Product Description
SALSA® MLPA® Probemix P102-D1 HBB

To be used with the MLPA General Protocol.

Version D1
For complete product history see page 13.

Catalogue numbers:
- **P102-025R:** SALSA MLPA Probemix P102 HBB, 25 reactions.
- **P102-050R:** SALSA MLPA Probemix P102 HBB, 50 reactions.
- **P102-100R:** SALSA MLPA Probemix P102 HBB, 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.

This product requires RNase sample treatment. For more information, see page 4.

Intended purpose
The SALSA MLPA Probemix P102 HBB 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 beta-globin (*HBB*) gene cluster and its regulatory region located on chromosome 11p15.4 in genomic DNA isolated from human peripheral whole blood specimens. P102 HBB is intended to confirm a potential cause for and clinical diagnosis of beta-thalassaemia or hereditary persistence of foetal haemoglobin (HPFH) and for molecular genetic testing of at-risk family members. In addition, this probemix can be used as confirmation of sequencing results for the presence of the mutation causing sickle cell anaemia (SCA) or sickle cell disease (SCD).

Copy number variations (CNVs) detected with P102 HBB 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 *HBB* gene region are point mutations, most of which will not be detected by MLPA. 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, SALSA Binning DNA SD067 and Coffalyser.Net analysis software.

Clinical background

The beta-globin gene cluster, consisting of five functional genes (*HBE1*, *HBG2*, *HBG1*, *HBD* and *HBB*), is located on the short arm of chromosome 11. Five nuclease hypersensitive sites are located upstream of this locus, constituting the beta-globin locus control region (LCR) which is responsible for correct transcription of the globin genes. Mutations involving one or more of the genes and/or the LCR may lead to a variety of haemoglobin disorders, together referred to as haemoglobinopathy.

Beta-thalassaemia is an autosomal recessive disorder, characterised by a reduction or absence of beta-globin chain production. Depending on the type of mutation, beta-thalassaemia patients experience a wide variety of symptoms, ranging from mild anaemia to severe transfusion-dependent haemolytic anaemia.

Beta-thalassaemia can be classified into three categories:

- Beta-thalassaemia minor: carrier of a single beta-thalassaemia mutation. Carriers present with microcytic hypochromic anaemia, however, in general they do not require any treatment.
- Beta-thalassaemia intermedia: both beta-globin genes are affected by (usually not completely inactivating) mutations. A substantial amount of functional beta-globin chains is still produced. Patients require sporadic blood transfusions, iron chelation therapy and folic acid supplementation. In some cases, a splenectomy may be necessary.
- Beta-thalassaemia major (also known as Cooley's anaemia): function of both beta-globin genes is completely disrupted, leading to severe haemolytic anaemia, jaundice, hepatosplenomegaly and dysmorphic features due to expansion of the bone marrow. Patients are dependent on life-long regular blood transfusions and iron chelation therapy to survive. The only option for cure at this moment is bone marrow transplantation from a matching donor.

Until now, more than 600 point mutations in the *HBB* gene have been described (http://globin.cse.psu.edu/hbvar/menu.html) that lead to structural variants of the beta-globin chain and subsequently to abnormal haemoglobin molecules. A relatively small number of these abnormal haemoglobins can cause severe disease in homozygous or compound heterozygous combinations. The most frequently occurring mutation is the Haemoglobin S (HbS; *HBB*:c.20A>T, p.Glu7Val) mutation. Other well-known haemoglobin variants are HbC, HbE, HbD-Punjab and HbO-Arab. Homozygosity for HbS or combined heterozygosity of HbS with a beta-thalassaemia mutation, a deletion of the *HBB* gene or with another haemoglobin variant causes sickle cell disease (SCD). SCD patients suffer from infarctions in the microcirculation which lead to severe pain crises and organ damage, particularly in the bones, spleen, heart and lungs. Treatment consists of pain management and regular blood transfusion or erythrocyte exchange transfusion. Like for beta-thalassaemia major, the only option for cure at this moment is bone marrow transplantation.

In addition to beta-thalassaemia, deletions in the beta-globin gene cluster may also lead to other types of haemoglobinopathy. Depending on the gene(s) involved, they include epsilon-gamma-delta-beta-thalassaemia, gamma-delta-beta-thalassaemia, delta-beta-thalassaemia and hereditary persistence of foetal haemoglobin (HPFH). These conditions cause a variety of phenotypes, ranging from borderline-normal haematological indices with high levels of HbF (up to 40%) to microcytic hypochromic anaemia comparable to 'regular' beta-thalassaemia. During embryonic and foetal development, carriership of epsilon-gamma-delta-beta-thalassaemia can lead to severe haemolytic anaemia which may require intra-uterine blood transfusion (http://www.ncbi.nlm.nih.gov/books/NBK1426/; Weatherall 2010; Steinberg et al. 2009; Galanello et al. 2010).

Gene structure and transcript variants

The human beta-globin gene cluster spans ~45 kb on chromosome 11p15.4 and contains five functional genes. The genes vary in size between 1 and 2 kb and all consist of 3 exons. The GenBank chromosomal sequence is NG_000007.3, which is available on http://www.ncbi.nlm.nih.gov/nuccore/NG_000007. For each of the genes, only one transcript variant has been described. Details on the coding sequence of the genes are described in the table below.

Gene	NM number	Size of transcript	Coding sequence start and end (NG_000007.3)	NCBI gene link
Locus control region			6245-21766	-
HBE1	NM_005330.4	623 nt	27726-29147	http://www.ncbi.nlm.nih.gov/gene/3046
HBG2	NM_000184.3	586 nt	42888-44339	http://www.ncbi.nlm.nih.gov/gene/3048
HBG1	NM_000559.3	587 nt	47812-49257	http://www.ncbi.nlm.nih.gov/gene/3047
HBD	NM_000519.4	620 nt	63183-64652	http://www.ncbi.nlm.nih.gov/gene/3045
HBB	NM_000518.5	628 nt	70595-72018	http://www.ncbi.nlm.nih.gov/gene/3043

Exon numbering
The exon numbering used in this P102-D1 HBB product description is the exon numbering from the NG_000007.3 sequence. As changes to the databases can occur after release of this product description, the NG_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P102-D1 HBB contains 49 MLPA probes with amplification products between 130 and 502 nucleotides (nt). This includes 40 probes for the beta-globin gene cluster and its flanking regions. In addition, nine reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

Twenty-six probes are present that detect sequences in or very close to the globin genes. This includes two probes (136 and 180 nt) that detect sequences present in both *HBG1* and *HBG2*. Furthermore, one probe is specific for the HbS mutation in *HBB* and will only generate a signal when the mutation is present. The probemix also contains seven probes for the locus control region. Finally, two probes detect a sequence centromeric of the locus control region and five probes detect a sequence telomeric of the beta-globin gene cluster. These flanking probes are included to delineate the extent of larger deletions/duplications in the beta-globin gene cluster.

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 peripheral blood, free from impurities known to affect MLPA reactions and free from RNA. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

RNase sample treatment (essential for HBA and HBB MLPA probemixes)
Since *HBB* is heavily expressed in red blood cells, an RNase treatment of samples is essential for whole-blood derived samples. Without RNase treatment, *HBB* mRNA can bind to probes that detect a sequence within the *HBB* exons, thereby reducing the effective concentration of probes. Please note that some automatic DNA purification methods (e.g. Roche MagNA Pure) do not include an RNase treatment. The following method can be used to treat RNA containing DNA samples:

Mix 4 µl sample and 1 µl 0.5 mg/ml RNase A. Incubate 30 minutes at 37°C. Continue with the 5 minutes 98°C DNA denaturation step of the MLPA General Protocol.

RNase A is extremely stable; it can be diluted in TE and stored at -20°C. We recommend RNase A from Promega (A7973; 4 mg/ml solution), diluted 8 fold in TE (1 ml of 4 mg/ml RNase is sufficient for ~8000 samples). Do not use more than the recommended amount.

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 beta-thalassaemia or HPFH. 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 NA06342, NA20480 and NA16267 from the Coriell Institute have been tested with this P102-D1 probemix at MRC-Holland and can be used as positive control samples to detect compound heterozygosity for the Hb Kenya deletion and the HbS mutation (NA06342), heterozygosity for the Hb Lepore deletion (NA20480) and heterozygosity for the HbS mutation (NA16267). The quality of cell lines can change; therefore samples should be validated before use.

SALSA Binning DNA SD067
The SD067 Binning DNA provided with this probemix can be used for binning of all probes including the HbS mutation (*HBB*:c.20A>T, p.Glu7Val) specific probe (21234-L29609). SD067 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD067 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal. It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. RNase treatment is not required for SD067. For further details, please consult the SD067 Binning DNA product description, available online: www.mrcholland.com.

Performance characteristics
Most of the beta-thalassaemia mutations are caused by point mutations in the beta-globin gene and should be identified by sequencing analysis. Approximately 10% of cases are caused by deletions of (a part of) the

beta-globin gene or beta-globin gene cluster (http://www.ncbi.nlm.nih.gov/books/NBK1426/). Screening for deletions in the beta-globin gene cluster by MLPA is recommended for beta-thalassaemia patients in whom sequence analysis of the beta-globin gene is negative. Sickle cell disease (SCD) and sickle cell anaemia (SCA) are caused by the *HBB*:c.20A>T mutation (HbS mutation) and all cases can be identified by sequencing analysis of the *HBB* gene (https://www.ncbi.nlm.nih.gov/books/NBK1377/). The P102 HBB probemix can be used to confirm presence of the HbS mutation. The addition of MLPA to a diagnostic routine for beta-thalassaemia and HPFH leads to a definitive diagnosis in nearly all patients presenting with a beta-thalassaemia or HPFH phenotype. The analytical sensitivity and specificity for the detection of the HbS mutation and copy number changes in the beta-globin gene cluster (based on a 2008-2021 literature review) is very high and can be considered >99%.

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.

Note: Slope correction in samples with large deletions
The slope correction algorithm in Coffalyser.Net has been optimised to give the best possible results in as many situations as possible. However, the slope correction algorithm may confuse a large deletion for sloping, which can lead to over- or under-correction. Since large deletions are often detected when using the P102 probemix, this issue may occur relatively frequent. Incorrectly applied slope correction can cause an FSLP warning in Coffalyser.Net or ambiguous results for multiple probes. If you suspect that slope correction was incorrectly applied we recommend to contact info@mrcholland.com for assistance.

Interpretation of results
The expected results for most of the globin-gene-specific probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion) or 3 (heterozygous duplication), corresponding to probe ratios of 1, 0.5, and 1.5, respectively. In rare cases, copy numbers of 0 (homozygous deletion) or 4 (heterozygous triplication/homozygous duplication), corresponding to probe ratios of 0 and 2, respectively, can be obtained.

Two probes (136 and 180 nt) detect a sequence that is present in both *HBG1* and *HBG2*. In case of normal copy numbers these probes detect 4 copies / cell. The expected results for these two probes are allele copy numbers of 0 (homozygous deletion of both *HBG1* and *HBG2*), 1, 2, 3, 4 (normal), 5, 6, 7 or 8, corresponding to probe ratios of 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75 and 2, respectively. The probe ratios of these probes should be interpreted together with the results of the surrounding probes to determine the copy number.

The presence of a clear signal for the 214 nt probe (at least 10% of the median peak height of all reference probes in the sample), indicates the presence of the HbS mutation, either heterozygous or homozygous. This probe will not generate a signal in the majority of the samples. In a heterozygous HbS sample, the ratio of the 220 nt wildtype-specific probe is ~0.5, whereas there will be no signal for this probe in a homozygous HbS sample. Please note that a lower signal for this probe can also be caused by a deletion or by other mutations near the ligation site of the 220 nt probe (e.g. HbC mutation).

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 (with the exception of the 136

nt, 180 nt and 214 nt 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

Copy number status for the 136 nt and 180 nt probes	Final ratio (FR)
Homozygous deletion (0 copies)	FR = 0
Heterozygous deletion of *HBG1* and homozygous deletion of *HBG2* or *vice versa* (1 copy)	FR ≈ 0.25
Heterozygous deletion of *HBG1* and *HBG2* (2 copies)	FR ≈ 0.5
Heterozygous deletion of *HBG1* or *HBG2* (3 copies)	FR ≈ 0.75
Normal (4 copies)	FR ≈ 1
Heterozygous duplication of *HBG1* or *HBG2* (5 copies)	FR ≈ 1.25
Heterozygous duplication of *HBG1* and *HBG2* (6 copies)	FR ≈ 1.5
Heterozygous duplication of *HBG1* and homozygous duplication of *HBG2* or *vice versa* (7 copies)	FR ≈ 1.75
Homozygous duplication of *HBG1* and *HBG2* (8 copies)	FR ≈ 2

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 one of the globin 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.

P102 specific notes:

- The presence of a clear signal (at least 10% of the median peak height of all reference probes in the sample) for the 214 nt probe, indicates the presence of the HbS mutation (*HBB*:c.20A>T, rs334). This probe will not generate a signal in the majority of samples. The 220 nt probe indicates the copy number of the wildtype allele at this location. HbS is the most frequent pathological haemoglobin variant worldwide and is predominantly found in Africans and African-Americans. Homozygosity for this mutation or compound heterozygosity (HbS and beta-thalassaemia) leads to sickle-cell disease (Weatherall 2010).
- Results of the 214 nt and 220 nt probes must be interpreted with caution. Unreliable results may be obtained due to presence of a mutation near the ligation site (e.g. the haemoglobin C mutation (*HBB*:c.19G>A), c.25_26delAA and c.17_18delCT), which might influence hybridisation of the probes to the target sequence and/or ligation of the probes. It is, therefore, strongly recommended to use the P102 probemix in combination with sequence analysis of the *HBB* gene. Results of the sequence analysis should always be leading; results obtained with the 214 nt and 220 nt probes should be used as confirmation of sequencing results.
- The different globin genes in this locus are being expressed at different stages of development. Therefore, phenotype is not only dependent on which gene is affected, but also on stage of development. For example, a deletion involving the *HBE1*, *HBG1*, *HBG2*, *HBD* and *HBB* genes can lead to severe haemolytic anaemia during embryonic and foetal development which may require intra-uterine blood transfusion. During adult life, this type of deletion causes mild microcytic hypochromic anaemia, comparable to beta-thalassaemia carriers (Harteveld et al. 2003; Game et al. 2003; Shalev et al. 2013).
- Rare cases of mosaic segmental uniparental isodisomy (UPID) of chromosome 11p have been reported. If a somatic mutation that causes UPID occurs in the haemotopoietic tissue, the number of cells that become homozygous for the mutated allele may outgrow the heterozygous haematopoietic cells during life (loss of heterozygosity, LOH). LOH may also be a consequence of acquired large deletions in the same region. This may lead to late-onset transfusion-dependent beta-thalassaemia. If consecutive probes show the same ambiguous ratio (for example, 0.7), presence of UPID should be considered (Badens et al. 2002; Chang et al. 2008; Bento et al. 2013; Harteveld et al. 2013).
- The human globin genes have evolved from a single ancestor gene; therefore, all globin genes have a similar structure. The *HBG1* and *HBG2* genes are almost identical, differing by only a few nucleotides. These genes are, therefore, prone to homologous recombination, leading to either deletions, duplications, gene conversions, or formation of hybrid genes, most of which can be detected by the P102 probemix. Deletions or duplications of the *HBG1* and *HBG2* globin genes must be interpreted with caution; the majority of these rearrangements is not clinically relevant (Neumann et al. 2010).
- The nuclease hypersensitive site sequences HS1, HS2, HS3, HS4 and HS5 constitute the locus control region (LCR) of the beta-globin gene cluster. This LCR directs correct expression of the globin genes during the different stages of development. A mutation or deletion in the LCR disrupts its function, which leads to a thalassaemia phenotype although the globin genes themselves are present and intact (Stamatoyannopoulos 2005).
- Expected results for a few known deletions in the beta-globin gene cluster are summarised in the Table included in the Appendix of this product description.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *HBB* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P102 HBB.

- 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.

Haemoglobin mutation databases

http://globin.cse.psu.edu/globin/hbvar/menu.html and http://HBB.lovd.nl. We strongly encourage users to deposit positive results in the Database of human haemoglobin variants and thalassaemias and/or the Leiden Open Variation Database (LOVD). 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 deletion of *HBB* exons 1 and 3 but not intron 2) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P102-D1 HBB

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*HBB*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 16316-L18705	3q21	
136 * °	**HBG2/HBG1 probe** 21231-L29607		**HBG2 Exon 2 & HBG1 Exon 2**
142	Reference probe 18900-L24495	1p36	
148	**HBB probe** 05827-L06319		**HBB promoter region**
154	**HBB probe** 11883-L12683		**HBB Intron 1**
160 ¬	**TRIM68 probe** 18244-L22953		**Downstream of HBB**
166 °	**HBB probe** 13619-L15073		**HBB Exon 3**
173	**HBB probe** 05836-L06321		**0.5 kb downstream of HBB**
180 * °	**HBG2/HBG1 probe** 21230-L30179		**HBG2 Exon 1 & HBG1 Exon 1**
189	**HBB probe** 05828-L05332		**HBB Exon 1**
196 ±	**HBB probe** 05833-L05335		**HBB Intron 2**
202 *	Reference probe 18560-L24870	8q24	
208	**HBB probe** 11885-L25666		**0.2 kb downstream of HBB**
214 § ° +	**HBB probe** 21234-L29609		*HBB:c.20A>T mutation-specific probe*
220 § ° +	**HBB probe** 21234-L29610		*HBB:c.20A>T wildtype-specific probe*
227 ¬ ¥	**OR52A5 REGION probe** 18246-L30180		**Downstream of HBB**
233 ¥	**HBB-HS3 REGION probe** 21238-L30181		**HS3 region**
240 ¥	**HBB-HS1 REGION probe** 12189-L30182		**HS1 region**
245	Reference probe 11213-L14694	15q26	
253 Ж ¬	**OR51M1 probe** 18247-SP0630-L27000		**Upstream of HBB**
261	**HBB-HS5 REGION probe** 05804-L27001		**Upstream of HS5**
268	**HBB-HS4 REGION probe** 05807-L22540		**HS4 region**
274 ¬ ±	**HBB probe** 11980-L12803		**Downstream of HBB**
283 Ж ±	**HBBP1 probe** 18248-SP0631-L27002		**Upstream of HBBP1**
292	**HBB-HS2 REGION probe** 06395-L05315		**HS2 region**
298 ¬	**OR51B2 probe** 18249-L24239		**Upstream of HBB**
304	**HBB-HS4 REGION probe** 05806-L22542		**HS4 region**
312 *	**HBD probe** 21555-L30397		**HBD Exon 1**
319	Reference probe 14404-L16086	12q13	
330 ¥	**HBB-HS3 REGION probe** 21240-L29616		**HS3 region**
337 ±	**HBB probe** 05824-L05328		**Upstream of HBB**
346 ° +	**HBD probe** 21235-L29611		**HBD Exon 3**
355 ¬	**OR52A1 probe** 18251-L27003		**Downstream of HBB**
365	**HBB probe** 11982-L24242		**Upstream of HBB**
373 ±	**HBG2 probe** 05815-L05319		**Upstream of HBG2**
382 ¥	**HBBP1 probe** 21239-L29615		**HBBP1 Exon 3**
391 Ж	**HBD probe** 18108-SP0126-L15076		**HBD Exon 3**
401 *	Reference probe 20870-L28888	14q24	
409	**HBG1 probe** 05817-L05321		**Upstream of HBG1**
420 ±	**HBD probe** 05821-L06327		**Upstream of HBD**
427 † ° +	**HBG2 probe** 21237-L29612		**HBG2 Exon 3**
436 † ° +	**HBG1 probe** 21237-L29613		**HBG1 Exon 3**
445	**HBBP1 probe** 06400-L05323		**HBBP1 Exon 1**
454	Reference probe 04075-L03310	17q11	
463 ±	**HBE1 probe** 05813-L05317		**HBE Exon 1**
472 Ж	**HBG2 probe** 18109-SP0127-L27005		**HBG2 Exon 3**
486 ¬	**OR51V1 probe** 18253-L22962		**Downstream of HBB**
494	Reference probe 04274-L25060	13q12	
502	Reference probe 18161-L25061	21q22	

[a] See section
Exon numbering on page 3 for more information.

For explanation of symbols, see below Table 2.

Table 2. Beta-globin gene cluster probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / Exon[a]	Ligation site NG_000007.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
Centromeric flanking probes					
253 ¬ Ж	18247-SP0630-L27000	OR51M1 gene	897-898 & 927-928 in NM_001004756.3	TGCTGGACCTGG-30 nt spanning oligo-TGCACACAGTAG	66.1 kb
298 ¬	18249-L24239	OR51B2 gene	281-282 in NM_033180.5	TAGCAGTGTGGG-CTGCTTCCTACA	28.9 kb
HS5: 6245-6302; HS4: 9048-9713; HS3: 12459-13097; HS2: 16671-17058; HS1: 21481-21766 in NG_000007.3					
261	05804-L27001	Upstream HS5	2504-2505	TCCATGAAGTAT-TACAGCATTTGG	6.8 kb
304	05806-L22542	HBB-HS4 region	9279-9280	CACTCAGCAGCT-ATGAGATGGCTT	0.4 kb
268	05807-L22540	HBB-HS4 region	9639-9640	CATTTCCATGTC-ATACTGAGAAAG	2.1 kb
330	21240-L29616	HBB-HS3 region	11754-11755	TTCCTGCCCATT-CAGGGCTCCAGC	1.1 kb
233	21238-L30181	HBB-HS3 region	12807-12808	TGGCCACCAGCT-ATCAGGGCCCAG	4.1 kb
292	06395-L05315	HBB-HS2 region	16893-16894	GTGCCCAGATGT-TCTCAGCCTAGA	4.8 kb
240	12189-L30182	HBB-HS1 region	21654-21655	TCCTGAGCTCTT-ATCTATATCCAC	6.0 kb
HBE1 gene; exon 1: 27473-27817; exon 2: 27940-28162; exon 3: 29019-29266 in NG_000007.3					
463 ±	05813-L05317	HBE1 Exon 1	27674-27675	GCAGCAGCACAT-ATCTGCTTCCGA	13.8 kb
HBG2 gene; exon 1: 42835-42979; exon 2: 43102-43324; exon 3: 44211-44425 in NG_000007.3					
373 ±	05815-L05319	Upstream HBG2	41485-41486	TGAGCAGATATA-AGCCTTACACAG	1.4 kb
180 °	21230-L30179	HBG1+HBG2 exon 1	42926-42925 reverse + 47850-47849 reverse	CCCCACAGGCTT-GTGATAGTAGCC	0.2 kb
136 °	21231-L29607	HBG1+HBG2 exon 2	43150-43149 reverse + 48074-48073 reverse	GAGGACAGGTTG-CCAAAGCTGTCA	1.2 kb
427 † °	21237-L29612	HBG2 Exon 3	44305-44304 reverse	CACTGGCCACTC-CAGTCACCATCT	0.2 kb
472 Ж	18109-SP0127-L27005	HBG2 Exon 3	44498-44497 & 44453-44452 reverse	CACATAAACACA-45 nt spanning oligo-TAAAAAAAGAAC	1.9 kb
HBG1 gene; exon 1: 47759-47903; exon 2: 48026-48248; exon 3: 49129-49344 in NG_000007.3					
409	05817-L05321	Upstream HBG1	46413-46414	CAATGAGCAGAT-ATAAGCTTTACA	1.4 kb
180	21230-L30179	HBG1+HBG2 exon 1	42926-42925 reverse + 47850-47849 reverse	CCCCACAGGCTT-GTGATAGTAGCC	0.2 kb
136	21231-L29607	HBG1+HBG2 exon 2	43150-43149 reverse + 48074-48073 reverse	GAGGACAGGTTG-CCAAAGCTGTCA	1.2 kb
436 † °	21237-L29613	HBG1 Exon 3	49223-49222 reverse	CACTGGCCACTG-CAGTCACCATCT	2.8 kb
HBBP1 pseudogene; exon 1: 54024-54166; exon 2: 54288-54510; exon 3: 55369-55662 in NG_000007.3					
283 Ж ±	18248-SP0631-L27002	Upstream HBBP1	51986-51987 & 52019-52020	GAAATGGGGAAC-33 nt spanning oligo-AAAGTGACTGCA	2.1 kb
445	06400-L05323	HBBP1 Exon 1	54132-54133	TGAAGCAAGGTT-AAGGTGAGAAGG	1.5 kb
382	21239-L29615	HBBP1 Exon 3	55582-55583	ATGGGGGAGGTT-GGGGAGAAGAGC	4.1 kb
HBD gene; exon 1: 62988-63274; exon 2: 63403-63625; exon 3: 64524-64787 in NG_000007.3					
420 ±	05821-L06327	Upstream HBD	59683-59684	CTCATCCTCCTT-ACATACATTTCC	3.4 kb
312	21555-L30397	HBD Exon 1	63033-63032 reverse	ATTAGTTTGTGA-GAATGAAAAATG	1.4 kb
346 ° #	21235-L29611	HBD Exon 3	64585-64584 reverse	CAGCCTGCATTT-GTGGGGTGAATT	0.2 kb
391 Ж	18108-SP0126-L15076	HBD Exon 3	64751-64750 & 64710-64709 reverse	TTATTAGGCAGA-41 nt spanning oligo-AAATAGAATCTA	3.0 kb
HBB gene; exon 1: 70545-70686; exon 2: 70817-71039; exon 3: 71890-72150 in NG_000007.3					
337 ±	05824-L05328	Upstream HBB	67724-67725	GATTTTCATGGA-GGAAGTTAATAT	1.9 kb
365	11982-L24242	Upstream HBB	69652-69653	TACCCCTACTTT-CTAAGTCACAGA	0.8 kb
148	05827-L06319	HBB Promoter	70423-70424	ACAGGTACGGCT-GTCATCACTTAG	0.1 kb
189	05828-L05332	Exon 1	70548-70547 reverse	GTCAGAAGCAAA-TGTAAGCAATAG	0.1 kb

Length (nt)	SALSA MLPA probe	Gene / Exon[a]	Ligation site NG_000007.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
220 § °	21234-L29610	*HBB:c.20A>T wildtype-specific*	70614-70613 reverse	CAGACTTCTCC**T**-CAGGAGTCAGGT	-
214 § °	21234-L29609	*HBB:c.20A>T mutation-specific*	70614-70613 reverse	CAGACTTCTCC**A**-CAGGAGTCAGGT	0.1 kb
154	11883-L12683	Intron 1	70727-70728	AGGAGACCAATA-GAAACTGGGCAT	0.4 kb
196 ±	05833-L05335	Intron 2	71150-71151	GGAAACAGACGA-ATGATTGCATCA	0.9 kb
166 °	13619-L15073	Exon 3	72080-72079 reverse	ATCCCCCAGTTT-AGTAGTTGGACT	0.3 kb
208	11885-L25666	0.2 kb after exon 3	72366-72367	GAAAAGGATTCA-AGTAGAGGCTTG	0.3 kb
173	05836-L06321	0.5 kb after exon 3	72672-72673	GTGAGCCCTTCT-TCCCTGCCTCCC	9.1 kb
Telomeric flanking probes					
274 ¬ ±	11980-L12803	Downstream HBB	9596 nt after HBB exon 3	AGAGTTGAGCAA-GGCCTGAAATTT	15.8 kb
486 ¬	18253-L22962	OR51V1 gene	646-645 (reverse) in NM_001004760.3	GAAAAGGATGAG-TATAGCATCCAA	48.3 kb
355 ¬	18251-L27003	OR52A1 gene	1202-1203 in NM_012375.3	ATTGTGAAACTA-GCAGCAGCAAAT	45.3 kb
227 ¬	18246-L30180	Downstream OR52A5 gene	25257 nt after OR52A5	GGCACTTCCTTA-AGAGCACAGGAA	504.3 kb
160 ¬	18244-L22953	TRIM68 gene	1018-1019 in NM_018073.8	AAAGAGAGGTCG-CAGAGGCCTGTC	

[a] See section Exon numbering on page 3 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.

* New in version D1.
\+ Changed in version D1. Probe redesigned on the reverse strand.
¥ Changed in version D1. Minor alteration, no change in sequence detected.
§ The 214 nt probe will only give a signal when the sickle cell-specific point mutation (rs334) is present. The 220 nt probe detects the wildtype sequence at the location of this mutation**. Warning: Signal of the 220 nt probe is reduced ~50% by the HbS mutation. This signal is reduced ~25-50% by other mutations near the ligation site (e.g. the haemoglobin C mutation (*HBB*:c.19G>A), c.25_26delAA and c.17_18delCT).**
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.
° This probe is expected to be more variable in samples that contain RNA (see page 4). RNase treatment of DNA samples is recommended for this product.
† Please disregard apparent copy number changes detected only by the 427 nt and 436 nt HBG1 and HBG2 probes. The sequences detected by these probes (17 nt after the stop codon), have only a single nucleotide difference. Due to their close proximity (5 kb), it is likely that the *HBG2* sequence at this position is changed in some healthy individuals due to gene conversion in a *HBG1* sequence (SNP rs200060381) and *vice versa* (SNP rs56205611), without any consequences. A single (benign) nucleotide change in the sample DNA can generate an apparent deletion detected by the 427 nt probe and a duplication detected by the 436 nt probe or vice versa.
± SNP rs554807922 is known to influence the 373 nt probe signal. SNP rs551352636 is known to influence the 337 nt probe signal. SNP rs543925348 is known to influence the 196 nt probe signal. SNP rs575180790 is known to influence the 420 nt probe signal. SNP rs112330205 is known to influence the 463 nt probe signal. SNP rs574030447 is known to influence the 283 nt probe signal. SNP rs189836443 could influence the 274 nt probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by these probes.
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.
¬ 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. 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 probemix

P140 HBA	Probes for the HBA gene region

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P102 product history	
Version	*Modification*
D1	Two target probes have been removed and three new target probes have been included. Five probes have been redesigned on the reverse strand, detecting the same ligation site. The length of four probes has been changed. Two reference probes have been replaced and one reference probe has been removed.
C1	Five probes in the *HBB* region removed and 13 new probes in the *HBB* region included. Several reference probes replaced.
B2	DNA denaturation control fragments at 88 and 96 nt replaced (QDX2).
B1	Several *HBB* probes replaced.
A	First release.

Implemented changes in the product description

Version D1-03 – 16 June 2021 (04P)
- Product description rewritten and adapted to a new template.
- Added table with expected FR for the 136 nt and 180 nt probes.
- Note on slope correction added.
- UK has been added to the list of countries in Europe that accept the CE mark.
- Updated the NM_ reference sequences to the most recent versions for *HBE1*, *HBG1*, *HBG2* and *HBD* in the section *Gene structure and transcript variants*.
- Ligation sites of the flanking probes updated according to new version of the NM_ reference sequence.
- New references added and references with publication date of 2014 and earlier removed from the list of selected publications.

Version D1-02 — 25 September 2019 (02P)

- Ligation site 346 nt probe 21235-L29611 corrected in Table 2.
- Product description adapted to a new template.
- Various minor textual or layout changes.
- Warning about off-scale peaks added to *Interpretation of results*.
- Israel added as country with IVD status.

Version D1-01 – 19 September 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 on Dutch I 12.6 kb-deletion has been corrected. The 337 nt probe is not involved in this deletion.
- Information on Hb-P Nilotic (anti-Lepore), Hb Kenya and *HBG2* duplication has been added to the interpretation table.
- Number of the SD Binning DNA has been changed from SD029 to SD067.
- Warning added for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- Warning added for effect of mutations near the ligation site on the 220 nt probe signal.

Version 31 – 23 May 2018 (03)
- Information on Filipino β^0-deletion has been corrected. The 391 nt probe is not involved in this deletion.

Version 30 – 23 November 2017 (03) – specific for a test version of the probemix
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Various minor textual and layout changes.

Version 29 – 28 April 2017 (03)
- Certificate of analysis adapted to a new lot.
- Several minor textual changes throughout the document.

Version 28 – 03 April 2017 (03)
- Warning added on RNase sample treatment on page 1.

Version 27 – 20 September 2016 (03)
- Textual changes in the intended use and Performance characteristics.

Version 26 – 15 September 2016 (03)
- Product description restructured and adapted to a new template.
- Warning added for the 214 nt and 219 nt probes.
- Probe remark on variability due to presence of RNA added to 214 nt, 219 nt, 427 nt and 436 nt probes.
- Interpretation table has been included.
- Various textual and layout changes.

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

IVD	EUROPE* CE ISRAEL
RUO	ALL OTHER COUNTRIES

*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.

Appendix - Interpretation HBB deletion types and expected probe ratios

Probe name	Length (nt)	Dutch I 12.6 kb β°-thal	Dutch III 112 kb ($\varepsilon^{G}\gamma$)Aγδβ°-thal	Dutch IV (εγ)δβ°-thal	Dutch V εγδβ°-thal	Belgian 50 kb $^{G}\gamma(^{A}\gamma\delta\beta)$°-thal	Chinese $^{G}\gamma(^{A}\gamma\delta\beta)$°-thal	Indian $^{G}\gamma^{A}\gamma$ (δβ)°-thal	HPFH-2 Ghanaian	HPFH-3 Indian	Filipino β°-thal	Hb Lepore δβ-fusion gene	Hb-P Nilotic (anti-Lepore)	Hb Kenya $^{A}\gamma\beta$-fusion gene	Sicilian 13.4 kb (δβ)°-thal	Turkish 7.6 kb β°-thal (double deletion)	Black 1,393 bp β°-thal	Indian 619 bp β°-thal	Duplication *HBG2*
OR51M1 probe	253	1	1	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
OR51B2 probe	298	1	0.5	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HS5 upstream	261	1	0.5	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HBB-HS4 region	304	1	0.5	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HBB-HS4 region	268	1	0.5	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HBB-HS3 region	330	1	0.5	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HBB-HS3 region	233	1	0.5	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HBB-HS2 region	292	1	0.5	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HBB-HS1 region	240	1	0.5	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HBE1 exon 1	463	1	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HBG2 upstream	373	1	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
HBG1 + HBG2 exon 1	180	1	0.75	0.5	1	0.75	1	1	1	1	1	1	1	1	1	1	1	1	1.25
HBG1 + HBG2 exon 2	136	1	0.75	0.5	1	0.75	1	1	1	1	1	1	1	1	1	1	1	1	1.25
HBG2 exon 3	427	1	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.5
HBG2 exon 3	472	1	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.5
HBG1 upstream	409	1	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.5
HBG1 + HBG2 exon 1	180	1	0.75	0.5	1	0.75	1	1	1	1	1	1	1	1	1	1	1	1	1.25
HBG1 + HBG2 exon 2	136	1	0.75	0.5	1	0.75	1	1	1	1	1	1	1	1	1	1	1	1	1.25
HBG1 exon 3	436	1	1	0.5	1	0.5	0.5	1	1	1	1	1	1	0.5	1	1	1	1	1
HBBP1 upstream	283	1	1	1	1	0.5	0.5	0.5	1	1	1	1	1	0.5	1	0.5	1	1	1
HBBP1 exon 1	445	1	1	1	1	0.5	0.5	0.5	1	0.5	1	1	1	0.5	1	0.5	1	1	1
HBBP1 exon 3	382	1	1	1	1	0.5	0.5	0.5	0.5	0.5	1	1	1	0.5	1	0.5	1	1	1
HBD upstream	420	1	1	1	1	0.5	0.5	0.5	0.5	0.5	1	1	1	0.5	1	1	1	1	1
HBD exon 1	312	1	1	1	1	0.5	0.5	0.5	0.5	0.5	1	1	1	0.5	1	1	1	1	1
HBD exon 3	346	1	1	1	1	0.5	0.5	0.5	0.5	0.5	1	0.5	1.5	0.5	0.5	1	1	1	1
HBD exon 3	391	1	1	1	1	0.5	0.5	0.5	0.5	0.5	1	0.5	1.5	0.5	0.5	1	1	1	1
HBB upstream	337	1	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.5	0.5	0.5	0.5	1	1	1
HBB upstream	365	0.5	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.5	0.5	0.5	0.5	1	1	1
HBB promoter	148	0.5	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.5	0.5	0.5	0.5	0.5	1	1
HBB exon 1	189	0.5	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.5	0.5	0.5	0.5	0.5	1	1
HBB exon 1	220	0.5	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.5	0.5	0.5	0.5	0.5	1	1
HBB intron 1	154	0.5	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.5	0.5	0.5	0.5	0.5	1	1
HBB intron 2	196	0.5	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	1	1	1	0.5	0.5	0.5	1	1
HBB exon 3	166	0.5	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	1	1	1	0.5	0.5	1	0.5	1
HBB downstream	208	0.5	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	1	1	1	0.5	0.5	1	0.5	1
HBB downstream	173	0.5	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	1	1	1	0.5	0.5	1	1	1
HBB downstream	274	1	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	1	1	1	1	1	1	1	1
OR51V1 probe	486	1	1	1	1	1	0.5	1	0.5	0.5	0.5	1	1	1	1	1	1	1	1
OR52A1 probe	355	1	1	1	1	1	1	1	1	1	0.5	1	1	1	1	1	1	1	1

Probe name	Length (nt)	Dutch I 12.6 kb β°-thal	Dutch III 112 kb ($\varepsilon^{G}\gamma$)Aγδβ°-thal	Dutch IV (εγ)δβ°-thal	Dutch V εγδβ°-thal	Belgian 50 kb $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thal	Chinese $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thal	Indian $^{G}\gamma^{A}\gamma$ (δβ)°-thal	HPFH-2 Ghanaian	HPFH-3 Indian	Filipino β°-thal	Hb Lepore δβ-fusion gene	Hb-P Nilotic (anti-Lepore)	Hb Kenya $^{A}\gamma\beta$-fusion gene	Sicilian 13.4 kb (δβ)°-thal	Turkish 7.6 kb β°-thal (double deletion)	Black 1,393 bp β°-thal	Indian 619 bp β°-thal	Duplication *HBG2*
OR52A5 downstream	227	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
TRIM68 probe	160	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Please note: this interpretation table is meant as an aid only. MLPA users are responsible for the correct interpretation of their results, and no rights can be derived from this table.

For references and descriptions of the deletions mentioned in this table, please refer to the Database of Human Haemoglobin Variants and Thalassaemias (http://globin.bx.psu.edu/hbvar/menu.html). Please note that deletion breakpoints mentioned in this database may not reflect the expected results in the interpretation table, as some breakpoint locations are estimated and do not reflect the exact breakpoints.

Please notify us of any mistakes and additional information to be included in this table: info@mrcholland.com.