Product Description SALSA® MLPA® Probemix P080-C2 Craniofacial

To be used with the MLPA General Protocol.

Version C2. As compared to version C1, one reference probe has been replaced and several probe lengths have been adjusted. For complete product history see page 9.

Catalogue numbers:
- **P080-025R:** SALSA MLPA Probemix P080 Craniofacial, 25 reactions.
- **P080-050R:** SALSA MLPA Probemix P080 Craniofacial, 50 reactions.
- **P080-100R:** SALSA MLPA Probemix P080 Craniofacial, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

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

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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 P080 Craniofacial is a **research use only (RUO)** assay for the detection of deletions or duplications in the *FGFR1*, *FGFR2*, *FGFR3*, *TWIST1*, *MSX2*, *ALX4*, *ALX3*, *ALX1*, *RUNX2* and *EFBN1* genes, which are associated with several craniofacial disorders. This probemix can also be used to detect the presence of the wild-type sequences of the *FGFR2* c.755C>G and the *FGFR3* c.749C>G point mutations.

The *FGFR1*, *FGFR2* and *FGFR3* genes encode growth factors and cause a diverse group of skeletal disorders. In general, mutations in *FGFR1* and *FGFR2* mostly cause craniosynostosis (premature fusion of the cranial sutures). Dwarfing syndromes are often associated with *FGFR3* mutations.

Deletion of the *TWIST1* gene is the cause of disease in an estimated 11% of Saethre-Chotzen syndrome patients. Also included is a probe for the *TWISTNB* (TWIST nearby) gene located at a distance of ~500 kb from *TWIST1*. Large deletions of the TWIST region often result in mental retardation.

Dosage of the *MSX2* gene is critical for human skull development. Enlarged parietal foramina and craniosynostosis can result, respectively, from loss and gain of activity in an *MSX2* pathway of calvarial osteogenic differentiation.

Mutations in *ALX4* can result in parietal foramina as well as craniosynostosis. Potocki-Shaffer syndrome, also known as the proximal 11p deletion syndrome, is a contiguous gene syndrome caused by deletion of the 11p13-p11 region. Mutations in the *ALX3* gene can result in frontonasal dysplasia. The *ALX1* gene is known to be essential for normal skull bone development; null mice are born with severe craniofacial defects such as a lacking cranium.

Defects in the *RUNX2* gene cause the dominant disorder cleidocranial dysplasia.

Loss-of-function mutations in the *EFNB1* gene cause craniofrontonasal syndrome.

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/

Probemix content: The SALSA MLPA Probemix P080-C2 Craniofacial contains 48 MLPA probes with amplification products between 122 and 504 nt. This includes 39 probes for the different genes involved in craniofacial disorders, as described in tables 1 and 2. In addition, nine reference probes are included and detect different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 121 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.mlpa.com.

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

No DNA controls results in only five major peaks shorter than 121 nucleotides (nt): four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

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

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: 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 unrelated individuals who are from families without a history of craniofacial disorders. It is recommended to use samples of the same sex to facilitate interpretation. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample 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.mlpa.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 all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the dosage quotient (DQ) of the probes can be used to interpret MLPA results when **reference samples of the same sex** have been used:

Copy number status		Dosage quotient
Autosomal sequences X-chromosome sequences (females)	**X-chromosome sequences in males**	
Normal	Normal	0.80 < DQ < 1.20
Homozygous deletion	Deletion	DQ = 0
Heterozygous deletion		0.40 < DQ < 0.65
Heterozygous duplication		1.30 < DQ < 1.65
Heterozygous triplication/homozygous duplication	Duplication	1.75 < DQ < 2.15
Ambiguous copy number		All other values

- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the gene. 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 (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 are unlikely to have any relation to the condition tested for.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the genes involved in craniofacial disorders are small (point) mutations, most of which will not be detected by using SALSA® MLPA® Probemix P080 Craniofacial.
- 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *RUNX2* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P080-C2 Craniofacial

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)						
		reference	*ALX1*	*ALX3*	*ALX4*	*EFNB1*	*RUNX2*	*TWIST1*	Other
64-105	Control fragments – see table in probemix content section for more information								
122	Reference probe 02844-L02274	18q11							
130	**EFNB1** probe 14408-L16113					**Exon 4**			
136	**ALX3** probe 14409-L16114			**Exon 2**					
142	**RUNX2** probe 02610-L04842						**Exon 2**		
148	Reference probe 07018-L06629	14q11							
154	**RUNX2** probe 14410-L16115						**Exon 4**		
160 « ¥	**RUNX2** probe 22020-L31270						**Exon 3**		
166	**ALX3** probe 14411-L16412			**Exon 1**					
172	**MSX2** probe 14412-L16117								***MSX2* exon 2**
178	**FGFR1** probe 04184-L13122								***FGFR1* exon 5**
184	**FGFR2** probe 14413-L16118								***FGFR2* exon 10**
193 «	**TWIST1** probe 02079-L01598							**Exon 2**	
198	**ALX4** probe 14425-L16415				**Exon 2**				
204	**FGFR3** probe 04182-L16417								***FGFR3* exon 6**
208	**ALX1** probe 14414-L16627		**Exon 1**						
214	**RUNX2** probe 02613-L16418						**Exon 5**		
220	**ALX4** probe 02608-L16414				**Exon 3**				
226	Reference probe 08588-L16628	17p11							
232	**TWISTNB** probe 02147-L16629								***TWISTNB***
238	**RUNX2** probe 02614-L02085						**Exon 6**		
249 ∞	**FGFR2** probe 19612-L24455								***FGFR2* exon 7**
256	**ALX3** probe 14415-L24456			**Exon 4**					
263	Reference probe 08812-L24457	2p13							
268 ¥	**RUNX2** probe 21486-L24458						**Exon 7**		
274	**EFNB1** probe 14416-L24459					**Exon 3**			
281	**ALX1** probe 14417-L24460		**Exon 3**						
288	**RUNX2** probe 14418-L24461						**Exon 9**		
295 «	**TWIST1** probe 01166-L24462							**Exon 2**	
301	**RUNX2** probe 02616-L24463						**Exon 8**		
310	**EFNB1** probe 14419-L16124					**Exon 2**			
319 «	**TWIST1** probe 01969-L02364							**Exon 1**	
334 ∞	**FGFR3** probe 19611-L24464								***FGFR3* exon 7**
346	Reference probe 11018-L11687	15q14							
353 «	**TWIST1** probe 14433-L24466							**Exon 1**	
364	**ALX3** probe 14420-L24467			**Exon 3**					
373	**ALX4** probe 14421-L24468				**Exon 4**				
381	**ALX1** probe 14422-L24469		**Exon 1**						
391	**FGFR1** probe 01046-L24470								***FGFR1* exon 2**
399 *	Reference probe 12453-L13454	22q12							
407	**ALX1** probe 14423-L24472		**Exon 4**						
416	Reference probe 07823-L07577	1q31							
427	**MSX2** probe 14426-L16131								***MSX2* exon 1**
432	**ALX1** probe 14427-L16132		**Exon 2**						
445	Reference probe 16286-L18578	13q14							
462	**EFNB1** probe 14428-L24473					**Exon 1**			
470 «	**RUNX2** probe 14429-L24474						**Exon 3**		
478	**EFNB1** probe 14430-L24475					**Exon 5**			
504	Reference probe 15203-L22928	3p12							

* New in version C2 (from lot C2-1118 onwards).
¥ Changed in version C2 (from lot C2-1118 onwards). Small change in length, no change in sequence detected.
∞ Wild type sequence detected. The presence of the mutation will result in a decreased probe signal.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

Table 2. P080-C2 probes arranged according to chromosomal location

Table 2a. *ALX3* gene

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_006492.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*61-63 (exon 1)*		
166	14411-L16412	Exon 1	85-86	ACTGCGCGCCTT-TCCGCGTGGGGC	5.7 kb
136	14409-L16114	Exon 2	347-348	AGCTGAGGAGAA-GACCTCCAAAGC	3.4 kb
364	14420-L24467	Exon 3	706-707	AGCGTTATGGGA-AGATCCAGGAGG	0.7 kb
256	14415-L24456	Exon 4	1020-1021	GATGGTGACTAT-AAGTCTCCAAGC	
		stop codon	*1090-1092 (exon 4)*		

Note: The exon numbering used in this P080-C2 Craniofacial product description is the exon numbering from the RefSeq transcript NM_006492.3, which is identical to the LRG_1265 sequence. The exon numbering and NM sequence used is from 01/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2b. *FGFR3* gene

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_000142.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*257-259 (exon 2)*		
204	04182-L16417	Exon 6	904-905	CTGGTCATGGAA-AGCGTGGTGCCC	0.2 kb
334 ∞	19611-L24464	Exon 7	1005-1006	AGAGCGCTCCCC-GCACCGGCCCAT	
		stop codon	*2675-2677 (exon 19)*		

∞ The 334 nt probe detects the wild type sequence at the site of the c.749C>G (p.Pro250Arg) mutation. A 50% reduced signal is expected when this exon is deleted or when samples contain one allele of this mutation.

Note: The exon numbering used in this P080-C2 Craniofacial product description is the exon numbering from the RefSeq transcript NM_000142.4, which is identical to the LRG_1021 sequence. The exon numbering and NM sequence used is from 01/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2b. *MSX2* gene

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_002449.5	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*79-81 (exon 1)*		
427	14426-L16131	Exon 1	457-intron 1	CGCCGCCGCCAA-GTGAGTGCGCGC	4.0 kb
172	14412-L16117	Exon 2	99 nt before exon 2	GGGAGGCCCGAA-AGGAAAAAACCT	
		stop codon	*880-882 (exon 2)*		

Note: The exon numbering used in this P080-C2 Craniofacial product description is the exon numbering from the RefSeq transcript NM_002449.5. The exon numbering and NM sequence used is from 01/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2d. *RUNX2* gene

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_001024630.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*198-200 (exon 2)*		
142	02610-L04842	Exon 2	216-217	CAAACAGCCTCT-TCAGCACAGTGA	93.6 kb
470 «	14429-L24474	Exon 3	268 nt before exon 3	GTGTTCCAAAGA-CTCCGGCAAAGA	0.3 kb
160 « ¥ +	22020-L31270	Exon 3	10 nt before exon 3	GTTGTGATGCGT-ATTCCCGTAGAT	9.3 kb
154	14410-L16115	Exon 4	626-627	ATGTAGGTGGTA-GCCCTCGGAGAG	6.1 kb
214	02613-L16418	Exon 5	799-800	CACCTTGACCAT-AACCGTCTTCAC	54.1 kb
238	02614-L02085	Exon 6	989-990	GTCCCGCCTCAG-AACCCACGGCCC	20.3 kb
268 ¥	21486-L24458	Exon 7	1127-1126 reverse	GACGGGGACGTC-ATCTGGCTCAGG	33.0 kb
301	02616-L24463	Exon 8	1269-1268 reverse	CTGGCTCTTCTT-ACTGAGAGTGGA	1.9 kb
288	14418-L24461	Exon 9	1607-1608	TCCAGAATGCTT-CCGCCATGCACC	
		stop codon	*1761-1763 (exon 9)*		

¥ Changed in version C2 (from lot C2-1118 onwards). Small change in length, no change in sequence detected.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
+ This RUNX2 probe partial sequence was changed; GTTGTGATGCGT-ATTCCTGTAGAT changed into GTTGTGATGCGT-ATTCCCGTAGAT.

Note: The exon numbering used in this P080-C2 Craniofacial product description is the exon numbering from the RefSeq transcript NM_001024630.4. The exon numbering and NM sequence used is from 01/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2e. *TWIST1* gene

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_000474.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
232	02147-L16629	*TWISTNB*	769-770 NM_001002926.2	AGCTAGCAGATG-ATGCAGATGACA	581.5 kb
		start codon	*316-318 (exon 1)*		
355 «	14433-L24466	Exon 1	532-531 reverse	CTTGCCGCGCTT-GCCCTGGGCCGG	0.3 kb
319 «	01969-L02364	Exon 1	823-824	ACGAGCTGGACT-CCAAGATGGCAA	0.7 kb
295 «	01166-L24462	Exon 2	983-984	ATTGTTTCCAGA-GAAGGAGAAAAT	0.3 kb
193 «	02079-L01598	Exon 2	1300-1301	TCGTGCCAATCA-GCCACTGAAAGG	
		stop codon	*922-924 (exon 1)*		

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

Note: The exon numbering used in this P080-C2 Craniofacial product description is the exon numbering from the RefSeq transcript NM_000474.4. The exon numbering and NM sequence used is from 01/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2f. *FGFR1* gene

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_023110.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*943-945 (exon 2)*		
391	01046-L24470	Exon 2	931-932	CAACCTCTAACT-GCAGAACTGGGA	29.5 kb
178	04184-L13122	Exon 5	1481-1482	CAAATGCCCTTC-CAGTGGGACCCC	
		stop codon	*3409-3411 (exon 18)*		

Note: The exon numbering used in this P080-C2 Craniofacial product description is the exon numbering from the RefSeq transcript NM_023110.2, which is identical to the LRG_993 sequence. The exon numbering and NM sequence used is from 01/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2g. *FGFR2* gene

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_000141.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*648-650 (exon 2)*		
249 ∞	19612-L24455	Exon 7	1402-1403	ACCAGAGCGATC-GCCTCACCGGCC	4.9 kb
184	14413-L16118	Exon 10	1826-1827	TGTATGGTGGTA-ACAGTCATCCTG	
		stop codon	*3111-3113 (exon 19)*		

∞ The 249 nt probe detects the wildtype sequence at the site of the c.755C>G (p.Ser252Trp) mutation. A 50% reduced signal is expected when this exon is deleted or when samples contain one allele of this mutation.

Note: The exon numbering used in this P080-C2 Craniofacial product description is the exon numbering from the RefSeq transcript NM_000141.4, which is identical to the LRG_994 sequence. The exon numbering and NM sequence used is from 01/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2h. *ALX4* gene

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_021926.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*78-80 (exon 1)*		
198	14425-L16415	Exon 2	32 nt after exon 2	AGTGAGGCTGGT-AAAGCAGAGCCT	7.7 kb
220	02608-L16414	Exon 3	901-902	GCGGGAGCGTTT-TGGGCAGATGCA	2.6 kb
373	14421-L24468	Exon 4	1243-1244	GCCGGACCGCAA-GACCTCGAGCAT	
		stop codon	*1311-1313 (exon 4)*		

Note: The exon numbering used in this P080-C2 Craniofacial product description is the exon numbering from the RefSeq transcript NM_021926.4. The exon numbering and NM sequence used is from 01/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2i. *ALX1* gene

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_006982.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*43-45 (exon 1)*		
381	14422-L24469	Exon 1	74-75	GTTTGCCCTCAA-GAGCCCTCCGAG	0.1 kb
208	14414-L16627	Exon 1	185-186	GTCTGCAGGCAA-ATGCGTGCAGGC	3.2 kb
432	14427-L16132	Exon 2	283-284	ACTATGGGATCA-CTAAAGTAGAAG	3.3 kb
281	14417-L24460	Exon 3	609-610	AAATGGAGAAAA-AGGGAACGTTAT	14.4 kb
407	14423-L24472	Exon 4	798-799	ATGACACCTTAT-TCTCACTCGCCT	
		stop codon	*1021-1023 (exon 4)*		

Note: The exon numbering used in this P080-C2 Craniofacial product description is the exon numbering from the RefSeq transcript NM_006982.3. The exon numbering and NM sequence used is from 01/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2j. *EFNB1* gene

Length (nt)	SALSA MLPA probe	Exon	Ligation site NM_004429.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*781-783 (exon 1)*		
462	14428-L24473	Exon 1	766-767	CCGAAGTGCAGT-CTGCCCCCGGGA	9.1 kb
310	14419-L16124	Exon 2	1174-1175	AGCACCATGATT-ACTACATTACCT	0.8 kb
274	14416-L24459	Exon 3	1189-1190	TTCCTGCAGCAA-CATCCAATGGAA	0.3 kb
130	14408-L16113	Exon 4	1315-1316	AGCTGACTACCA-GCAGGCCCAGCA	0.4 kb
478	14430-L24475	Exon 5	1571-1572	CCTACTACTGAA-GCTACGCAAGCG	
		stop codon	*1819-1821 (exon 5)*		

Note: The exon numbering used in this P080-C2 Craniofacial product description is the exon numbering from the RefSeq transcript NM_004429.4. The exon numbering and NM sequence used is from 01/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

- P310 TCOF1: Contains probes for *TCOF1*, involved in Treacher Collins-Franceschetti 1.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P080 Craniofacial

- Bir FD et al. (2017). Cleidocranial dysplasia: Clinical, endocrinologic and molecular findings in 15 patients from 11 families. *Eur J Med Genet.* 60:163-8.

- Di Rocco F et al. (2015). Y-craniosynostosis by premature fusion of the metopic and coronal sutures: a new nosological entity or a variety of Saethre-Chotzen syndrome? *Birth Defects Res A Clin Mol Teratol*. 103:306-10.
- Plaisancié J et al. (2015). MSX2 gene duplication in a patient with eye development defects. *Ophthalmic Genet*. 36:353-8.
- Babbs C et al. (2011). Duplication of the EFNB1 Gene in Familial Hypertelorism: Impalance in Ephrin-B1 Expression and Abnormal Phenotypes in Humans and Mice. *Hum Mutat*. 32:930-8.
- Jehee FS et al. (2008). High frequency of submicroscopic chromosomal imbalances in patients with syndromic craniosynostosis detected by a combined approach of microsatellite segregation analysis, multiplex ligation-dependent probe amplification and array-based comparative genome hybridisation. *J Med Genet*. 45:447-50.

P080 Product history	
Version	*Modification*
C2	One reference probe has been replaced and several probe lengths have been adjusted.
C1	One of the two ALX4 exon 2 probes is removed, the two FGFR2 and FGFR3 probes at the location of the APERT mutation have been replaced and several reference probes have been replaced. In addition control fragments have been adjusted (QDX2).
B1	21 probes have been removed, 33 probes added.
A1	First release.

Implemented changes in the product description

Version C2-02 – 25 March 2019 (01P)
- The probe sequence of the RUNX2 probe for exon 3 (160 nt) was adjusted. The old sequence contained a mistake: GTTGTGATGCGT-ATTCCTGTAGAT changed into GTTGTGATGCGT-ATTCCCGTAGAT.

Version C2-01 – 25 January 2019 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).
- Ligation sites of the probes targeting the *ALX3*, *MSX2*, *RUNX2*, *TWIST1*, *ALX4* and *ALX1* genes have been 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.

Version 18 – 09 August 2017 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- New references added on page 1.
- Exon numbering of the *FGFR1* and *FGFR2* genes has been changed.
- Minor layout changes.

Version 17 (53)
- This product description has been changed to incorporate a new product version (lot number added, new pictures included).
- Table 2 has been arranged according to chromosomal location.

Version 16 (49)
- Warning added in Table 1, 335 nt probe 02841-L02272.

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