Product Description
SALSA® MLPA® Probemix P250-B2 DiGeorge

To be used with the MLPA General Protocol.

Version B2
For complete product history see page 12.

Catalogue numbers:
- **P250-025R:** SALSA MLPA Probemix P250 DiGeorge, 25 reactions.
- **P250-050R:** SALSA MLPA Probemix P250 DiGeorge, 50 reactions.
- **P250-100R:** SALSA MLPA Probemix P250 DiGeorge, 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 P250 DiGeorge is an in vitro diagnostic (IVD)[1] or a research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the human 22q11.2 region in genomic DNA isolated from human peripheral whole blood specimens, buccal swabs, (un)cultured amniotic fluid obtained in week 16 of the pregnancy or later and free from blood contamination, (un)cultured chorionic villi free from maternal contamination, or fetal blood. P250 DiGeorge is intended to confirm a potential cause for and clinical diagnosis of 22q11.2 Deletion/Duplication Syndrome or Cat Eye Syndrome (CES). It further contains probes for the detection of copy number status on 4q, 8p, 9q, 10p, and 17p regions to confirm a potential cause for and clinical diagnosis of DiGeorge (DGS) type II or disorders with phenotypic features of DGS. This assay is suitable for initial detection of deletions or duplications of above-mentioned regions or for confirmation of results obtained with a different (MLPA) assay.

Copy number variations (CNVs) detected with P250 DiGeorge should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method.

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, parental evaluation, 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, 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 use (IVD) 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

One of the most common genetic disorders causing learning disabilities and mild mental retardation is 22q11.2 Deletion Syndrome (DS). 22q11.2 DS is an autosomal dominant contiguous gene deletion syndrome with a live birth prevalence estimated between 1:3,000 and 1:6,000 among the general population. Cardiac abnormalities are present in ~75% of 22q11.2 DS patients and are the major cause of mortality. Developmental delay, facial dysmorphia, palatal dysfunction, and feeding difficulties are also observed in most individuals with this syndrome. It is now recognized that 22q11.2 DS encompasses the phenotypes previously described as DiGeorge syndrome (DGS), velocardiofacial syndrome (VCFS), conotruncal anomaly face syndrome (CTAF), and Cayler cardiofacial syndrome (asymmetric crying facies), (GeneReview: https://www.ncbi.nlm.nih.gov/books/NBK1523/).

22q11.2 Duplication syndrome (DupS) is a condition caused by an extra copy of a part of chromosome 22 (~3 Mb). The features of this condition vary widely, even among members of the same family (intrafamilial variability). Affected individuals may have intellectual or learning disability, developmental delay, slow growth leading to short stature, and hypotonia. Many individuals with a 22q11.2 duplication have no apparent physical or intellectual phenotype (Draaken et al. 2010, Sedghi et al. 2015). 22q11.2 DupS is much rarer than 22q11.2 DS, and its prevalence remains undetermined.

Cat Eye Syndrome (CES) has a large phenotypic variability, ranging from near normal to severe physical malformations, predominately affecting the eyes. CES is caused by the presence of an extra 22q11.2 copy between the DGS region and centromere, which usually presents as a small extra chromosome, frequently having two centromeres (https://www.omim.org/entry/115470). In many cases this chromosomal abnormality is mosaic.

The relatively high frequency of 22q11.2 copy number changes is prompted by low-copy number repeat (LCR22) sequences situated in this region. The span of the 22q11.2 deletions is variable, although the most common one (~85%) extends from the first (LCR22-A) until the fourth (LCR22-D) repeat (Table 2), leading to the typical 2.54 Mb deletion. In this P250 DiGeorge probemix, 14 probes target this repeat region. Two other deletions, the proximal nested 1.5-Mb (LCR22A–LCR22B) and 2-Mb (LCR22A–LCR22C) have a combined relative frequency of 5–10%. The distal nested (LCR22B–LCR22D and LCR22C–LCR22D) and other more distal deletions flanked by LCRs C–E; D–E; D–F; E–F; F–G are rare, and combined they only explain about 2% of cases (Lima et al. 2010; Michaelovsky et al. 2012; McDonald-McGinn et al. 2015). Haploinsufficiency of the *TBX1* gene, situated between LCR22A and -B, is particularly responsible for most of the physical phenotype observed in 22q11.2 DS. Point mutations in *TBX1* have also been observed in individuals with DGS-like phenotypes. Although the majority of DGS cases are explained by 22q11.2 deletions, chromosome defects on 4q, 8p, 9q, 10p, and 17p have also been associated with DiGeorge-like symptoms (see Table 2 for more information).

Probemix content

The SALSA MLPA Probemix P250 DiGeorge contains 48 MLPA probes with amplification products between 129 and 487 nucleotides (nt): 29 probes are located in the 22q11.2 region; four of the 29 probes for 22q11.2 and one probe for 22q11.1 target the Cat Eye Syndrome region. In addition, 17 probes are present for regions relevant for DGS type II or disorders with phenotypic features similar to DGS, and target chromosomal regions 4q, 8p, 9q, 10p, 17p. Two probes are included that target 22q13 (Table 1 and 2). These 19 probes also serve as reference probes. Complete probe sequences 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 blood, buccal swabs, (un)cultured amniotic fluid obtained in week 16 of the pregnancy or later and free from blood contamination, (un)cultured chorionic villi free from maternal contamination, or fetal blood 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 DiGeorge or DiGeorge-like syndromes. 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. Multiple samples from the Coriell Institute have been tested with this P250-B2 probemix at MRC Holland and can be used as positive control samples (see table below). See the MRC Holland Support portal for additional positive samples: https://www.mrcholland.com/r/ifu/positive-samples. The quality of cell lines can change; therefore samples should be validated before use.

Coriell sample ID	Affected region	Copy number status
NA07215; NA10382; NA17942	22q11.2 - LCR-A to LCR-D	Heterozygous deletion
NA05401	22q11.2 LCR-A to LCR-B + CES region; with the exception of the probe target in *DGCR8*	Heterozygous deletion
NA02944	22q11.2 LCR-A to LCR-B + CES region	Heterozygous deletion
NA07106	22q11.2 + 22q13	Heterozygous duplication
NA13284	22q13.33	Heterozygous deletion
NA00501; NA10313	4q35	Heterozygous duplication
NA03013	4q35	Heterozygous deletion
NA02030; NA03255	8p23	Heterozygous duplication
NA12721	8p23	Heterozygous deletion
NA13685	9q34	Heterozygous duplication

Coriell sample ID	Affected region	Copy number status
NA06936	10p14; with the exception of the probe target in *NEBL*	Heterozygous deletion
NA06047; NA09208	17p13.3	Heterozygous deletion

Performance characteristics

Deletions on 22q11.2 explain 100% of 22q11.2 DS cases (22q11.2 Deletion Syndrome GeneReview: www.ncbi.nlm.nih.gov/books/NBK1523/). Duplications on 22q11.2 explain 100% of 22q11.2 DupS cases (Wentzel et al. 2008).

CES is caused by duplication or triplication of 22q11.2 (OMIM #115470). In many cases this chromosomal abnormality is mosaic. MLPA measures the average copy number of the CES region and may not be able to detect CES in mosaic samples that contain predominantly normal cells.

Deletions of the 9q telomeric gene *EHMT1* are associated with Kleefstra syndrome, a disorder with phenotypic features similar to DGS such as cardiac anomalies, epileptic seizures and mental retardation. The syndrome is caused by a microdeletion in chromosomal region 9q34.3 in 50% of the cases (GeneReview: https://www.ncbi.nlm.nih.gov/books/NBK47079/).

The percentage of deletions/duplications on 4q35, 8p23, 10p14 and 17p13 resulting in DGS II or disorders with phenotypic features similar to DGS is unknown. However, the association between 10p and DGS type II, and the association between 4q, 8p, 9q, 17p and disorders with phenotypic features of DGS is well established.

The analytical sensitivity and specificity for the detection of deletions/duplications in the 4q35, 8p23, 9q34.3, 10p14, 17p13 and 22q11.2 region is very high and can be considered >99% (based on a 2008-2023 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 the 22q11.2 region specific MLPA probes as well as the reference probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication), or 4 (heterozygous triplication).

The standard deviation of all probes in the reference samples should be ≤0.10 and the final ratio (FR) of the reference probes in the patient samples should be between 0.80 and 1.20. In rare cases, one region covered by reference probes can deviate in patient samples in the same manner (deleted or duplicated). 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

Copy number status	Final ratio (FR)
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.
- 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.

P250-specific notes:

- Cat Eye Syndrome is usually the result of a small supernumerary chromosome of the 22q11 CES region. **WARNING:** The extra chromosome present in CES patients is easily lost during postzygotic divisions, resulting in mosaicism. Detection of CES in mosaic samples might be better done by standard karyotype analysis or by FISH (see below). MLPA measures the average copy number of the CES region and may not be able to detect CES in mosaic samples that contain predominantly normal cells.
- Please note that not all abnormalities detected by the SALSA MLPA Probemix P250 DiGeorge can be confirmed by FISH: FISH probe D22S553 is located around the *CLTCL1* gene, FISH probe D22S609 is located between *CLTCL1* and *HIRA*, and FISH probe D22S942 is located between *HIRA* and *CDC45L*. The clinical consequences of deletions/duplications which cannot be confirmed by FISH may require further investigation.
- This P250 DiGeorge probemix contains 17 probes for relevant regions of DGS type II or disorders with phenotypic features of DGS on chromosomes 4q, 8p, 9q, 10p, 17p. Furthermore, two probes on 22q13 are present. These 19 probes are also used as reference probes for sample normalisation. Please inform MRC Holland (info@mrcholland.com) when a deletion of one or more of the probes outside the 22q11.2 region is detected in a patient with a DiGeorge phenotype.

Limitations of the procedure

- 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.
- For use on (un)cultured amniocytes, contamination of the sample with maternal DNA may lead to wrong conclusions.
- For use on (un)cultured chorionic villi, discrepancies in chromosomal patterns between DNA from chorionic villi and fetus have been described due to maternal contamination, postzygotic nondisjunction, postzygotic isochromosome formation, mosaic situations, and complications in DNA sampling in twin pregnancies (Van den Berg et al. 2006).

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.

Table 1. SALSA MLPA Probemix P250-B2 DiGeorge

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)	
		Reference	22q11
64-105	Control fragments – see table in probemix content section for more information		
129	**PPIL2 probe** 07529-L04870		**22q11.2 D-E***
136	Reference probe 05059-L07380	*EHMT1*, 9q34.3	
142	**SLC25A18 probe** 05457-L07613		**22q11.2 CES**
148 +	**DGCR8 probe** 08475-L08486		**22q11.2 A-B**
154	Reference probe 05058-L07382	*EHMT1*, 9q34.3	
160 +	**HIRA probe** 01214-L02328		**22q11.2 A-B**
166	**SNRPD3 probe** 08481-L08492		**22q11.2 G-H**
172 « + ±	**TBX1 probe** 05408-L07614		**22q11.2 A-B**
178	**MICAL3 probe** 05458-L04861		**22q11.2 CES**
184	Reference probe 01217-L00694	*KLKB1*, 4q35.2	
191 +	**CLTCL1 probe** 05462-L05809		**22q11.2 A-B**
196 +	**CLDN5 probe** 01218-L06270		**22q11.2 A-B**
202 +	**ZNF74 probe** 05927-L07395		**22q11.2 B-C**
208 « +	**GP1BB probe** 05464-L10114		**22q11.2 A-B**
214	**GNAZ probe** 08478-L08489		**22q11.2 E-F**
220	**SMARCB1 probe** 05928-L07969		**22q11.2 F-G**
226	**USP18 probe** 07528-L04863		**22q11.2 CES**
232	Reference probe 06787-L07383	*SHANK3*, 22q13.33	
238 +	**TXNRD2 probe** 01223-L05814		**22q11.2 A-B**
245 « +	**TBX1 probe** 10810-L14347		**22q11.2 A-B**
255	Reference probe 01735-L07385	*RPH3AL*, 17p13.3	
261 «	**RSPH14 probe** 08484-L09139		**22q11.2 E-F**
267	Reference probe 01225-L09140	*GATA3*, 10p14	
274	Reference probe 01226-L03844	*GATA4*, 8p23.1	
283 +	**KLHL22 probe** 01227-L05815		**22q11.2 B-C**
292 ~	**TOP3B probe** 13299-L14649		**22q11.2 D-E**
301	Reference probe 07636-L07321	*GATA3*, 10p14	
308	**HIC2 probe** 13302-L15009		**22q11.2 D-E**
316 +	**MED15 probe** 01231-L15877		**22q11.2 B-C**
326	Reference probe 12093-L15011	*SLC25A4*, 4q35.1	
335	**IL17RA probe** 01082-L15012		**22q11.1 CES**
342	**RAB36 probe** 05932-L04872		**22q11.2 E-F**
350	Reference probe 01232-L17068	*TCEB1P3 region*, 10p14	
357	**BID probe** 01767-L07389		**22q11.2 CES**
364	Reference probe 01234-L00781	*CELF2*, 10p14	
373 +	**SNAP29 probe** 01235-L00773		**22q11.2 C-D**
382	Reference probe 01522-L00952	*CELF2*, 10p14	
391	Reference probe 13603-L03531	*YWHAE*, 17p13.3	
400 ±	**SMARCB1 probe** 05933-L05812		**22q11.2 F-G**
409	Reference probe 01238-L07390	*GEMIN4*, 17p13.3	
418 +	**LZTR1 probe** 01521-L00951		**22q11.2 C-D**
427	Reference probe 01240-L00787	*MSRA*, 8p23.1	
434	Reference probe 04081-L25903	*RPH3AL*, 17p13.3	
445	Reference probe 01093-L00661	*ARSA*, 22q13.33	
454	**RSPH14 probe** 08479-L08490		**22q11.2 E-F**
466 +	**CDC45 probe** 05463-L05808		**22q11.2 A-B**
472	Reference probe 01243-L07392	*PPP1R3B*, 8p23.1	
487	Reference probe 08480-L15878	*NEBL*, 10p12.31	

* The name of the regions of low-copy number repeats (LCRs) is based on Burnside (2015).

« 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.

+ Probes that are expected to have approximately 35-50% reduced peak height in most DiGeorge patients (LCR22A – LCR22D deletion).
~ This probe has been reported to have deviating copy numbers in healthy individuals. An apparent deletion or duplication detected by this single probe will not result in DGS, DGS type II or disorders with phenotypic features of DGS.
± SNP rs72646950 could influence the 172 nt probe signal (05408-L07614). SNP rs372348692 could influence the 400 nt probe signal (05933-L05812). In case of apparent deletions, it is recommended to sequence the region targeted by these probes.

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. P250 probes arranged according to chromosomal location

Table 2a. 22q11.1-22q11.2

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
Cat Eye Syndrome (CES) region:				
335	01082-L15012	*IL17RA*	GCAGAGTTATCT-GTCCTGCAGCTG	464 kb
142	05457-L07613	*SLC25A18*	GCAGTGAGAAGA-GTCGAGTGAAGC	183 kb
357	01767-L07389	*BID*	CTACTGGTGTTT-GGCTTCCTCCAA	98 kb
178	05458-L04861	*MICAL3*	GAACTACCGCCT-GTCCCTGAGGCA	308 kb
226	07528-L04863	*USP18*	CTCAGTCCCGAC-GTGGAACTCAGC	609 kb
End of CES region; Start DiGeorge (DGS) region; probes in region LCR22A – LCR22B*:				
191	05462-L05809	*CLTCL1*	TGTTGCCTTGGT-GACCGAGACCGC	77 kb
160	01214-L02328	*HIRA*	GGAGCTGCTGAA-GGAGCTGCTACC	148 kb
466	05463-L05808	*CDC45*	ATGTTCGTGTCC-GATTTCCGCAAA	44 kb
196	01218-L06270	*CLDN5*	TTCGCCAACATT-GTCGTCCGCGAG	200 kb
208 «	05464-L10114	*GP1BB*	CACAACCGAGCT-GGTGCTGACCGG	36 kb
172 « ±	05408-L07614	*TBX1*	CCGGGTGAAGCT-TCGCTGGCTGCC	6 kb
245 «	10810-L14347	*TBX1*	TCCCTTCGCGAA-AGGCTTCCGGGA	133 kb
238	01223-L05814	*TXNRD2*	GGAGGGTCAGGA-GAGGAGCTGCAG	187 kb
148	08475-L08486	*DGCR8*	GGTAATGGACGT-TGGCTCTGGTGG	626 kb
Probes in region LCR22B – LCR22C*:				
202	05927-L07395	*ZNF74*	CAGGCAGATTAT-TCCTCGATGCTG	94 kb
283 «	01227-L05815	*KLHL22*	TCTTCGATGTTG-TGCTGGTGGTGG	93 kb
316	01231-L15877	*MED15 (PCQAP)*	TGGCATTTGGAT-GAAGACACAGGT	305 kb
Probes in region LCR22C – LCR22D:				
373	01235-L00773	*SNAP29*	AGGAGCAAGATG-ACATTCTTGACC	107 kb
418	01521-L00951	*LZTR1*	ATGATGAAGGAG-TTCGAGCGCCTC	450 kb
End of the commonly-deleted DiGeorge (DGS) region; probes in region LCR22D – LCR22E:				
308	13302-L15009	*HIC2*	GTTCCAGCAGAT-CTTGGACTTCAT	250 kb
129	07529-L04870	*PPIL2*	GAAGAGCCCTCA-ACCAGTGCCACT	273 kb
292 ~	13299-L14649	*TOP3B*	GAGACATGATAA-AATCCAGTCCTT	1081 kb
Probes in region LCR22E – LCR22F*:				
261 «	08484-L09139	*RSPH14 (RTDR1)*	GGTGTGTCATTT-TGACGTCATCCC	61 kb
214	08478-L08489	*GNAZ*	TCACCATCTGCT-TTCCCGAGTACA	17 kb
454	08479-L08490	*RSPH14 (RTDR1)*	CTCCTTGGAGCT-TCCCATTAACAT	5 kb
342	05932-L04872	*RAB36*	AGCTGGATGCTT-GGACGCGCCGCT	642 kb
Probes in region LCR22F – LCR22G*:				
220 «	05928-L07969	*SMARCB1*	CTTCGGGCAGAA-GCCCGTGAAGTT	47 kb
400 ±	05933-L05812	*SMARCB1*	CATCAGCACACG-GCTCCCACGGAG	777 kb
Probe in region LCR22G – LCR22H*:				

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
166	08481-L08492	*SNRPD3*	CCGGTGAGGTAT-ATCGGGGGAAGC	

* The name of the regions of low-copy number repeats (LCRs) is based on Burnside (2015).

Table 2b. 4q35-qter

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
326	12093-L15011	*SLC25A4*	CATCAAGATCTT-CAAGTCTGATGG	1087 kb
184	01217-L00694	*KLKB1*	ATGCCCAATACT-GCCAGATGAGGT	3880 kb to 4q telomere
4q telomere				

An association of the 4q34.2 to 4qter deletions and the phenotype of velocardiofacial syndrome has been suggested by several authors (Caliebe et al. 1997, Descartes et al. 1996, Tsai et al. 1999, Fernandez et al. 2008).

Table 2c. 8p23

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
472	01243-L07392	*PPP1R3B*	ACCGAGCTCCTA-GACAACATTGTG	1067 kb
427	01240-L00787	*MSRA*	GCAACAGAACAG-TCGAACCTTTCC	1551 kb
274	01226-L03844	*GATA4*	TGGATTTTCTCA-GATGCCTTTACA	

Deletions in the 8p region are associated with congenital heart malformations according to several authors (Devriendt et al. 1999, Paez et al. 2008, Wat et al. 2009). More GATA4 probes are present in the P234 GATA3-GATA4 probemix.

Table 2d. 9q34.3

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
136	05059-L07380	*EHMT1*	AAATGCTGCAAA-GCACACTCAGGA	74 kb
154	05058-L07382	*EHMT1*	GGACCCCGTTGA-TGGAAGCAGCCG	~ 465 kb to 9q telomere
9q telomere				

Deletions of the 9q telomeric gene *EHMT1* are associated with Kleefstra syndrome, a disorder with phenotypic features of DGS such as cardiac anomalies, epileptic seizures and mental retardation (OMIM #610253). More EHMT1 probes are present in the P340 EHMT1 probemix.

Table 2e. 10p14

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
267	01225-L09140	*GATA3*	GAGTGCCTCAAG-TACCAGGTGCCC	15 kb
301	07636-L07321	*GATA3*	AACAGCTCGTTT-AACCCGGCCGCC	2433 kb
350	01232-L17068	*TCEB1P3*	TGTAGACCACAT-GATGGAGATTTG	428 kb
364	01234-L00781	*CELF2 (CUGBP2)*	GACATTCACTGT-GGAAATTTGGTG	231 kb
382	01522-L00952	*CELF2 (CUGBP2)*	TCCCCCGGTCAT-GGTCGGAAAAGG	9979 kb
487	08480-L15878	*NEBL*	CTGGGATCCTTT-TCTGTTCACTCA	

The *GATA3* gene is the centre of the HDR (hypoparathyroidism, sensorineural deafness, and renal insufficiency) syndrome region and *CELF2* should be in the centre of the DGS type II region according to the report of Lichtner et al. (2000). More GATA3 probes are present in the P234 GATA3-GATA4 probemix.
The *NEBL* gene is a long gene (~500 kb) located at almost 10 Mb centromeric of the *CELF2* gene. The *NEBL* gene was noticed to be deleted in two patients with a DGS type II deletion by Villanueva et al. (2002). A patient with a 5.5 Mb deletion on 10p was also found to have a deleted *NEBL* gene by Yatsenko et al. (2004). Please note that the distance between the DGS type II region and *NEBL* is much more than 5.5 Mb. The significance of *NEBL* for DiGeorge syndrome is unclear.

Table 2f. 17p13.3

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
255	01735-L07385	*RPH3AL*	AGGCGGAATGTG-ATGGGGAACGGC	14 kb
434	04081-L25903	*RPH3AL*	GTAGTGGACACT-TGTACGTGCACT	413 kb
409 «	01238-L07390	*GEMIN4*	AAACAGTGATAG-ACGTCAGCACAG	615 kb
391 #	13603-L03531	*YWHAE*	GCCACAGGAAAC-GACAGGAAGGAG	~1361 kb to q-telomere
17p telomere				

Deletions of the 17p telomeric region have been suggested to result in a DiGeorge-like phenotype (Greenberg et al. 1988). More probes in the 17p region are present in the P061 Lissencephaly probemix.

Table 2g. 22q13.3

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
445	01093-L00661	*ARSA*	GGAGGATCAGAT-CTCCGCTCGAGA	94 kb
232 «	06787-L07383	*SHANK3*	ACCAACTGTGAT-CAGTGAGCTCAG	~190 kb to q-telomere
22q telomere				

When requesting molecular analysis of chromosome 22q, it is not always clearly specified if 22q11.2 or 22q13 should be investigated, for this reason two 22q13 probes have been included. The 22q13.3 deletion syndrome (also called Phelan-McDermid syndrome) has overlapping symptoms with velocardiofacial syndrome. More probes for 22q13 are present in the P188 22q13 probemix. Distance between these 22q13 probes and the DiGeorge region is more than 25 Mb.

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

~ This probe has been reported to have deviating copy numbers in healthy individuals. An apparent deletion or duplication detected by this single probe will not result in DGS, DGS type II or disorders with phenotypic features of DGS.
« 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'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.
± SNP rs72646950 could influence the 172 nt probe signal (05408-L07614). SNP rs372348692 could influence the 400 nt probe signal (05933-L05812). In case of apparent deletions, it is recommended to sequence the region targeted by these probes.

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 SALSA MLPA probemixes

P061 Lissencephaly	Contains probes for 17p13.
P188 22q13	Contains probes for 22q13.
P234 GATA3	Contains probes for *GATA3*.
P245 Microdeletion syndromes	Contains probes for 21 different microdeletion syndromes, including 22q11.2 DiGeorge and 10p14 DGSII probes.
P258 SMARCB1	Contains probes for the *SMARCB1* gene region, frequently deleted in rhabdoid tumours.
P324 22q11	Contains probes for 22q11.
P340 EHMT1	Contains probes for *EHMT*.

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P250 product history	
Version	*Modification*
B2	Control fragments have been adjusted.
B1	Six probes have been replaced.
A1	Two denaturation control fragments, X chromosome and Y chromosome control fragments have been added and small change in length, not in detected sequence, is applied for one probe since first release.
A0	First release.

Implemented changes in the product description
Version B2-08 – 15 May 2024 (04P) - Table 1 updated: salt sensitivity warning removed for probes SLC25A4-D02-GG-326-M, ARSA-D01-CC-445-M, DGCR8-D01-GT-148-M, and SHANK3-D20-GA-232-M, as well as from Tables 2a, 2b, and 2g for probes SLC25A4-D02-GG-326-M, ARSA-D01-CC-445-M, and DGCR8-D01-GT-148-M. *Version B2-07 – 21 June 2023 (04P)* - Clinical background section updated. - Layout of tables with Positive samples and Related probemixes updated. - Table 1 and 2 updated: warnings for salt sensitivity were updated; column with distances to telomeres in Table 2 were removed as the relevant distances to telomeres are already present in last column. - Sections *References* and *Selected publications* curated. - Minor textual and layout changes. *Version B2-06 – 22 January 2020 (04P)* - Product description restructured and adapted to a new template. - Updated intended purpose. - Various minor textual changes. - Positive DNA sample information is updated, nine new samples added and three removed; all samples can be found on the Support portal. - Warning for the SNPs rs72646950 and rs372348692 added to Table 1 and Table 2. - In Table 1 the name DGR2 is removed behind the genes *GATA3*, *TCEB1P3*, and *NEBL*. - Probemixes P234 and P340 added to Related SALSA MLPA Probemixes section. - Added references below Table 2b and 2c, to the reference list, and the selected publications list. - UK has been added to the list of countries in Europe that accept the CE mark.

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

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