Product Description

SALSA® MLPA® Probemix P420-B1 MPN mix 1

To be used with the MLPA General Protocol.

Version B1
For complete product history see page 8.

The **P420-B1 MPN mix 1** contains eight MLPA probes allowing the detection diagnostically relevant gene mutations frequently detected in myeloproliferative neoplasm (MPN) samples. A minimum of 10-20% allelic burden in the patient sample is required for detection of these mutations. For lower allelic burden detection of these mutations **P520-A2 MPN mix 2** is available, containing the same mutation-specific probes as the P420-B1 probemix. **P520-A2** allows higher detection sensitivity for point mutations, as low as 1% allelic burden, for reliable detection of these eight specific mutations.

For data binning in the fragment analysis procedure, and as an artificial positive control for all the mutation-specific probes, an artificial DNA sample (SD069) is supplied with each P420-B1 order and should be used in each P420 experiment.

Please note that this probemix should be used only as a tool for determining mutation presence (or absence) and should NOT used as a quantitative tool for measuring the exact mutation burden. In addition, positive controls should be included both in the validation phase and in further experiments with P420.

Catalogue numbers:

- **P420-025R:** SALSA MLPA Probemix P420 MPN mix 1, 25 reactions.
- **P420-050R:** SALSA MLPA Probemix P420 MPN mix 1, 50 reactions.
- **P420-100R:** SALSA MLPA Probemix P420 MPN mix 1, 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 MLPA probemix cannot detect any mutations that are outside the target sequences of the MLPA probes. Neither can it be used for copy number detection of the aforementioned target genes. Even when MLPA analysis did not detect any aberrations, the possibility remains that changes in the corresponding gene(s) do exist but remain undetected. This probemix covers only the most frequent mutations of clinical and diagnostic relevance in MPNs. Rare or infrequent mutations are not covered. **If mutation calling with P420 MLPA probemix is negative for a patient sample, we recommend first to repeat analysis with P520 MLPA probemix, and then if still negative to perform sequencing of the *JAK2*, *MPL*, *CALR* and *KIT* genes to detect the rare or unknown mutations possibly present in a sample.**

General information
The SALSA MLPA Probemix P420 MPN mix 1 is a **research use only (RUO)** assay for detection of eight different mutations frequently found in MPNs in the *JAK2, MPL, CALR* and *KIT* genes.

MPNs are clonal hematopoietic stem cell malignancies, characterized by excessive production of blood cells. MPNs are subdivided in polycytemia vera (PV), essential thrombocytemia (ET), primary myelofibrosis (PMF) and less common conditions like chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL), hypereosinophilic syndrome (HES) and systemic mastocytosis (SM).

Discovery of a frequent *JAK2* mutation (9p24.1), common to classic MPNs (PV, ET and PMF), has linked these diseases on a molecular level. The current WHO diagnostic criteria for classic MPNs include presence of *JAK2, CALR* or *MPL* mutations. The *JAK2* V617F point mutation is detected in ~98% of PV patients, and in ~60% of patients with ET and PMF, whereas other *JAK2* exon 12 mutations are commonly found in V617F-negative PV patients. P420-B1 MPN mix 1 probemix contains three mutation-specific *JAK2* probes: one probe for V617F and two probes for the most common exon 12 mutations N542_E543del and E543_D544del.

Mutations in the *MPL* gene (1p34.2) are found in 4-11% of *JAK2* V617F negative ET and PMF patients. This problemix contains two mutation-specific probes for *MPL*, W515K and W515L, that are diagnostically relevant in PV, ET and PMF according to the WHO classification.

The discovery of novel *CALR* gene (19p13.13) mutations in ET and PMF provides additional diagnostic tools for MPNs. Patients with ET and PMF but negative for *JAK2* and *MPL* mutations, have been reported to harbour somatic insertions and deletions in exon 9 of the *CALR* gene. A 52-bp deletion (type 1) and a 5-bp insertion (type 2) are the most common mutations found in the *CALR* gene (53% and 32%, respectively). These mutations result in a frameshift to an alternative reading frame (Klampfl et al. 2013, Nangalia et al. 2013). *CALR* mutation-specific probes for the 52-bp deletion (L367fs*46, type 1) and 5-bp insertion (K385fs*47, type 2) are included in this probemix.

In addition, a probe specific for the D816V mutation in the *KIT* gene (4q12) is present. This is the most common *KIT* mutation and is present in >90% of patients with SM. Consequently, the presence of this mutation is considered a diagnostic criterion of SM according to the WHO classification.

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

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
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The *JAK2, MPL, CALR* and *KIT* exon numbering used in this P420-B1 MPN mix 2 product description is the exon numbering from the LRG_612 (*JAK2*), LRG_510 (*MPL*), LRG_828 (*CALR*) and LRG_307 (*KIT*). The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P420-B1 MPN mix 1 contains 25 MLPA probes with amplification products between 115 and 338 nucleotides (nt). This includes eight mutation-specific probes for the following mutations:

***JAK2* p.V617F**	= c.1849G>T
***JAK2* p.E543_D544del**	= c.1627_1632delGAAGAT
***JAK2* p.N542_E543del**	= c.1624_1629delAATGAA
***MPL* p.W515L**	= c.1544G>T
***MPL* p.W515K**	= c.1543_1544TG>AA
***KIT* p.D816V**	= c.2447A>T
***CALR* p.L367fs*46**	= c.1092_1143del52
***CALR* p.K385fs*47**	= c.1154_1155insTTGTC

These above mentioned eight probes will only generate a signal when the specific mutation is present. In addition, 17 reference probes are included that target relatively copy number stable regions in various cancer types including MPNs. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

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

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different healthy individuals without a history of MPN or other relevant hematologic condition. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive and negative control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. The quality of cell lines can change; therefore samples should be validated before use. Please find below some sources for commercial positive reference samples, tested by MRC Holland:

- Reference standard sample for the *JAK2* p.V617F point mutation (Catalog IDs: HD649 for mutant and HD652 for WT) available at www.horizondiscovery.com (Horizon Discovery).
- *JAK2* p.V617F WHO reference panel (code 16/120) (nominal values 100% [100%], 90% [89.5%], 30% [29.6%], 10% [10.8%], 1% [1.0%], 0.1% [0.03%] and 0% [0%] VAF – in square brackets is the proposed consensus value by WHO committee) - www.nibsc.org **Error! Hyperlink reference not valid.**(The National Institute for Biological Standards and Control - NIBSC).

- Quan-Plex™ NGS Reference Standard Genomic DNA (Catalog ID: ARF-1001G-1 for mutant) including *KIT* p.D816V and Onco-Ref™ Genomic DNA Reference Standard HCT116 WT (Catalog ID: ASO-6052-1) – www.accuref.com (AccuRef).

We highly recommend inclusion of **negative control samples** from healthy individuals in the experiments to evaluate the background signal for the mutation-specific probes in the MLPA peak pattern for your specific types of samples and DNA extraction method used.

Additionally, we highly recommend to also include **mutation-positive samples (cell line or patient derived) extracted with the same DNA extraction method as the test samples** in MLPA experiments to optimise mutation calling for the test samples.

SALSA Binning DNA SD069

The SD069 Binning DNA provided with this probemix can be used for binning of all probes including the following eight mutation-specific probes: *CALR* L367fs*46 (S0999-L26702; 124 nt), *CALR* K385fs*47 (S1001-L26517; 130 nt), *JAK2* N542_E543del (16924-L21237; 167 nt), *JAK2* E543_D544del (16924-L21238; 172 nt), *MPL* W515K (S1048-SP0405-L29870; 181 nt), *MPL* W515L (S1048-SP0405-L29871; 186 nt), *KIT* D816V (17722-SP0542-L23707; 200 nt), and *JAK2* V617F (13190-L21572; 240 nt). SD069 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains target sequences detected by the above mentioned probes. Inclusion of one reaction with 5 µl SD069 Binning DNA in each MLPA experiment 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(s). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD069 Binning DNA product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

Data analysis

Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results

The standard deviation of each individual probe over all the reference samples should be ≤0.10. Comparison of the relative peak height of a mutation-specific probe (vs. reference probes) on a positive sample with the relative peak height obtained with that probe on the SD069 Binning DNA provides an indication of the presence for the corresponding mutation. SD069 contains an estimated 50% allele burden for each aforementioned mutations.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios are affected both by percentage of tumour cells and by possible subclonality.

- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure

- This SALSA MLPA probemix P420 is designed to detect the presence of the aforementioned mutations in a DNA sample. Note that a mutation or polymorphism in the sequence detected by a probe can also cause a reduction in relative peak height, even when not located exactly on the ligation site, and this can lead to false positive or negative results! In addition, some probe signals can be more sensitive to sample purity and small changes in experimental conditions. Unlike majority of MLPA probemixes this P420 does not provide copy number information of the target probes.
- MLPA cannot detect mutations that are not included in this assay. 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.
- MLPA 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 final 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

Confirmation of the presence of a specific point mutation can be done by e.g. Sanger sequencing, next generation sequencing, qPCR, or digital droplet PCR. In particular, it is recommended to confirm positive mutation calls with low mutation burden with the above mentioned methods.

We recommended to use P520 MPN mix 2 for test samples when testing with P420 MPN mix 1 is negative or when the mutation specific peak ratios are low in order to confirm the result. And if still negative to perform sequencing of the *JAK2, MPL, CALR* and *KIT* genes to detect the rare or unknown mutations possibly present in a sample.

The P420 assay is a conventional MLPA assay allowing detection of mutations on samples with >10-20% allele burden (up to 100% allele burden). For samples with estimated 1-10% allele burden we recommend to use P520 as qualitative tool for mutation detection.

COSMIC mutation database

http://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in the COSMIC database. 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 or false negative results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P420-B1 MPN mix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)	
		Reference	**Mutation**
64-105	Control fragments – see table in probemix content section for more information		
115	Reference probe S0973-L26704	4p13	
124 §	**CALR probe** S0999-L26702		**p.L367fs*46**=c.1092_1143del52
130 §	**CALR probe** S1001-L26517		**p.K385fs*47**=c.1154_1155insTTGTC
136	Reference probe 16316-L25926	3q21	
142	Reference probe 07387-L26769	12q13	
148	Reference probe 10663-L11245	6p12	
154	Reference probe 13781-L15275	11p14	
160	Reference probe 17621-L21665	10q22	
167 § £	**JAK2 probe** 16924-L21237		**p.N542_E543del**=c.1624_1629delAATGAA
172 § £	**JAK2 probe** 16924-L21238		**p.E543_D544del**=c.1627_1632delGAAGAT
181 § Ж	**MPL probe** S1048-SP0405-L29870		**p.W515K**=c.1543_1544TG>AA
186 § Ж	**MPL probe** S1048-SP0405-L29871		**p.W515L**=c.1544G>T
193	Reference probe 11556-L26606	5q31	
200 § Ж	**KIT probe** 17722-SP0542-L23707		**p.D816V**=c.2447A>T
230	Reference probe 17130-L26574	11p11	
240 § ∑	**JAK2 probe** 13190-L21572		**p.V617F**=c.1849G>T
250	Reference probe 05386-L29196	12p11	
256	Reference probe 13572-L30062	1q23	
265	Reference probe 12434-L26073	14q24	
277	Reference probe 16270-L26771	20q11	
287	Reference probe 05713-L20268	2p11	
297	Reference probe 04570-L20036	16q13	
313	Reference probe 04833-L20693	5p13	
323	Reference probe 20640-L26608	6q12	
338	Reference probe 12785-L15496	2q13	

§ Mutation-specific probe. This probe will generate a signal when the mutation is present.
Ж This probe consists of three parts and has two ligation sites.
£ **When probe signals at 167 nt and 172 nt are both present in the MLPA peak pattern, it is indicative for the *JAK2* E543_D544del mutation. When only the probe signal at 167 nt is present, it is indicative for the *JAK2* N542_E543del mutation**.
∑ This probe can have a very low unspecific background signal detected also in healthy control samples.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. P420-B1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / exon[a] / mutation	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)
***MPL* gene**, at 1p34.2, indicated ligation sites are in NM_005373.3. Mutations in the thrombopoietin receptor (*MPL*) gene, most commonly W515L and W515K substitutions in exon 10, are detected in patients with ET and PMF, which can support diagnosis when *JAK2* is not mutated. W515L and W515K are reported to be the predominant *MPL* mutations in MPNs (Ma W et al. 2011).				
186 § Ж	S1048-SP0405-L29871	***MPL***, exon 10, **p.W515L=c.1544G>T**	1575-1576 and 25 nt after exon 10	GCTGCTGATGT**T**-46nt spanning oligo-TGGCGGTGGACC
181 § Ж	S1048-SP0405-L29870	***MPL***, exon 10, **p.W515K=c.1543_1544TG>AA**	1575-1576 and 25 nt after exon 10	GCTGCTGAGG**AA**-46nt spanning oligo-TGGCGGTGGACC
***KIT* gene**, at 4q12, indicated ligation sites are in NM_000222.3. Somatic activating *KIT* D816V mutation is present in more than 97% of SM patient samples (Erben P et al. 2014). This D816V mutation results in ligand-independent activation of c-KIT tyrosine kinase, and detection of this point mutation aids in prediction of response to tyrosine kinase inhibitor (TKI) therapy (imatinib).				
200 § Ж	17722-SP0542-L23707	***KIT***, exon 17, **p.D816V=c.2447A>T**	2505-2504 and 2467-2466 *reverse*	CATTCTTGATG**A**-38nt spanning oligo-CCATGAGTAAGG
***JAK2* gene,** at 9q24.1, indicated ligation sites are in NM_004972.4. Discovery of a *JAK2* mutation common to MF, PV and ET has linked these diseases on a molecular level, and diagnostic criteria for MPN include detection of a clonal marker e.g. *JAK2* V617F mutation in exon 14 or exon 12 mutations. *JAK2* is mutated in 97% of all patients with PV, 55% of patients with ET and 65% of patients with PMF (Tefferi A, 2010). The most common *JAK2* mutations in exon 12 are the following 6-bp deletions: N542_E543del and E543_D544del.				
167 § £	16924-L21237	***JAK2*,** exon 12, **p.N542_E543del** **=c.1624_1629delAATGAA**	2099-2100	AAAATCAGAGAT-TTGATATTTGTA
172 § £	16924-L21238	***JAK2*,** exon 12, **p.E543_D544del** **=c.1627_1632delGAAGAT**	2099-2100	AAAATCAGAAAT-TTGATATTTGTA
240 § ∑	13190-L21572	***JAK2*,** exon 14, **p.V617F=c.1849G>T**	2316-2315 *reverse*	GTCTCCACAGA**A**-ACATACTCCATA
***CALR* gene**, at 19p13.13, indicated ligation sites are in NM_004343.4. Majority of patients with ET or PM that are negative for *JAK2* and *MPL* carry a somatic mutation in *CALR* gene. A 52-bp deletion (type 1=L367fs*46) and 5-bp insertion (type 2=K385fs*47) are the most common mutations found in *CALR* (53% and 32%, respectively), resulting in a frameshift to an alternative reading frame (Klampfl T et al. 2013, Nangalia J et al. 2013).				
124 §	S0999-L26702	***CALR***, exon 9, **p.L367fs*46** **=c.1092_1143del52**	1163-1214 *reverse*	CTTGTCCTCTGC-TCCTCGTCCTGT
130 §	S1001-L26517	***CALR***, exon 9, **p.K385fs*47** **=c.1154_1155insTTGTC**	1225-1226	CAGAGGACAA**TT-GTC**GGAGGATGA

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

[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

§ Mutation-specific probe. This probe will generate a signal when the mutation is present.
Ж This probe consists of three parts and has two ligation sites.
£ When probe signals at 167 nt and 172 nt both are present in the MLPA peak pattern, it is indicative for the *JAK2* E543_D544del mutation. When only the probe signal at 167 nt is present, it is indicative for the *JAK2* N542_E543del mutation.
∑ This probe can have a very low unspecific background signal detected also in healthy control samples.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Complete probe sequences are available at www.mrcholland.com.

Related SALSA MLPA probemixes

P520 MPN mix 2	This probemix is a high sensitivity MLPA probemix allowing detection of the same mutations as included in P420 on samples with >1% allele burden.
P414 MDS	This probemix contains probes for chromosome 3, 5q (*EGR1, MIR145, SPARC, MIR146A*), 7q (*EZH2*), 8q (*MYC*), 11q (*KMT2A*), 12p (*ETV6*), chromosome 17 (*TP53, NF1, SUZ12*), chromosome 19, 20q (*ASXL1*), Y-chromosome, and for *JAK2* V617F mutation.
P377 Hematologic malignancies	This probemix contains probes for 2p (*MYCN, ALK*), 5q (*MIR145, EBF1, MIR146A*), 6q, 7p12 (*IKZF1*), 7q, 8q24 (*MYC*), 9p (*MTAP, CDKN2A, CDKN2B, PAX5*), 10q23 (*PTEN*), 11q22 (*ATM*), 12p13 (*ETV6, CCND2, MDM2*), 12q, 13q14 (*RB1, MIR15A, DLEU2, DLEU1*), 17p13 (*TP53*), 17q, Chr 18, Chr 19, 21q22 (*RUNX1*) and for *JAK2* V617F mutation.

References

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P420 product history	
Version	*Modification*
B1	One target probe and one reference probe have been removed, and several probes have a change in length but no change in the sequences detected.
A1	First release.

Implemented changes in the product description
Version B1-07 - 13 July 2022 (04P) - Text corrected on page 1 and 4: SD069 should be used in each P420 experiment *Version B1-06 – 07 July 2021 (04P)* - Product description rewritten and adapted to a new template. - Various minor textual or layout changes. - Ligation sites of the probes targeting the name gene updated according to newest versions of the NM_ reference sequence for *JAK2, MPL, CALR* and *KIT* genes. - Small changes of probe lengths in Table 1 in order to better reflect the true lengths of the amplification products.

Version 05 (T08) – 16 February 2021
- Details about the SD069 adjusted: plasmid DNA used instead of synthetic DNA.
- Small change of probe length for the two MPL mutation-specific probes (in Table 1 and 2) in order to better reflect the true lengths of the amplification products.
- Sample pictures updated, because of changed probes lengths.

Version 04 (T08) – 18 October 2018
- Use of P520 MPN mix 2 changed in the related SALSA MLPA probemixes section.
- Minor textual and layout changes.

Version 03 (T08) – 26 September 2017
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new pictures included).
- Various textual changes throughout the document.

More information: www.mrcholland.com; www.mrcholland.eu	
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