Product Description
SALSA® MLPA® Probemix P220-B3 Obesity

To be used with the MLPA General Protocol.

Version B3
For complete product history see page 10.

Catalogue numbers:
- **P220-025R:** SALSA MLPA Probemix P220 Obesity, 25 reactions.
- **P220-050R:** SALSA MLPA Probemix P220 Obesity, 50 reactions.
- **P220-100R:** SALSA MLPA Probemix P220 Obesity, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General information
The SALSA MLPA Probemix P220 Obesity is a **research use only (RUO)** assay for the detection of deletions or duplications in the *LEPR*, *POMC*, *SIM1*, *LEP*, *MC4R*, *MC2R* and *MC3R* genes and the 16p11.2 region, which are associated with obesity.

Obesity is known to have a genetic basis and is often accompanied by hyperglycemia, hypertension and hyperlipidemia. Leptin deficiency, leptin receptor deficiency, and proopiomelanocortin deficiency, all involved in obesity, can be inherited in an autosomal recessive fashion. Leptin (encoded by *LEP*) is an adipocyte-specific hormone which plays a major role in the regulation of body weight. Leptin acts through the leptin receptor (*LEPR*), a single-transmembrane-domain receptor of the cytokine receptor family, which is found in many tissues in several alternatively spliced forms. The proopiomelanocortin (*POMC*) gene encodes a polypeptide hormone precursor that undergoes extensive, tissue-specific, post-translational processing via cleavage by subtilisin-like enzymes known as prohormone convertases.

The *LEPR* gene (23 exons) spans ~215 kb of genomic DNA and is located on 1p31.3, ~66 Mb from the p-telomere. The *POMC* gene (4 exons) spans ~8.0 kb of genomic DNA and is located on chromosome 2p23.3, ~25 Mb from the p-telomere. The *LEP* gene (3 exons) spans ~16 kb of genomic DNA and is located on chromosome 7q32.1, ~128 Mb from the p-telomere.

Obesity can also be caused by mutations in single-minded homolog 1 (*SIM1*; Holder et al. 2000), the melanocortin-4 receptor gene (*MC4R*; Emmerson et al. 2007), *MC3R* (Tao and Segaloff 2004) and *MC2R*. MC4R deficiency is the most common form of monogenic obesity (Faroogi et al. 2003).

The *SIM1* gene (11 exons) spans ~80 kb of genomic DNA and is located on chromosome 6q16.3, ~101 Mb from the p-telomere. The *MC4R* gene (1 exon) spans ~1.7 kb of genomic DNA and is located on chromosome 18q21.32, ~56 Mb from the p-telomere. The *MC2R* gene (2 exons) spans ~33 kb of genomic DNA and is located on chromosome 18p11.21, ~14 Mb from the p-telomere. The *MC3R* gene (1 exon) spans ~1.1 kb of genomic DNA and is located on chromosome 20q13.2, ~54 Mb from the p-telomere.

Deletions of chromosome 16p11.2 are also found to be associated with obesity (Bochukova et al. 2010). The 16p11.2 region harbours *SH2B1*, involved in leptin and insulin signalling. Aside from *SH2B1*, the whole 16p11.2 region (including *SEZ6L2*) is associated with obesity.

The *SH2B1* gene (10 exons) spans ~10 kb of genomic DNA and is located on chromosome 16p11.2, ~29 Mb from the p-telomere. The *SEZ6L2* gene (18 exons) spans ~28 kb of genomic DNA and is located on chromosome 16p11.2, ~30 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK11167/ (16p11.2 recurrent deletion).

This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The exon numbering used in this P220-B3 Obesity product description is the exon numbering from the sequences indicated in the table below.

Gene	LRG or NG sequence
LEPR	LRG_283
POMC	NG_008997.1
SIM1	NG_008230.1
LEP	NG_007450.1
SH2B1	NG_029706.2
SEZ6L2	NG_029737.2
MC4R	LRG_1346
MC2R	NG_011819.1
MC3R	NG_012200.1

The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG or NG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P220-B3 Obesity contains 47 MLPA probes with amplification products between 130 and 495 nucleotides (nt). This includes 11 probes for *LEPR*, four probes for *POMC*, eight probes for *SIM1*, four probes for genes flanking *SIM1*, three probes for *LEP*, four probes for the 16p11.2 region, two probes for *MC4R*, one probe for *MC2R*, and two probes for *MC3R*. In addition, eight reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens
Extracted DNA, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different unrelated individuals who are from families without a history of obesity. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

Data analysis
Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results
The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term "dosage quotient", used in older product description versions, has been replaced by "final ratio" to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure

- Genetic defects in the above-listed genes can also be caused by small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P220 Obesity.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

LOVD Mutation database

https://databases.lovd.nl/shared/genes/. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *SIM1* exons 9 and 11 but not exon 10) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P220-B3 Obesity

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	Target exon	Target region
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 13351-L14781	8q		
137	**SIM1 probe** 07292-L19213		**Exon 1**	
142	**MC4R probe** 02848-L02278		**Exon 1**	
148 ¬	CDK19 probe 03945-L03403		upstream of *SIM1*	
154	**SIM1 probe** 16068-L19470		**Exon 1**	
160	**MC3R probe** 08885-L08941		**Exon 1**	
166	Reference probe 12534-L14278	9q		
171	**SIM1 probe** 07294-L17071		**Exon 2**	
178	**LEPR probe** 21572-L30446		**Exon 11**	
184	**LEPR probe** 08873-L19206		**Exon 18**	
196	**LEPR probe** 08867-L08923		**Exon 5**	
202	**LEP probe** 08880-L08936		**Exon 2**	
208	**SEZ6L2 probe** 11668-L12439			16p11.2
214 ¬	COQ3 probe 16153-L19387		downstream of *SIM1*	
220	**POMC probe** 08879-L08935		**Exon 4**	
226	**SH2B1 probe** 15155-L16929			16p11.2
233	**MC4R probe** 15154-L25342		**Exon 1**	
238 ±	**MC3R probe** 08884-L08940		**Exon 1**	
244	Reference probe 08051-L07832	5p		
250	**LEPR probe** 08868-L16799		**Exon 7**	
265	**LEPR probe** 08874-L19305		**Exon 20**	
270	**SH2B1 probe** 15158-L20075			16p11.2
276	Reference probe 08545-L20076	3q		
283	**SIM1 probe** 07299-L21259		**Exon 7**	
295	**SH2B1 probe** 15157-L19310			16p11.2
302 ¬	NR2E1 probe 03939-L09973		upstream of *SIM1*	
310 ∧	**LEP probe** 15151-L21098		**Exon 3**	
316	**SIM1 probe** 07300-L21099		**Exon 8**	
322	**LEPR probe** 08875-L21100		**Exon 23**	
328	**LEP probe** 08881-L08937		**Exon 3**	
337	Reference probe 09073-L09242	19p		
347 ◎	**POMC probe** 08877-L19217		**Exon 2**	
355 ◎	**LEPR probe** 08866-L08922		**Exon 2**	
364	**SIM1 probe** 07302-L06939		**Exon 9**	
373	**POMC probe** 08878-L08934		**Exon 3**	
384	**LEPR probe** 08872-L08928		**Exon 16**	
392	**SIM1 probe** 07303-L09975		**Exon 10**	
400	**LEPR probe** 08871-L08927		**Exon 14**	
409 ¬	GRIK2 probe 16154-L18338		upstream of *SIM1*	
420	**SIM1 probe** 16155-L18783		**Exon 11**	
427	**LEPR probe** 08869-L08925		**Exon 9**	
436	**MC2R probe** 16156-L18688		**Exon 2**	
445	Reference probe 12526-L13576	4q		
454 ◎	**POMC probe** 08876-L19667		**Exon 1**	
472 ◎	**LEPR probe** 15153-L17804		**Exon 1**	
484	Reference probe 11603-L12350	13q		
495 *	Reference probe 22488-L24472	12q		

[a] See section Exon numbering on page 2 for more information.

* New in version B3.

± SNP rs61736060 (non-validated) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
∧ SNPs rs200893300, rs188857005 and rs563847116 (all validated) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
֍ The significance of *LEPR* and *POMC* exon 1 and 2 deletions is not clear as these exons are non-coding and alternative transcript variants using other transcription start sites are known.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. P220-B3 probes arranged according to chromosomal location

Table 2a. *LEPR* gene

Length (nt)	SALSA MLPA probe	*LEPR* exon[a]	Ligation site NM_001003679.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*186-188 (Exon 4)*		
472 ֍	15153-L17804	Exon 1	84 nt before exon 1	GCAGGCGCCGCG-TTTGCGAGCTAA	4.8 kb
355 ֍	08866-L08922	Exon 2	132-131 *reverse*	CCAGCATAAGAA-AAGTCAGTCCAA	145.4 kb
196	08867-L08923	Exon 5	464-465	TGCTTTCGGAGT-GAGCAAGATAGA	22.1 kb
250	08868-L16799	Exon 7	788-789	GTGCCTGTGCCA-ACAGCCAAACTC	5.9 kb
427	08869-L08925	Exon 9	1091-1092	CTTCCTGGGTCT-TCGTATGAGGTT	3.2 kb
178	21572-L30446	Exon 11	1544-1545	TCAACCAGTACA-ATCCAGTCACTT	8.1 kb
400	08871-L08927	Exon 14	2004-2005	TGTGTGCAGTCT-ATGCTGTTCAGG	6.1 kb
384	08872-L08928	Exon 16	2320-2321	AGCACATACTGT-TACGGTTCTGGC	3.8 kb
184	08873-L19206	Exon 18	2620-2619 *reverse*	TAAATATTGGGT-AAAGACTGAACT	3.0 kb
265	08874-L19305	Exon 20	2825-2826	AACCCCAAGAAT-TGTTCCTGGGCA	10.9 kb
322	08875-L21100	Exon 23	3593-3592 *reverse*	TATGTAGGGTCA-TCCTGATATGAA	
		stop codon	*2874-2876 (Exon 23)*		

Table 2b. *POMC* gene

Length (nt)	SALSA MLPA probe	*POMC* exon[a]	Ligation site NM_001035256.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*154-156 (Exon 3)*		
454 ֍	08876-L19667	Exon 1	30 nt before exon 1	AGCTCGGCAAGT-ATATAAGGACAG	2.9 kb
347 ֍	08877-L19217	Exon 2	103-104	TGGATTCTCCAA-AAGTATCTGCAG	0.9 kb
373	08878-L08934	Exon 3	206-207	GGCCTTGCTGCT-TCAGGCCTCCAT	3.8 kb
220	08879-L08935	Exon 4	1078-1079	TCAGCCTCTTAA-AGCTGCCTGTAG	
		stop codon	*955-957 (Exon 4)*		

Table 2c. *SIM1* gene

Length (nt)	SALSA MLPA probe	Gene / *SIM1* exon[a]	Ligation site NM_005068.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
148 ¬	03945-L03403	*CDK19* gene		CCAGATTATTCA-ATTCTCCTCTAA	2451.8 kb
302 ¬	03939-L09973	*NR2E1* gene		GGAGAGAACTGT-TTGTTCTAGGAA	6654.4 kb
409 ¬	16154-L18338	*GRIK2* gene		GGTGCGCCTGAA-GACTGGATTACT	935.6 kb
		start codon	*776-778 (Exon 1)*		
137	07292-L19213	Exon 1	543-544	AAATTCGGTGGA-TCAGCAACTTTC	0.4 kb
154	16068-L19470	Exon 1	3 nt after exon 1	TCCCAGAAGGTA-AGTGACTTTGCC	9.5 kb
171	07294-L17071	Exon 2	1011-1012	CGTTGGCCGAGA-ACTGGGCTCCCA	5.6 kb
283	07299-L21259	Exon 7	1597-1598	GGCTGCGACACC-TTCCACCTGCGC	0.8 kb
316	07300-L21099	Exon 8	1661-1662	CCAAGTACTACA-GGTTCCTGGCGA	26.6 kb
364	07302-L06939	Exon 9	1917-1918	CTCAAAGTCAAA-ATCCAGGACTTC	27.1 kb
392	07303-L09975	Exon 10	2127-2128	TGGCTTTGCGCT-TGACCACTCGAG	3.2 kb
420	16155-L18783	Exon 11	2964-2965	CATTAGAAACTA-TTCCTTGGGCTG	1014.4 kb
		stop codon	*3074-3076 (Exon 11)*		
214 ¬	16153-L19387	*COQ3* gene		TCATTTGATCCA-GTCCTGGATAAG	

Table 2d. *LEP* gene

Length (nt)	SALSA MLPA probe	*LEP* exon[a]	Ligation site NM_000230.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*58-60 (Exon 2)*		
202	08880-L08936	Exon 2	192-191 *reverse*	ACCGTGTGTGAA-ATGTCATTGATC	2.3 kb
328	08881-L08937	Exon 3	3 nt before exon 3	CTTCCTCCTGCA-TAGCAGTCAGTC	2.0 kb
310 ∧	15151-L21098	Exon 3	2241-2242	GAAGCTCACCCA-ATAAACATTAAG	
		stop codon	*559-561 (Exon 3)*		

Table 2e. 16p11.2 region

Length (nt)	SALSA MLPA probe	Gene / Exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		SH2B1	**NM_001145797.2**		
		start codon	*187-189 (Exon 2)*		
226	15155-L16929	Exon 2	1076-1077	GCTTCGAAGTGA-AGGAGAAGGAGG	2.0 kb
295	15157-L19310	Exon 4	1260-1261	ATCATGGAGACA-GTGGATGCCCAG	4.6 kb
270	15158-L20075	Exon 10	2448-2449	CCCGTGGTTGAA-TTGGAAGAGGCC	1025.4 kb
		stop codon	*2236-2238 (Exon 10)*		
		SEZ6L2	**NM_012410.4**		
		start codon	*532-534 (Exon 1)*		
208	11668-L12439	Exon 1	491-492	GCAGCCAGATTA-CTTAGAGAGGCA	
		stop codon	*3091-3093 (Exon 18)*		

Table 2f. *MC4R*/*MC2R* genes

Length (nt)	SALSA MLPA probe	Gene / Exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		MC4R	**NM_005912.3**		
		start codon	*427-429 (Exon 1)*		
233	15154-L25342	Exon 1	299-300	GACATTTACTCA-CAGCAGGCATGG	0.5 kb
142	02848-L02278	Exon 1	828-829	AGCTCCTTGCTT-GCATCCATTTGC	42.3 **M**b
		stop codon	*1423-1425 (Exon 1)*		
		MC2R	**NM_000529.2**		
		start codon	*178-180 (Exon 2)*		
436	16156-L18688	Exon 2	730-731	TGTTCCCGCTGA-TGCTGGTCTTCA	
		stop codon	*1069-1071 (Exon 2)*		

Table 2g. *MC3R* gene

Length (nt)	SALSA MLPA probe	*MC3R* exon[a]	Ligation site NM_019888.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*113-115 (Exon 1)*		
238 ±	08884-L08940	Exon 1	281-282	TGCTGGAAAACA-TCCTGGTTATCC	0.7 kb
160	08885-L08941	Exon 1	962-963	ACTTCAACACCT-ACCTGGTCCTCA	
		stop codon	*1082-1084 (Exon 1)*		

[a] See section Exon numbering on page 2 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

± SNP rs61736060 (non-validated) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
∧ SNPs rs200893300, rs188857005 and rs563847116 (all validated) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
⍟ The significance of *LEPR* and *POMC* exon 1 and 2 deletions is not clear as these exons are non-coding and alternative transcript variants using other transcription start sites are known.

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Related SALSA MLPA probemixes

P343 Autism-1 Contains more probes for the 16p11.2 region.

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P220 product history	
Version	*Modification*
B3	One reference probe has been replaced.
B2	One reference probe has been removed and two probe lengths have been adjusted.
B1	Four probes for *SIM1* and one probe for *MC2R* have been removed, and two probes (for *LEP* and *MC4R*) have been replaced. One probe for *LEPR*, one probe for *MC2R* and four probes for the 16p11.2 region have been added. Nine reference probes and four control fragments at 88, 96, 100 and 105 nt have been included (QDX2).
A1	First release.

Implemented changes in the product description

Version B3-02 – 28 July 2022 (04P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting the *POMC*, *SIM1* and *SH2B1* genes updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- SALSA MLPA Probemix P224 PPARG removed from the Related SALSA MLPA probemixes section as the probemix was discontinued.

Version B3-01 — 18 October 2019 (02P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *SEZ6L2* and *MC4R* genes updated according to the new versions of the NM_ reference sequences.
- NM_ reference sequence for the *LEP* gene updated.
- New publications using SALSA MLPA probemix P220 Obesity added.

Version 14 – 12 January 2018 (55)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- New reference added on page 2.
- Various minor textual changes on pages 1 and 2.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

- Exon numbering of the *LEPR* gene has been changed.
- Ligation sites of the probes targeting the *POMC* gene have been updated according to a new version of the NM_ reference sequence.

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