Product Description
SALSA® MLPA® Probemix
P385-A3 DOCK8 & P386-A4 DOCK8-STAT3

To be used with the MLPA General Protocol.

P385 version A3
As compared to version A2, five reference probes have been replaced
P386 version A4
As compared to version A3, five reference probes have been replaced and one probe length has been adjusted.
For complete products history see page 9.

Catalogue numbers:
- **P385-025R:** SALSA MLPA Probemix P385 DOCK8, 25 reactions.
- **P385-050R:** SALSA MLPA Probemix P385 DOCK8, 50 reactions.
- **P385-100R:** SALSA MLPA Probemix P385 DOCK8, 100 reactions.

- **P386-025R:** SALSA MLPA Probemix P386 DOCK8-STAT3, 25 reactions.
- **P386-050R:** SALSA MLPA Probemix P386 DOCK8-STAT3, 50 reactions.
- **P386-100R:** SALSA MLPA Probemix P386 DOCK8-STAT3, 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 Probemixes P385 DOCK8 and P386 DOCK8-STAT3 are **research use only (RUO)** assays for the detection of deletions or duplications in the *DOCK8* and *STAT3* genes, which are associated with Hyper-IgE syndrome.

Hyper-IgE syndrome (HIES) is a rare immunodeficiency disease, often accompanied by high serum IgE. It is often characterized by facial features, repeated skin infections, eczema and pulmonary infection, including autosomal dominant HIES (AD-HIES) and autosomal recessive HIES (AR-HIES). AD-HIES is caused by mutations in the *STAT3* gene. AR-HIES is mainly caused by mutations in the *DOCK8* gene.

The *DOCK8* gene (48 exons) spans ~192 kb of genomic DNA and is located on chromosome 9p24.3, 0.2 Mb from the p-telomere. The *STAT3* gene (24 exons) spans ~75 kb of genomic DNA and is located on chromosome 17q21.2, 38 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK25507/.

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 *DOCK8* exon numbering used in the P385-A3 DOCK8 and P386-A4 DOCK8-STAT3 product descriptions is the exon numbering from the LRG_196 sequence. The *STAT3* exon numbering used in the P386-A4 DOCK8-STAT3 product description is the exon numbering from the LRG_112 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 LRG 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 P385-A3 DOCK8 contains 48 MLPA probes with amplification products between 130 and 490 nucleotides (nt). This includes 38 probes for the *DOCK8* gene and one flanking probe detecting the *DMRT1* gene. In addition, nine reference probes are included that detect autosomal chromosomal locations.

The SALSA MLPA Probemix P386-A4 DOCK8-STAT3 contains 46 MLPA probes with amplification products between 131 and 493 nucleotides (nt). This includes 13 probes for the *DOCK8* gene, 24 probes for the *STAT3* gene and one flanking probe detecting the *DMRT1* gene. 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).

These probemixes contain 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 HIES. 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. Sample ID numbers from the Coriell Institute in the table below have been tested with this P385-A3/P386-A4 probemix at MRC Holland and can be used as positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Altered target genes in P385-A3/P386-A4	Expected copy number alteration
NA02819	*DOCK8*	Heterozygous duplication
NA03226	*DOCK8*	Heterozygous duplication
NA05347	*DOCK8*	Heterozygous deletion
NA10989	*DOCK8*	Heterozygous deletion

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 *DOCK8* and *STAT3* genes are small (point) mutations, most of which will not be detected by using these SALSA MLPA Probemixes P385 DOCK8 and P386 DOCK8-STAT3.
- 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.

***DOCK8* and *STAT3* mutation databases**

https://databases.lovd.nl/shared/genes/DOCK8 and https://databases.lovd.nl/shared/genes/STAT3. 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 *DOCK8* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1a. SALSA MLPA Probemix P385-A3 DOCK8

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*DOCK8*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 19616-L26704	4p	
136	**DOCK8 probe** 16108-L18278		**Exon 22**
142	**DOCK8 probe** 16109-L18279		**Exon 44**
148	**DOCK8 probe** 16110-L18280		**Exon 35**
154	**DOCK8 probe** 16111-L18281		**Exon 11**
160	**DOCK8 probe** 16112-L18282		**Exon 5**
168 *	Reference probe 09267-L31310	10q	
172	**DOCK8 probe** 16113-L18283		**Exon 40**
178 Ж	**DOCK8 probe** 16114-SP0326-L18284		**Exon 24**
184	**DOCK8 probe** 16115-L18285		**Exon 30**
190	**DOCK8 probe** 13721-L15202		**Exon 25**
196 Ж	**DOCK8 probe** 16116-SP0327-L18286		**Exon 18**
202 *	Reference probe 15105-L16876	1q	
208	**DOCK8 probe** 16117-L18287		**Exon 36**
214	**DOCK8 probe** 16118-L18288		**Exon 14**
220	**DOCK8 probe** 16119-L18289		**Exon 48**
226	**DOCK8 probe** 16120-L18915		**Exon 1**
232 Ж	**DOCK8 probe** 16121-SP0328-L18291		**Exon 26**
238 *	Reference probe 19519-L26013	19p	
244	**DOCK8 probe** 16122-L28214		**Exon 15**
250	**DOCK8 probe** 16123-L18293		**Exon 7**
256	**DOCK8 probe** 16124-L18294		**Exon 39**
265	**DOCK8 probe** 16125-L18295		**Exon 10**
273 Ж	**DOCK8 probe** 16126-SP0329-L18296		**Exon 20**
283	Reference probe 06754-L06358	8q	
292	**DOCK8 probe** 16127-L18297		**Exon 12**
301	**DOCK8 probe** 16128-L18298		**Exon 34**
310	**DOCK8 probe** 16129-L18299		**Exon 8**
316	**DOCK8 probe** 20259-L15671		**Exon 21**
327	**DOCK8 probe** 16130-L18300		**Exon 3**
336 *	Reference probe 22062-L31024	13q	
346	**DOCK8 probe** 16131-L18301		**Exon 43**
353	**DOCK8 probe** 16132-L18302		**Exon 29**
364	**DOCK8 probe** 16133-L18303		**Exon 6**
373	**DOCK8 probe** 16134-L18304		**Exon 37**
382	**DOCK8 probe** 16135-L18305		**Exon 45**
392	Reference probe 13587-L15044	1q	
400	**DOCK8 probe** 16136-L18306		**Exon 16**
409	**DOCK8 probe** 16137-L18307		**Exon 2**
418	Reference probe 18479-L23656	6q	
427 Ж	**DOCK8 probe** 16139-SP0331-L18309		**Exon 33**
436 ¬ »	DMRT1 probe 13080-L14299		downstream
444	**DOCK8 probe** 20258-L18310		**Exon 4**
454	**DOCK8 probe** 16141-L18311		**Exon 41**
462	**DOCK8 probe** 16142-L18312		**Exon 27**
472	**DOCK8 probe** 16143-L18313		**Exon 1**
481 Ж	**DOCK8 probe** 20121-SP0330-L28347		**Exon 46**
490 *	Reference probe 21097-L29349	11p	

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

* New in version A3.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

¬ 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.

» Detects the same sequence as the *DMRT1* flanking probe in SALSA MLPA Probemix P386.

Table 1b. SALSA MLPA Probemix P386-A4 DOCK8-STAT3

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*DOCK8*	*STAT3*
64-105	Control fragments – see table in probemix content section for more information			
131 ¥	Reference probe 00797-L25925	5q		
136	**STAT3 probe** 16070-L18240			**Exon 9**
142	**DOCK8 probe** 16071-L18241		**Exon 17**	
148	**STAT3 probe** 16072-L18242			**Exon 21**
154	**STAT3 probe** 16073-L19096			**Exon 6**
160	**DOCK8 probe** 16074-L19103		**Exon 38**	
168 *	Reference probe 09267-L31310	10q		
173 Ж	**STAT3 probe** 16075-SP0319-L23086			**Exon 2**
178 Ж	**DOCK8 probe** 16076-SP0320-L19104		**Exon 23**	
184 Ж	**STAT3 probe** 16077-SP0321-L18247			**Exon 8**
190 Ж	**STAT3 probe** 16078-SP0322-L19097			**Exon 13**
196	**STAT3 probe** 16079-L18249			**Exon 1**
202 *	Reference probe 15105-L16876	1q		
208	**STAT3 probe** 16080-L28231			**Exon 20**
214	**DOCK8 probe** 16081-L18251		**Exon 42**	
220	**DOCK8 probe** 16082-L18252		**Exon 13**	
226	**STAT3 probe** 16083-L19098			**Exon 4**
234	**STAT3 probe** 16084-L28232			**Exon 24**
244 Ж	**STAT3 probe** 16085-SP0323-L18255			**Exon 14**
251	**DOCK8 probe** 16086-L28233		**Exon 2**	
256	**STAT3 probe** 16087-L19099			**Exon 12**
266 *	Reference probe 04483-L03872	1p		
274	**STAT3 probe** 16089-L18259			**Exon 18**
282	Reference probe 21259-L29867	15q		
292	**STAT3 probe** 16090-L18260			**Exon 5**
302	**STAT3 probe** 16091-L18261			**Exon 15**
310	**STAT3 probe** 16092-L19101			**Exon 10**
317	**DOCK8 probe** 21730-L31468		**Exon 31**	
328	**STAT3 probe** 16094-L18264			**Exon 19**
336 *	Reference probe 22062-L31024	13q		
344	**STAT3 probe** 16095-L18265			**Exon 7**
353 Ж	**DOCK8 probe** 16096-SP0324-L18266		**Exon 28**	
368	**STAT3 probe** 16097-L30968			**Exon 11**
376	**STAT3 probe** 16098-L30971			**Exon 3**
382	**DOCK8 probe** 16099-L18269		**Exon 1**	
391	Reference probe 16035-L18212	12p		
400	**DOCK8 probe** 16100-L18270		**Exon 9**	
409	**STAT3 probe** 16101-L18271			**Exon 16**
418	**DOCK8 probe** 16102-L18272		**Exon 19**	
427 Ж	**STAT3 probe** 16103-SP0325-L18273			**Exon 23**
436 ¬ »	DMRT1 probe 13080-L14299		downstream	
445	**DOCK8 probe** 16104-L18274		**Exon 47**	
459	**DOCK8 probe** 16105-L30969		**Exon 32**	
474	**STAT3 probe** 16106-L31552			**Exon 17**

484	**STAT3 probe** 16107-L30972			**Exon 22**
493 *	Reference probe 21097-L32726	11p		

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

* New in version A4.

¥ Changed in version A4. Minor alteration, no change in sequence detected.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

¬ 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.

» Detects the same sequence as the *DMRT1* flanking probe in SALSA MLPA Probemix P385.

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. P385-A3/P386-A4 probes arranged according to chromosomal location

Table 2a. *DOCK8* gene

Length (nt) P385 / P386	SALSA MLPA probe	*DOCK8* exon[a]	Ligation site NM_203447.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*113-115 (Exon 1)*		
382	16099-L18269	Exon 1	32 nt before exon 1	TTTCGGCTTAGA-AGGTGGAAATGC	0.1 kb
226	16120-L18915	Exon 1	91-92	CGACCCTAGAAG-CCACCGAACCGC	1.0 kb
472	16143-L18313	Exon 1	931 nt after exon 1	TGTTAATCTTCA-CTTAGCTGAGCC	55.7 kb
251	16086-L28233	Exon 2	210-211	TCTCCCACCAAA-CCTTGGCCAGTA	0.1 kb
409	16137-L18307	Exon 2	30 nt after exon 2	TTTACTTAGCGA-TTGGTCAAGTGC	14.9 kb
327	16130-L18300	Exon 3	431-432	TGCAGCCCTCTT-TGCCGGAGGAAG	2.9 kb
444	20258-L18310	Exon 4	486-487	TGTTCAGACCTA-CATCCGTGAGTG	15.1 kb
160	16112-L18282	Exon 5	599-600	CGAAACAGACGT-TTGAGTCGGAAA	7.4 kb
364	16133-L18303	Exon 6	762-763	CCTGCAGCAAGT-GAGTGCCGAGGA	5.0 kb
250	16123-L18293	Exon 7	885-886	TCCAGTACCAGA-ATGTCCCAAGGA	8.6 kb
310	16129-L18299	Exon 8	974-975	TGTTTGCCAGCA-TTGCCCTCTACG	2.5 kb
400	16100-L18270	Exon 9	11 nt after exon 9	GGTAATTCAGTA-CGATCTGATTTG	4.3 kb
265	16125-L18295	Exon 10	1192-1193	GAGATTGGAGAC-TGTGCAGAGCCC	1.9 kb
154	16111-L18281	Exon 11	1391-1392	ATGTGGACTCTG-TGGTTGGTAAGA	2.2 kb
292	16127-L18297	Exon 12	1427-1428	AACGGAGGACAT-TGGCCCAATCTA	2.5 kb
220	16082-L18252	Exon 13	1622-1623	GAGTCAAGTCAA-TTCCAGGTGTGA	1.1 kb
214	16118-L18288	Exon 14	1674-1675	GATCATCAATTG-CTGTCTGACTCC	27.9 kb
244	16122-L28214	Exon 15	1836-1837	CTTTGTAAACAA-ACTAGCATCAGC	2.2 kb
400	16136-L18306	Exon 16	1953-1954	TCTGCAGGAAGT-GTACACAGCTGT	1.2 kb
142	16071-L18241	Exon 17	2066-2067	CCTTCTACCATA-TCAGCTGTCAGC	0.7 kb
196 Ж	16116-SP0327-L18286	Exon 18	2144-2145; 2175-2176	TCTTAAATGAAC-31 nt spanning oligo-TGCCTTGGAAAA	4.0 kb
418	16102-L18272	Exon 19	2249-2250	ATCCTCCCATTA-AGTGGGCTGAAG	0.8 kb
273 Ж	16126-SP0329-L18296	Exon 20	2347-2348; 2374-2375	TTCTTCACCCTC-27 nt spanning oligo-TTCCCCATCCGC	2.8 kb
316	20259-L15671	Exon 21	2578-2579	TTTGCCTTCGAG-TCCGTGGTGGCC	2.8 kb
136	16108-L18278	Exon 22	2769-2770	GTATGGCCGCAC-ATCAGCTGCTGC	3.8 kb
178 Ж	16076-SP0320-L19104	Exon 23	2938-2939; 2968-2969	TGCTCTGGCAGT-30 nt spanning oligo-AGGCCAGCCAGC	4.2 kb
178 Ж	16114-SP0326-L18284	Exon 24	3065-3066; 19 nt after exon 24	AGTATGCCTGGT-36 nt spanning oligo-GTATCTGTGCTC	6.3 kb
190	13721-L15202	Exon 25	3162-3163	TGACCGTTTCAT-GGATGACATAAC	2.3 kb
232 Ж	16121-SP0328-L18291	Exon 26	3258-3259; 3288-3289	GGAAAAGATGAA-30 nt spanning oligo-TCTCTCCCTCAT	5.8 kb

Length (nt) P385 / P386	SALSA MLPA probe	*DOCK8* exon[a]	Ligation site NM_203447.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
462	16142-L18312	Exon 27	3369-3370	GCTCAGTAACCT-TCCAACGCTCAT	2.1 kb
353 Ж	16096-SP0324-L18266	Exon 28	3607-3608; 3634-3635	CTCCTCTTCACA-27 nt spanning oligo-GGGGAAGGGTAT	7.8 kb
353	16132-L18302	Exon 29	3722-3723	CACGCTGTGTCA-AACCAGAGGTGA	3.3 kb
184	16115-L18285	Exon 30	3856-3857	TCGGATGAAGAA-CAAGAAGGAGCC	2.3 kb
317	21730-L31468	Exon 31	3988-3989	AACGCGGACACT-ACTCGCAACCTC	0.6 kb
459	16105-L30969	Exon 32	4190-4191	AGTCAAGGGATG-TCAAGGCCCGGC	1.1 kb
427 Ж	16139-SP0331-L18309	Exon 33	4308-4309; 4335-4336	TTTGAGATGGAA-27 nt spanning oligo-TAATGAGAAGCT	4.8 kb
301	16128-L18298	Exon 34	4393-4394	TTGATCAGTGGC-AATCTGGCTACA	1.5 kb
148	16110-L18280	Exon 35	4535-4536	GTGATCAGAGTA-CCACCTACCTGA	1.3 kb
208	16117-L18287	Exon 36	4642-4643	CTATGTCACCAA-GTCCTGCACCAC	2.5 kb
373	16134-L18304	Exon 37	4807-4808	GCACCAGACTTT-AATGAAGAGCAC	1.7 kb
160	16074-L19103	Exon 38	4924-4925	TGTAATCTGAAT-AGCATCTTATAT	0.8 kb
256	16124-L18294	Exon 39	37 nt before exon 39	TCATTAACCCAC-TGTCCTCAAAAC	4.5 kb
172	16113-L18283	Exon 40	23 nt before exon 40	CCTGTTCTCCAG-GCTTATACTGTG	2.1 kb
454	16141-L18311	Exon 41	5412-5413	AGAATTCCGGAA-GCTGACACTCAC	0.5 kb
214	16081-L18251	Exon 42	5488-5489	AGAATGTTTGGA-ACCTACTTCCGA	1.6 kb
346	16131-L18301	Exon 43	5647-5648	GTGGAAGTGATT-AAAGACTCCACT	3.1 kb
142	16109-L18279	Exon 44	5870-5871	ACACAGTCCTGA-CCACTATGCACG	3.4 kb
382	16135-L18305	Exon 45	6073-intron 45	ACTGTAAATCAG-GTAAGCAAAACC	2.1 kb
481 Ж	20121-SP0330-L28347	Exon 46	6094-6095; 6124-6125	GAAGTAGCCCAA-30 nt spanning oligo-AAACTCTATCGA	11.5 kb
445	16104-L18274	Exon 47	6237-6238	CCAGAGGGAATA-TCAGCAGGAACT	0.6 kb
220	16119-L18289	Exon 48	6381-6382	TAGTTTCAGGAA-ATGTGAAACCCA	377.7 kb
		stop codon	*6410-6412 (Exon 48)*		
436 ¬ 436 ¬	13080-L14299	*DMRT1* gene		AGGCATTCAGCA-AGCCCTCTACAC	

Table 2b. *STAT3* gene

Length (nt) P386	SALSA MLPA probe	*STAT3* exon[a]	Ligation site NM_139276.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*188-190 (Exon 2)*		
196	16079-L18249	Exon 1	94 nt after exon 1	GGGATGTTGTGA-TGGACGCTGCAG	39.7 kb
173 Ж	16075-SP0319-L23086	Exon 2	217-218; 254-255	CTACAGCAGCTT-37 nt spanning oligo-GTGACAGCTTCC	1.8 kb
376	16098-L30971	Exon 3	388-389	ATTGACCAGCAG-TATAGCCGCTTC	1.1 kb
226	16083-L19098	Exon 4	534-535	ATCACGCCTTCT-ACAGACTGCAGC	6.3 kb
292	16090-L18260	Exon 5	645-646	GGATGTCCGGAA-GAGAGTGCAGGT	0.6 kb
154	16073-L19096	Exon 6	699-700	TCTCCAGGATGA-CTTTGATTTCAA	0.9 kb
344	16095-L18265	Exon 7	767-768	GAAACAACCAGT-CAGTGACCAGGC	0.3 kb
184 Ж	16077-SP0321-L18247	Exon 8	833-834; 860-861	TTTCCAACCAGA-27 nt spanning oligo-TGTCAGCGATGG	3.5 kb
136	16070-L18240	Exon 9	1011-1012	AGAATCTCAACT-TCAGACCCGTCA	0.3 kb
310	16092-L19101	Exon 10	1230-1231	GTTCACTACTAA-AGTCAGGTAGGC	2.0 kb
368	16097-L30968	Exon 11	123 nt before exon 11	TGTGAGCCTGTA-ATTATAGACAGC	1.9 kb
256	16087-L19099	Exon 12	5 nt after exon 12 *reverse*	CTCTAGGCTGAA-CTTACCCTCTGA	0.2 kb
190 Ж	16078-SP0322-L19097	Exon 13	1378-1379; 1405-1406	ATGAACATGGAA-27 nt spanning oligo-GAATTCAAACAC	0.1 kb
244 Ж	16085-SP0323-L18255	Exon 14	13 nt before exon 14; 1437-1438	CACCTGCCTTTT-30 nt spanning oligo-ATGTGGGAATGG	3.2 kb
302	16091-L18261	Exon 15	1469-1470	CGTTACTGTAGG-CTTCCCTGATTG	1.2 kb
409	16101-L18271	Exon 16	1595-1596	ACATCTGTCAGA-TGCCAAATGCCT	0.2 kb

474	16106-L31552	Exon 17	31 nt before exon 17	CACCATCCCTCA-TCTAAACAAGCA	1.3 kb
274	16089-L18259	Exon 18	36 nt after exon 18	TGGGAATGGGAA-TGCTCACCTGCA	0.1 kb
328	16094-L18264	Exon 19	81 nt before exon 19	CGGGCCTGAGGA-TTTGGGTGATGC	0.3 kb
208	16080-L28231	Exon 20	22 nt before exon 20	CAATAACAACAT-TGTTCCTCCTCC	0.8 kb
148	16072-L18242	Exon 21	2174-2175	TCATGGATGCTA-CCAATATCCTGG	5.2 kb
484	16107-L30972	Exon 22	2313-2314	CCTGAAGACCAA-GTTTATCTGTGT	0.4 kb
427 Ж	16103-SP0325-L18273	Exon 23	2400-2401; 2430-2431	GATGCAGTTTGG-30 nt spanning oligo-AGGAGGGCAGTT	1.0 kb
234	16084-L28232	Exon 24	2471-2472	ACATGGAGTTGA-CCTCGGAGTGCG	
		stop codon	*2498-2500 (Exon 24)*		

[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.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ 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.

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.

Selected publications using SALSA MLPA Probemix P385 DOCK8 & P386 DOCK8-STAT3

- Capkova Z et al. (2021). Duplication of 9p24. 3 in three unrelated patients and their phenotypes, considering affected genes, and similar recurrent variants. *Mol Genet Genomic Med*. 9:e1592.
- Seo E et al. (2021). Hematopoietic stem cell transplantation in an infant with dedicator of cytokinesis 8 (DOCK8) deficiency associated with systemic lupus erythematosus: A case report. *Medicine (Baltimore)*. 100.
- Suspitsin E et al. (2020). Next generation sequencing analysis of consecutive Russian patients with clinical suspicion of inborn errors of immunity. *Clin Genet*. 98:231-239.
- Tóth B et al. (2013). Novel dedicator of cytokinesis 8 mutations identified by multiplex ligation-dependent probe amplification. *Eur J Haematol*. 91:369-375.
- Tsuge I et al. (2014). Acute eosinophilic pneumonia occurring in a dedicator of cytokinesis 8 (DOCK8) deficient patient. *Pediatr Pulmonol*. 49:E52-E55.

P385 product history	
Version	*Modification*
A3	Five reference probes have been replaced.
A2	Control fragments have been adjusted (QDX2).
A1	First release.

P386 product history	
Version	*Modification*
A4	Five reference probes have been replaced and one probe length has been adjusted.
A3	One reference probe has been removed and six reference probes have been replaced. In addition, several probe lengths have been adjusted.
A2	Control fragments have been adjusted (QDX2).
A1	First release.

Implemented changes in the product description
Version A3/A4-01 – 12 October 2021 (04P) - 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 DOCK8 and STAT3 genes updated according to new versions of the NM_ reference sequences. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. Version A2/A3-02 – 19 September 2019 (01P) - Correcting information regarding mode of inheritance, it was stated that AR-HIES is caused by STAT3 and AD-HIES by DOCK8. This is corrected to AR-HIES caused by DOCK8 and AD-HIES by STAT3. Version A2/A3-01 – 01 February 2019 (01P) - Product description restructured and adapted to a new template. - Product description adapted to a new product version of P386. - Small changes in Table 1 and Table 2.

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