

# Product Description

## SALSA® digitalMLPA™ Probemix D006-A1 Multiple Myeloma

To be used with the digitalMLPA General Protocol.

### Version A1

First release.

### Catalogue numbers

- **D006-025R:** SALSA® digitalMLPA™ Probemix D006 Multiple Myeloma, 25 reactions
- **D006-050R:** SALSA® digitalMLPA™ Probemix D006 Multiple Myeloma, 50 reactions
- **D006-100R:** SALSA® digitalMLPA™ Probemix D006 Multiple Myeloma, 100 reactions

SALSA® digitalMLPA™ Probemix D006-A1 Multiple Myeloma (hereafter: D006 Multiple Myeloma) is to be used in combination with:

1. SALSA® digitalMLPA™ Reagent Kit (Cat No: DRK01-IL, DRK05-IL, DRK20-IL)
2. One or multiple barcode plates:  
SALSA® digitalMLPA™ Barcode Plate 1 (Cat No: BP01-IL (from lot 03-009-xxxxxx and higher))  
SALSA® digitalMLPA™ Barcode Plate 2 (Cat No: BP02-IL (from lot 03-008-xxxxxx and higher))

**N.B.** The three-digit number between dashes (e.g. -008-) will increase with every new barcode plate lot.



3. Data analysis software Coffalyser digitalMLPA™ (Cat No: n.a.)

### Volumes and ingredients

Volumes			Ingredients
D006-025R	D006-050R	D006-100R	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, Tris-HCl, EDTA, DTT

The digitalMLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

### Storage and handling

Recommended storage conditions		
--------------------------------	---	---

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

### Certificate of Analysis

Information regarding storage conditions and quality tests from the current sales lot is available at [www.mrcholland.com](http://www.mrcholland.com).

### Precautions and warnings

For professional use only. Always consult the most recent product description AND the digitalMLPA General Protocol before use: [www.mrcholland.com](http://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.

Due to high numbers of copy number alterations (CNAs) in multiple myeloma samples, the control probes included for sample identification will not be able to indicate sample DNA contamination, but can be used for reliable sample identification (SNP code in Coffalyser digitalMLPA reports).

## General information

SALSA® digitalMLPA™ Probemix D006-A1 Multiple Myeloma is a **research use only (RUO)** assay for the detection of deletions, gains or amplifications of genes and chromosomal regions mentioned in Table 2 that are recurrently altered in multiple myeloma, such as 1p, 1q, 13q and 17p, as well as for the detection of *BRAF* p.V600E point mutation.

Multiple myeloma (MM) is a clonal B-cell disorder characterised by malignant proliferation of monoclonal plasma cells. MM cases present with a common histological and morphological diagnosis, however simultaneously displaying enormous genetic and molecular complexity as well as marked variations in clinical characteristics and patient survival. Recent progress in molecular cytogenetics has improved the understanding of pathogenesis of MM and also provided reasoning for molecular sub-classification of MM. Genetic alterations in MM are well characterised and include gross chromosomal rearrangements such as fusion genes, hyper-/hypodiploidy and also focal deletions. This probemix is designed to detect the majority of the primary and secondary CNAs in MM.

**This probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.**

## Probemix content

A total number of 644 probes is included in D006 Multiple Myeloma consisting of:

- 189 target probes detecting copy number alterations involved in multiple myeloma (Table 2).
- One mutation-specific probe which will only generate probe reads when *BRAF* p.V600E point mutation is present (Table 2).
- 278 karyotyping probes, covering all existing chromosome arms (at the middle, near the centromeres and near the telomeres). 81 karyotyping probes are used as reference probes. See Table 3 for all chromosomal regions and genes included.
- More than 160 control probes and fragments are included: probes to aid in normalisation in case of copy number changes in tumour samples, probes for sample identification and probes for detection of errors or deviations when performing digitalMLPA assays, impurities in and fragmentation of the DNA samples, ligase and polymerase activity and extent of hybridisation.

The total number of probes can be used to calculate the number of reactions that can be combined into one sequencer run. See chapter “Amplicon Quantification by Illumina Sequencers” in the digitalMLPA General Protocol or the calculator tool available at [support.mrcholland.com](http://support.mrcholland.com).

More information on the location, mutation details and warnings of the probes present in this probemix can be found in the Probe Information File (PIF) available at [www.mrcholland.com](http://www.mrcholland.com).

## Reference probes

The selected reference probes are a subset of karyotyping probes in regions that show minimal copy number changes in MM. This was determined using information from the Progenetix oncogenomic online resource, Database of Genomic Variants (DGV), Broad Institute TCGA Copy Number Portal, and Catalogue of Somatic Mutations in Cancer (COSMIC) Cancer Gene Census. As long as more than 50% of the sequences targeted by the reference probes have a normal copy number (CN=2) in the test sample, the correct baseline will be detected and data will be correctly normalised using Coffalyser digitalMLPA.

## Gene structure and transcript variants:

Entrez Gene shows transcript variants of each gene: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene>

For NM\_ mRNA reference sequences: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide>

Matched Annotation from NCBI and EMBL-EBI (MANE): <http://www.ncbi.nlm.nih.gov/refseq/MANE/>

Tark – Transcript Archive: <http://tark.ensembl.org/>

## digitalMLPA technique

SALSA® digitalMLPA™ (Benard-Slagter et al. 2017) combines the robustness and simplicity of the trusted SALSA® MLPA® technology (Schouten et al. 2002) with next-generation sequencing. The principles of digitalMLPA are described in the digitalMLPA General Protocol ([www.mrcholland.com](http://www.mrcholland.com)).

## digitalMLPA technique validation

Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using digitalMLPA for the first time, or when pre-analytical steps, DNA extraction method or the instruments used are

changed. This validation experiment should result in a standard deviation  $\leq 0.10$  for all probes with the exception of SNP- and mutation-specific probes.

#### **Required specimens**

Extracted DNA from human bone marrow plasma cells free from impurities known to affect digitalMLPA reactions. For more information see the digitalMLPA General Protocol, section DNA sample treatment. The minimum percentage of tumour cells required for reliable analysis is 30% (Al Zaabi et al. 2010, Coll-Mulet et al. 2008). We advise to use tumour samples with at least 50% tumour cell content. Therefore, tumour samples should be evaluated by a pathologist before extraction of DNA.

#### **Reference samples**

As X- and Y-chromosome specific probes are included in this probemix, at least three male reference samples AND three female reference samples need to be used per experiment. In instances where an experiment only contains test samples of one gender, at least three reference samples of the same gender should be used. Pooled DNA from different genders can never be used as reference samples for D006 Multiple Myeloma analysis.

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. More information regarding the selection and use of reference samples can be found in the digitalMLPA General Protocol.

#### **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/home.html>) have a diverse collection of biological resources which may be used as a positive control DNA sample in your digitalMLPA experiments. In addition, reference standards from Horizon Discovery (<https://horizondiscovery.com/>) may also be used for *BRAF* p.V600E point-mutation detection. The quality of cell lines can change, therefore positive samples should be either acquired from quality assessed biological sample repositories or validated before use. Tables 1a and 1b contain a list of positive control samples that have been tested with D006 Multiple Myeloma at MRC Holland.

**Table 1. Positive samples from biobanks tested by MRC Holland****Table 1a. Germline samples**

Coriell sample ID	Genomic aberration * ^ *
NA22977	1p36.33 heterozygous deletion
NA00803	1q23.1-q23.3 heterozygous deletion (including <i>FCRL5</i> , <i>SLAMF7</i> , <i>PBX1</i> -area, <i>PBX1</i> )
NA00214	1q31.3 heterozygous deletion
NA10401	Trisomy 2
NA11428	3p26.1-p26.2 heterozygous deletion (including <i>CRBN</i> ) & 3q23-q29 heterozygous duplication
NA04127	3p24.1-p26.2 heterozygous duplication (including <i>CRBN</i> )
NA03563	3q23-q29 heterozygous duplication (including <i>ATR</i> )
NA10175	3q29 heterozygous duplication
NA10947	4p heterozygous duplication (including <i>FGFR3</i> , <i>NSD2</i> )
NA14489	4p16.3 heterozygous deletion (including <i>FGFR3</i> , <i>NSD2</i> ) & 4p15.1-p15.32 heterozygous duplication (including <i>ATR</i> )
NA10313	4q33-q35.2 heterozygous duplication & 7q36.23 heterozygous deletion
NA00782	4q13.1-q31.21 heterozygous duplication
NA14131	5p15.2-p15.33 heterozygous deletion & <i>IGHD/M</i> heterozygous deletion on 14q32.33
NA14230	5q23.2-q31.1 heterozygous deletion
NA04371	5q35.3 heterozygous duplication
NA06801	6p25.2-p25.3 heterozygous duplication (including <i>IRF4</i> )
NA12721	6p22.3-p25.3 heterozygous duplication (including <i>JARID2</i> , <i>IRF4</i> ) & 8p23.3 and <i>IGHD/M</i> heterozygous deletion on 14q32.33
NA06802	6q25.3-q27 heterozygous deletion (including <i>PRKN</i> )
NA21698	6q27 heterozygous deletion
NA07081	7p heterozygous duplication (including <i>IKZF1</i> )
NA10925	7p12.2-p12.3 heterozygous deletion (including <i>IKZF1</i> )
NA05067	9p heterozygous duplication & <i>IGHD/M</i> heterozygous deletion on 14q32.33
NA10989	9p24.1-p24.3 heterozygous deletion & <i>IGHD/M</i> heterozygous deletion on 14q32.33
NA02819	9p22.3-p24.3 heterozygous duplication & 12q24.33 heterozygous deletion
NA13685	9q34.3 heterozygous duplication (including <i>TRAF2</i> ) & <i>IGHD/M</i> heterozygous deletion on 14q32.33
NA03047	10p13-p14 heterozygous deletion
NA11672	10q11.22 heterozygous deletion
NA09596	11q21-q22.3 heterozygous deletion (including <i>BIRC2/3</i> , <i>ATM</i> ) & <i>IGHD/M</i> heterozygous deletion on 14q32.33
NA15099	11q21-q25 heterozygous duplication (including <i>BIRC2/3</i> , <i>ATM</i> , <i>NCAPD3</i> )
NA07981	12p heterozygous triplication/homozygous duplication (including <i>LTBR</i> , <i>NCAPD2</i> , <i>CHD4</i> , <i>ETV6</i> , <i>CDKN1B</i> , <i>GPRC5D</i> )
NA07891	12q24.33 heterozygous duplication & 18q21.1-q23 heterozygous deletion
NA13721	13q14.11-q21.33 heterozygous deletion (including <i>RB1</i> , <i>DLEU1</i> , <i>DIS3</i> )
NA08254	13q34 heterozygous deletion
NA13410	14q32.31-q32.33 heterozygous duplication (including <i>TRAF3</i> , <i>IGHD/M</i> )
NA03184	Trisomy 15 (including <i>GABRB3</i> )
NA20375	15q12 heterozygous deletion (including <i>GABRB3</i> )
NA06226	16p13.12-p13.3 heterozygous duplication (including <i>TNFRSF17</i> ( <i>BCMA</i> ))
NA12074	16q22.1 heterozygous deletion
NA09687	16q23.1-q24.3 heterozygous duplication (including <i>WWOX</i> )
NA09209	17p13.3 heterozygous deletion
NA16445	17q25.3 heterozygous duplication
NA01359	Trisomy 18
NA02944	20p heterozygous duplication & 22q11.1-q11.21 heterozygous deletion
NA09868	21q22.2-q22.3 heterozygous deletion
NA07106	22q heterozygous duplication (including <i>SMARCB1</i> )
NA04626#	Trisomy X (including <i>KDM6A</i> )

**Table 1b. Multiple myeloma cell line samples**

DSMZ sample ID (name)	CNAs detected by D006 Multiple Myeloma probes * ^ +
<b>ACC-163 (NCI-H929) #</b>	<b>gains:</b> 1q21.1-q23.3 (including <i>ANP32E, MCL1, ADAR, CKS1B, FCRL5, SLAMF7, PBX1-area, PBX1</i> ), 8p23.3-q24.21 (including <i>MYC</i> ), 11q21-q25 (including <i>BIRC2/3, ATM, NCAPD3</i> ), 18q21.1-q23, 19p13.13-p13.3, 20q (including <i>MAFB</i> ) <b>deletions:</b> 1p21.2-p22.1 (including <i>EVI5, RPL5, CDC14A</i> ), 1p12 (including <i>TENT5C</i> ), 6q25.3-q27 (including <i>PRKN</i> ), 7p22.1-p22.2, 10q11.21-q11.22, 12p11.22-p13.31 (including <i>LTBR, NCAPD2, CHD4, ETV6, CDKN1B, GPRC5D</i> ), 13q (including <i>RB1, DLEU1/2/7, DIS3</i> ), <i>IGHD/M</i> on 14q32.33 (homozygous), 19q13.42-q13.43, 20p, Xp (including <i>KDM6A</i> ), Xq11.1-q22.1
<b>ACC-430 (SK-MM-2)</b>	<b>gains:</b> 8q, 11q13.3-q25 (including <i>CCND1, BIRC2/3, ATM, NCAPD3</i> ), 18p, 18q21.1 <b>deletions:</b> 1p31.3-p32.3 (including <i>FAF1, CDKN2C</i> (homozygous), <i>DAB1</i> ), 6q22.31-q27 (including <i>PRKN</i> ), 8p, 9p22.3-p24.3, 13q12.3-q14.3 (including <i>RB1, DLEU1/2/7</i> ), <i>IGHD/M</i> on 14q32.33, 16q (including <i>CYLD, WWOX</i> ), 17p13.1-p13.3 (including <i>TP53</i> ), 18q23, 22q11.21-q12.2 (including <i>SMARCB1</i> ), chr. Y (homozygous)~
<b>ACC-606 (KMS-12-PE) #</b>	<b>gains:</b> 1q21.1-q23.3 (including <i>ANP32E, MCL1, ADAR, CKS1B, FCRL5, SLAMF7, PBX1-area, PBX1</i> ), 1q44, 3q29, IRF4 at 6p25.3, 7p11.2-qter (including <i>IKZF1</i> ), 8q24.21-q24.3 (including <i>MYC</i> ), 9q21.12-q31.1, 10p13-p15.3, 11q13.3-q25 (including <i>CCND1, BIRC2/3, ATM, NCAPD3</i> ), 13q34, 14q32.33 (excluding <i>IGHD/M</i> ), 18p <b>deletions:</b> 1p32.3 (including <i>FAF1, CDKN2C</i> (homozygous)), 1p21.3-p22.1 (including <i>EVI5, RPL5</i> ), 1p12-p13.1 (including <i>TENT5C</i> ), 1q31.3, 4q31.21-q35.2, 5q, 9p22.3-p24.3, 10q11.21-q26.3, 11q11.2-q22.2, 13q12.3-q21.33 (including <i>RB1, DLEU1/2/7, DIS3</i> ), 14q11.2-q22.2, <i>IGHM</i> on 14q32.33, 16q23.1-q24.3 (including <i>WWOX</i> ), 17p (including <i>TP53</i> ), 18q11.2, 18q21.1-q23, 19q, 20p12.3-p13, 22q11.1-q11.21, 22q12.2-q13.33, Xp (including <i>KDM6A</i> , homozygous for exon 4 probe), Xq11.1-q22.1

\* Single probe findings are not shown in the tables above; only CNAs detected by two or more consecutive probes are listed.

+ Only gene names listed in Table 2 with two or more probes are indicated, however, the chromosomal bands with CNAs do contain probes (listed in Table 3).

^ Indicated CNA region is based on the chr. band (hg38) targeted by D006 Multiple Myeloma probes, however, the exact extent of CNA cannot be determined by this probemix.

# Analysis of this female sample was done using >3 female reference samples.

~ Y chromosome CN status was determined based on the knowledge of the sample gender from public resources and checking the read counts for probes targeting the Y-chromosome in Coffalyser digitalMLPA "Ratios" excel output file. In this case, the median read count for Y-probes was 0, indicating a homozygous deletion.

### Data analysis

Coffalyser digitalMLPA must be used for data analysis in combination with the appropriate lot-specific product sheet. Coffalyser digitalMLPA software is freely downloadable at [www.mrcholland.com](http://www.mrcholland.com)<sup>1</sup>. Use of other non-proprietary software may lead to inconclusive or false results. Normalisation of results should be performed within one experiment. The digitalMLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

### Interpretation of results

The expected results for (pseudo)autosomal probes are allele copy numbers of 2 (normal), 1 or 0 (deletion), ≥3 (gain). The same results can be expected for the X-chromosome-specific probes in female samples. For the X-chromosome-specific probes in male samples, expected copy numbers are 1 (normal), 0 (deletion) or 2 (gain).

The standard deviation of all probes in the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the inter ratio of the probes can be used to interpret digitalMLPA results when **reference samples of the same sex** have been used:

Copy number status	Inter ratio	
	autosomal probes / X-chromosome-specific probes in female samples	X- and Y-chromosome-specific probes in male samples
Normal	0.85 < ratio < 1.15	0.60 < ratio < 1.40
Deletion *	ratio < 0.75	ratio < 0.40
Gain ±	ratio > 1.25	ratio > 1.60
Ambiguous copy number	All other values	All other values

\* Ratios might indicate a (subclonal) biallelic deletion when autosomal inter ratios are ≤0.30.

± Ratios might indicate an amplification when inter ratios are ≥2.15.

**Please note that these above mentioned inter ratios are affected both by percentage of tumour cells and by possible subclonality.** In case of a deletion that is subclonal and/or a lower percentage of tumour cells, the inter

<sup>1</sup> In case a reference sample quality warning or error is reported due to high standard deviation for X and Y probes, more information can be found on [our website](#).

ratio may be higher than expected. For example, a monoallelic deletion in a sample with 50% tumour cell content or a monoallelic deletion present in 50% of the tumour cells, will result in an inter ratio around 0.75. However, the same (ambiguous) inter ratio of 0.75 will also be found in a sample with a biallelic deletion and a tumour cell percentage of 25%, or a subclone harbouring a biallelic deletion comprising 25% of all tumour cells. The digitalMLPA technique cannot discriminate between these two scenarios.

More information on this can be found on [our website](#).

#### General notes on digitalMLPA interpretation:

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in subclonal cases.
- 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 lead to false-positive results. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe read count, in particular for probes located in or near a GC-rich region. The use of an alternative DNA extraction method or sample clean up (e.g. with ethanol precipitation or silica column based-kits) may resolve such cases. Control probes are present in all digitalMLPA probemixes that provide a warning for incomplete DNA denaturation.
- False-positive results (gains): Contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe read count (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 digitalMLPA are pathogenic. 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. In some genes, intragenic deletions are known that result in very mild, or no disease (Schwartz et al. 2007). Gains that include the first or last exon of a gene (e.g. exons 1-3) might in some cases not result in the inactivation of that gene copy.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

#### **D006 Multiple Myeloma specific notes**

- The use of enrichment of CD138+ plasma cells is highly recommended as it increases the proportion of malignant myeloma cells in the sample and enhances sensitivity in detecting copy number aberrations compared to analysing samples with mixed cell populations (Boyle et al. 2015).
- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood-derived germline samples, which could result in warnings in Coffalyser digitalMLPA about reference probe quality.
- Due to high numbers of CNAs in MM samples, the control probes included for sample identification will not be able to indicate sample DNA contamination, but can still be used for reliable sample identification (SNP code in Coffalyser digitalMLPA reports).

#### **Limitations of the procedure**

- Translocations involving the IgH locus are the most common primary genetic events in MM, however digitalMLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect most copy number neutral inversions or translocations. Even when digitalMLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- In majority of MM samples, the main cause of genetic defects in the *ATM*, *DIS3*, *TENT5C* (*FAM46C*) genes covered by D006 Multiple Myeloma are small (point) mutations, which will not be detected by using this probemix.
- Sequence changes (e.g. SNVs, point mutations, small indels) 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 read count by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- digitalMLPA analysis on tumour samples provides information on the average situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage

of tumour cells is low. In addition, subclonality of the aberration affects the inter ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample especially in samples with more chaotic karyotypes.

### **Confirmation of results**

Copy number changes and point mutation detected with D006 Multiple Myeloma must be verified by another method. SALSA® MLPA® probemixes are available for several genes and chromosomal regions in D006 Multiple Myeloma. Most of these SALSA® MLPA® probemixes contain probes with a different ligation site that can be used for initial confirmation of results (see section 'Related SALSA® MLPA® probemixes' in this product description). Alternatively, copy number changes can be confirmed by another independent technique such as long range PCR, qPCR, array CGH, FISH, WES/WGS/NGS-based methods or Southern blotting.

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/SNV/other variation that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or SNVs are present in the probe target sequence. The finding of a heterozygous mutation or SNVs indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

### **COSMIC mutation database**

<http://cancer.sanger.ac.uk/cosmic>. We strongly encourage users to deposit positive results in the COSMIC. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on <http://varnomen.hgvs.org/>.

Please report false positive results due to SNVs and unusual results to MRC Holland: [info@mrcholland.com](mailto:info@mrcholland.com).

**Table 2. D006-A1 Multiple Myeloma**

Chromosomal position (hg38)	Gene	NM sequence <sup>a)</sup>	# probes / # exons in gene	Gene / region length	Remarks	
1p (33 probes)	1p36.33	<i>TMEM240</i>	1 probe per gene	43 Mb		
		<i>CFAP74</i>				
	1p35.1	<i>YARS1</i>				
	1p34.1	<i>HECTD3</i>				
	1p32.3	<i>FAF1</i>	NM_007051.3	2/19	523 kb	
		<i>CDKN2C</i>	NM_078626.3	3/2	4.9 kb	
	1p32.2	<i>ORC1</i>	NM_001365792.1	1 probe per gene	4.2 Mb	
		<i>PLPP3</i>				
		<i>DAB1</i>				
	1p31.3	<i>LEPR</i>		1 probe		
	1p22.1	<i>EVI5</i>	NM_001350197.2	4/20	276 kb	
		<i>RPL5</i>	NM_000969.5	3/8	9.9 kb	
	1p21.3	<i>DPYD</i>	NM_003672.4	1 probe per gene	3.0 Mb	
1p21.2	<i>DBT</i>					
	<i>CDC14A</i>		4/16	168 kb		
1p13.1	<i>SLC22A15</i>		1 probe			
1p12	<i>TENT5C</i>	NM_017709.4	2/2	22.3 kb	previous name <i>FAM46C</i>	
	<i>SPAG17</i>		1 probe			
1q (29 probes)	1q21.1	<i>PDZK1</i>	1 probe per gene	1.9 Mb		
		<i>BCL9</i>				
	1q21.2	<i>ANP32E</i>	NM_030920.5	2/7	17.7 kb	
		<i>RPRD2</i>		1 probe		
		<i>MCL1</i>	NM_021960.5	2/3	5.1 kb	
	1q21.3	<i>NUP210L</i>		1 probe		
		<i>ADAR</i>	NM_001111.5	2/15	26.1 kb	
		<i>CKS1B</i>	NM_001826.3	3/3	4.6 kb	
	1q23.1	<i>FCRL5</i>	NM_031281.3	3/17	39.1 kb	
	1q23.3	<i>SLAMF7</i>	NM_021181.5	2/7	15.6 kb	
		<i>NUF2</i>		1 probe		
		<i>PBX1</i> -area		5 probes	81.3 Mb	gene-poor region; probes are targeting 113-238 kb upstream of <i>PBX1</i>
		<i>PBX1</i>		1 probe		
1q31.3	<i>KCNT2</i>		1 probe per gene			
	<i>DENND1B</i>					
1q44	<i>ADSS2</i>		1 probe per gene			
	<i>DES12</i>					
3p26.2	<i>CRBN</i>	NM_016302.4	3/11	29.7 kb		
3q23	<i>ATR</i>	NM_001184.4	3/47	129.5 kb		
4p16.3	<i>FGFR3</i>	NM_000142.5	5/18	15.6 kb		
	<i>NSD2</i>	NM_001042424.3	2/22	111 kb	previous name <i>WHSC1</i>	
6p25.3	<i>IRF4</i>	NM_002460.4	2/9	19.7 kb		
6p22.3	<i>JARID2</i>	NM_004973.4	2/18	276.0 kb		
6q26	<i>PRKN</i>	NM_004562.3	2/12	1.4 Mb	previous name <i>PARK2</i>	
7p12.2	<i>IKZF1</i>	NM_006060.6	3/8	100.4 kb		
7q34	<i>BRAF</i> <sup>+</sup>	NM_004333.6	1 mutation-specific probe	205.6 kb	<b>p.V600E<sup>+</sup></b> (c.1799T>A)	
8q24.21	<i>MYC</i>	NM_002467.6	3/3	6.7 kb		
9q34.3	<i>TRAF2</i>	NM_021138.4	2/11	40.1 kb		
11q13.3	<i>CCND1</i>	NM_053056.3	3/5	13.3 kb		
11q22.2	<i>BIRC3</i>		1 probe	31.5 kb		
	<i>BIRC2</i>	NM_001166.5	2/9	21.9 kb		
11q22.3	<i>ATM</i>	NM_000051.4	4/63	146.0 kb		



Chromosomal position (hg38)	Gene	NM sequence <sup>a)</sup>	# probes / # exons in gene	Gene / region length	Remarks	
11q25	<b>NCAPD3</b>	NM_015261.3	2/35	73.9 kb		
12p13.31	<b>LTBR</b>	NM_002342.3	2/10	7.4 kb		
	<b>NCAPD2</b>	NM_014865.4	2/32	37.9 kb		
	<b>CHD4</b>	NM_001273.5	2/40	37.3 kb		
12p13.2	<b>ETV6</b>	NM_001987.5	3/8	245.7 kb		
12p13.1	<b>CDKN1B</b>	NM_004064.5	2/3	5.0 kb		
	<b>GPRC5D</b>	NM_018654.2	3/4	11.6 kb		
13q (23 probes)	13q12.3	<b>KATNAL1</b>		1 probe per gene	13.2 Mb	
	13q14.11	<b>ENOX1</b>				
	13q14.2-q14.3	<b>RB1</b>	NM_000321.3	5/27	1 probe per gene	21.2 Mb
		<b>RCBTB2</b>				
		<b>DLEU2</b>				
		<b>KCNRG</b>				
		<b>MIR15A</b>				
		<b>DLEU1</b>	NR_109973.1	2/7		
		<b>DLEU7</b>				
		<b>RNASEH2B</b>				
		<b>ATP7B</b>				
	13q21.33	<b>VPS36</b>			1 probe per gene	
		<b>PCDH8</b>				
		<b>KLHL1</b>				
13q34	<b>DIS3</b>	NM_014953.5	2/21	29.7 kb		
	<b>ARHGEF7</b>			1 probe per gene	2.5 Mb	
	<b>GRK1</b>					
14q32.32	<b>TRAF3</b>	NM_145725.3	2/12	134.0 kb		
14q32.33	<b>IGHD</b>	NG_001019.6	2/7	8.9 kb		
	<b>IGHM</b>		4/6	4.5 kb		
15q12	<b>GABRB3</b>	NM_000814.6	2/9	229.5 kb		
16p13.13	<b>TNFRSF17</b>	NM_001192.3	4/3	2.9 kb	BCMA in literature	
16q12.1	<b>CYLD</b>	NM_001378743.1	2/19	59.9 kb		
16q23.1	<b>WWOX</b>	NM_016373.4	2/9	1.1 Mb		
17p (20 probes)	17p13.3	<b>VPS53</b>		1 probe per gene	1.7 Mb	
		<b>NXN</b>				
	17p13.1	<b>TP53</b>	NM_000546.6	14/11	1 probe per gene	9.0 Mb
		<b>PIK3R6</b>				
		<b>USP43</b>				
	17p11.2	<b>RAI1</b>			1 probe per gene	
		<b>MIR33B</b>				
17q12-q21.1	<b>IKZF3</b>	NM_012481.5	2/8	106.6 kb		
17q21.31	<b>MAP3K14</b>	NM_003954.5	2/16	53.9 kb		
20q12	<b>MAFB</b>	NM_005461.5	2/1	3.4 kb		
22q11.23	<b>SMARCB1</b>	NM_003073.5	5/9	51.0 kb		
Xp11.3	<b>KDM6A</b>	NM_001291415.2	2/30	239.6 kb		

Genes covered by two or more probes are indicated in **bold**.

**(a) NM sequence and MANE exon numbering:** The exon numbering and NM\_ sequence used are based on MANE project (release version 1.0) retrieved on 10/2022. As changes to the MANE database can occur after release of this product description, exon numbering may not be up-to-date. Exon numbering used here may differ from literature. When a MANE transcript is not available, then the exon numbering is based on the NM, NR or NG sequence.

+ Please note that due to high nucleotide sequence similarity of mutated V600E (GTG to GAG single nucleotide variation) and V600K (GTG to AAG double nucleotide variation) codons, the BRAF V600E probe included in this probemix might result in small number of reads on a sample with V600K mutation.

More information on the location and warnings of the probes present in this probemix can be found in the [Probe Information File \(PIF\)](#) available at [www.mrcholland.com](http://www.mrcholland.com).

**Table 3. Karyotyping probes**

Chromosomal position (hg38)	Gene
2p25.3	<i>TMEM18</i>
2p25.3	<i>COLEC11</i> §
2p22.3	<i>SPAST</i> §
2p22.2	<i>VIT</i> §
2p15	<i>PEX13</i> §
2p11.2	<i>REEP1</i>
2q11.2	<i>CNNM3</i> §
2q11.2	<i>NPAS2</i> §
2q24.3	<i>SCN1A</i> §
2q31.1	<i>ABCB11</i> §
2q32.2	<i>COL3A1</i> §
2q37.3	<i>CAPN10</i>
2q37.3	<i>KIF1A</i>
3p26.2	<i>TRNT1</i>
3p26.1	<i>SUMF1</i>
3p24.2	<i>NR1D2</i>
3p24.1	<i>NEK10</i>
3p12.3	<i>CNTN3</i>
3p11.1	<i>HTR1F</i>
3q11.2	<i>CPOX</i>
3q23	<i>CLSTN2</i>
3q24	<i>SLC9A9</i>
3q29	<i>OPA1</i>
3q29	<i>ACAP2</i>
3q29	<i>RUBCN</i>
4p16.3	<i>LETM1</i> §
4p16.3	<i>ADD1</i> §
4p15.32	<i>LDB2</i>
4p15.31	<i>KCNIP4</i> §
4p15.2	<i>SEL1L3</i>
4p15.1	<i>PCDH7</i> §
4p13	<i>ATP8A1</i> §
4q13.1	<i>TECRL</i> §
4q13.3	<i>UGT2A1</i>
4q21.1	<i>G3BP2</i> §
4q25	<i>HADH</i> §
4q31.21	<i>INPP4B</i> §
4q31.21	<i>ZNF827</i> §
4q31.23	<i>NR3C2</i> §
4q33	<i>CLCN3</i> §
4q35.1	<i>CYP4V2</i>
4q35.2	<i>TRIM11</i>
5p15.33	<i>IRX4</i>
5p15.31	<i>NSUN2</i>
5p15.2	<i>CTNND2</i>
5p15.2	<i>DNAH5</i>
5p13.3	<i>NPR3</i>
5p13.2	<i>TTC23L</i>
5q11.2	<i>IL31RA</i>
5q11.2	<i>MIER3</i>
5q13.3	<i>CERT1</i>
5q14.3	<i>ADGRV1</i>
5q23.2	<i>SNCAIP</i>
5q23.2	<i>MARCHF3</i>
5q31.1	<i>SLC22A5</i>
5q31.2	<i>MYOT</i>
5q31.2	<i>GFRA3</i>
5q31.2	<i>CTNNA1</i>
5q31.3	<i>PCDHA1</i>

Chromosomal position (hg38)	Gene
5q31.3	<i>PCDHAC1</i>
5q31.3	<i>PCDHAC2</i>
5q31.3	<i>PCDHB2</i>
5q31.3	<i>PCDHB10</i>
5q31.3	<i>SLC25A2</i>
5q31.3	<i>TAF7</i>
5q31.3	<i>PCDHGA11</i>
5q32	<i>SH3TC2</i>
5q35.3	<i>COL23A1</i>
5q35.3	<i>MAPK9</i>
6p25.2	<i>GMDS-DT</i> §
6p25.2	<i>SERPINB6</i>
6p25.2	<i>PXDC1</i> §
6p25.2	<i>ECI2</i> §
6p22.3	<i>KIAA0319</i>
6p22.1	<i>ZFP57</i> §
6p21.33	<i>TNF</i> §
6p12.3	<i>PKHD1</i>
6p12.1	<i>RAB23</i> §
6p11.2	<i>PRIM2</i> §
6q12	<i>EYS</i>
6q13	<i>COL19A1</i>
6q13	<i>RIMS1</i>
6q22.31	<i>TBC1D32</i>
6q22.33	<i>LAMA2</i>
6q23.3	<i>TNFAIP3</i>
6q25.3	<i>TFB1M</i>
6q25.3	<i>WTAP</i>
6q25.3	<i>IGF2R</i>
6q27	<i>SMOC2</i>
6q27	<i>ERMARD</i>
7p22.2	<i>SDK1</i>
7p22.1	<i>RADIL</i>
7p15.3	<i>RAPGEF5</i>
7p15.3	<i>STK31</i>
7p12.3	<i>ADCY1</i>
7p12.3	<i>ABCA13</i>
7p11.2	<i>LANCL2</i>
7q11.21	<i>KCTD7</i>
7q11.22	<i>GALNT17</i>
7q31.1	<i>PNPLA8</i>
7q31.1	<i>IFRD1</i>
7q36.3	<i>RBM33</i>
7q36.3	<i>DYNC211</i>
8p23.3	<i>FBXO25</i>
8p23.3	<i>CLN8</i>
8p23.1	<i>GATA4</i>
8p21.3	<i>GFRA2</i>
8p21.3	<i>TNFRSF10B</i>
8p21.3	<i>TNFRSF10A</i>
8p21.2	<i>NEFL</i>
8p12	<i>RBPMS</i>
8p12	<i>GSR</i>
8p11.23	<i>ZNF703</i>
8q11.21	<i>SNTG1</i> §
8q12.2	<i>CHD7</i> § +
8q21.3	<i>RMDN1</i> §
8q21.3	<i>CPNE3</i> §
8q22.3	<i>RRM2B</i>

Chromosomal position (hg38)	Gene
8q24.3	SLC39A4 §
9p24.3	DOCK8
9p24.1	JAK2
9p24.1	GLDC
9p22.3	FREM1
9p22.3	BNC2
9p13.2	FBXO10
9p13.2	DCAF10
9q21.12	TRPM3
9q31.1	ALDOB
9q34.3	COL5A1
9q34.3	GRIN1
9q34.3	EHMT1
10p15.3	ZMYND11 §
10p15.3	DIP2C §
10p15.2	PFKP
10p15.2	PITRM1
10p14	ECHDC3 §
10p13	NMT2 §
10p13	ITGA8
10p11.21	CUL2 §
10p11.21	ZNF25 §
10q11.21	MARCHF8 §
10q11.22	ARHGAP22 §
10q22.2	KAT6B §
10q25.2	ADD3 §
10q26.3	INPP5A §
10q26.3	KNDC1
11p15.5	RIC8A
11p15.5	DEAF1
11p14.3	ANO5 +
11q12.3	BEST1
11q21	MTMR2
11q25	NTM
11q25	JAM3
12p13.33	WNK1
12p13.33	CACNA2D4
12p13.33	TSPAN9
12p13.31	CD27
12p13.31	VAMP1
12p13.2	BCL2L14
12p13.2	LRP6
12p13.2	BORCS5
12p12.3	AEBP2
12p11.22	REP15
12p11.22	MANSC4
12p11.22	PTHLH
12p11.22	TMTC1
12q12	KIF21A §
12q12	NELL2 §
12q15	MDM1 §
12q21.2	E2F7 §
12q23.1	NEDD1 §
12q23.1	SLC17A8 §
12q24.22	NOS1 §
12q24.33	GALNT9
12q24.33	PGAM5
14q11.2	CHD8 §
14q22.1	DDHD1 §
14q22.2	SAMD4A §

Chromosomal position (hg38)	Gene
14q24.3	NPC2 §
14q32.31	DYNC1H1 §
14q32.33	COA8
14q32.33	MTA1
14q32.33	TEDC1
15q21.1	SPG11
15q21.1	FBN1
15q22.2	VPS13C
15q22.31	USP3
15q23	CLN6
15q26.3	IGF1R
15q26.3	CHSY1
15q26.3	TM2D3
16p13.3	DECR2 §
16p13.3	IFT140 §
16p13.13	TXNDC11 §
16p13.12	CPPED1
16p11.2	HIRIP3
16p11.2	ITGAL §
16q11.2	GPT2
16q12.1	LONP2
16q13	SLC12A3
16q22.1	SLC12A4
16q22.1	DUS2
16q23.2	MAF
16q24.3	ANKRD11
16q24.3	GAS8
17q11.2	PSMD11
17q23.2	MED13
17q25.3	CCDC57
17q25.3	CSNK1D
18p11.31	LPIN2 §
18p11.31	TGIF1 §
18p11.21	GNAL §
18p11.21	SPIRE1 §
18p11.21	RNMT §
18q11.2	NPC1 § +
18q21.1	LOXHD1 §
18q21.1	LIPG §
18q23	CTDP1 §
18q23	TXNL4A
19p13.3	PLPP2
19p13.2	TEX45
19p13.13	GET3
19p13.13	GCDH
19p13.13	STX10
19p13.11	GMIP
19q13.11	SLC7A9
19q13.2	ACP7
19q13.2	COQ8B
19q13.42	DNAAF3
19q13.43	SLC27A5
20p13	RBCK1
20p13	RSPO4
20p13	TGM6 §
20p12.3	TRMT6 §
20p12.2	PLCB4 §
20p11.23	RIN2 §
20p11.21	APMAP §
20q11.22	ACSS2 §

Chromosomal position (hg38)	Gene
20q11.22	<i>EDEM2</i> §
20q11.23	<i>SAMHD1</i> §
20q13.12	<i>SLC13A3</i> §
20q13.13	<i>STAU1</i> §
20q13.33	<i>OSBPL2</i>
20q13.33	<i>UCKL1</i>
21q11.2	<i>RBM11</i>
21q11.2	<i>HSPA13</i>
21q22.11	<i>ITSN1</i>
21q22.2	<i>PSMG1</i>
21q22.3	<i>PDE9A</i>
21q22.3	<i>PWP2</i>
21q22.3	<i>TSPEAR</i>
22q11.1	<i>GAB4</i>
22q11.21	<i>CECR2</i>
22q11.21	<i>HIRA</i>
22q12.2	<i>NF2</i>
22q12.2	<i>ZMAT5</i>

Chromosomal position (hg38)	Gene
22q12.2	<i>SFI1</i>
22q12.3	<i>LARGE1</i>
22q13.2	<i>EP300</i>
22q13.31	<i>TRMU</i>
22q13.33	<i>BRD1</i>
Xp22.31	<i>ANOS1</i>
Xp22.11	<i>ACOT9</i>
Xp11.22	<i>FGD1</i>
Xq11.1	<i>ARHGEF9</i>
Xq13.3	<i>ZDHHC15</i>
Xq22.1	<i>NXF3</i>
Xq28	<i>CLIC2</i>
Xq28	<i>TMLHE</i>
Yp11.2	<i>PCDH11Y</i>
Yp11.2	<i>TBL1Y +</i>
Yq11.221	<i>USP9Y</i>
Yq11.223	<i>KDM5D</i>
Yq11.223	<i>RPS4Y2</i>

Odd numbered chromosomes are highlighted grey.

§ Used as reference probe for normalisation purposes in data analysis.

+ Two probes are included for this gene.

Note: No karyotyping probes are present in the acrocentric chromosome p-arms.

More information on the location, mutation details and warnings of the probes present in this probemix can be found in the [Probe Information File \(PIF\)](#) available at [www.mrcholland.com](http://www.mrcholland.com).

**Table 4. Related SALSA® MLPA® probemixes**

Related probemix	Coverage ±	Can be used for confirmation ^
P425 Multiple Myeloma	Contains probes for 1p, 1q, 5q31, chr. 9, 12p13, 13q14-q22, <i>TRAF3</i> , chr. 15, <i>CYLD</i> , <i>WWOX</i> , <i>TP53</i> .	no
P056 TP53	Contains probes for each exon of <i>TP53</i> .	no
P047 RB1	Contains probes for each exon of <i>RB1</i> , except exon 15.	yes *
P041/P042 ATM-1/-2	Contain probes for each exon of <i>ATM</i> .	yes *
P088 Oligodendroglioma 1p-19q	Contains probes for chromosomal arms 1p, 1q, 19p, 19q.	yes *
P380 Wilms' tumour	Contains probes for 1p, 1q, 16p, 16q, <i>TP53</i> .	yes *
P480 WHS & Achondroplasia	Contains probes for 4p16.3 including <i>NSD2</i> and <i>FGFR3</i> .	yes *
P244 AIP-MEN1-CDKN1B	Contains six probes on chromosomal arm 12p (including <i>CDKN1B</i> ).	yes *
P202 IKZF1-ERG	Contains probes for each exon of <i>IKZF1</i> and 14q32.33, among others.	yes *
P335 ALL- <i>IKZF1</i>	Contains probes for <i>IKZF1</i> , <i>ETV6</i> and <i>RB1</i> , among others.	yes *
P037/P038/P040 CLL-1/-2	Contain probes for 2p, 6q, 8p, 8q ( <i>MYC</i> ), 11q ( <i>ATM</i> ), chr. 12, 13q14 ( <i>RB1</i> , <i>DLEU1/2</i> ), <i>TP53</i> , 14q32.33, 17p ( <i>TP53</i> ), chr. 19.	yes *
P258 SMARCB1	Contains probes for each exon of <i>SMARCB1</i> and flanking regions on 22q.	yes *
P414 MDS	Contains probes for chr. 3, 5q, 7q, 8q, 11q, 12p ( <i>ETV6</i> ), chr. 17 ( <i>TP53</i> ), chr. 19, 20q and Y chromosome.	yes *
P323 CDK4-HMGA-MDM2	Contains probes on chromosomal arms 12p and 12q.	yes *
P105 Glioma-2	Contains nine probes for <i>TP53</i> , among others.	no
P051/P052 Parkinson mix 1/2	Contain probes for each exon of <i>PRKN</i> .	yes *
P064 Microdeletion Syndromes-1B	Contains nine probes for <i>NSD2</i> , among others.	yes *
P377 Hematologic Malignancies	Contains several probes for <i>IKZF1</i> , <i>MYC</i> , <i>ATM</i> , <i>ETV6</i> , 13q14, <i>TP53</i> , among others.	yes *
P078 Breast tumour	Contains probes for <i>MYC</i> , <i>CCND1</i> , among others.	yes
P451 Chromosome 16	Contains probes on chromosomal arms 16p and 16q.	yes *
P343 Autism 1	Contains 26 probes on chromosomal arm 15q.	yes *
P298 BRAF-HRAS-KRAS-NRAS	Contains a mutation-specific probe for <i>BRAF</i> p.V600E, among others.	yes
P301/P302/P303 Medulloblastoma mix 1/2/3	Contain probes for chr. 1, 2, 3, 4q, 5q, 6, 7, 8, 9, 10, 14q, 16, 17, 20.	yes *

± Only genes or chromosomal regions included in D006 Multiple Myeloma are mentioned in this table. SALSA® MLPA® probemixes additionally contain probes for genes not mentioned in this table.

^ Probemixes can be used for confirmation when ligation sites are different between D006 Multiple Myeloma probes and the probes in the corresponding probemixes. Of note, this statement concerns the majority of the probes in a probemix and does not mean that all probes always have a different ligation site. For more information, please contact [info@mrcolland.com](mailto:info@mrcolland.com).

\* The reference probes included in this SALSA® MLPA® probemix have not been optimised for MM samples. If the sample derived from MM harbours multiple copy number alterations on the genomic locations of the reference probes, the normalisation can be compromised and reliable result interpretation will not be possible with this probemix.

## References

- Al Zaabi EA et al. (2010). Multiplex Ligation-Dependent Probe Amplification Versus Multiprobe Fluorescence in Situ Hybridization to Detect Genomic Aberrations in Chronic Lymphocytic Leukemia: A Tertiary Center Experience. *J Mol Diagn.* 12:197-203.
- Benard-Slagter A et al. (2017). Digital multiplex ligation-dependent probe amplification for detection of key copy number alterations in T- and B-cell lymphoblastic leukemia. *J Mol Diagn.* 19: 659-72.
- Boyle EM et al. (2015). A molecular diagnostic approach able to detect the recurrent genetic prognostic factors typical of presenting myeloma. *Genes Chromosomes Cancer.* 54:91-8.
- Coll-Mulet L et al. (2008). Multiplex Ligation-Dependent Probe Amplification for Detection of Genomic Alterations in Chronic Lymphocytic Leukaemia. *Br J Haematol.* 142:793-801.

- Hömig-Hölzel C and Savola S (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res*. 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat*. 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem*. 421:799-801.

## Selected publications using D006 Multiple Myeloma

- Croft J et al. (2021). Copy number evolution and its relationship with patient outcome—an analysis of 178 matched presentation-relapse tumor pairs from the Myeloma XI trial. *Leukemia*. 35:2043-53.
- Kosztolanyi S et al. (2018). High-Throughput Copy Number Profiling by Digital Multiplex Ligation-Dependent Probe Amplification in Multiple Myeloma. *J Mol Diagn*. 20:777-88.
- Menezes K et al. (2020). High-Throughput Molecular Cancer Cell Line Characterization Using Digital Multiplex Ligation-Dependent Probe Amplification for Improved Standardization of in Vitro Research. *J Mol Diagn*. 22:1179-88.

D006 Multiple Myeloma history	
Version	Modification
A1	First release.

Implemented changes in the product description
Version A1-04 – 24 January 2025 (03) <ul style="list-style-type: none"> <li>- Added inter ratio values for interpretation of X- and Y-chromosome-specific probe results in 'Interpretation of results' section on page 5.</li> <li>- Minor textual corrections.</li> </ul>
Version A1-03 – 15 November 2024 (03) <ul style="list-style-type: none"> <li>- Specified the total number of probes and added a paragraph regarding the calculation of the number of reactions that can be combined into one sequencer run in 'Probemix content' section on page 2.</li> <li>- "Interpretation of results" section completely rewritten: new cut off values for deletions and gains introduced on page 5.</li> <li>- Minor textual changes.</li> </ul>
Version A1-02 – 08 January 2024 (03) <ul style="list-style-type: none"> <li>- Replaced "SALSA digitalMLPA" with "SALSA® digitalMLPA™" where applicable.</li> <li>- To be used with: section restructured and reagent kits (Cat No: DRK05-IL, DRK20-IL) added.</li> <li>- Barcode plate names and lot numbers updated.</li> <li>- Added sections: Ingredients, SDS note, Storage and handling, information on shelf life and safe disposal.</li> <li>- Added note on page 9 about <i>BRAF</i> V600E mutation-specific probe giving small read count also on samples with V600K mutation.</li> <li>- Small rearrangement of CNA region order for ACC-606 cell line in Table 1b on page 4.</li> <li>- Updated links to our website to <a href="https://www.mrcholland.com">https://www.mrcholland.com</a> throughout the document.</li> <li>- Various minor textual changes.</li> </ul>
Version A1-01 – 08 November 2022 (03) <ul style="list-style-type: none"> <li>- Not applicable, new document.</li> </ul>

More information: <a href="http://www.mrcholland.com">www.mrcholland.com</a> ; <a href="http://www.mrcholland.eu">www.mrcholland.eu</a>	
	MRC Holland BV; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	<a href="mailto:info@mrcholland.com">info@mrcholland.com</a> (information & technical questions) <a href="mailto:order@mrcholland.com">order@mrcholland.com</a> (orders)
Phone	+31 888 657 200