binning procedure in Coffalyser.Net. As a result, peaks may be misidentified or missed completely, which can result in warnings for missing probes.

Experiments where none of the samples have a signal for a particular probe are relatively prone to binning issues. We supply all probemixes with mutation-specific probes with a SALSA Binning DNA that can aid in determination of bin boundaries.

Coffalyser.Net also provides warnings if signals are too far from the centre of a bin. These warnings can help detect an incorrect bin set, and can also help identify issues with nonspecific peaks or electrophoresis.

Tags

Coffalyser.Net

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