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.

To be used in combination with:

1. SALSA® digitalMLPA reagent kit DRK01-IL (100 reactions) or DRK05-IL (500 reactions).
2. SALSA® Barcode Plates for Illumina instruments BP01-IL or BP02-IL (768 reactions/plate) with an expiry date of 2022-12 and later.
3. Coffalyser digitalMLPA software.

Certificate of Analysis
Information regarding storage conditions and quality tests 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 digitalMLPA 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.

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
The 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. The D006 Multiple Myeloma digitalMLPA probemix is designed to detect the majority of the primary and secondary CNAs in MM.

This SALSA digitalMLPA 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 ~630 probes is included in this D006-A1 Multiple Myeloma probemix 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 most chromosome arms at the centromeres, middle of the chromosome arms and telomere regions. No probes are present for the short p-arm of the five acrocentric chromosomes (13, 14, 15, 21 and 22). 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.

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.

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
The digitalMLPA technique is a variant of the widely used conventional MLPA technique (Schouten et al. 2002, Benard-Slagter et al. 2017). In digitalMLPA, Illumina sequencers, instead of capillary electrophoresis instruments, are used for relative quantification of amplicons. The principles of the digitalMLPA technique are described in the digitalMLPA General Protocol (www.mrcholland.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

digitalMLPA technique validation
Internal validation of the digitalMLPA technique using 16 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 ≤0.10 for all probes with the exception of SNV- and mutation-specific probes.

Required specimens
Extracted DNA from 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 the D006-A1 probemix 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 *FCRL5, SLAMF7, PBX1-area, PBX1*)
NA00214	1q31.3 heterozygous deletion
NA10401	Trisomy 2
NA11428	3p26.1-p26.2 heterozygous deletion (including *CRBN*) & 3q23-q29 heterozygous duplication
NA04127	3p24.1-p26.2 heterozygous duplication (including *CRBN*)
NA03563	3q23-q29 heterozygous duplication (including *ATR*)
NA10175	3q29 heterozygous duplication
NA10947	4p heterozygous duplication (including *FGFR3*, *NSD2*)
NA14489	4p16.3 heterozygous deletion (including *FGFR3*, *NSD2*) & 4p15.1-p15.32 heterozygous duplication (including *ATR*)
NA10313	4q33-q35.2 heterozygous duplication & 7q36.23 heterozygous deletion
NA00782	4q13.1-q31.21 heterozygous duplication
NA14131	5p15.2-p15.33 heterozygous deletion & *IGHD/M* 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 *IRF4*)
NA12721	6p22.3-p25.3 heterozygous duplication (including *JARID2, IRF4*) & 8p23.3 and *IGHD/M* heterozygous deletion on 14q32.33
NA06802	6q25.3-q27 heterozygous deletion (including *PRKN*)
NA21698	6q27 heterozygous deletion
NA07081	7p heterozygous duplication (including *IKZF1*)
NA10925	7p12.2-p12.3 heterozygous deletion (including *IKZF1*)
NA05067	9p heterozygous duplication & *IGHD/M* heterozygous deletion on 14q32.33
NA10989	9p24.1-p24.3 heterozygous deletion & *IGHD/M* heterozygous deletion on 14q32.33
NA02819	9p22.3-p24.3 heterozygous duplication & 12q24.33 heterozygous deletion
NA13685	9q34.3 heterozygous duplication (including *TRAF2*) & *IGHD/M* heterozygous deletion on 14q32.33
NA03047	10p13-p14 heterozygous deletion
NA11672	10q11.22 heterozygous deletion
NA09596	11q21-q22.3 heterozygous deletion (including *BIRC2/3, ATM*) & *IGHD/M* heterozygous deletion on 14q32.33
NA15099	11q21-q25 heterozygous duplication (including *BIRC2/3, ATM, NCAPD3*)
NA07981	12p heterozygous triplication/homozygous duplication (including *LTBR, NCAPD2, CHD4, ETV6, CDKN1B, GPRC5D*)
NA07891	12q24.33 heterozygous duplication & 18q21.1-q23 heterozygous deletion
NA13721	13q14.11-q21.33 heterozygous deletion (including *RB1*, *DLEU1*, *DIS3*)
NA08254	13q34 heterozygous deletion
NA13410	14q32.31-q32.33 heterozygous duplication (including *TRAF3*, *IGHD/M*)
NA03184	Trisomy 15 (including *GABRB3*)
NA20375	15q12 heterozygous deletion (including *GABRB3*)
NA06226	16p13.12-p13.3 heterozygous duplication (including *TNFRSF17* (BCMA))
NA12074	16q22.1 heterozygous deletion
NA09687	16q23.1-q24.3 heterozygous duplication (including *WWOX*)
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 *SMARCB1*)
NA04626#	Trisomy X (including *KDM6A*)

Table 1b. Multiple myeloma cell line samples

DSMZ sample ID (name)	CNAs detected by D006 probes * ^ +
ACC-163 (NCI-H929) #	**gains**: 1q21.1-q23.3 (including *ANP32E, MCL1, ADAR, CKS1B, FCRL5, SLAMF7, PBX1-area, PBX1*), 8p23.3-q24.21 (including *MYC*), 11q21-q25 (including *BIRC2/3, ATM, NCAPD3*), 18q21.1-q23, 19p13.13-p13.3, 20q (including *MAFB*) **deletions**: 1p21.2-p22.1 (including *EVI5, RPL5, CDC14A*), 1p12 (including *TENT5C*), 6q25.3-q27 (including *PRKN*), 7p22.1-p22.2, 10q11.21-q11.22, 12p11.22-p13.31 (including *LTBR, NCAPD2, CHD4, ETV6, CDKN1B, GPRC5D*), 13q (including *RB1, DLEU1/2/7, DIS3*), *IGHD/M* on 14q32.33 (homozygous), 19q13.42-q13.43, 20p, Xp (including *KDM6A*), Xq11.1-q22.1
ACC-430 (SK-MM-2)	**gains**: 8q, 11q13.3-q25 (including *CCND1, BIRC2/3, ATM, NCAPD3*), 18p, 18q21.1 **deletions**: 1p31.3-p32.3 (including *FAF1, CDKN2C* (homozygous), *DAB1*), 6q22.31-q27 (including *PRKN*), 8p, 9p22.3-p24.3, 13q12.3-q14.3 (including RB1, DLEU1/2/7) , IGHD/M on 14q32.33, 16q (including *CYLD, WWOX*), 17p13.1-p13.3 (including *TP53*), 18q23, 22q11.21-q12.2 (including *SMARCB1*), chr. Y (homozygous)~
ACC-606 (KMS-12-PE) #	**gains**: 1q21.1-q23.3 (including *ANP32E, MCL1, ADAR, CKS1B, FCRL5, SLAMF7, PBX1-area, PBX1*), 1q44, 3q29, IRF4 at 6p25.3, 7p11.2-qter (including *IKZF1*), 8q24.21-q24.3 (including *MYC*), 9q21.12-q31.1, 10p13-p15.3, 11q13.3-q25 (including *CCND1, BIRC2/3, ATM, NCAPD3*), 13q34, 14q32.33 (excluding *IGHD/M*), 18p **deletions**: 1p32.3 (including *FAF1, CDKN2C* (homozygous)), 1p21.3-p22.1 (including *EVI5, RPL5*), 1p12-p13.1 (including *TENT5C*), 1q31.3, 4q31.21-q35.2, 5q, 9p22.3-p24.3, 10q11.21-q26.3, 13q12.3-q21.33 (including *RB1, DLEU1/2/7, DIS3*), 11q11.2-q22.2, 14q11.2-q22.2, *IGHM* on 14q32.33, 16q23.1-q24.3 (including WWOX), 17p (including *TP53*), 18q11.2, 18q21.1-q23, 19q, 20p12.3-p13, 22q11.1-q11.21, 22q12.2-q13.33, Xp (including *KDM6A*, 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 probes, however, the exact extent of CNA cannot be determined by D006 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 software must be used for data analysis in combination with the appropriate lot-specific Coffalyser digitalMLPA product sheet. Coffalyser digitalMLPA software is freely downloadable at www.mrcholland.com [1] . 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 autosomal probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 0 (homozygous deletion), 3 (heterozygous gain), 4 (heterozygous triplication / homozygous gain) or ≥5 (amplification). 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 indication		Inter ratio
Autosomal sequences / X-chromosome sequences in females	**X-chromosome sequences in males**	
Normal	Normal	0.80 < ratio < 1.20
Homozygous deletion	Deletion	ratio = 0
Heterozygous deletion		0.40 < ratio < 0.65
Heterozygous duplication / gain		1.30 < ratio < 1.65
Heterozygous triplication / Homozygous duplication / gain	Gain	1.75 < ratio < 2.15
Ambiguous*		All other values

[1] 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.

Please note that these above mentioned inter ratios are affected both by the percentage of tumour cells and by possible subclonality. In case of a heterozygous deletion that is subclonal and/or the sample contains a lower percentage of tumour cells, the inter ratio might not be in the expected range of 0.40-0.65. For example, in case of 50% tumour cell content or a copy number alteration present in 50% of the tumour cells, the inter ratio of a gene or region with a heterozygous deletion will be around 0.75. However, the same (ambiguous) inter ratio (0.75) will also be found for a sample harbouring a homozygous deletion with a tumour cell percentage of 25% or a subclone comprising 25% of all tumour cells. The digitalMLPA technique cannot discriminate between these two scenarios.

* Ambiguous ratios might indicate a (subclonal) homozygous deletion when inter ratios are between 0 and 0.30 or an amplification when inter ratios are > 2.15.

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 MLPA 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-A1 probemix 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 the D006 probemix 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 the D006 Multiple Myeloma probemix must be verified by another method. Conventional MLPA probemixes are available for several genes and chromosomal regions in the D006-A1 Multiple Myeloma probemix. Most of these conventional MLPA probemixes contain probes with a different ligation site that can be used for initial confirmation of results (see section 'Related conventional 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.

Table 2. digitalMLPA Probemix D006-A1 Multiple Myeloma

Chromosomal position (hg38)		Gene	NM sequence [a)]	# probes / # exons in gene	Gene / region length	Remarks
1p (33 probes)	1p36.33	*TMEM240*		1 probe per gene	43 **M**b	
		CFAP74				
	1p35.1	*YARS1*				
	1p34.1	*HECTD3*				
	1p32.3	***FAF1***	NM_007051.3	2/19	523 kb	
		CDKN2C	NM_078626.3	3/2	4.9 kb	
		ORC1		1 probe per gene	4.2 **M**b	
	1p32.2	*PLPP3*				
		DAB1	NM_001365792.1	4/15	429 kb	
	1p31.3	*LEPR*		1 probe		
	1p22.1	***EVI5***	NM_001350197.2	4/20	276 kb	
		RPL5	NM_000969.5	3/8	9.9 kb	
	1p21.3	*DPYD*		1 probe per gene	3.0 **M**b	
	1p21.2	*DBT*				
		CDC14A	NM_003672.4	4/16	168 kb	
	1p13.1	*SLC22A15*		1 probe		
	1p12	***TENT5C***	NM_017709.4	2/2	22.3 kb	previous name *FAM46C*
		SPAG17		1 probe		
1q (29 probes)	1q21.1	*PDZK1*		1 probe per gene	1.9 **M**b	
	1q21.2	*BCL9*				
		ANP32E	NM_030920.5	2/7	17.7 kb	
		RPRD2		1 probe		
		MCL1	NM_021960.5	2/3	5.1 kb	
	1q21.3	*NUP210L*		1 probe		
		ADAR	NM_001111.5	2/15	26.1 kb	
		CKS1B	NM_001826.3	3/3	4.6 kb	
	1q23.1	***FCRL5***	NM_031281.3	3/17	39.1 kb	
	1q23.3	***SLAMF7***	NM_021181.5	2/7	15.6 kb	
		NUF2		1 probe	81.3 **M**b	
		PBX1-area		5 probes		gene-poor region; probes are targeting 113-238 kb upstream of *PBX1*
		PBX1		1 probe		
	1q31.3	*KCNT2*		1 probe per gene		
		DENND1B				
	1q44	*ADSS2*				
		DESI2				
3p26.2		***CRBN***	NM_016302.4	3/11	29.7 kb	
3q23		***ATR***	NM_001184.4	3/47	129.5 kb	
4p16.3		***FGFR3***	NM_000142.5	5/18	15.6 kb	
		NSD2	NM_001042424.3	2/22	111 kb	previous name *WHSC1*
6p25.3		***IRF4***	NM_002460.4	2/9	19.7 kb	
6p22.3		***JARID2***	NM_004973.4	2/18	276.0 kb	
6q26		***PRKN***	NM_004562.3	2/12	1.4 **M**b	previous name *PARK2*
7p12.2		***IKZF1***	NM_006060.6	3/8	100.4 kb	
7q34		***BRAF***	NM_004333.6	**1 mutation-specific probe**	205.6 kb	**p.V600E** (c.1799T>A)
8q24.21		***MYC***	NM_002467.6	3/3	6.7 kb	
9q34.3		***TRAF2***	NM_021138.4	2/11	40.1 kb	
11q13.3		***CCND1***	NM_053056.3	3/5	13.3 kb	
11q22.2		***BIRC3***		1 probe	31.5 kb	
		BIRC2	NM_001166.5	2/9	21.9 kb	
11q22.3		***ATM***	NM_000051.4	4/63	146.0 kb	

Chromosomal position (hg38)		Gene	NM sequence [a)]	# probes / # exons in gene	Gene / region length	Remarks
11q25		***NCAPD3***	NM_015261.3	2/35	73.9 kb	
12p13.31		***LTBR***	NM_002342.3	2/10	7.4 kb	
		NCAPD2	NM_014865.4	2/32	37.9 kb	
		CHD4	NM_001273.5	2/40	37.3 kb	
12p13.2		***ETV6***	NM_001987.5	3/8	245.7 kb	
12p13.1		***CDKN1B***	NM_004064.5	2/3	5.0 kb	
		GPRC5D	NM_018654.2	3/4	11.6 kb	
13q (23 probes)	13q12.3	*KATNAL1*		1 probe per gene	13.2 **M**b	
	13q14.11	*ENOX1*				
	13q14.2-q14.3	***RB1***	NM_000321.3	5/27	178.1 kb	
		RCBTB2		1 probe per gene	21.2 **M**b	
		DLEU2				
		KCNRG				
		MIR15A				
		DLEU1	NR_109973.1	2/7		
		DLEU7		1 probe per gene		
		RNASEH2B				
		ATP7B				
		VPS36				
		PCDH8				
	13q21.33	*KLHL1*				
		DIS3	NM_014953.5	2/21	29.7 kb	
	13q34	*ARHGEF7*		1 probe per gene	2.5 **M**b	
		GRK1				
14q32.32		***TRAF3***	NM_145725.3	2/12	134.0 kb	
14q32.33		***IGHD***	NG_001019.6	2/7	8.9 kb	
		IGHM		4/6	4.5 kb	
15q12		***GABRB3***	NM_000814.6	2/9	229.5 kb	
16p13.13		***TNFRSF17***	NM_001192.3	4/3	2.9 kb	BCMA in literature
16q12.1		***CYLD***	NM_001378743.1	2/19	59.9 kb	
16q23.1		***WWOX***	NM_016373.4	2/9	1.1 **M**b	
17p (20 probes)	17p13.3	*VPS53*		1 probe per gene	1.7 **M**b	
		NXN				
	17p13.1	***TP53***	NM_000546.6	14/11	19.1 kb	
		PIK3R6		1 probe per gene	9.0 **M**b	
		USP43				
	17p11.2	*RAI1*				
		MIR33B				
17q12-q21.1		***IKZF3***	NM_012481.5	2/8	106.6 kb	
17q21.31		***MAP3K14***	NM_003954.5	2/16	53.9 kb	
20q12		***MAFB***	NM_005461.5	2/1	3.4 kb	
22q11.23		***SMARCB1***	NM_003073.5	5/9	51.0 kb	
Xp11.3		***KDM6A***	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.

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.

Table 3. Karyotyping probes

Chromosomal position (hg38)	Gene
2p25.3	*TMEM18*
2p25.3	*COLEC11* §
2p22.3	*SPAST* §
2p22.2	*VIT* §
2p15	*PEX13* §
2p11.2	*REEP1*
2q11.2	*CNNM3* §
2q11.2	*NPAS2* §
2q24.3	*SCN1A* §
2q31.1	*ABCB11* §
2q32.2	*COL3A1* §
2q37.3	*CAPN10*
2q37.3	*KIF1A*
3p26.2	*TRNT1*
3p26.1	*SUMF1*
3p24.2	*NR1D2*
3p24.1	*NEK10*
3p12.3	*CNTN3*
3p11.1	*HTR1F*
3q11.2	*CPOX*
3q23	*CLSTN2*
3q24	*SLC9A9*
3q29	*OPA1*
3q29	*ACAP2*
3q29	*RUBCN*
4p16.3	*LETM1* §
4p16.3	*ADD1* §
4p15.32	*LDB2*
4p15.31	*KCNIP4* §
4p15.2	*SEL1L3*
4p15.1	*PCDH7* §
4p13	*ATP8A1* §
4q13.1	*TECRL* §
4q13.3	*UGT2A1*
4q21.1	*G3BP2* §
4q25	*HADH* §
4q31.21	*INPP4B* §
4q31.21	*ZNF827* §
4q31.23	*NR3C2* §
4q33	*CLCN3* §
4q35.1	CYP4V2
4q35.2	*TRIML1*
5p15.33	*IRX4*
5p15.31	*NSUN2*
5p15.2	*CTNND2*
5p15.2	*DNAH5*
5p13.3	*NPR3*
5p13.2	*TTC23L*
5q11.2	*IL31RA*
5q11.2	*MIER3*
5q13.3	*CERT1*
5q14.3	*ADGRV1*
5q23.2	*SNCAIP*
5q23.2	*MARCHF3*
5q31.1	*SLC22A5*
5q31.2	*MYOT*
5q31.2	*GFRA3*
5q31.2	*CTNNA1*
5q31.3	*PCDHA1*

Chromosomal position (hg38)	Gene
5q31.3	*PCDHAC1*
5q31.3	*PCDHAC2*
5q31.3	*PCDHB2*
5q31.3	*PCDHB10*
5q31.3	*SLC25A2*
5q31.3	*TAF7*
5q31.3	*PCDHGA11*
5q32	*SH3TC2*
5q35.3	*COL23A1*
5q35.3	*MAPK9*
6p25.2	*GMDS-DT* §
6p25.2	*SERPINB6*
6p25.2	*PXDC1* §
6p25.2	*ECI2* §
6p22.3	*KIAA0319*
6p22.1	*ZFP57* §
6p21.33	*TNF* §
6p12.3	*PKHD1*
6p12.1	*RAB23* §
6p11.2	*PRIM2* §
6q12	*EYS*
6q13	*COL19A1*
6q13	*RIMS1*
6q22.31	*TBC1D32*
6q22.33	*LAMA2*
6q23.3	*TNFAIP3*
6q25.3	*TFB1M*
6q25.3	*WTAP*
6q25.3	*IGF2R*
6q27	*SMOC2*
6q27	*ERMARD*
7p22.2	*SDK1*
7p22.1	*RADIL*
7p15.3	*RAPGEF5*
7p15.3	*STK31*
7p12.3	*ADCY1*
7p12.3	*ABCA13*
7p11.2	*LANCL2*
7q11.21	*KCTD7*
7q11.22	*GALNT17*
7q31.1	*PNPLA8*
7q31.1	*IFRD1*
7q36.3	*RBM33*
7q36.3	*DYNC2I1*
8p23.3	*FBXO25*
8p23.3	*CLN8*
8p23.1	*GATA4*
8p21.3	*GFRA2*
8p21.3	*TNFRSF10B*
8p21.3	*TNFRSF10A*
8p21.2	*NEFL*
8p12	*RBPMS*
8p12	*GSR*
8p11.23	*ZNF703*
8q11.21	*SNTG1* §
8q12.2	*CHD7* § +
8q21.3	*RMDN1* §
8q21.3	*CPNE3* §
8q22.3	*RRM2B*

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	*EDEM2* §
20q11.23	*SAMHD1* §
20q13.12	*SLC13A3* §
20q13.13	*STAU1* §
20q13.33	*OSBPL2*
20q13.33	*UCKL1*
21q11.2	*RBM11*
21q11.2	*HSPA13*
21q22.11	*ITSN1*
21q22.2	*PSMG1*
21q22.3	*PDE9A*
21q22.3	*PWP2*
21q22.3	*TSPEAR*
22q11.1	*GAB4*
22q11.21	*CECR2*
22q11.21	*HIRA*
22q12.2	*NF2*
22q12.2	*ZMAT5*

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

Odd numbered chromosomes are highlighted grey.

§ Used as reference probe for normalisation purposes in data analysis.
+ Two probes are included for this gene.

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.

Table 4. Related conventional 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, *TRAF3*, chr. 15, *CYLD*, *WWOX*, *TP53*	no
P056 TP53	Contains probes for each exon of *TP53*.	no
P047 RB1	Contains probes for each exon of *RB1*, except exon 15.	yes *
P041/P042 ATM-1/-2	Contain probes for each exon of *ATM*.	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, *TP53*.	yes *
P480 WHS & Achondroplasia	Contains probes for 4p16.3 including *NSD2* and *FGFR3*.	yes *
P244 AIP-MEN1-CDKN1B	Contains six probes on chromosomal arm 12p (including *CDKN1B*).	yes *
P202 IKZF1-ERG	Contains probes for each exon of *IKZF1* and 14q32.33, among others.	yes *
P335 ALL-IKZF1	Contains probes for *IKZF1*, *ETV6* and *RB1*, among others.	yes *
P037/P038/P040 CLL-1/-2	Contain probes for 2p, 6q, 8p, 8q (*MYC*), 11q (*ATM*), chr. 12, 13q14 (*RB1*, *DLEU1/2*), *TP53*, 14q32.33, 17p (*TP53*), chr. 19.	yes *
P258 SMARCB1	Contains probes for each exon of *SMARCB1* and flanking regions on 22q.	yes *
P414 MDS	Contains probes for chr. 3, 5q, 7q, 8q, 11q, 12p (*ETV6*), chr. 17 (*TP53*), 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 *TP53*, among others.	no
P051/P052 Parkinson mix 1/2	Contain probes for each exon of *PRKN*.	yes *
P064 Microdeletion Syndromes-1B	Contains nine probes for *NSD2*, among others.	yes *
P377 Hematologic Malignancies	Contains several probes for *IKZF1*, *MYC*, *ATM*, *ETV6*, 13q14, *TP53*, among others.	yes *
P078 Breast tumour	Contains probes for *MYC*, *CCND1*, among others.	
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 *BRAF* 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 the D006-A1 digitalMLPA probemix are mentioned in this table. 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-A1 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@mrcholland.com.
* The reference probes included in this conventional 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.

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Selected publications using digitalMLPA Probemix D006 Multiple Myeloma

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- 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 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-01 – 08 November 2022 (03) - Not applicable, new document.

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