Product Description
SALSA® MLPA® Probemix P493-A1 SMCHD1

To be used with the MLPA General Protocol.

Version A1
For complete product history see page 7.

Catalogue numbers:
- **P493-025R:** SALSA MLPA Probemix P493 SMCHD1, 25 reactions.
- **P493-050R:** SALSA MLPA Probemix P493 SMCHD1, 50 reactions.
- **P493-100R:** SALSA MLPA Probemix P493 SMCHD1, 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 P493 SMCHD1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SMCHD1* gene which is associated with Fascioscapulohumeral muscular dystrophy 2 (FSHD2) and Bosma arhinia microphthalmia syndrome (BAMS).

The *SMCHD1* gene (48 exons), spans ~149 kb of genomic DNA and is located on chromosome 18p11.32, ~2.7 Mb from the p-telomere. The *SMCHD1* gene encodes the structural maintenance of chromosomes hinge domain containing protein 1, an epigenetic modifier that plays a role in the regulation of the expression of several genes across the genome. Heterozygous pathogenic *SMCHD1* variants are associated two distinct disorders: FSHD2 and BAMS (Gurzau et al. 2020).

FSHD is form of muscular dystrophy characterised by a distinctive pattern of muscle weakness, starting with the facial, scapular and humeral muscles, later progressing the trunk and lower extremities (Pandya et al. 2008). Two types of FSHD have been described: FSHD1 (>95% of FSHD) and FSHD2 (<5% of FSHD). FSHD1 is caused by a contraction of the D4Z4 repeat array on chromosome 4 *in cis* with the 4qA haplotype. 80% of FSHD2 cases is caused by a heterozygous *SMCHD1* pathogenic variant, leading to hypomethylation of the D4Z4 repeat array (Zernov and Skoblov 2019).

BAMS is a rare syndrome characterised by among others severe hypoplasia of the nose and eyes, palatal abnormalities and deficient taste and smell (OMIM 603457).

More information on FSHD is available at https://www.ncbi.nlm.nih.gov/books/NBK1443/

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 *SMCHD1* exon numbering used in this P493-A1 SMCHD1 product description is the exon numbering from the NG_031972.1 sequence. 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 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 P493-A1 SMCHD1 contains 57 MLPA probes with amplification products between 121 and 500 nucleotides (nt). This includes 48 probes for the *SMCHD1* gene. In addition, nine 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 FSHD or BAMS. 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

- In most populations, the major cause of genetic defects in the *SMCHD1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P493 SMCHD1.
- 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.

***SMCHD1* mutation database**

https://databases.lovd.nl/shared/genes/SMCHD1. 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 *SMCHD1* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P493-A1 SMCHD1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*SMCHD1*
64-105	Control fragments – see table in probemix content section for more information		
121	Reference probe 19616-L27455	4p	
125	**SMCHD1 probe** S1292-L32833		**Exon 1**
130	**SMCHD1 probe** 22874-L31562		**Exon 17**
136	**SMCHD1 probe** 22875-L31563		**Exon 21**
143	**SMCHD1 probe** 22876-L31589		**Exon 38**
149	**SMCHD1 probe** 22877-L32260		**Exon 4**
154	**SMCHD1 probe** 22878-L32261		**Exon 43**
158	**SMCHD1 probe** 22879-L32262		**Exon 6**
163	Reference probe 10694-L32708	6p	
170	**SMCHD1 probe** 23002-L32263		**Exon 3**
176	**SMCHD1 probe** 22881-L32264		**Exon 10**
181	**SMCHD1 probe** 22882-L32265		**Exon 25**
186	**SMCHD1 probe** 22883-L32266		**Exon 12**
192	**SMCHD1 probe** 23003-L32649		**Exon 32**
197	**SMCHD1 probe** 22887-L32270		**Exon 8**
202	Reference probe 15424-L17583	7p	
208	**SMCHD1 probe** 22885-L32268		**Exon 5**
214	**SMCHD1 probe** 22888-L32271		**Exon 9**
220	**SMCHD1 probe** 22889-L32272		**Exon 2**
228	**SMCHD1 probe** 22886-L32269		**Exon 7**
233	**SMCHD1 probe** 22913-L32349		**Exon 26**
240	**SMCHD1 probe** 22914-L32350		**Exon 14**
247	Reference probe 21928-L32709	15q	
252	**SMCHD1 probe** 22979-L32650		**Exon 41**
258	**SMCHD1 probe** 22915-L32351		**Exon 29**
265	**SMCHD1 probe** 22917-L32651		**Exon 11**
270	**SMCHD1 probe** 22916-L32352		**Exon 23**
274	**SMCHD1 probe** 22918-L32354		**Exon 45**
281	**SMCHD1 probe** 22976-L32415		**Exon 35**
290	**SMCHD1 probe** 22920-L32356		**Exon 33**
295	Reference probe 04437-L32710	8p	
301	**SMCHD1 probe** 22919-L32652		**Exon 15**
310	**SMCHD1 probe** 22921-L32357		**Exon 48**
319	**SMCHD1 probe** 22922-L32358		**Exon 40**
325	**SMCHD1 probe** 22923-L32653		**Exon 47**
333	**SMCHD1 probe** 22924-L32360		**Exon 36**
340	Reference probe 21099-L30929	11p	
351	**SMCHD1 probe** 22970-L32409		**Exon 22**
356	**SMCHD1 probe** 22925-L32361		**Exon 19**
364	**SMCHD1 probe** 22977-L32416		**Exon 37**
373	**SMCHD1 probe** 22973-L32412		**Exon 30**
382	**SMCHD1 probe** 22969-L32408		**Exon 18**
389	**SMCHD1 probe** 22974-L32413		**Exon 31**
399	Reference probe 19323-L27531	12p	
407	**SMCHD1 probe** 22971-L32410		**Exon 27**
414	**SMCHD1 probe** 22926-L32362		**Exon 24**
421	**SMCHD1 probe** 22927-L32363		**Exon 16**
427	**SMCHD1 probe** 22975-L32414		**Exon 34**
436	**SMCHD1 probe** 22928-L32364		**Exon 42**
445	**SMCHD1 probe** 22929-L32365		**Exon 20**
452	Reference probe 19175-L25143	13q	

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*SMCHD1*
463	**SMCHD1 probe** 22981-L32420		**Exon 46**
470	**SMCHD1 probe** 22930-L32366		**Exon 13**
479	**SMCHD1 probe** 22980-L32419		**Exon 44**
488	**SMCHD1 probe** 22978-L32417		**Exon 39**
492	**SMCHD1 probe** 22972-L32411		**Exon 28**
500	Reference probe 19555-L27674	2p	

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

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. *SMCHD1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*SMCHD1* exon[a]	Ligation site NM_015295.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*351-353 (Exon 1)*		
125	S1292-L32833	Exon 1	4 nt after exon 1	TGTGTCAGGTAC-GCGAAGGGGCGA	9.9 kb
220	22889-L32272	Exon 2	583-584	TACAACAACAAG-TAGGAAAGAAAT	0.8 kb
170	23002-L32263	Exon 3	708-709	ACTTCTTACCTC-ACTATGACACAC	6.4 kb
149	22877-L32260	Exon 4	814-815	TTCATTGTCTGC-TACTTCTCGTAA	0.8 kb
208	22885-L32268	Exon 5	919-920	AAGAGGAATGAC-CTCTAAACAGCT	14.3 kb
158	22879-L32262	Exon 6	1017-1018	TTCGTCCAGTAC-CAGTGCCACGCA	0.2 kb
228	22886-L32269	Exon 7	1130-1131	GCAGATTCCCAA-GATGTTCACGAG	6.0 kb
197	22887-L32270	Exon 8	1316-1317	TTTACTGCTGTG-GTTATCACAGGG	2.4 kb
214	22888-L32271	Exon 9	1417-1418	CTATATTCATGG-CCCAAAAGGAAA	0.9 kb
176	22881-L32264	Exon 10	1659-1660	TATATGATAGAG-AAACTTACCCTG	2.6 kb
265	22917-L32651	Exon 11	1736-1737	TTCATACTTGAG-AAAGCAGCTAGA	0.3 kb
186	22883-L32266	Exon 12	1914-1915	AATTCCAGGTCA-GCACAAATAAAT	3.0 kb
470	22930-L32366	Exon 13	2138-2139	CAAGGTCCCTGG-GCAACATATGCA	1.9 kb
240	22914-L32350	Exon 14	2269-2270	TCATGATGGAGA-AGTATATGCTAC	0.6 kb
301	22919-L32652	Exon 15	2354-2355	CCAATTGCAAAG-CTGGATAGGACA	1.2 kb
421	22927-L32363	Exon 16	2438-2439	TTGTCAGTAACT-TGGCCTGAAGGA	0.3 kb
130	22874-L31562	Exon 17	2564-2565	GGAACAAGCCAT-GGAGGGTCAAAG	10.3 kb
382	22969-L32408	Exon 18	2652-2653	ATATTAGTCAAC-ATGGAGGAAAAT	0.2 kb
356	22925-L32361	Exon 19	2763-2764	CAGACACTTATG-CAGGAAGACCAC	4.2 kb
445	22929-L32365	Exon 20	2926-2927	TCAACTAGTAAC-TGATATTCAGCC	2.3 kb
136	22875-L31563	Exon 21	3037-3036 reverse	TGCCTTGACAAG-AGTTTACAGGGC	1.5 kb
351	22970-L32409	Exon 22	3089-3090	GGCTTAAAAGAA-GACTCACAGATT	2.1 kb
270	22916-L32352	Exon 23	3214-3215	GGAAGTTTTAGA-TGAATCAGACAA	0.7 kb
414	22926-L32362	Exon 24	3281-3282	GGTGCTCCAAAC-CTTCCAGTCTAT	3.0 kb
181	22882-L32265	Exon 25	3479-3480	CAAATATTCAGT-GTAGAAGGACAA	6.1 kb
233	22913-L32349	Exon 26	3681-3682	GTCTGCTTCCTG-ATGTGCAAGTAC	1.1 kb
407	22971-L32410	Exon 27	3851-3850 reverse	ACTTCTCCTTGA-AGCTCTTGGCCC	1.2 kb
492	22972-L32411	Exon 28	3910-3911	TCAAGCATTTTC-ACCAAGTTCTTT	3.1 kb
258	22915-L32351	Exon 29	4105-4106	ACTAATTTCTGG-ACCTCCTGCTAA	3.7 kb
373	22973-L32412	Exon 30	4180-4181	TGGAAGAGATTT-ACAGAACCCTAT	2.6 kb
389	22974-L32413	Exon 31	4346-4347	GTATTCAGTGTT-TTTGCCCCTAGG	0.3 kb
192	23003-L32649	Exon 32	4399-4400	CTATAACAAAAG-TATCATAGAAGG	1.0 kb
290	22920-L32356	Exon 33	4588-4589	TATTTCCATGAA-AATGTGGAAGCT	1.2 kb
427	22975-L32414	Exon 34	4681-4682	CAAAGAAGATGG-CTGCTTCTATTT	8.2 kb
281	22976-L32415	Exon 35	4728-4729	AAGTGGGGACAT-ATTGTATCCAGT	1.5 kb
333	22924-L32360	Exon 36	4879-4880	CTCAGTTGCCAG-TAGGACCTTGGT	1.5 kb

Length (nt)	SALSA MLPA probe	*SMCHD1* exon[a]	Ligation site NM_015295.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
364	22977-L32416	Exon 37	5020-5021	TTTTATTGGGAA-AGTTAGAACACT	6.0 kb
143	22876-L31589	Exon 38	5115-5116	ATAGTACTGAAT-ATTTTATTGTAT	0.3 kb
488	22978-L32417	Exon 39	5239-5240	TACAAAAGAAAA-GGACCAATTATC	1.6 kb
319	22922-L32358	Exon 40	5385-5384 reverse	TGTTGTAGGAAT-GTCAATATTATG	0.7 kb
252	22979-L32650	Exon 41	5432-5433	CTTCTGAAAAGA-AAGCTATCAGAA	3.5 kb
436	22928-L32364	Exon 42	5579-5580	GTTATTTCTTGG-CATCTGGCAAGT	2.1 kb
154	22878-L32261	Exon 43	5787-5786 reverse	AAATGTTAACAA-GTCTCGAGCAAA	0.3 kb
479	22980-L32419	Exon 44	5864-5863 reverse	GCATCCAGATTA-TCCAAAATAATG	6.3 kb
274	22918-L32354	Exon 45	5938-5939	GCTGACCAGAGA-TGGAGATCGAAT	11.5 kb
463	22981-L32420	Exon 46	6116-6117	CTAGACAGTGTG-AATAAGGATCTT	0.5 kb
325	22923-L32653	Exon 47	6294-6295	AGACGACAGATT-GTCCAGTTCCTC	6.2 kb
310	22921-L32357	Exon 48	6468-6469	CAGACTGAGTAT-TTCTGGGGACAA	
		stop codon	*6366-6368 (Exon 48)*		

[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.

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.

Related SALSA MLPA probemixes

P048 LMNA/MYOT/ZMPSTE24	Contains probes for *LMNA* and *CAV3*, involved in limb-girdle muscular dystrophy (LGMD) type 1B and 1C.
P061 Lissencephaly	Contains probes for *POMT1* and *POMGNT1*, involved in LGMD2K and LGMD2O.
P116 SGC	Contains probes for the *SGCA*, *SGCB*, *SGCD*, *SGCG* and *FKRP* genes, involved in LGMDs.
P176 CAPN3	Contains probes for the *CAPN3* gene, involved in LGMD2A.
P268 DYSF	Contains probes for the *DYSF* gene, involved in LGMD2B.
P436 ANO5	Contains probes for the *ANO5* gene, involved in LGMD2L.

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P493 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-01 – 15 February 2022 (04P) - Not applicable, new document.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200