Product Description SALSA® MLPA® Probemix P419-B1 CDKN2A/2B-CDK4

To be used with the MLPA General Protocol.

Version B1. As compared to version A2, two probes for *CDK4* are replaced and one is added, one probe for *CDKN2A* is replaced, one probe for *CDKN2B* is added, several reference probes are replaced, and several differences in lengths but not in the sequences detected. For complete product history see page 10.

Catalogue numbers:
- **P419-025R:** SALSA MLPA Probemix P419 CDKN2A/2B-CDK4, 25 reactions.
- **P419-050R:** SALSA MLPA Probemix P419 CDKN2A/2B-CDK4, 50 reactions.
- **P419-100R:** SALSA MLPA Probemix P419 CDKN2A/2B-CDK4, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions, and Coffalyser.Net. 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.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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.

General information: The SALSA MLPA Probemix P419 CDKN2A/2B-CDK4 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *CDKN2A, CDKN2B* and *CDK4* genes, which are associated with familial cutaneous melanoma and pancreatic cancer syndromes. This probemix can also be used to detect the presence of a *MITF* p.E318K (c.952G>A) point mutation and status of codon 24 of the *CDK4* gene with a WT probe.

Malignant melanoma is estimated to be hereditary in 5-10% of the cases. Familial cutaneous melanoma arises in an autosomal-dominant pattern within the affected families and germline alterations in *CDKN2A* gene (9p21.3) are detected in up to 40% of cases (Goldstein et al. 2007). The *CDKN2A* gene encodes for two proteins read in alternative reading frames, namely $p16^{INK4A}$ and $p14^{ARF}$. The majority of *CDKN2A* mutations detected in familial melanoma patients affect exons 2 and 3, which are coding for $p16^{INK4A}$. However, exon 1 (previously known as 1β) mutations specific for $p14^{ARF}$ are also reported (Hewitt et al. 2002), as well as larger genomic deletions of *CDKN2A* (Randerson-Moor et al. 2001; Knappskog et al. 2006; Lesueur et al. 2008). Moreover, both heterozygous and homozygous deletions harbouring both *CDKN2A* and *CDKN2B* are frequently detected in somatic melanoma samples (Flores et al. 1996).

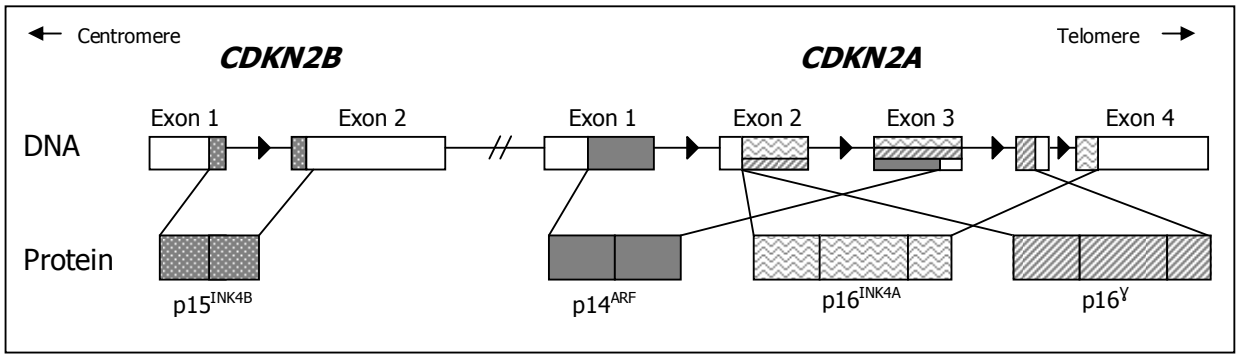
Another high-penetrance, but low-frequency melanoma susceptibility gene is *CDK4* (12q14.1), which is mutated in 2% of the melanoma families (Goldstein et al. 2007). Two point mutations (p.R24H and p.R24K) of *CDK4* have been detected in familial melanoma cases (Zuo et al. 1996). In addition, a germline mutation p.E318K (c.952G>A) of *MITF* gene has been suggested to be associated with predisposition to familial melanoma (Yokoyama et al. 2011; Bertolotto et al. 2011).

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 *CDKN2A* exon numbering used in this P419 CDKN2A/2B-CDK4 product description is the exon numbering from LRG_11, which is based on NG_007485.1 and RefSeq transcripts NM_000077 ($p14^{ARF}$) and NM_058195 ($p16^{INK4A}$). The exon numbering for *CDKN2B* is from the RefSeq transcript NM_078487 ($p15^{INK4B}$) identical to NG_023297.1. The exon numbering for *CDK4* is from LRG_490, based on NG_007484.2 and RefSeq transcript NM_000075. The exon numbering and NM sequence used have been retrieved in 12/2018. As changes to the NCBI or LRG databases can occur after release of this product description, exon numbering may not be up-to-date.

Schematic presentation 1. *CDKN2A* and *CDKN2B* gene structures and proteins encoded



Note for schematic presentation 1: An alternative exon (330 nt before exon 4) for *CDKN2A* is present in NM_001195132.1 transcript variant 5. In this P419 probemix two probes for this alternative exon are included, probe 17807-L22411 at 157 nt and probe 17811-L21945 at 184 nt. The clinical significance of this transcript variant, also known as $p16^{\gamma}$ (Lin et al. 2007) is not yet know. Transcript variant 5 includes an additional exon that causes a frameshift in the 3' coding region when compared to variant 1 (encoding $p16^{INK4A}$). The resulting isoform $p16^{\gamma}$ has a distinct C-terminus, which is longer than $p16^{INK4A}$. However, this transcript is candidate for nonsense-mediated mRNA decay and it is not known if endogenous protein $p16^{\gamma}$ is expressed *in vivo*.

Probemix content: The SALSA MLPA Probemix P419-B1 CDKN2A/2B-CDK4 contains 57 MLPA probes with amplification products between 121 and 504 nucleotides (nt). This includes 14 probes for the *CDKN2A* gene, nine probes for the *CDKN2B* gene, nine probes for *CDK4* gene and in total 10 flanking probes for *CDKN2A* and *CDKN2B* genes. Furthermore, this probemix contains one probe specific for the *MITF* p.E318K (c.952G>A) point mutation, which will only generate a signal when the mutation is present, and one wildtype probe for *CDK4* codon 24, which will have a drop in signal in case of a *CDK4* codon 24 mutation. In addition, 13 reference probes are included that detect relatively copy number stable regions in various cancer types including cutaneous melanoma. Complete probe sequences are available online (www.mlpa.com) and the identity of the genes detected by the reference probes is available in Table 2d.

This probemix contains nine quality control fragments generating amplification products between 64 and 120 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.mlpa.com.

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

No DNA controls result in only five major peaks shorter than 121 nt: four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-250 ng) is used.

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

Required specimens: Extracted DNA, which includes DNA derived from paraffin-embedded tissues, 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. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. 2017.

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 unrelated individuals who are from families without a history of familial melanoma, pancreatic cancer syndrome or other conditions related to *CDKN2A/2B* or *CDK4* genes. More information regarding the selection and use of reference samples can be found in the MLPA 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 MLPA experiments. Samples from the Coriell Institute have been tested at MRC-Holland with the P419-B1 probemix and can be used to detect a heterozygous duplication of 9p21.3 region (NA03226 and NA05067) and *MITF* p.E318K (c.952G>A) point mutation (HG00259 and HG01498). The following samples from Leibniz Institute DSMZ have been tested at MRC-Holland with P419-B1 probemix and can be used as positive control samples: ACC-47/DOHH-2 for a homozygous deletion of *MTAP, CDKN2A/2B* and *DMRTA1;* ACC-264/COLO-679 for a heterozygous deletion of *MLLT3, MIR31, MTAP* and *CDKN2B,* and homozygous deletion of *CDKN2A;* and ACC-573/SU-DHL-8 for a heterozygous duplication of *CDK4.* The quality of cell lines can change, therefore positive samples should either be acquired from quality assessed biological sample repositories or be validated before use.

SALSA Binning DNA SD008: The SD008 Binning DNA provided with this probemix can be used for binning of *MITF* mutation-specific probe (probe 17808-L23191 at 161 nt). SD008 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD008 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal, as for this purpose true mutation positive patient samples or cell lines should be used. 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 SD008 Binning DNA product description provided. **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.mlpa.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. When this criteria is fulfilled, the following cut-off values for the dosage quotient (DQ) of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

Please note that these above mentioned dosage quotients are only valid for germline testing. Dosage quotients are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic or subclonal cases. Analysis of parental samples may be necessary for correct interpretation of complex results of hereditary 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. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *CDK4* gene. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.

P419 specific note:

- In samples from tumour tissues, reference probes are more prone to have deviating copy number results than in blood derived germline samples. When regions targeted by reference probes are affected by copy number alterations it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct interpretation of the target region.

Limitations of the procedure:

- In most populations, the most genetic alterations in *CDKN2A, CDKN2B* and *CDK4* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P419 CDKN2A/2B-CDK4.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

- 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 solid tumours with more chaotic karyotypes.

Confirmation of results: Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

LOVD and COSMIC mutation databases:
CDKN2A database (LOVD) - https://databases.lovd.nl/shared/genes/CDKN2A
CDK4 database (LOVD) - https://databases.lovd.nl/shared/genes/CDK4
COSMIC database for somatic mutations in cancer - https://cancer.sanger.ac.uk/cosmic
We strongly encourage users to deposit positive results in the LOVD and 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 SNPs and unusual results (e.g. a duplication of *CDKN2A* exons 2 and 4 but not exon 3) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P419-B1 CDKN2A/2B-CDK4

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)				
		Reference	CDKN2A	CDKN2B	CDK4	Other
64-105	Control fragments – see table in probemix content section for more information					
121 *	Reference probe 19616-L27455	4p13				
128 *	Reference probe 21566-L30677	18q21				
133 *	**CDKN2B probe** 22023-L30944			Exon 2		
140	**CDKN2B probe** 11867-L23435			Exon 1		
145	Reference probe 14199-L22409	2q13				
151 «	**CDK4 probe** 03173-L22410				Exon 3	
157 Ø	**CDKN2A probe** 17807-L22411		Int 3			
161 §	**MITF probe** 17808-L23191					**p.E318K** (c.952G>A)
166 «	**CDK4 probe** 17809-L21943				Exon 2	
172	Reference probe 12741-L21983	21q22				
178 «	**CDK4 probe** 17810-L21944				Exon 1	
184 Ø	**CDKN2A probe** 17811-L21945		Int 3			
190 «	**CDK4 probe** 17812-L23573				Exon 6	
196 *	Reference probe 05268-L30942	2p22				
202 ¬	**MLLT3 probe** 01287-L23185					9p21.3
208	**CDKN2B probe** 16066-L23293			Exon 1		
216 ∞« Ж	**CDK4 probe** 17813-SP0552-L22575				**WT codon 24**	
222	**CDKN2B probe** 11871-L22416			Exon 1		
229	**CDKN2B probe** 16059-L18233			Exon 2		
236 ¥	**CDKN2A probe** 21912-L19021		Exon 4			
242 ¥	**CDKN2A probe** 01289-L31535		Exon 2			
250 *	Reference probe 18056-L31125	16q23				
256 ¥	**CDKN2A probe** 16060-L31124		Exon 2			
263 ¥	**CDKN2A probe** 15674-L31123		Exon 3			
269 ¥	**CDKN2A probe** 15675-L31122		Exon 4			
275 ¥ «	**CDK4 probe** 17735-L31121				Exon 8	
283	**CDKN2A probe** 01291-L22469		Exon 4			
288 *	Reference probe 08834-L30725	2p13				
294 ¬	**DMRTA1 probe** 18242-L23192					9p21.3
301 ¬	**MTAP probe** 15677-L21991					9p21.3
307 ¬	**TMC1 probe** 08987-L23572					9**q**21.13
314	Reference probe 17876-L23179	19q13				
319 ¬	**MTAP probe** 01293-L22907					9p21.3
328 ¬	**MTAP probe** 01294-L13278					9p21.3
335	Reference probe 09776-L23522	15q15				
341 ¥	**CDKN2A probe** 14004-L31117		Exon 1			
349 ¬	**DMRTA1 probe** 18243-L23511					9p21.3
357 «	**CDK4 probe** 17815-L23512				Exon 4	
364 *	**CDKN2A probe** 21951-L30767		Exon 3			
373	**CDKN2B probe** 15992-L23660			Exon 1		
379	Reference probe 05288-L23294	14q22				
385	**CDKN2A probe** 17817-L23295		Exon 3			
394 ¥	**CDKN2A probe** 08659-L30724		Exon 2			
400 * «	**CDK4 probe** 21952-L30768				Exon 7	
409	**CDKN2B probe** 03814-L03851			Exon 1		
418 * «	**CDK4 probe** 21950-L30943				Exon 8	
425 ¥	**CDKN2A probe** 15680-L30801		Exon 1			
432 ¬	**PTENP1 probe** 17311-L23180					9p13.3
440 ¬	**MTAP probe** 17819-L23181					9p21.3
448	**CDKN2B probe** 17820-L23182			Exon 2		
454	Reference probe 09107-L21996	4q25				
463	**CDKN2B probe** 17821-L21955			Exon 2		
470 ¥ ¬	**MIR31 probe** 13665-L22879					9p21.3
477 *	Reference probe 14956-L31118	6q22				
483 * «	**CDK4 probe** 21953-L30769				Exon 5	
490 ¥	**CDKN2A probe** 12475-L30941		Exon 1			
504 *	Reference probe 21229-L30802	10p11				

* New in version B1.

¥ Changed in version B1. Small change in length, no change in sequence detected.

§ Mutation-specific probe. This probe will only generate a signal when the *MITF* p.E318K (c.952G>A) mutation is present. It has been tested on artificial DNA and on positive cell line samples listed on page 3.

∞ Wild type sequence detected. The presence of the *CDK4* codon 24 mutation will result in a decreased probe signal.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Ø This probe is targeting an alternative exon present in NM_001195132.1 transcript variant 5, also known as p16$^{\gamma}$. Clinical and/or diagnostic significance of copy number alterations in this alternative exon is not yet known, please see Schematic representation 1 and the note below this figure on page 2 for further information.

Table 2a. CDKN2A and CDKN2B probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / Exon	Chromosomal band / Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
202 ¬	01287-L23185	MLLT3	9p21.3	GGTCCGGAGCAC-AGTAACATACAG	891.4 kb
470 ¬	13665-L22879	MIR31	9p21.3	GTCCTTCGTGTA-TTGCTGTGTATG	290.7 kb
328 ¬	01294-L13278	MTAP, ex 1	NM_002451.3; 123-122 reverse	GGTGGTGGTGCC-AGAGGCCATGTC	35.2 kb
440 ¬ #	17819-L23181	MTAP, ex 5	NM_002451.3; 502-503	TGGAAGTCATTC-TTGTGCCAGAGG	16.8 kb
319 ¬	01293-L22907	MTAP, ex 6	NM_002451.3; 725-726	GAGGTGGTTCTT-GCTAAGGAGGCT	4.5 kb
301 ¬ #	15677-L21991	MTAP, ex 7	NM_002451.3; 825-826	ACCGGGTCTTAA-AGACCCTGAAAG	108.2 kb
269	15675-L31122	**CDKN2A**, ex 4	NM_000077.4 & NM_058195.3; 181 nt after ex 4	TGAAATGCGGTT-AAAATGATGAAT	0.5 kb
236	21912-L19021	**CDKN2A**, ex 4	NM_000077.4; 932-933 NM_058195.3; 829-830	CCTTTTAACGTA-GATATATGCCTT	0.1 kb
283	01291-L22469	**CDKN2A**, ex 4	NM_000077.4; 786-787 NM_058195.3; 683-684	TGAAAGAACCAG-AGAGGCTCTGAG	0.4 kb
		stop codon	*775-777 (ex 4) of NM_000077.4*		
157 Ø	17807-L22411	**CDKN2A**, int 3	NM_000077.4 & NM_058195.3; 358 nt before ex 4; NM_001195132.1; 934-935	GGGAAAGGCCAC-ATCTTCACGCCT	0.1 kb
184 Ø	17811-L21945	**CDKN2A**, int 3	NM_000077.4 & NM_058195.3; 446 nt before ex 4; NM_001195132.1; 846-847	AGCGCCAGAGCC-TGAGGCGCCCTT	2.3 kb
385	17817-L23295	**CDKN2A**, ex 3	NM_000077.4; 706-707 NM_058195.3; 603-604	TGCGCGCGGCTG-CGGGGGGCACCA	0.2 kb
		stop codon	*557-559 (ex 3) of NM_058195.3*		
364 #	21951-L30767	**CDKN2A**, ex 3	NM_000077.4; 549-548 reverse NM_058195.3; 446-445 reverse	GCGTCGTGCACG-GGTCGGGTGAGA	0.1 kb
263	15674-L31123	**CDKN2A**, ex 3	NM_000077.4 & NM_058195.3; 45 nt before ex 3	TCCTTTCCGTCA-TGCCGGCCCCCA	3.5 kb
242	01289-L31535	**CDKN2A**, ex 2	NM_000077.4; 434-433 reverse NM_058195.3; 3.5 kb before ex 3	TCCGACCGTAAC-TATTCGGTGCGT	0.2 kb
		start codon	*307-309 (ex 2) of NM_000077.4*		
394	08659-L30724	**CDKN2A**, ex 2	NM_000077.4; 205-206 NM_058195.3; 3.7 kb before ex 3	GCACCGGAGGAA-GAAAGAGGAGGG	0.1 kb
256	16060-L31124	**CDKN2A**, ex 2	NM_000077.4; 138-139 NM_058195.3; 3.8 kb before ex 3	GCCTGGAAAGAT-ACCGCGGTCCCT	19.3 kb
341	14004-L31117	**CDKN2A**, ex 1	NM_000077.4; 19.4 kb to ex 2 NM_058195.3; 191-192	TGACCCTCCGGA-TTCGGCGCGCGT	0.1 kb
		start codon	*161-163 (ex 1) of NM_058195.3*		
425	15680-L30801	**CDKN2A**, ex 1	NM_000077.4; 19.3 kb to ex 2 NM_058195.3; 76-77	AGTCTGCAGTTA-AGGGGGCAGGAG	0.2 kb
490	12475-L30941	**CDKN2A**, ex 1	NM_000077.4; 19.2 kb to ex 2 NM_058195.3; 76 nt before ex 1	CGCAGGGCTCAG-AGCCGTTCCGAG	8.4 kb
448	17820-L23182	**CDKN2B**, ex 2	NM_078487.2; 3907-3908	GACATTACCAAG-GTTTGTACAAAT	1.9 kb
463	17821-L21955	**CDKN2B**, ex 2	NM_078487.2; 2054-2055	TCAGGGTGCAGA-GGTCAGACTAAG	1.0 kb
229	16059-L18233	**CDKN2B**, ex 2	NM_078487.2; 1030-1031	GCCTGTCTGAGA-CTCACAGGAAGG	0.2 kb
133 #	22023-L30944	**CDKN2B**, ex 2	NM_078487.2; 822-821 reverse	ACGGGCAGACGA-CCCCAGGCATCG	2.8 kb

Length (nt)	SALSA MLPA probe	Gene / Exon	Chromosomal band / Ligation site	**Partial** sequence (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*595-597 (ex 1)*		
409	03814-L03851	**CDKN2B**, ex 1	NM_078487.2; 470-471	CCTGGAAGCCGG-CGCGGATCCCAA	0.1 kb
		start codon	*361-363 (ex 1)*		
140	11867-L23435	**CDKN2B**, ex 1	NM_078487.2; 327-328	CCAACGGTGGAT-TATCCGGGCCGC	0.1 kb
222	11871-L22416	**CDKN2B**, ex 1	NM_078487.2; 260-261	TCCTAGGAAGGA-GAGAGTGCGCCG	0.3 kb
208	16066-L23293	**CDKN2B**, ex 1	NM_078487.2; 83 nt before ex 1	CCTCCCGGCGAT-CACAGCGGACAG	0.1 kb
373	15992-L23660	**CDKN2B**, ex 1	NM_078487.2; 155 nt before ex 1	GTCTCTGGCGCA-TGCGTCCTAGCA	437.6 kb
294 ¬	18242-L23192	DMRTA1, ex 1	9p21.3	GCGGTCAGCGCA-CTTTCCACTTGG	4.4 kb
349 ¬	18243-L23511	DMRTA1, ex 2	9p21.3	GGCTAGAAGGCA-TTCTACGGTTCT	11.2 **M**b
432 ¬	17311-L23180	PTENP1	9p13.3	CAAATCTAATTA-CAGAGTTGCGCA	40.9 **M**b
307 ¬	08987-L23572	TMC1	9**q**21.13	ATTCCTGAGGTT-TCTGGCTAACTT	-

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Ø This probe is targeting an alternative exon present in NM_001195132.1 transcript variant 5, also known as p16ɣ. Clinical and/or diagnostic significance of copy number alterations in this alternative exon is not yet known, please see Schematic representation 1 and the note below this figure on page 2 for further information.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

Note I: The *CDKN2A* exon numbering was changed from product description version 4 onwards (March 2015); we have adopted the LRG exon numbering that is present in the LGR_11 sequence identical to NG_007485.1 for *CDKN2A*. This exon numbering used here may differ from literature! The *CDKN2A* ligation sites used in this P419 CDKN2A/2B-CDK4 product description are according to the RefSeq transcript NM_000077.4 ($p14^{ARF}$) and NM_058195.3 ($p16^{INK4A}$), corresponding to the LRG_11 sequence. Exon numbering and ligations sites for *CDKN2B* are from the RefSeq transcript NM_078487.2 ($p15^{INK4}$) identical to NG_023297.1. The exon numbering and NM_ sequence used have been retrieved in 12/2018. As changes to the NCBI or LRG databases can occur after release of this product description, ligation site and exon numbering may not be up-to-date. Please notify us of any mistakes: info@mlpa.com.

Table 2b. CDK4 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / Exon	Ligation site	**Partial** sequence (24 nt adjacent to ligation site)	Distance to next probe
275 «	17735-L31121	**CDK4**, ex 8	NM_000075.4; 1157-1158	TCTCTGAGGCTA-TGGAGGGTCCTC	0.2 kb
		stop codon	*1065-1067 (ex 8)*		
418 «	21950-L30943	**CDK4**, ex 8	NM_000075.4; 976-977	CTCCCCTCAGGA-AATGCTGACTTT	0.6 kb
400 «	21952-L30768	**CDK4**, ex 7	NM_000075.4; 959-960	GAGGAGTCGGGA-GCACAGCTGCTG	0.3 kb
190 «	17812-L23573	**CDK4**, ex 6	NM_000075.4; 827-828	GACCAGTTGGGC-AAAATCTTTGAG	1.3 kb
483 «	21953-L30769	**CDK4**, ex 5	NM_000075.4; 689-690	GTTGTTACACTC-TGGTACCGAGCT	0.2 kb
357 «	17815-L23512	**CDK4**, ex 4	NM_000075.4; 644-645	GGCCTGGCCAGA-ATCTACAGCTAC	0.3 kb
151 «	03173-L22410	**CDK4**, ex 3	NM_000075.4; 433-434	AACCCTGGTGTT-TGAGCATGTAGA	0.3 kb
166 «	17809-L21943	**CDK4**, ex 2	NM_000075.4; 303-304	GAGGAGGCCTTC-CCATCAGCACAG	0.1 kb
216 ∞ Ж «	17813-SP0552-L22575	**CDK4**, ex 2	**WT for codon 24 -** NM_000075.4; 225-224 and 197-196 reverse	GTGGGGATCACG-28nt spanning oligo-ACACCAATTTCA	0.7 kb
		start codon	*156-158 (ex 2)*		
178 «	17810-L21944	**CDK4**, ex 1	NM_000075.4; 74 nt before ex 1	TCCGGCGTTCGC-CCCGCCCTCCCA	-

∞ Wild type sequence detected. The presence of the *CDK4* codon 24 mutation will result in a decreased probe signal.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

Note II: The exon numbering for *CDK4* is from LRG_490 (based on NG_007484.2 and RefSeq transcript NM_000075.2) and ligation sites are indicated according to newest RefSeq transcript NM_000075.4. The exon numbering and NM sequence used have been retrieved in 12/2018. As changes to the NCBI or LRG databases can occur after release of this product description, ligation site and exon numbering may not be up-to-date. Please notify us of any mistakes: info@mlpa.com.

Table 2c. MITF mutation specific probe

Length (nt)	SALSA MLPA probe	*MITF* point mutation	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
161 §	17808-L23191	**p.E318K (c.952G>A)**	NM_000248.3; 1075-1076	TCATCAAGCAA**A**-AACCCGTTCTTG	-

§ Mutation-specific probe. This probe will only generate a signal when the *MITF* p.E318K (c.952G>A) mutation is present. It has been tested on artificial DNA and on positive cell line samples listed on page 3.

Table 2d. Reference probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Location (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
196	05268-L30942	SPAST	2p22	GGACGTCTATAA-TGACAGTACTAA	02-032.177
288	08834-L30725	DYSF	2p13	TATTATCTGGAA-TACCAGAGATGT	02-071.750
145	14199-L22409	EDAR	2q13	GAGAGTTCTGTG-GGTGGAGAGAAG	02-108.894
121	19616-L27455	ATP8A1	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042.278
454	09107-L21996	CFI	4q25	ACGATGCATGTA-TCATTAGGTTGG	04-110.887
477	14956-L31118	LAMA2	6q22	GAATGCTGTATG-TTGGTGGGTTAC	06-129.868
504	21229-L30802	CCDC7	10p11	ATCGCCTTAAAC-AGAGGTCTAAAT	10-032.800
379	05288-L23294	ATL1	14q22	AAGCTAACAATT-TAGCAGCCGTGG	14-050.160
335	09776-L23522	SPG11	15q15	TCAGCAGAACAA-ATGGCCCCTTCT	15-042.700
250	18056-L31125	PLCG2	16q23	GATCCAGCAGTA-CTTCCCATCCAA	16-080.518
128	21566-L30677	DCC	18q21	GAGTTGTGGCTT-ACAATGAATGGG	18-048.959
314	17876-L23179	SLC7A9	19q13	CCTAAGACCACC-AGTCTCCAAAAG	19-038.051
172	12741-L21983	RIPK4	21q22	AAGCCAAGAAGA-TGGAGATGGCCA	21-042.050

Related SALSA MLPA probemixes

- ME024 9p21 CDKN2A/2B region: Contains probes for detection of methylation and/or copy number status of the chromosomal region 9p21.3 *(CDKN2A/2B, CDKN2B-AS1, MTAP, MIR31,* and *PAX5).*

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Selected publications using SALSA MLPA probemix P419 CDKN2A/2B-CDK4

- Betti M et al. (2016). CDKN2A and BAP1 germline mutations predispose to melanoma and mesothelioma. *Cancer Lett*. 378:120-30.
- Pramio DT et al. (2016). LINE-1 hypomethylation and mutational status in cutaneous melanomas. *J Investig Med*. 64:899-904.

P419 Product history	
Version	*Modification*
B1	Two probes for *CDK4* are replaced and one is added, one probe for *CDKN2A* is replaced, one probe for *CDKN2B* is added, several reference probes are replaced, and several differences in lengths but not in the sequences detected.
A2	Several probes have a small change in length, but no change in sequence detected.
A1	First release.

Implemented changes in the product description

Version B1-01 — 17 December 2018 (01P)
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Product description adapted to a new template.
- For uniformity, the chromosomal positions and bands in this document are now all based on hg18(NCBI36).

Version 05 – 14 March 2016 (T08)
- Various minor textual and layout changes.
- Schematic presentation 1 at page 7 has been updated to include alternative exon 4 of the NM_001195132.1 transcript, and information about probes covering this alternative exon has been included in note III.

Version 04 – 26 March 2015 (T07)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Various minor textual and formatting changes.
- Warning added below Table 1 and 2 that the exon numbering used for CDKN2A is different from the product description version 3.
- Tables 2a to 2d are reordered and numbers adapted accordingly.
- Ligation sites of the probes targeting the *MITF* and *MLLT3* genes are updated according to new version of the NM_reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Schematic presentation 1 updated for the new *CDKN2A* exon numbering according to NG_007485.1 sequence.
- Note III updated according to the latest information on NM_001195132.1 transcript variant 5 coding for p16$^{\gamma}$.
- New figure 3 added to pinpoint peaks at 376 nt and 407 nt in no DNA reaction.

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