Product Description
SALSA® MLPA® Probemix P091-D2 CFTR

To be used with the MLPA General Protocol.

Version D2
For complete product history see page 10.

Catalogue numbers:
- **P091-025R:** SALSA MLPA Probemix P091 CFTR, 25 reactions.
- **P091-050R:** SALSA MLPA Probemix P091 CFTR, 50 reactions.
- **P091-100R:** SALSA MLPA Probemix P091 CFTR, 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.

Intended purpose
The SALSA MLPA Probemix P091 CFTR is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the *CFTR* gene and the wild type allele of the *CFTR* p.Phe508del and p.Ile507del mutations in genomic DNA isolated from human peripheral whole blood specimens. P091 CFTR is intended to confirm a potential cause for and clinical diagnosis of cystic fibrosis or congenital absence of the vas deferens, and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P091 CFTR should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *CFTR* gene are point mutations, which will not be detected by MLPA, with the exception of the two aforementioned mutations. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1]Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).
[2]To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background
Cystic fibrosis is a childhood-onset, multisystem disorder that affects epithelia of the respiratory tract, exocrine pancreas, intestine, hepatobiliary system, exocrine sweat glands and reproductive organs. The major clinical characteristics of cystic fibrosis are progressive obstructive pulmonary disease, pancreatic insufficiency, failure to thrive, meconium ileus, elevated sweat chloride levels and male infertility due to

congenital absence of the vas deferens (CAVD). There is, however, a high variability in cystic fibrosis phenotypes, which range from severe forms affecting multiple organ systems (i.e. classical phenotype) to mild disease manifestations affecting a single organ (e.g. only CAVD).

Cystic fibrosis is the most common life-shortening autosomal recessive disorder in individuals of northern European (Caucasian) background, with a disease incidence of 1:3,200 live births and a carrier frequency of 1:28. The disease frequency is much lower in other ethnic populations, e.g. 1:15,000 in African Americans and 1:31,000 in Asian Americans. Cystic fibrosis and CAVD are both caused by homozygous or compound heterozygous mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*), which encodes a cAMP-regulated chloride channel expressed at the apical membrane of exocrine/secretory epithelial cells (Kerem et al. 1989; Riordan et al. 1989; Rommens et al. 1989). The defective protein impairs ion transport and water movement across epithelia, which among other things leads to an increased salt concentration in sweat and the formation of viscous mucus obstructing the airways of the lungs and ducts of the pancreas.

The most common *CFTR* variants are point mutations and small insertions/deletions, which are detected in 97-98% of the probands with cystic fibrosis and 79% of the probands with CAVD (Yu et al. 2012). The p.Phe508del (F508del; c.1521_1523del) mutation is the variant most commonly found in the general population, affecting ~66% of the *CFTR* alleles worldwide (Bobadilla et al. 2002). Other mutations have a much lower frequency, e.g. the p.Ile507del (I507del; c.1519_1521del) mutation constitutes ~0.5-1.3% of all *CFTR* mutations identified (Bobadilla et al. 2002). Large deletions and duplications are found in <3% of patients with cystic fibrosis and CAVD. The frequency and spectrum of *CFTR* mutations vary widely among different populations and depend upon the geographical and ethnic origin of patients (Estivill et al. 1997). Some mutations tend to be seen more frequently in specific populations. For example, a deletion of *CFTR* exon 2 and 3 (CFTRdele2,3 (21 kb)) is rather common among patients of German or Eastern European origin (Dörk et al. 2000). More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1250/.

Gene structure
The *CFTR* gene spans ~189 kilobases (kb) on chromosome 7q31.2 and contains 27 exons. The *CFTR* LRG_663 is available at www.lrg-sequence.org and is identical to GenBank NG_016465.4.

Transcript variants
For *CFTR,* one transcript variant has been described encoding the full length protein (NM_000492.4; 6070 nt; coding sequence 71-4513; https://www.ncbi.nlm.nih.gov/gene/1080). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 1 and the stop codon is located in exon 27.

Exon numbering
The *CFTR* exon numbering used in this P091-D2 CFTR product description is the exon numbering from the LRG_663 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 P091-D2 CFTR contains 44 MLPA probes with amplification products between 130 and 481 nucleotides (nt). This includes 32 probes for the *CFTR* gene, one flanking probe for the *ASZ1* gene and one flanking probe for the *CTTNBP2* gene. Each exon of *CFTR* is covered by at least one probe. The ligation site of the exon 10 probe is located in intron 9. Exons 1, 11, 13 and 27 are covered by two probes and one probe for the upstream region of *CFTR* is present. One of the probes for exon 11 detects the wild type allele of the p.Phe508del and p.Ile507del mutations. A reduced signal for this probe can indicate the presence of the p.Phe508del mutation, the p.Ile507del mutation or a partial deletion of exon 11. In addition, 10 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 from human peripheral whole blood specimens, 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 cystic fibrosis or congenital absence of the vas deferens. 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 described in the table below have been tested with this P091-D2 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 ID number	Genotype	Probes affected		Expected final ratio
		Length (nt)	Target	
NA01531	Homozygous p.Phe508del	330	Exon 11; p.Phe508del WT	0
NA18668	Compound heterozygous deletion exons 2-3 & p.Phe508del	199	Exon 2	~0.5
		220	Exon 3	~0.5
		330	Exon 11; p.Phe508del WT	~0.5
NA11277	Heterozygous p.Ile507del	330	Exon 11; p.Phe508del WT	~0.5

Sample ID number	Genotype	Probes affected		Expected final ratio
		Length (nt)	Target	
HG02461	Heterozygous deletion exons 19-21	256	Exon 19	~0.5
		283	Exon 20	~0.5
		310	Exon 21	~0.5
HG04131	Heterozygous deletion exons 4-11	247	Exon 4	~0.5
		346	Exon 5	~0.5
		274	Exon 6	~0.5
		301	Exon 7	~0.5
		337	Exon 8	~0.5
		400	Exon 9	~0.5
		391	Exon 10	~0.5
		465	Exon 11	~0.5
		330	Exon 11; p.Phe508del WT	~0.5
HG01565	Heterozygous deletion *CFTR* gene	All CFTR probes (excluding flanking probes)		~0.5
NA01059	Heterozygous deletion 7q22.1-7q31.33 (1 copy of *CFTR*)*	All target probes (including flanking probes)		~0.5
NA12519	Heterozygous triplication 7q31.1-7q36.2 (4 copies of *CFTR*)*	All target probes (including flanking probes)		~2.0

* The whole extent of the CNV present in this cell line cannot be determined by the P091-D2 CFTR probemix.

Performance characteristics

Deletions or duplications in the *CFTR* gene are found in <3% of patients with cystic fibrosis and CAVD (https://www.ncbi.nlm.nih.gov/books/NBK1250/; Lucarelli et al. 2017; Paracchini et al. 2008; Schneider et al. 2007; Terzic et al. 2019). The p.Phe508del mutation accounts for ~66% of all *CFTR* mutations identified worldwide (Bobadilla et al. 2002). The incidence of this variant varies between 30-80% among different ethnic groups; p.Phe508del is most common among people from northwest European descent and less common in people from southeast European descent and in other populations (Estivill et al. 1997; Morral et al. 1994). The p.Ile507del mutation constitutes ~0.5-1.3% of all *CFTR* mutations identified (Bobadilla et al. 2002). The analytical sensitivity and specificity for the detection of deletions or duplications in the *CFTR* gene and the wild type allele of the *CFTR* p.Phe508del and p.Ile507del mutations is very high and can be considered >99% (based on a 2006-2022 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

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 expected results for *CFTR* region specific MLPA probes are allele copy number of 2 (normal), 0 (homozygous deletion), 1 (heterozygous deletion), 3 (heterozygous duplication) and occasionally 4 (homozygous duplication or heterozygous triplication). 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 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.

P091 specific notes:

- Deletion or duplication of the flanking genes *ASZ1* and *CTTNPB2* are not expected to be the cause of cystic fibrosis or CAVD. These probes have only been included to delineate the extent of large deletions and duplications.
- The probe at 330 nt detects the wild type allele of the p.Phe508del and p.Ile507del mutations. A final ratio of ~0.5 can be caused by a heterozygous mutation of either p.Phe508del or p.Ile507del or a heterozygous (partial) deletion of exon 11. A final ratio of 0 can correspond to a homozygous mutation of either

p.Phe508del or p.Ile507del, a homozygous (partial) deletion of exon 11, or compound heterozygosity for the mutations or for a mutation and a deletion.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *CFTR* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P091 CFTR.
- SALSA MLPA Probemix P091 CFTR cannot discriminate between p.Phe508del, p.Ile507del and a partial deletion of *CFTR* exon 11, as all three mutations will lead to an equal reduction in signal of the 330 nt probe.
- 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 the 330 nt *CFTR* exon 11 probe can be the result of the p.Phe508del mutation, the p.Ile507del mutation, or a partial deletion of exon 11. These results need to be confirmed with sequence analysis.

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.

Cystic fibrosis mutation database

http://www.genet.sickkids.on.ca/. We strongly encourage users to deposit positive results in the Cystic Fibrosis Mutation 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 *CFTR* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P091-D2 CFTR

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*CFTR*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q	
136 ¬	ASZ1 probe 03571-L03264		58 kb before *CFTR*
142	**CFTR probe** 02956-L13079		**Exon 14**
148	**CFTR probe** 03842-L03315		**Exon 26**
154	**CFTR probe** 02944-L02376		**Exon 1**
160 ±	**CFTR probe** 02957-L02389		**Exon 15**
167	Reference probe 16255-L19128	13q	
173	**CFTR probe** 03578-L02939		**Exon 27**
178	**CFTR probe** 02958-L02390		**Exon 16**
184	Reference probe 14817-L16525	1p	
190	**CFTR probe** 03574-L02400		**Exon 27**
199	**CFTR probe** 02946-L13077		**Exon 2**
206	**CFTR probe** 02959-L13651		**Exon 17**
214	Reference probe 03791-L05919	21q	
220	**CFTR probe** 02947-L02379		**Exon 3**
226 Δ	**CFTR probe** 18196-L22807		**Exon 13**
238	**CFTR probe** 03839-L03312		**Exon 1**
247	**CFTR probe** 02948-L02380		**Exon 4**
256	**CFTR probe** 02961-L02393		**Exon 19**
265	Reference probe 02318-L01809	19p	
274	**CFTR probe** 13916-L15981		**Exon 6**
283	**CFTR probe** 02962-L02394		**Exon 20**
292	**CFTR probe** 03841-L03314		**Exon 13**
301	**CFTR probe** 13627-L15081		**Exon 7**
310	**CFTR probe** 03576-L13076		**Exon 21**
319	Reference probe 17454-L21210	12p	
330 ∞	**CFTR probe** 03322-L14978		**Exon 11; p.Phe508del WT**
337	**CFTR probe** 02951-L13653		**Exon 8**
346	**CFTR probe** 03840-L03313		**Exon 5**
355 ±	**CFTR probe** 13629-L15083		**Exon 22**
364	**CFTR probe** 18197-L22808		**Exon 18**
373	**CFTR probe** 12113-L23339		**Upstream**
382	**CFTR probe** 02965-L02397		**Exon 23**
391 +	**CFTR probe** 02953-L30560		**Exon 10**
400	**CFTR probe** 18198-L22809		**Exon 9**
409	**CFTR probe** 02966-L02398		**Exon 24**
418	**CFTR probe** 02955-L02387		**Exon 12**
427	Reference probe 13981-L15550	17q	
436	**CFTR probe** 02967-L02399		**Exon 25**
445 ¬	CTTNBP2 probe 03572-L03267		58 kb after *CFTR*
454	Reference probe 00605-L00018	15q	
465	**CFTR probe** 02954-L23460		**Exon 11**
474	Reference probe 02757-L02206	11p	
481	Reference probe 16290-L18582	20q	

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

∞ Wild type sequence detected. A lowered probe signal can be due to the p.Phe508del mutation, the p.Ile507del mutation, or a partial deletion of exon 11. The p.Phe508Cys mutation (F508C; c.1523T>G; rs74571530) is not expected to influence the signal of this probe. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

± The p.Trp846X mutation (W846X; rs397508393 or rs267606722) could influence the signal of the 160 nt probe. The c.3528delC mutation (3659delC; rs78984783) could influence the signal of the 355 nt probe. In case of apparent deletions, it is recommended to sequence the region targeted by these probes.
¬ 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.
+ The ligation site of this probe is located in intron 9.

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. *CFTR* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*CFTR* exon[a]	Ligation site NM_000492.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
136 ¬	03571-L03264	*ASZ1* gene		TATGCTGCTAGT-GTTGCCAATGCA	57.1 kb
		start codon	*71-73 (Exon 1)*		
373	12113-L23339	Upstream of *CFTR*	676 nt before exon 1; 746 nt before ATG start codon	AGCGCTAAGGTA-AATGCATCAGAC	0.5 kb
154	02944-L02376	Exon 1	164 nt before exon 1	GCTAGAGCAAAT-TTGGGGCCGGAC	0.1 kb
238	03839-L03312	Exon 1	18 nt before exon 1	AGAAAAAGGGTT-GAGCGGCAGGCA	24.3 kb
199	02946-L13077	Exon 2	180-179 reverse	GGATTTGGTATA-TGTCTGACAATT	4.8 kb
220	02947-L02379	Exon 3	263-264	TGGCTTCAAAGA-AAAATCCTAAAC	21.9 kb
247	02948-L02380	Exon 4	387-388	GGGAAGAATCAT-AGCTTCCTATGA	3.4 kb
346	03840-L03313	Exon 5	598-599	GATAAAATAAGT-ATTGGACAACTT	1.0 kb
274	13916-L15981	Exon 6	726-727	CTGGGAGTTGTT-ACAGGCGTCTGC	1.3 kb
301	13627-L15081	Exon 7	886-885 reverse	CAGTATGCCTTA-ACAGATTGGATA	3.5 kb
337	02951-L13653	Exon 8	988-989	AGATACTTCAAT-AGCTCAGCCTTC	1.9 kb
400	18198-L22809	Exon 9	1220-1221	ATAAGACATTGG-AATATAACTTAA	6.1 kb
391 +	02953-L30560	Exon 10	480 nt before exon 10 reverse	CTACTCCATCAC-ACTGGTAGCACC	11.4 kb
465	02954-L23460	Exon 11	1521-1520 reverse	TTCTTCCACTGT-GCTTAATTTTAC	0.1 kb
330 ∞	03322-L14978	Exon 11	1590-1591	AGAAAATATCAT-CTTTGGTGTTTC	28.2 kb
418	02955-L02387	Exon 12	1678-1679	TTTGCAGAGAAA-GACAATATAGTT	2.6 kb
226 Δ	18196-L22807	Exon 13	1783-1784	TTGTATTTATTA-GACTCTCCTTTT	0.2 kb
292	03841-L03314	Exon 13	81 nt after exon 13	AGATTGCATTTT-ACCTCTTGAGAA	1.9 kb
142	02956-L13079	Exon 14	2296-2297	GTACCAGATTCT-GAGCAGGGAGAG	2.6 kb
160 ±	02957-L02389	Exon 15	2610-2609 reverse	GAAGGTATGTGT-TCCATGTAGTCA	7.9 kb
178	02958-L02390	Exon 16	2701-2702	GTGGCTGCTTCT-TTGGTTGTGCTG	0.8 kb
206	02959-L13651	Exon 17	2855-2856	CTTTGCTTGCTA-TGGGATTCTTCA	3.0 kb
364	18197-L22808	Exon 18	3010-3011	TCCAAAGATATA-GCAATTTTGGAT	3.9 kb
256	02961-L02393	Exon 19	3117-3116 reverse	CTGTTGCAACAA-AGATGTAGGGTT	1.1 kb
283	02962-L02394	Exon 20	3322-3321 reverse	TTGGCAGTATGT-AAATTCAGAGCT	3.0 kb
310	03576-L13076	Exon 21	3484-3485	TTAGCCATGAAT-ATCATGAGTACA	12.9 kb
355 ±	13629-L15083	Exon 22	3592-3593	ACAGAAGGTAAA-CCTACCAAGTCA	15.0 kb
382	02965-L02397	Exon 23	3895-3896	GTGTCTTGGGAT-TCAATAACTTTG	10.4 kb
409	02966-L02398	Exon 24	4020-4019 reverse	CATCTGCAACTT-TCCATATTTCTT	11.8 kb
436	02967-L02399	Exon 25	4062-4063	GATAGAACAGTT-TCCTGGGAAGCT	0.8 kb
148	03842-L03315	Exon 26	4288-4287 reverse	CATTCCAGCATT-GCTTCTATCCTG	1.4 kb
190	03574-L02400	Exon 27	4356-4357	TTCCATCCAGAA-ACTGCTGAACGA	0.1 kb
173	03578-L02939	Exon 27	4473-4472 reverse	CTGTCTCCTCTT-TCAGAGCAGCAA	58.1 kb
		stop codon	*4511-4513 (Exon 27)*		
445 ¬	03572-L03267	*CTTNBP2* gene		CAAGAAGCAATA-TTGTCAAGAGCC	

[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.

∞ Wild type sequence detected. A lowered probe signal can be due to the p.Phe508del mutation, the p.Ile507del mutation, or a partial deletion of exon 11. The p.Phe508Cys mutation (F508C; c.1523T>G; rs74571530) is not expected to influence the signal of this probe. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
± The p.Trp846X mutation (W846X; rs397508393 or rs267606722) could influence the signal of the 160 nt probe. The c.3528delC mutation (3659delC; rs78984783) could influence the signal of the 355 nt probe. In case of apparent deletions, it is recommended to sequence the region targeted by these probes.
¬ 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.
+ The ligation site of this probe is located in intron 9.

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.

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P091 product history	
Version	*Modification*
D2	One CFTR probe has been adjusted. No change in sequence detected.
D1	Three CFTR and four reference probes have been replaced. In addition, the 88 and 96 nt control fragments have been replaced (QDX2).
C1	Four CFTR probes and one reference probe have been replaced.
B1	The wild type specific probe for p.Phe508del and control fragments at 88, 96, 100 and 105 nt have been included.
A1	First release.

Implemented changes in the product description
Version D2-05 – 17 November 2022 (04P) - P091 specific notes updated; information already present in the Clinical background section removed and note about the 330 nt probe rewritten and clarified. - Partial sequence of probe 12113-L23339 (373 nt) corrected in Table 2; one nucleotide changed. - Morocco removed as country with IVD status. - Various minor textual changes. Version D2-04 – 11 June 2021(04P) - Product description rewritten and adapted to a new template. - Intended purpose updated. - Clinical background and performance characteristics updated. - Cystic fibrosis mutation database website link changed. - Ligation sites of the probes targeting the *CFTR* gene updated according to new version of the NM_ reference sequence. - Warning added to Table 1 and 2 for sensitivity to experimental variation of the 226 nt probe. - Note added to Table 1 and 2 specifying that the ligation site of the 391 nt probe is located in intron 9. - SNP number for c.3528delC (3659delC) mutation updated from rs121908747 to rs78984783. - Probe number of 391 nt CFTR exon 10 probe corrected in Table 2. - List of selected publications using SALSA MLPA probemix P091 CFTR updated. - UK has been added to the list of countries in Europe that accept the CE mark. Version D2-03 – 11 May 2020 (04) - Colombia added as country with IVD status. Version D2-02 – 29 January 2019 (04) - Product is now registered for IVD use in Morocco and Israel. Version D2-01 – 13 March 2018 (04) - 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). - Information for “Gene structure” updated: LRG number added and NG number updated. - Recommendations for positive control DNA samples added. - Information about polymorphisms effecting probe signals was updated (footnotes of Table 1 and 2). - Previous names of the genes ASZ1 and CTTNBP2 (flanking probes), GASZ and CORTBP2 respectively, removed from Table 2. - The position of upstream and exon 1 probes relative to the coding sequence clarified (Table 1 and 2). - Various minor textual or layout changes.

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*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.