

Product Description

SALSA® MLPA® Probemix P049-C2 SLC6A8-ABCD1

To be used with the MLPA General Protocol.

Version C2

As compared to version C1, one reference probe has been replaced. For complete product history see page 8.

Catalogue numbers:

- **P049-025R:** SALSA MLPA Probemix P049 SLC6A8-ABCD1, 25 reactions.
- **P049-050R:** SALSA MLPA Probemix P049 SLC6A8-ABCD1, 50 reactions.
- **P049-100R:** SALSA MLPA Probemix P049 SLC6A8-ABCD1, 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 P049 SLC6A8-ABCD1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SLC6A8* gene, the *ABCD1* gene and the Xq28 chromosomal region.

Defects in the *SLC6A8* gene, encoding the X-linked creatine transporter, are a relatively frequent cause of intellectual disability in males. Several other genes located at a short distance from *SLC6A8* have also been implicated in neurological syndromes, including *L1CAM* (X-linked hydrocephalus, this probemix), *MECP2* (RETT syndrome; P015 probemix), *FLNA* (lissencephaly; P061 probemix) and *ABCD1* (X-linked adrenoleukodystrophy; this probemix).

Defects in the *ABCD1* gene result in adrenoleukodystrophy due to defective peroxisomal beta oxidation and the accumulation of the saturated very long chain fatty acids (VLCFA) in all tissues of the body. The manifestations of the disorder occur primarily in the adrenal cortex, in the myelin of the central nervous system, and in the Leydig cells of the testes.

More information is available at <https://www.ncbi.nlm.nih.gov/books/NBK3794/> and <https://www.ncbi.nlm.nih.gov/books/NBK1315/>.

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 *SLC6A8* exon numbering used in this P049-C2 SLC6A8-ABCD1 product description is the exon numbering from the NG_012016.2 sequence. The *ABCD1* exon numbering used in this P049-C2 SLC6A8-ABCD1 product description is the exon numbering from the LRG_1017 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 or 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 P049-C2 SLC6A8-ABCD1 contains 43 MLPA probes with amplification products between 130 and 472 nucleotides (nt). This includes nine probes for the *SLC6A8* gene, nine probes for the *ABCD1* gene (one for each exon except exon 9) and 16 more probes for the Xq28 chromosomal region. In addition, nine reference probes are included that detect locations on the X-chromosome. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains ten 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 two chromosome Y-specific fragments (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)
105-121	Y-fragments (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 of the same sex 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 mental retardation, X-linked hydrocephalus, and/or adrenoleukodystrophy. 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 (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:

Copy Number status: Male samples	Final ratio
Normal	$0.80 < FR < 1.20$
Deletion	$FR = 0$
Duplication	$1.65 < FR < 2.25$
Ambiguous copy number	All other values

Copy Number status: Female samples	Final ratio
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 or in or near the *SLC6A8*, *PNCK* or *GDI1* genes. 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 aforementioned genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P049 SLC6A8-ABCD1.
- 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.

SLC6A8 and ABCD1 mutation databases

<https://databases.lovd.nl/shared/genes/SLC6A8> and <https://databases.lovd.nl/shared/genes/ABCD1>. 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 *ABCD1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P049-C2 SLC6A8-ABCD1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^a			
		Reference	SLC6A8	ABCD1	Xq28
64-121	Control fragments – see table in probemix content section for more information				
130	Reference probe 13499-L02104	Xp			
137 «	GDI1 probe 16875-L19669				Xq28
142	Reference probe 05861-L08942	Xq			
148	BCAP31 probe 01882-L05470				Xq28
154 «	PNCK probe 04285-L03677				Xq28
160 «	SLC6A8 probe 01869-L01438		Exon 1		
166 «	SLC6A8 probe 01879-L01448		Exon 11		
172	Reference probe 07664-L07370	Xp			
178	BCAP31 probe 03488-L14008				Xq28
184	Reference probe 13928-L15467	Xq			
190	FLNA probe 04135-L03492				Xq28
196	L1CAM probe 07048-L06657				Xq28
202 «	SLC6A8 probe 01871-L01440		Exon 3		
208	ABCD1 probe 11183-L11868			Exon 1	
214	ABCD1 probe 11185-L11870			Exon 3	
222 ± «	SLC6A8 probe 01881-L05472		Exon 13		
229	L1CAM probe 07050-L08340				Xq28
237	L1CAM probe 07051-L06660				Xq28
244	ABCD1 probe 11184-L13913			Exon 2	
250	Reference probe 01894-L01054	Xp			
257	FLNA probe 04136-L03493				Xq28
265	ABCD1 probe 17304-L20813			Exon 6	
278	MECP2 probe 01768-L21151				Xq28
292 «	SLC6A8 probe 02113-L21152		Exon 5		
301 «	SLC6A8 probe 01876-L01445		Exon 8		
310	ABCD1 probe 03493-L02870			Exon 10	
319	L1CAM probe 00819-L00337				Xq28
328 «	SLC6A8 probe 01875-L01444		Exon 7		
346	Reference probe 05125-L04515	Xq			
355	MECP2 probe 01348-L00895				Xq28
364 «	SLC6A8 probe 01877-L01446		Exon 9		
371	ABCD1 probe 11186-L14009			Exon 4	
378	ABCD1 probe 11189-L13912			Exon 8	
387	IDH3G probe 01887-L21148				Xq28
393	FLNA probe 17262-L21149				Xq28
402 ± «	SLC6A8 probe 01878-L01447		Exon 10		
409	Reference probe 00820-L00338	Xq			
427	ABCD1 probe 03491-L02868			Exon 5	
436 +	ABCD1 probe 11188-L11873			Exon 7	
445 «	PNCK probe 04902-L04286				Xq28
454 «	PNCK probe 04903-L08341				Xq28
463	Reference probe 07875-L07689	Xp			
472	Reference probe 02915-L02309	Xq			

^a See section Exon numbering on page 1 for more information.

± SNP rs4065272 (01881-L05472) and SNP rs143916832 (01878-L01447) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

« 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 probe has been found to generate a small background signal. It resulted in a background signal of approximately 10-20% in a male sample with an exon 6-10 *ABCD1* deletion that was tested at MRC-Holland.

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

Table 2. Xq28 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene exon ^a	Ligation site	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
154 «	04285-L03677	PNCK Exon 13		GCCAAGTGGACT-GACCCCCAGATT	2.0 kb
454 «	04903-L08341	PNCK Exon 5		ATGGAAGTGTGA-GGAGGGCCTGGG	0.9 kb
445 «	04902-L04286	PNCK Exon 3		AGAAACACACGG-AGGACATCAGCA	15.6 kb
		SLC6A8	NM_005629.4		
		start codon	650-652 (Exon 1)		
160 « #	01869-L01438	Exon 1	698-699	TGTCCGGCGACG-AGAAGAAGGGCC	2.9 kb
202 « #	01871-L01440	Exon 3	1224-1225	AGACTGTGCCAA-TGCCAGCCTGGC	1.6 kb
292 « #	02113-L21152	Exon 5	1510-1511	GGCGCCTGGAT-GGCATCATTAC	0.4 kb
328 « #	01875-L01444	Exon 7	1763-1764	CAGAGCAGGGCG-TGCACATCTCCA	0.4 kb
301 « #	01876-L01445	Exon 8	1819-1820	ATCGCCTACCCG-CGGGCTGTACG	0.2 kb
364 « #	01877-L01446	Exon 9	1940-1941	CCGGCCTCCTCG-ACCTCCTCCCGG	0.3 kb
402 ± « #	01878-L01447	Exon 10	2143-2144	GCCTGGGTGTAC-GGTAGGTCATGG	0.2 kb
166 « #	01879-L01448	Exon 11	2239-2240	ACCCCGCTGGTC-TGCATGGTAAGG	0.6 kb
222 ± « #	01881-L05472	Exon 13	2555-2556	AGAGTGTGTCATGT-GACAACTCAGCT	20.4 kb
		stop codon	2555-2557 (Exon 13)		
178	03488-L14008	BCAP31 Exon 4		CTTCCACATGAA-GCTTTTCCGTGC	7.6 kb
148	01882-L05470	BCAP31 Exon 2		CAGTTGCCACCT-TCCTCTATGCGG	2.3 kb
		ABCD1	NM_000033.4		
		start codon	412-414 (Exon 1)		
208	11183-L11868	Exon 1	622-623	TGAACCGGGTAT-TCCTGCAGCGGC	3.8 kb
244	11184-L13913	Exon 2	1377-1378	CTCATCCTTCTG-GAAGCCTGTGG	6.9 kb
214	11185-L11870	Exon 3	1545-1546	GAGGAGCTGGTG-AGCGAGCGCACA	0.3 kb
371	11186-L14009	Exon 4	1732-1733	CCAGGGAGCTAG-AGGACGCTCAGG	0.7 kb
427	03491-L02868	Exon 5	1830-1831	GTGGAACAGGGG-ATCATCTGCGAG	3.1 kb
265	17304-L20813	Exon 6	2 nt after exon 6	TCCCGCAGAGGT-AAGGAAGCCCGT	0.5 kb
436 + #	11188-L11873	Exon 7	2155-2156	CCATCCTGGACG-TCGTGCACCTGC	2.3 kb
378 #	11189-L13912	Exon 8	2227-2226 reverse	CTCGCCACCCGA-CAGGACGTCCTT	0.5 kb
	No probe	Exon 9			
310 #	03493-L02870	Exon 10	2406-2407	CTGGCCAGGAAA-TACCACACACAC	50.9 kb
		stop codon	2647-2649 (Exon 10)		
387	01887-L21148	IDH3G Exon 1		TCCCCGAAACTT-CGCACCCCGTCG	70.3 kb
237	07051-L06660	L1CAM Exon 23		CAGCGGGTGAAA-ACTACAGTGTGCG	2.1 kb
229	07050-L08340	L1CAM Exon 18		TATGAGATCAAA-GTCCAGGCCGTC	5.5 kb
319	00819-L00337	L1CAM Exon 4		AACAGCAACTTT-GCTCAGAGGTTTC	3.6 kb
196	07048-L06657	L1CAM Exon 1		CTCTCCTCCTCT-GCAGCCCTGCC	154.6 kb
278	01768-L21151	MECP2 Exon 3 (4)		TTTCATCCTCCA-TGCCAAGGCCAA	2.0 kb
355	01348-L00895	MECP2 Exon 2 (3)		GCCCACCACTCT-GCTGAGCCCGCA	283.5 kb
393	17262-L21149	FLNA Exon 38		GCCTGCAGAGTT-TATCATTGATAC	12.2 kb
257	04136-L03493	FLNA Exon 11		GGCTTCGAGTAT-TACCCCATGGTC	2.5 kb
190	04135-L03492	FLNA Exon 4		AGCAAGCCCGTT-ACCAATGC CGCA	69.6 kb
137 «	16875-L19669	GDI1 Exon 1		CCTGACCATGGA-CGAGGAATACGA	

The exon numbering used in previous versions of this product description can be found in between brackets.

^a See section Exon numbering on page 1 for more information.

^b Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

± SNP rs4065272 (01881-L05472) and SNP rs143916832 (01878-L01447) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

« 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 probe has been found to generate a small background signal. It resulted in a background signal of approximately 10-20% in a male sample with an exon 6-10 *ABCD1* deletion that was tested at MRC-Holland.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

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

Related products

For related products, see the [product page](#) on our website.

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 P049 SLC6A8-ABCD1

- Cervera-Acedo C et al. (2015). A novel SLC6A8 mutation associated with motor dysfunction in a child exhibiting creatine transporter deficiency. *Hum Genome Var.* 2:15037.
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- Horn MA et al. (2016). Mild phenotype in an adult male with X-linked adrenoleukodystrophy—case report. *Clin Case Rep.* 4:177-181.
- Van de Kamp JM et al. (2013). Phenotype and genotype in 101 males with X-linked creatine transporter deficiency. *J Med Genet.* jmedgenet-2013.
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- Nicholl J et al (2013). Epilepsy with cognitive deficit and autism spectrum disorders: prospective diagnosis by array CGH. *Am J Med Genet B Neuropsychiatr Genet.* 162:24-35.
- Reardon W et al. (2010). Progressive cerebellar degenerative changes in the severe mental retardation syndrome caused by duplication of MECP2 and adjacent loci on Xq28. *Eur J Pediatr.* 169:941-949.
- Salsano E et al. (2012). Preferential expression of mutant ABCD1 allele is common in adrenoleukodystrophy female carriers but unrelated to clinical symptoms. *Orphanet J Rare Dis.* 7:10.

P049 product history	
Version	Modification
C2	One reference probe has been replaced.
C1	One probe for <i>GDI1</i> and one probe for <i>FLNA</i> have been replaced and one probe for exon 6 of <i>ABCD1</i> has been added. Two reference probes have been replaced and three have been removed.
B2	The 88, 96 and 118 nt control fragments have been replaced (QDX2).
B1	One less reliable <i>SLC6A8</i> probe has been removed, six new <i>ABCD1</i> probes have been added, and several probes telomeric of <i>ABCD1</i> have been removed. Four extra control fragments at 88-96-100-105 nt have been included.
A1	First release.

Implemented changes in the product description
Version C2-04 – 10 September 2025 (04P) - Removed Related SALSA MLPA products section. - Exon numbering of the 278 nt and 355 nt MECP2 probes is adapted to the MANE project and is based on a MANE Select transcript. Version C2-03 – 21 September (04P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the <i>SLC6A8</i> and <i>ABCD1</i> genes updated according to new versions of the NM_ reference sequences. - Remark below Table 1 and 2 about SNPs that could influence the probe signal updated. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. Version C2-02 – 06 June 2018 (01P) - Warning added to Table 2 for probe detecting <i>ABCD1</i> exon 7 (436 nt). Version C2-01 – 30 April 2018 (01P) - Product description restructured and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Various minor textual or layout changes. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

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