How do I adjust the Coffalyser sheet for SALSA (MS-)MLPA Probemix P200 Reference-1?

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SALSA MLPA Probemix P200 Reference-1 contains thoroughly tested reference probes and control fragments to which you can add your own synthetic (MS-)MLPA probes. Most SALSA® MLPA® probemixes have a benchmark fragment at 92 nt, which is used for the normalisation of several quality control fragments. This fragment has been left out of the P200 probemix in order to leave as much room for synthetic probes as possible. The median signal of all reference probes in the probemix can be used as a substitute for the 92 nt benchmark fragment by Coffalyser.Net. Due to technical limitations, this requires manual adjustment of the Coffalyser sheet.

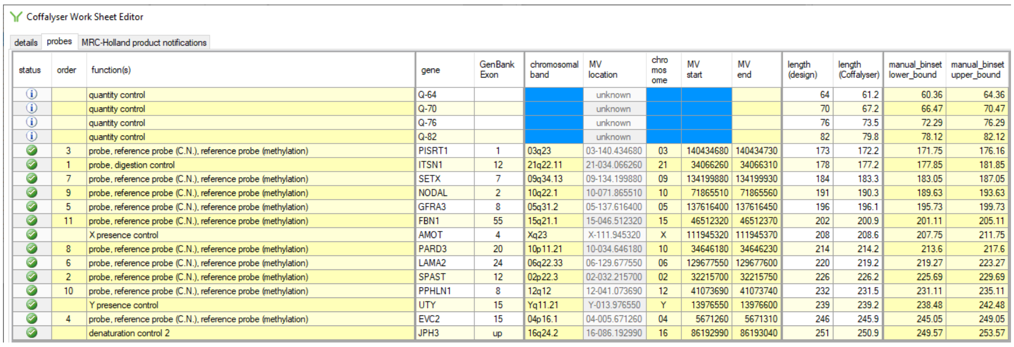
Warning

The adjustments to the Coffalyser sheet described here are specific for the P200 probemix and should *not* be applied to any other probemix. SALSA MLPA Probemix P300 Reference-2 also does not require adjustments to probe functions in the Coffalyser sheet as it does have the 92 nt benchmark fragment. The use of the P300 probemix is recommended unless the extra room for synthetic probes offered by the P200 probemix is required.

Required adjustments to the Coffalyser sheet

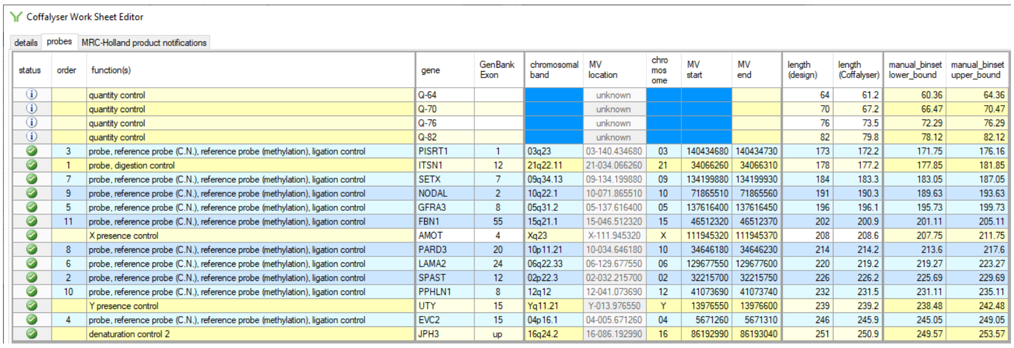
The probe functions of all reference probes should be changed to also include the ligation control function, which is the name used in Coffalyser.Net for the benchmark fragment. This can be done as follows:

- Open the sheet library in Coffalyser.Net.
- Either right-click an existing analysis sheet and choose Open, or create a new sheet by right-clicking on an empty area and choosing Add. More details about adding Coffalyser sheets.
- The Coffalyser Work Sheet Editor should open. Navigate to the probes tab. You should see something similar to the screenshot below. Details may differ between versions and lots.



status	order	function(s)	gene	GenBank Exon	chromosomal band	MV location	chromosome	MV start	MV end	length (design)	length (Coffalyser)	manual_binset lower_bound	manual_binset upper_bound
ⓘ		quantity control	Q-64			unknown				64	61.2	60.36	64.36
ⓘ		quantity control	Q-70			unknown				70	67.2	66.47	70.47
ⓘ		quantity control	Q-76			unknown				76	73.5	72.29	76.29
ⓘ		quantity control	Q-82			unknown				82	79.8	78.12	82.12
✓	3	probe, reference probe (C.N.), reference probe (methylation)	PISRT1	1	03q23	03-140.434680	03	140434680	140434730	173	172.2	171.75	176.16
✓	1	probe, digestion control	ITSN1	12	21q22.11	21-034.066260	21	34066260	34066310	178	177.2	177.85	181.85
✓	7	probe, reference probe (C.N.), reference probe (methylation)	SETX	7	09q34.13	09-134.199880	09	134199880	134199930	184	183.3	183.05	187.05
✓	9	probe, reference probe (C.N.), reference probe (methylation)	NODAL	2	10q22.1	10-071.865510	10	71865510	71865560	191	190.3	189.63	193.63
✓	5	probe, reference probe (C.N.), reference probe (methylation)	GFRA3	8	05q31.2	05-137.616400	05	137616400	137616450	196	196.1	195.73	199.73
✓	11	probe, reference probe (C.N.), reference probe (methylation)	FBN1	55	15q21.1	15-046.512320	15	46512320	46512370	202	200.9	201.11	205.11
✓		X presence control	AMOT	4	Xq23	X-111.945320	X	111945320	111945370	208	208.6	207.75	211.75
✓	8	probe, reference probe (C.N.), reference probe (methylation)	PARD3	20	10p11.21	10-034.646180	10	34646180	34646230	214	214.2	213.6	217.6
✓	6	probe, reference probe (C.N.), reference probe (methylation)	LAMA2	24	06q22.33	06-129.677550	06	129677550	129677600	220	219.2	219.27	223.27
✓	2	probe, reference probe (C.N.), reference probe (methylation)	SPAST	12	02p22.3	02-032.215700	02	32215700	32215750	226	226.2	225.69	229.69
✓	10	probe, reference probe (C.N.), reference probe (methylation)	PPHLN1	8	12q12	12-041.073690	12	41073690	41073740	232	231.5	231.11	235.11
✓		Y presence control	UTY	15	Yq11.21	Y-013.976550	Y	13976550	13976600	239	239.2	238.48	242.48
✓	4	probe, reference probe (C.N.), reference probe (methylation)	EVC2	15	04p16.1	04-005.671260	04	5671260	5671310	246	245.9	245.05	249.05
✓		denaturation control 2	JPH3	up	16q24.2	16-086.192990	16	86192990	86193040	251	250.9	249.57	253.57

- Double-click on each probe with probe, reference probe (C.N.), reference probe (methylation) in the function(s) column to display a dropdown menu. Add the ligation control function to these probes. Do *not* remove any existing probe functions.
- Some older, existing sheets may have the probe function defined for the X presence control probe, the Y presence control probe and the denaturation control 2 probe. If you use an existing sheet and this is the case, remove the probe function from these probes (but leave the other functions). This avoids unnecessary variability warnings, e.g. when analysing samples from different genders.
- When done, the values in the function(s) column should resemble the screenshot below.



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- Optionally proceed to add your custom synthetic probes to the analysis sheet as described in the Reference Manual.
- Click OK to save the adjustments.

More information

- More information about adding, modifying and removing probes can be found in the Reference Manual.
- For additional assistance, you can contact us. Please note that we no longer offer any support on the design of synthetic probes.

Tags

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