SALSA MLPA probemix P370-B1 BRAF-IDH1-IDH2

Lot B1-0317 and B1-0114. As compared to version A1 (lot A1-1110), several new probes have been included for FGFR1, MYB and MYBL1, several probes have been replaced for the 3p, 7q and 9p arms and most of the reference probes have been replaced. Also, the control fragments have been replaced (QDX2).

Gliomas, which include astrocytomas, oligodendrogliomas, mixed oligoastrocytomas and ependymomas, are the most common malignant CNS tumours with often a poor survival. This probemix can be used to detect genomic duplications leading to the KIAA1549-BRAF, SRGAP3-RAF1 and FGFR1-TACC1 fusion genes, to identify the BRAF V600E & four predominant IDH1 and IDH2 point mutations, and to detect copy number aberrations in the BRAF, CDKN2A/B, FGFR1, MYB and MYBL1 genes.

Activation of the MAPK pathway has been detected with high frequency in pilocytic astrocytomas, in particular via a 2 Mb tandem duplication leading to an oncogenic KIAA1549-BRAF fusion gene at 7q34 (Jones DTW et al. 2008, *Cancer Res.* 68:8673-7). Detection of this duplication is of help in differentiating these tumours from diffuse astrocytomas. Alternative MAPK pathway activation mechanisms include: 1) the formation of a similar SRGAP3-RAF1 fusion gene at 3p25, through a 3.6 Mb tandem duplication (Jones DTW et al. 2009, *Oncogene* 28:2119-23), 2) intragenic duplications of FGFR1 and FGFR1-TACC1 microamplifications (Zhang J et al. 2013, *Nat Genet*. 45:602-12; Jones DTW et al. 2013, *Nat Genet*. 45:927-32), and 3) certain BRAF mutations, in particular the V600E mutation (Schiffman JD et al. 2010, *Cancer Res.* 70:512-9; Dougherty MJ et al. 2010, *Neuro Oncol.* 12:621-30). The BRAF V600E activating mutation in combination with deletion of CDKN2A is suggested to define a clinical distinct subgroup of childhood glioma: the presence of these genetic alterations was found to be significantly enriched in cases of low grade glioma that are undergoing transformation to secondary high grade gliomas (Mistry M et al. 2015, *J Clin Oncol*. 33:1015-22).

The IDH1/2 mutations represent frequent genetic abnormalities in gliomas. Their identification facilitates distinguishing the different glioma entities leading to a more accurate prognosis and treatment (Riemenschneider MJ et al. 2010, *Acta Neuropathol.* 120:567-84). IDH1 and IDH2 point mutations have been detected with high frequency in diffuse gliomas (Hartmann C et al. 2009, *Acta Neuropathol.* 118:469-74; Yan H et al. 2009, *N Engl J Med.* 360:765-73), and the presence of these mutations is associated with a longer survival (Sanson M et al. 2009, *J Clin Oncol.* 27:4150-4; van den Bent MJ et al. 2010, *Clin Cancer Res.* 16:1597-604). This MLPA probemix contains probes that are specific for the two most frequent IDH1 (p.R132H and p.R132C) and the two most frequent IDH2 (p.R172M and p.R172K) mutations.

This probemix also includes probes for the FGFR1, MYB and MYBL1 genes and for the 9p21.3 region (CDKN2A/2B, MIR31). All these genes and regions are suggested to help in differentiating molecular subtypes of gliomas (see Table 2 for more detailed information). Furthermore, this probemix contains 12 reference probes detecting autosomal chromosomal locations that are regarded as relatively stable in gliomas and brain tumours. However, it should be noticed that glioma karyotypes can harbour multiple numerical and structural aberrations, which can complicate interpretation of these reference probes.

SALSA Binning DNA SD043

Please note that the mutation-specific probes have only been tested on artificial sample DNA and not on positive human DNA samples with the BRAF (p.V600E=c.1799T>A), IDH1 (p.R132H=c.395G>A and p.R132C=c.394C>T) or IDH2 (p.R172M=c.515G>T and p.R172K=c.515G>A) point mutations! The SD043 Binning DNA is provided with each probemix vial and can be used in data binning in the fragment analysis and as an artificial positive control for the mutation-specific probes (see page 3).

This SALSA® MLPA® probemix is designed to detect copy number changes of one or more sequences in the above mentioned genes and chromosomal regions and to detect the presence of the aforementioned point mutations in a DNA sample. Heterozygous deletions of recognition sequences should give a 35-50% reduced relative peak height of the amplification product of that probe. 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! In addition, some probe signals are more sensitive to sample purity and small changes in experimental conditions. Therefore, deletions and duplications detected by MLPA should always be confirmed by other methods. Not all deletions and duplications detected by MLPA will be pathogenic;

users should always verify the latest scientific literature when interpreting their findings. We have no information on what percentage of defects in these genes is caused by deletions/duplications of complete exons. Finally, note that majority of defects in these genes are expected to be small (point) mutations which will not be detected by this SALSA® MLPA® test.

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.

The use of this SALSA® MLPA® probemix and reagents requires a thermocycler with heated lid and sequence type electrophoresis equipment. Different fluorescent PCR primers are available. The MLPA technique has been first described in Nucleic Acids Research 30, e57 (2002).

Related SALSA® MLPA® probemixes

- P088 Oligodendroglioma: Contains probes for the 1p arm, the 19q arm, IDH1/2 and CDKN2A/2B.
- P105 Glioma-2: Contains probes for EGFR, TP53, PTEN, CDKN2A/2B, PDGFRA, CDK4, MDM2 and NFKBIA.
- ME012 MGMT-IDH1-IDH2: Contains probes for detection of MGMT methylation status and the most common IDH1/2 point mutations.

References for SALSA® MLPA® P370 BRAF-IDH1-IDH2

- Trabelsi S et al. 2015. Adult recurrent pilocytic astrocytoma: Clinical, histopathological and molecular study. *Neurochirurgie.* 61:392-7.
- Lhotska H et al. 2015. Genetic and epigenetic characterization of low-grade gliomas reveals frequent methylation of the MLH3 gene. *Genes Chromosomes Cancer*. 54:655-67.
- Gessi M et al. 2016. Intramedullary gangliogliomas: histopathologic and molecular features of 25 cases. *Hum Pathol.* 49:107-13.
- Lhotska H et al. 2016. Primary and recurrent diffuse astrocytomas: genomic profile comparison reveals acquisition of biologically relevant aberrations. *Mol Cytogenet*. 9:13.
- Gessi M et al. 2016. Dysembryoplastic neuroepithelial tumor of the septum pellucidum and the supratentorial midline: histopathologic, neuroradiologic, and molecular features of 7 cases*. Am J Surg Pathol.* 40:806-11.
- Trabelsi S et al. 2017. Molecular Diagnostic and Prognostic Subtyping of Gliomas in Tunisian Population. *Mol Neurobiol.* 54:2381-94.

More information

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Data analysis

The P370-B1 BRAF-IDH1-IDH2 probemix contains 58 MLPA probes with amplification products between 124 nt and 500 nt. This includes five probes, specific for the BRAF V600E (c.1799T>A), IDH1 R132H (c.395G>A), IDH1 R132C (c.394C>T), IDH2 R172M (c.515G>T) and IDH2 R172K (c.515G>A) point mutations, which will only generate a signal when the mutation is present. In addition, it contains 9 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt, three DNA Denaturation control fragments (D-fragments) at 88-92-96 nt, one X-fragment at 100 nt and one Y-fragment at 105 nt. More information on how to interpret observations on these control fragments can be found in the MLPA protocol.

SALSA Binning DNA SD043

The SD043 Binning DNA provided with this probemix can be used as Binning DNA sample for binning of the following five mutation-specific probes: BRAF probe 08780-SP0039-L08904 (p.V600E=c.1799T>A), IDH1 probe 19529-L16492 (p.R132H=c.395G>A), IDH1 probe 14787-L16493 (p.R132C=c.394C>T), IDH2 probe 14788-L17032 (p.R172M=c.515G>T) and IDH2 probe 14788-L17560 (p.R172K=c.515G>A). SD043 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 SD043 DNA in MLPA experiments is recommended as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software and as an artificial positive control for the specific point mutations. 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), as for this purpose true mutation-positive patient samples or cell lines should be used. It is strongly advised to use DNA samples and reference DNA samples extracted with the same method that have been derived from the same source of tissue. For further details, please consult the SD043 Binning DNA product description provided.
This product is for research use only (RUO).

Data generated by this probemix should be normalised with a more robust method, as the target sites of the reference probes maybe gained or lost. (1) Intra-sample normalisation should be performed by dividing the signal of each target-specific probe by the signal of every single reference probe in that sample, thus creating as many ratios per target-specific probe as there are reference probes. Subsequently, the median of all these produced ratios per probe should be taken; this is the probe's Normalisation Constant. (2) Secondly, inter-sample comparison should be performed by dividing the Normalisation Constant of each probe in a given sample by the average Normalisation Constant of that probe in all the reference samples.

Data normalisation should be performed within one experiment. Always use sample and reference DNA extracted with the same method and derived from the same source of tissue. Confirmation of deletions, duplications and amplifications can be done by e.g. Southern blotting, long range PCR, qPCR, FISH.

Note that Coffalyser.Net, the MLPA analysis tool developed at MRC-Holland, can be downloaded free of charge from our website www.mlpa.com.

Warning: 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. Furthermore, although reference probes are located in 'silent' regions that are not frequently altered in copy number in glioma, there is always a possibility that one or more reference probes *do* show a copy number alteration in a sample. Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. When in doubt, users should always verify the latest updates of the database and scientific literature when interpreting their findings.

This probemix was developed at MRC-Holland.

Info/remarks/suggestions for improvement: info@mlpa.com.

Table 1. SALSA MLPA P370-B1 BRAF-IDH1-IDH2 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position						
		reference	IDH1/2	3p	6q	Chr. 7	Chr. 8	9p
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA							
88-92-96	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation							
100	X-fragment: Specific for the X chromosome							
105	Y-fragment: Specific for the Y chromosome							
124 ¥	Reference probe 18709-L21056	5q31						
130 *	Reference probe 16316-L18705	3q21						
136 *	**SRGAP3 probe** 19365-L25758			3p25.3				
142 *	Reference probe 17857-L22116	2p21						
148 * «	**MYB probe** 12500-L25742				6q23.3			
155 * ¬	**LAMA2 probe** 14924-L25757				6q22.33			
160 *	**SRGAP3 probe** 19366-L25759			3p25.3				
166	**KIAA1549 probe** 15251-L17567					7q34		
172 *	**MYBL1 probe** 07915-L07628						8q13.1	
179 ¥	**BRAF probe** 10509-L25743					7q34		
184 *	**FGFR1 probe** 04184-L25753						8**p**12	
190 *	**TACC1 probe** 19368-L25761						8**p**11.23	
196 *	Reference probe 18049-L22439	16q23						
203 ¥ §	**IDH1 probe** 19529-L16492		**R132H**					
208	**KIAA1549 probe** 15252-L17007					7q34		
214 *	Reference probe 10730-L25930	6p12						
220 §	**IDH1 probe** 14787-L16493		**R132C**					
226 § Ж	**BRAF probe** 08780-SP0039-L08904					**V600E**		
232 *	Reference probe 16428-L25931	18q21						
238 §	**IDH2 probe** 14788-L17032		**R172M**					
242 §	**IDH2 probe** 14788-L17560		**R172K**					
249	**CDKN2A probe** 10333-L17690							9p21.3
254	**RAF1 probe** 15332-L17816			3p25.1				
261 *	**MYBL1 probe** 07914-L26455						8q13.1	
266	**KIAA1549 probe** 15253-L17561					7q34		
274 * ¬	**CNTNAP2 probe** 12947-L25756					7q35		
280 *	Reference probe 13350-L26120	9q21						
285 *	**CDKN2A probe** 16533-L26121							9p21.3
292 * ¬	**IKZF1 probe** 16911-L15654					7**p**12.2		
299 *	**MYB probe** 17265-L26123				6q23.3			
304 * ¬	**SLC26A3 probe** 17066-L26124					7q22.3		
310 *	Reference probe 16559-L26125	11q13						
319	**KIAA1549 probe** 15255-L17010					7q34		
326 *	**MYBL1 probe** 19605-L26457						8q13.1	
333 * ¬	**PLAGL1 probe** 18472-L26458				6q24.2			
339 *	**FGFR1 probe** 17635-L26228						8**p**12	
346 *	Reference probe 05388-L26459	12p11						
353 *	**FGFR1 probe** 04439-L26460						8**p**12	
360 ¥	**MKRN1 probe** 15257-L26461					7q34		
366 ¥	**RAF1 probe** 14692-L25749			3p25.1				
373 *	**FGFR1 probe** 18296-L25750						8**p**12	
380 *	**BRAF probe** 19322-L26031					7q34		
388 *	**MIR31 probe** 19508-L26462							9p21.3
395 ¥	**HIPK2 probe** 15259-L26463					7q34		
401 *	**FGFR1 probe** 04440-L26464						8**p**12	
409 *	**BRAF probe** 19324-L25551					7q34		
417 *	Reference probe 13817-L15311	2q13						
424 *	**FGFR1 probe** 04441-L21311						8**p**12	
430 *	**MYB probe** 19369-L26102				6q23.3			
437 *	**TACC1 probe** 19370-L25763						8**p**11.23	
447 *	**BRAF probe** 19328-L25555					7q34		
454	**CDKN2B probe** 01531-L13742							9p21.3
463 *	**MYBL1 probe** 19606-L26231						8q13.1	

Length (nt)	SALSA MLPA probe	Chromosomal position
471 *	**MYB probe** 19371-L26746	6q23.3
478 ¥	**BRAF probe** 10502-L26466	7q34
484 ¥ ¬	**CRBN probe** 06312-L26467	3p26.3
494 *	Reference probe 19137-L26747	21q22
500 *	Reference probe 19675-L26275	4p13

* New in version B1 (from lot B1-0114 onwards).
¥ Changed in version B1 (from lot B1-0114 onwards). Small change in length, no change in the sequence detected.
§ Mutation-specific probe. This probe will only generate a signal when the indicated mutation is present. It has been tested on artificial test DNA **but not on positive human samples!**
« This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.
Ж This probe consists of three parts and has two ligation sites.
¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.

Note: The identity of the genes detected by the reference probes is available in Table 2.

Table 2. P370 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / Exon	Location / Ligation site	**Partial** sequence (24 nt adjacent to ligation site)	Distance to next probe
Reference probes at 2p and 2q.					
142	17857-L22116	SLC3A1	2p21	TATGCAGAGAGT-ATTGACAGGACC	64.5 **M**b
417	13817-L15311	EDAR	2q13	TGGCCAGGTGAA-CCAGCGACAGCA	-
IDH1, at 2q33.3 These two probes will only give a signal when either the R132H or the R132C mutation is present in the sample. The R132H (G395A) mutation has been detected by sequencing in 664 samples covering 92.7% of IDH1 mutations, and the R132C (C394T) mutation in 29 samples covering 4.2% of the IDH1 mutations in a cohort of 1010 diffuse gliomas (Hartmann C et al, 2009, *Acta Neuropathol.* 118:469-74). No probes are present in this probemix for the IDH1 R132S (C394A), R132G (C394G) and R132L (G395T) mutations which were detected in 11 (1.5%), 10 (1.5%) and 2 (0.2%) samples, respectively, in the same study by Hartmann C et al.					
203 §	19529-L16492	**IDH1**, ex 6; **c.395G>A=p.R132H**	NM_005896.3; 690-691	CATCATAGGTC**A**-TCATGCTTATGG	0.0 kb
220 §	14787-L16493	**IDH1**, ex 6; **c.394C>T=p.R132C**	NM_005896.3; 689-688 *reverse*	ATAAGCATGAC**A**-ACCTATGATGAT	-
SRGAP3-RAF1 fusion, at 3p25 Activation of the RAF1 gene by formation of a SRGAP3-RAF1 fusion gene in a similar way as the KIAA1549-BRAF fusion gene has been described in pilocytic astrocytoma patients (Jones DTW et al. 2009, *Oncogene.* 28:2119-23; Forshew T et al. 2009, *J Pathol.* 218:172–81; Tatevossian RG et al. 2010, *Acta Neuropathol.* 120:731-43). It is indicated in the 4th column whether the probe is located ***within*** or *outside* the duplicated region.					
484 ¬	06312-L26467	**CRBN**	3p26.3; *outside*	CCGATGGCAGCT-TATGTGAATCCT	5.8 **M**b
160	19366-L25759	**SRGAP3**, ex 20	3p25.3; NM_014850.3; 2972-2973; *outside*	GAAGTGGAGCAG-ATCGAGGCTATT	130.4 kb
136	19365-L25758	**SRGAP3**, ex 2	3p25.3; NM_014850.3; 851-852; ***within***	GAGCAGCAATCA-GAGTCGCGACTG	3.5 **M**b
254	15332-L17816	**RAF1**, ex 17	3p25.1; NM_002880.3; 3033-3034; ***within***	TCATGCTGAATT-TTGTCTTCCAGG	28.2 kb
366	14692-L25749	**RAF1**, ex 3	3p25.1; NM_002880.3; 675-676; *outside*	GAAAGCACTCAA-GGTGAGGGGCCT	117.4 **M**b
Reference probes at 3q, 4p, 5q and 6p.					
130	16316-L18705	RAB7A	3q21	CACAATAGGAGC-TGACTTTCTGAC	-
500	19675-L26275	ATP8A1	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	-
124	18709-L21056	IL4	5q31	ATCGACACCTAT-TAATGGGTCTCA	-
214	10730-L25930	PKHD1	6p12	GCTTCCCTGGAA-GGAGCATCAGAC	77.9 **M**b
MYB aberrations, at 6q23.3 MYB is an oncogenic transcription factor that can act both as a transcriptional activator and repressor. Both amplifications of the whole MYB gene and 3' deletions of the MYB gene have been described in paediatric low-grade gliomas (Tatevossian RG et al. 2010, *Acta Neuropathol.* 120:731-43; Zhang J et al. 2013, *Nat Genet.* 45:602-12).					
155 ¬	14924-L25757	**LAMA2**	6q22.33	GGGTTTCAAACA-GATGTCAGAGTT	6.0 **M**b
148 «	12500-L25742	**MYB**, ex 2	NM_001130173.1; 258-259	TGAGGACTTTGA-GATGTGTGACCA	11.2 kb
471	19371-L26746	**MYB**, ex 10	NM_001130173.1; 1597-1598	CCACCCAAGGTC-TTACCTCCTGCA	6.1 kb
430	19369-L26102	**MYB**, ex 15	NM_001130173.1; 2278-2279	CTTACAAGCTCC-GTTTTAATGGCA	15.2 kb
299	17265-L26123	**MYB**, ex 16	NM_001130173.1; 2962-2963	CATTTAATCCAG-ATTGTAAATGCT	8.7 **M**b
333 ¬	18472-L26458	**PLAGL1**	6q24.2	AAGTTTGTCTGA-AGATTCAAACCT	-

Length (nt)	SALSA MLPA probe	Gene / Exon	Location / Ligation site	**Partial** sequence (24 nt adjacent to ligation site)	Distance to next probe
KIAA1549-BRAF fusion, at 7q34 MAPK pathway activation through a 2 Mb tandem duplication leading to an oncogenic KIAA1549-BRAF fusion gene is suggested to be a very frequent event in pilocytic astrocytomas (Jones DTW et al. 2008, *Cancer Res.* 68:8673-7). The BRAF V600E mutation, which can be detected by the appearance of the 226 nt probe, is also found regularly in this type of tumours. It is indicated in column 4 whether the probe is located ***within*** or *outside* the commonly duplicated region. Note that several different fusion variants have been described in the literature. The most common is the KIAA1549 exon 16-BRAF exon 9 fusion. For more information on other fusion variants see e.g. Table 1 in Forshew T et al. 2009, *J Pathol.* 218:172–81. When the BRAF V600E point mutation is detected in combination with deletion of CDKN2A, paediatric low grade glioma patients have an increased risk for transformation to secondary high grade glioma; therefore, therapy may need other preventive measures for this defined clinical distinct subgroup (Mistry M et al. 2015, *J Clin Oncol.* 33:1015-22).					
7p arm					
292 ¬	16911-L15654	**IKZF1**	7p12.2; *outside*	AGACATGTCCCA-AGTTTCAGGTGA	56.9 **M**b
7q arm					
304 ¬	17066-L26124	**SLC26A3**	7q22.3; *outside*	CCACTTCCTGCA-TGTGGCAGAAAG	31.0 **M**b
166	15251-L17567	**KIAA1549**, ex 19	NM_001164665.1; 5546-5547; *outside*	TTGCCAGCAGAA-TTGGAGCTCAGC	12.0 kb
266	15253-L17561	**KIAA1549**, ex 17	NM_001164665.1; 5316-5317; *outside*	AGACTATGGAAT-GACTCCCCCGAC	29.3 kb
208	15252-L17007	**KIAA1549**, ex 11	NM_001164665.1; 4141-4142; ***within***	GGTCAGCACAAT-AAAGACGACATA	29.7 kb
319	15255-L17010	**KIAA1549**, ex 4	NM_001164665.1; 3135-3136; ***within***	CCAGACTCCTTT-AATCCTGTCTGT	703.2 kb
395	15259-L26463	**HIPK2**, ex 8	NM_022740.4; 2123-2124; ***within***	ACTACCCATCTA-CACTCTACCAGC	855.8 kb
360	15257-L26461	**MKRN1**, ex 6	NM_013446.3; 11 nt before ex 6;***within***	ACCACCCTCTTA-CACATTTTCAGC	280.6 kb
179	10509-L25743	**BRAF**, ex 17	NM_004333.4; 8 nt after ex 17; ***within***	CCAAGTAAGTAA-AAGCTTCATGCT	13.5 kb
226 § Ж	08780-SP0039-L08904	**BRAF**, ex 15; **c.1799T>A=p.V600E**	NM_004333.4; 1820-1821 and 1860-1861	TTCTTCATGAAG-ACCTCACAGTAAA AATAGGTGATTTTGGTCTAGCTACAG**A**-GAAATCTCGATG	0.9 kb
447	19328-L25555	**BRAF**, ex 14	NM_004333.4; 1799-1800; ***within***	ACCTCAAGAGTA-ATAGTATCCTTC	23.9 kb
409	19324-L25551	**BRAF**, ex 12	NM_004333.4; 1523-1524; ***within***	TGTTGAATGTGA-CAGCACCTACAC	16.4 kb
478	10502-L26466	**BRAF**, ex 8	NM_004333.4; 1050-1051; *outside*	CAGGCCCCAAAT-TCTCACCAGTCC	14.5 kb
380	19322-L26031	**BRAF**, ex 4	NM_004333.4; 593-594; *outside*	GAGTTACAGTCC-GAGACAGTCTAA	6.2 **M**b
274 ¬	12947-L25756	**CNTNAP2**	7q35; *outside*	GTGCCTCTGGAT-TGGAATGGAGAA	-
Intragenic duplications of FGFR1 and FGFR1-TACC1 microamplifications, at 8p11-p12 Intragenic duplications of exons 10-18 or exons 11-18 of the FGFR1 gene are detected both in paediatric low grade diffuse gliomas and in pilocytic astrocytomas (Zhang J et al. 2013, *Nat Genet.* 45:602-12; Jones DTW et al. 2013, *Nat Genet.* 45:927-32). These intragenic duplications are suggested to produce autophosphorylation of FGFR1 and upregulation of the MAPK/ERK and PI3K pathways. In addition, microamplifications of FGFR1 and TACC1 leading to in-frame FGFR1-TACC1 fusions by joining exon 18 of FGFR1 with exon 7 of TACC1, have been described in in low-grade gliomas (Zhang J et al. 2013, *Nat Genet.* 45:602-12).					
424	04441-L21311	**FGFR1**, ex 18	NM_023110.2; 3729-3730	AGCCAATGAACA-GGCATGCAAGTG	1.6 kb
401	04440-L26464	**FGFR1**, ex 14	NM_023110.2; 2808-2809	TGCATACACCGA-GACCTGGCAGCC	1.1 kb
353	04439-L26460	**FGFR1**, ex 13	NM_023110.2; 2710-2711	ACCCCAGCCACA-ACCCAGAGGAGC	2.4 kb
373	18296-L25750	**FGFR1**, ex 10	NM_023110.2; 2259-2260	TCCATGAACTCT-GGGGTTCTTCTG	9.7 kb
184	04184-L25753	**FGFR1**, ex 5	NM_023110.2; 1481-1482	CAAATGCCCTTC-CAGTGGGACCCC	29.4 kb
339	17635-L26228	**FGFR1**, ex 2	NM_023110.2; 955-956	TGTGGAGCTGGA-AGTGCCTCCTCT	271.0 kb
190	19368-L25761	**TACC1**, ex 1	NM_001146216.2; 237-238	GCTGACTTCGCA-CTTGAGCTCCAG	113.9 kb
439	19370-L25763	**TACC1**, ex 11	NM_001146216.2; 2350-2351	ATGGAGAAGGAA-CAGGCCCTGGCT	28.8 **M**b to MYBL1
Duplications of MYBL1, at 8q13.1 MYBL1 belongs to the Myb family of transcription factors. Gain of 8q13.1 is detected in 28% of paediatric diffuse astrocytomas resulting in partial duplication of MYBL1 with truncation of its C-terminal negative-regulatory domain (Ramkissoon LA et al. 2013, *PNAS.* 110:8188-93; Zhang J et al. 2013, *Nat Genet.* 45:602-12).					
172	07915-L07628	**MYBL1**, ex 14	NM_001080416.3; 2343-2344	CTGTTGACTGAA-GACATTTCAGAC	25.8 kb
326	19605-L26457	**MYBL1**, ex 8	NM_001080416.3; 1258-1259	AGAATGAAGTTA-GAAGAAAGCGAA	4.9 kb
463	19606-L26231	**MYBL1**, ex 5	NM_001080416.3; 867-868	ATCATCTATGAA-GCACATAAGCGG	5.1 kb
261	07914-L26455	**MYBL1**, ex 2	NM_001080416.3; 474-475	GATCATGATTAT-GAAGTACCACAA	-
CDKN2A/CDKN2B/MIR31 genes, at 9p21.3 Loss of 9p, and especially deletions of the 9p21.3 region including CDKN2A, are common in high-grade gliomas. In contrast, CDKN2A deletions are rare in anaplastic astrocytomas and glioblastomas with mutated IDH1 or IDH2 genes but are more frequent in these tumours without IDH1/IDH2 mutations (Yan H et al. 2009, *N Eng J Med.* 360:765-73). Additionally, homozygous deletions of CDKN2A have been reported to define a subset of malignant astrocytomas in children (Schiffman JD et al. 2010, *Cancer Res.* 70:512-9). When deletion of CDKN2A is detected in combination with the BRAF V600E mutation in paediatric low grade glioma, there is an increased risk for transformation to secondary high grade glioma; therefore, therapy may need other preventive measures for this defined clinical distinct subgroup (Mistry M et al. 2015, *J Clin Oncol.* 33:1015-22).					
388	19508-L26462	**MIR31**, ex 1	NR_029505.1; 58-57 *reverse*	AAAGATGGCAAT-ATGTTGGCATAG	456.1 kb
285	16533-L26121	**CDKN2A**, ex 4	NM_000077.4; 33 nt before ex 4	TTGACCTCAGGT-TTCTAACGCCTG	6.7 kb
249	10333-L17690	**CDKN2A**, ex 2	NM_000077.4; 138-139	GCCTGGAAAGAT-ACCGCGGTCCCT	25.6 kb

Length (nt)	SALSA MLPA probe	Gene / Exon	Location / Ligation site	**Partial** sequence (24 nt adjacent to ligation site)	Distance to next probe
454	01531-L13742	**CDKN2B**, down ex 2	NM_078487.2; 2341 nt after ex 2 *reverse*	CCTAGGAAAGGT-GATAGAGCTTAG	56.0 **M**b
Reference probes at 9q, 11q and 12p.					
280	13350-L26120	PCSK5	9q21	CATTAGCAAGCA-TTAGAACATCTC	-
310	16559-L26125	SHANK2	11q13	TGGTGCCAACAA-GGACTCACTCTC	-
346	05388-L26459	PKP2	12p11	TGAGAAACTTAG-TATTTGAAGACA	-
IDH2 gene, at 15q26.1 These two probes will only give a signal when either the R172K or the R172M mutation is present in the sample. The R172K (G515A) mutation has been detected by sequencing in 20 samples, and the R172M (G515T) mutation in 6 samples, in a cohort of 1010 diffuse gliomas (Hartmann C et al. 2009, *Acta Neuropathol.* 118:469-74). The same study suggests that IDH2 mutations occur predominantly in oligodendroglial tumours. No probe is present for the IDH2 R172W (A514T) mutation, which was detected in 5 samples in this study of 1010 patients.					
242 §	14788-L17560	**IDH2**, ex 5; **c.515G>A=p.R172K**	NM_002168.3; 679-680	CACCATTGGCA**A**-GCACGCCCATGG	0.0 kb
238 §	14788-L17032	**IDH2**, ex 5; **c.515G>T=p.R172M**	NM_002168.3; 679-680	CACCATTGGCA**T**-GCACGCCCATGG	-
Reference probes at 16q, 18q and 21q.					
196	18049-L22439	PLCG2	16q23	TCCTGTCGCCAG-CTGAGGAGGCGG	-
232	16428-L25931	MYO5B	18q21	TGGACCCTGATT-GATTTTTATGAT	-
494	19137-L26747	PSMG1	21q22	TGGAAGCTTTTA-AGCCTATACTTT	-

§ Mutation-specific probe. This probe will only generate a signal when the indicated mutation is present. It has been tested on artificial test DNA **but not on positive human samples!**
« This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.
Ж This probe consists of three parts and has two ligation sites.
¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.

Note: Exon numbering used here may differ from literature! Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA probemix P370-B1 BRAF-IDH1-IDH2 sample pictures

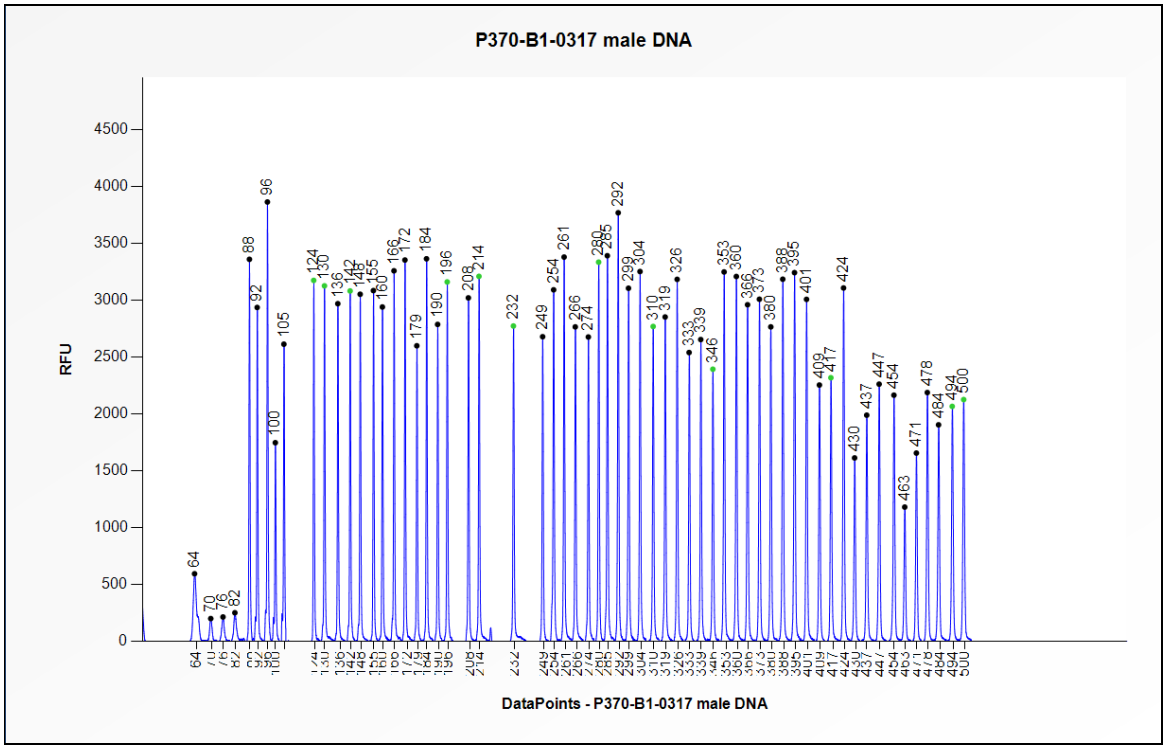

Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P370-B1 BRAF-IDH1-IDH2 probemix (lot B1-0317).

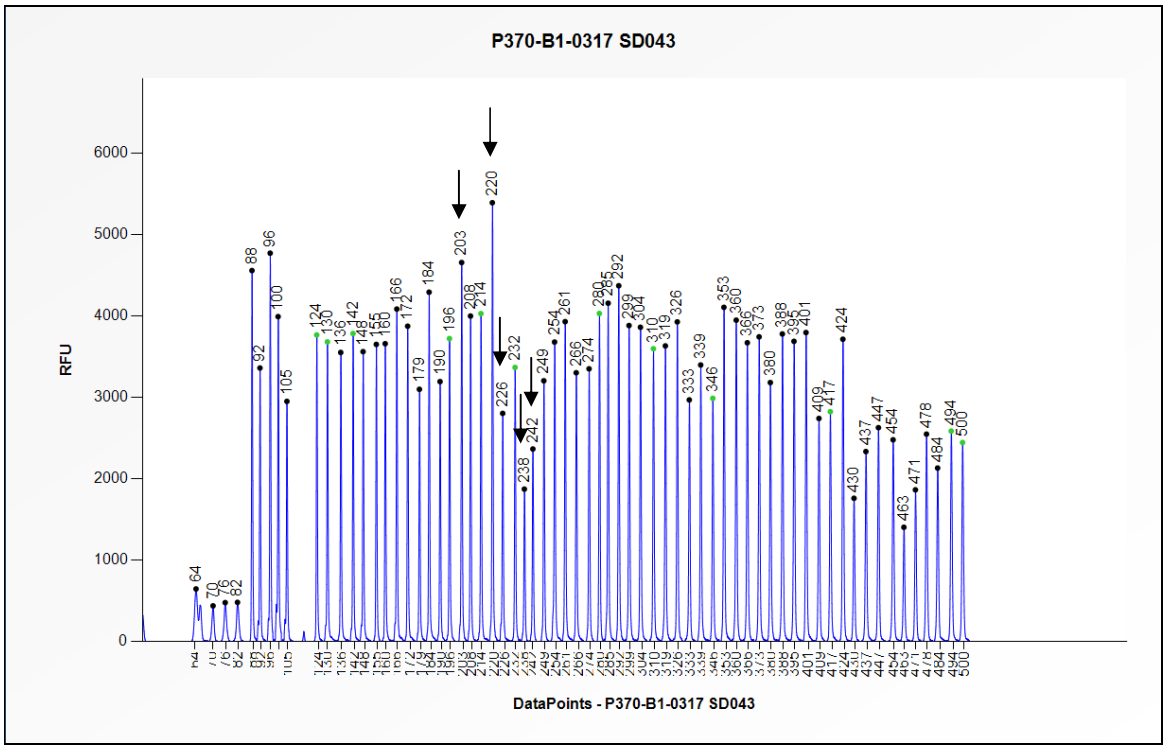

Figure 2. Capillary electrophoresis pattern of SALSA Binning DNA SD043 (approximately 50 ng human female control DNA) analysed with SALSA MLPA probemix P370-B1 BRAF-IDH1-IDH2 (lot B1-0317). The locations of the BRAF, IDH1 and IDH2 mutations specific probes at 203 nt, 220 nt, 226 nt, 238 nt and 242 nt are indicated with an arrow.

Implemented Changes – compared to the previous product description version(s).
Version 10 – 20 September 2017 (T08) - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - New related MLPA probemix and a reference added on page 2. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. - NM sequences and ligations sites for the MYBL1 and IDH2 genes have been updated in Table 2. - Various small textual changes throughout the document. *Version 09 – 03 January 2017 (T08)* - Various textual changes on page 1. - New references added on page 2. - Table 2: information on which exon is targeted by the flanking probes removed. *Version 08 – 22 May 2015 (T07)* - Information on the R172M (G515T) mutation corrected in Table 2. *Version 07 – 07 May 2015 (T07)* - New information on BRAF mutation and CDKN2A deletion added on page 1 and in Table 2. *Version 06 (T06)* - Small textual changes and correction of typos. - New related probemix (ME011) added on page 2. *Version 05 (T06)* - 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). - Several textual changes. *Version 04 (48)* - Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added. *Version 03 (48)* - P088 product name corrected in Related probemixes. - Small textual changes. *Version 02 (46)* - Product description adapted to a new lot (lot number added, new picture added). *Version 01 (46)* - Not applicable, new document.