Product Description
SALSA® MLPA® Probemix P080-C2 Craniofacial

To be used with the MLPA General Protocol.

Version C2
For complete product history see page 8.

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 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 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 *EFNB1* genes, which is 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 intellectual disability.

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.

More information is available at https://www.ncbi.nlm.nih.gov/books/.

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 *FGFR1*, *FGFR2*, *FGFR3*, *TWIST1*, *MSX2*, *ALX4*, *ALX3*, *ALX1*, *RUNX2* and *EFNB1* exon numbering used in this P080-C2 Craniofacial product description is the exon numbering from the LRG_993, LRG_994, LRG_1021, NG_008114.2, NG_008124.1, LRG_1256, LRG_1265, NG_023202.1, NG_008020.2 and NG_008887.1 sequences respectively. The exon numbering of the NM_ sequences that were used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the 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 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 that detect different 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 craniofacial disorders. 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 when **reference samples of the same sex** have been used:

Copy number status		Final ratio (FR)
Autosomal sequences and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < FR < 1.20
Homozygous deletion	Deletion	FR = 0
Heterozygous deletion		0.40 < FR < 0.65
Heterozygous duplication		1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	Duplication	1.75 < FR < 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. 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

- In most populations, the major cause of genetic defects in the *FGFR1*, *FGFR2*, *FGFR3*, *TWIST1*, *MSX2*, *ALX4*, *ALX3*, *ALX1*, *RUNX2* and *EFNB1*genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P080-C2 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. 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.

***FGFR1*, *FGFR2*, *FGFR3*, *TWIST1*, *MSX2*, *ALX4*, *ALX3*, *ALX1*, *RUNX2* and *EFNB1* mutation databases**

https://databases.lovd.nl/shared/genes/EFNB1. We strongly encourage users to deposit positive results in the Leiden Open Variation Database. 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 *EFNB1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P080-C2 Craniofacial

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]							
		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	18q							
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	14q							
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	17p							
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	2p							
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	15q							
355 «	**TWIST1** probe 14433-L24466							**Exon 1**	
364	**ALX3** probe 14420-L24467			**Exon 3**					
373	**ALX4** probe 14421-L24468				**Exon 4**				
382	**ALX1** probe 14422-L24469		**Exon 1**						
391	**FGFR1** probe 01046-L24470								***FGFR1* exon 2**
399	Reference probe 12453-L13454	22q							
407	**ALX1** probe 14423-L24472		**Exon 4**						
416	Reference probe 07823-L07577	1q							
427	**MSX2** probe 14426-L16131								***MSX2* exon 1**
432	**ALX1** probe 14427-L16132		**Exon 2**						
445	Reference probe 16286-L18578	13q							
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	3p							

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

« 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.
∞ Wild type sequence detected. A lowered probe signal can be due to a presence of a mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

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

Table 2a. *ALX3* gene

Length (nt)	SALSA MLPA probe	*ALX3* Exon[a]	Ligation site NM_006492.3	Partial sequence[b] (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)*		

Table 2b. *FGFR3* gene

Length (nt)	SALSA MLPA probe	*FGFR3* Exon[a]	Ligation site NM_000142.4	Partial sequence[b] (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.

Table 2c. *MSX2* gene

Length (nt)	SALSA MLPA probe	*MSX2* Exon[a]	Ligation site NM_002449.5	Partial sequence[b] (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)*		

Table 2d. *RUNX2* gene

Length (nt)	SALSA MLPA probe	*RUNX2* Exon[a]	Ligation site NM_001024630.4	Partial sequence[b] (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)*		

+ This RUNX2 probe partial sequence was changed; GTTGTGATGCGT-ATTCCTGTAGAT changed into GTTGTGATGCGT-ATTCCCGTAGAT.

Table 2e. *TWIST1* gene

Length (nt)	SALSA MLPA probe	*TWIST1* Exon[a]	Ligation site NM_000474.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
232	02147-L16629	*TWISTNB*		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)*		

Table 2f. *FGFR1* gene

Length (nt)	SALSA MLPA probe	*FGFR1* Exon[a]	Ligation site NM_023110.2	Partial sequence[b] (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)*		

Table 2g. *FGFR2* gene

Length (nt)	SALSA MLPA probe	*FGFR2* Exon[a]	Ligation site NM_000141.4	Partial sequence[b] (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.

Table 2h. *ALX4* gene

Length (nt)	SALSA MLPA probe	*ALX4* Exon[a]	Ligation site NM_021926.4	Partial sequence[b] (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)*		

Table 2i. *ALX1* gene

Length (nt)	SALSA MLPA probe	*ALX1* Exon[a]	Ligation site NM_006982.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*43-45 (exon 1)*		
382	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)*		

Table 2j. *EFNB1* gene

Length (nt)	SALSA MLPA probe	*EFNB1* Exon[a]	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)*		

[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.

« 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.
∞ Wild type sequence detected. A lowered probe signal can be due to a presence of a mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

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.

Related SALSA MLPA probemixes

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

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.
- Twigg SRF et al. (2006). The Origin of EFNB1 Mutations in Craniofrontonasal Syndrome: Frequent Somatic Mosaicism and Explanation of the Paucity of Carrier Males. *The Am J of Hum Genet*. 78:999-1010.
- LA Mavrogiannis et al. (2006). Enlarged parietal foramina caused by mutations in the homeobox genes ALX4 and MSX2: from genotype to phenotype. *Eur J Med Genet*. 14:151–158.

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 C2-04 –20 November 2024 (04P) - The term 'mental retardation' is considered outdated and was updated to 'intellectual disability' where appropriate. Version C2-03 – 02 September 2021 (04P) - Product description restructured and adapted to a new template.

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