SALSA MLPA probemix P105-D2 Glioma-2

Lot D2-0217. As compared to the previous version D1 (lot D1-0413) two probes have a small change in length, but no change in the sequence targeted.

Gliomas are the most common primary brain tumours and account for one third of central nervous system (CNS) tumours. Gliomas comprise a very heterogeneous group of CNS neoplasms derived from glial cells. There are several oncogenes and tumour suppressor genes, which have been shown to undergo copy number changes in these tumours. Somatic mutations, disruptions, or copy number aberrations in three critical signalling pathways, a) the RTK/PI3K pathway (involving e.g. EGFR, PDGFRA and PTEN genes), b) the p53 pathway (involving e.g. CDKN2A, MDM2 and TP53 genes) and c) the RB pathway (involving e.g. CDKN2A and CDK4 genes), are suggested to contribute to the development of gliomas (TCGA, 2008. *Nature*. 455:1061-8).

Epidermal growth factor receptor (EGFR) and its ligands are cell signalling molecules involved in diverse cellular functions. These include cell proliferation, differentiation, motility and survival, and tissue development. Glioblastomas often express EGFRvIII, a constitutively active genomic deletion variant of EGFR which is characterised by deletions of exons 2-7 of the EGFR gene (Humphrey PA et al. 1990. *PNAS.* 87:4207-11; Sugawa N et al. 1990. *PNAS.* 87:8602-6).

This P105-D2 Glioma-2 probemix can be used to determine aberrant copy numbers of the PDGFRA (4q12), EGFR (7p11.2), CDKN2A (9p21.3), PTEN (10q23.31), TP53 (17p13.1), CDK4-MIR26A2-MDM2 (12q14-q15) and NFKBIA (14q13.2) genes. This probemix also allows detection of deletions of EGFR that result in EGFRvIII. In addition, 12 reference probes have been included in this probemix, detecting several different autosomal chromosomal locations, which are relatively stable in copy number in gliomas.

This SALSA® MLPA® probemix is designed to detect deletions/duplications of one or more sequences in the aforementioned genes 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 most defects in this gene 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).

More information

Website : www.mlpa.com
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Related SALSA® MLPA® probemixes

- P088 Oligodendroglioma 1p-19q: Contains probes for chromosomes 1p and 19q, CDKN2A/B and IDH1 R132H/C and IDH2 R172M/K point mutations.
- P370 BRAF-IDH1-IDH2: Contains probes for chromosomes 3p, 6q, 7q, 8p, 8q, CDKN2A/B and BRAF V600E, IDH1 R132H/C and IDH2 R172M/K point mutations.
- P315 EGFR: Contains more probes for the EGFR gene and EGFR T790M and L858R point mutations.
- P225 PTEN: Contains more probes for the PTEN gene.
- P056 TP53: Contains more probes for the TP53 gene.
- ME012 MGMT-IDH1-IDH2: Contains probes for detection of methylation status of MGMT, and for IDH1 R132H/C and IDH2 R172M/K point mutations.
- ME024 9p21: Contains more probes for the CDKN2A/2B genes and 9p21.3 region and also allows methylation detection in this region.
- P419 CDKN2A/2B-CDK4: Contains more probes for the CDKN2A, CDKN2B and CDK4 genes.
- P323 CDK4-HMGA2-MDM2: Contains more probes for the CDK4 and MDM2 genes.

References for SALSA® MLPA® P105 Glioma-2

- Shibahara I et al. (2018). Glioblastoma in neurofibromatosis 1 patients without IDH1, BRAF V600E, and TERT promoter mutations. *Brain Tumor Pathol*. 35:10-8.
- Andrade RC et al. (2017). TP53 and CDKN1A mutation analysis in families with Li-Fraumeni like syndormes. *Fam Cancer* 16:243-248.
- Kanamori M et al. (2016). Malignany transformation of diffuse astrocytoma to glioblastoma associated with newly developed BRAFV600E mutation. *Brain Tumor Pathol*. 33:50-6.
- Trabelsi S et al. (2015). Adult recurrent pilocytic astrocytoma: Clinical, histopathological and molecular study. *Neurochirurgie.* 61:392-7.
- Franceschi S et al. (2015). Investigating molecular alterations to profile short- and long-term recurrence-free survival in patients with primary glioblastoma. *Oncol Lett.* 10:2599-3606.
- Westphal M et al. (2015). A randomised, open label phase III trial with nimotuzumab, an anti-epidermal growth factor monoclonal antibody in the treatment of newly diagnosed adult glioblastoma*. Eur J Cancer*. 51:522-32.
- Navarro L et al. (2015). Alteration of major vault protein in human glioblastoma and its relation with EGFR and PTEN status. *Neuroscience.* 297:243-51.
- Molenaar RJ et al. (2014). Rhabdoid glioblastoma in a child: case report and literature review Neuro Oncol. 16:1263-73.
- Serna E et al. (2014). Correlation between EGFR amplification and the expression of microRNA-200c in primary glioblastoma multiforme. *PLoS One.* 9(7):e102927.
- Weller M et al. (2014). Assessment and prognostic significance of the epidermal growth factor receptor vIII mutation in glioblastoma patients treated with concurrent and adjuvant temozolomide radiochemotherapy. *Int J Cancer.* 134:2437-47.
- Shibahara I et al. (2013). The expression status of CD133 is associated with the pattern and timing of primary glioblastoma recurrence. *Neuro-Oncology*. 15:1151-9.
- García-Claver A et al. (2013). Gene expression changes associated with erlotinib response in glioma cell lines. *Eur J Cancer.* 49:1641-53.
- Gessi M et al. (2013). H3.3 G34R mutations in pediatric primitive neuroectodermal tumors of central nervous system (CNS-PNET) and pediatric glioblastomas: possible diagnostic and therapeutic implications? *J Neurooncol.* 112:67-72.
- Boots-Sprenger S et al. (2013). Significance of complete 1p/19q co-deletion, IDH1 mutation and MGMT promoter methylation in gliomas: use with caution. *Mod Pathol*. 26:922-9.
- Garcia-Claver A et al. (2013). Gene expression changes associated with erlotinib response in glioma cell lines. *Eur J Cancer.* 49:1641-53.
- Motomura K et al. (2012). Immunohistochemical analysis-based proteomic subclassification of newly diagnosed glioblastomas. *Cancer Science.* 103:1871–9.
- Guillaudeau A. et al. (2012). EGFR soluble isoforms and their transcripts are expressed in meningiomas. *PLoS One*. 7(5):e37204.

Data analysis

The P105-D2 Glioma-2 probemix contains 55 MLPA probes with amplification products between 126 and 500 nt. 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.

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, 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 gliomas, 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 P105-D2 Glioma probemix

Length (nt)	SALSA MLPA probe	Chromosomal position				
		Reference	EGFR	PTEN	Other targets	Location (HG 18) in kb
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					
126	Reference probe 18709-L21698	5q31				05-132.038
131	Reference probe 16316-L22397	3q21				03-130.000
137	**EGFR** probe 06121-L20393		Exon 6			07-055.188
142	**CDKN2A** probe 18753-L24594				Exon 1	09-021.985
148	Reference probe 14279-L15949	15q13				15-025.951
157	**NFKBIA** probe 18758-L24126				Exon 3	14-034.942
161	**EGFR** probe 05438-L24607		Exon 5			07-055.186
166	**TP53** probe 01588-L06028				Exon 1	17-007.531
172	**EGFR** probe 06405-L24605		Exon 1			07-055.054
178	**PTEN** probe 17314-L20922			Exon 3		10-089.675
184	**EGFR** probe 05440-L04856		Exon 7			07-055.189
190	**PTEN** probe 06729-L06339			Exon 2		10-089.644
196	**TP53** probe 01996-L09268				Exon 2a	17-007.521
202	Reference probe 04542-L03931	2q24				02-166.567
208 ±	**PTEN** probe 17391-L21278			Exon 4		10-089.681
214	**PTEN** probe 07686-L15591			Exon 9		10-089.716
220	**EGFR** probe 17208-L23059		Exon 4			07-055.182
226	**PTEN** probe 17387-L24118			Exon 1		10-089.614
231	**NFKBIA** probe 18757-L24608				Exon 5	14-034.942
238	Reference probe 15174-L16949	3p13				03-072.510
244	Reference probe 12431-L13432	22q12				22-032.003
250	**CDKN2A** probe 16060-L22417				Exon 2	09-021.965
256	**EGFR** probe 05959-L24612		Exon 13			07-055.197
263 ǂ	**TP53** probe 02376-L21409				Exon 4b	17-007.519
269	**EGFR** probe 05969-L24610		Exon 23			07-055.234
277	**NFKBIA** probe 18759-L24127				Exon 4	14-034.942
283 ǂ ±	**TP53** probe 01999-L21074				Exon 7	17-007.518
292	Reference probe 11900-L12706	6p12				06-052.028
299 ¥	**TP53** probe 17420-L29693				Exon 3	17-007.520
309	**CDKN2A** probe 17814-L22631				Exon 4	09-021.958
316	**TP53** probe 17421-L24613				Exon 5	17-007.519
323	**PTEN** probe 03639-L24076			Exon 6		10-089.702
331	Reference probe 08905-L24614	11p11				11-047.316
339 «	**CDK4** probe 17815-L22422				Exon 4	12-056.431
346	**EGFR** probe 17210-L24618		Exon 16			07-055.206
353	**TP53** probe 17422-L24617				Exon 10	17-007.515
359	**PTEN** probe 17397-L24616			Exon 8		10-089.711
366	Reference probe 06760-L24615	8q12				08-061.928
373	**PTEN** probe 03638-L22839			Exon 5		10-089.683
385	**CDKN2A** probe 17817-L23295				Exon 3	09-021.961
391	**PDGFRA** probe 03107-L02038				Exon 3	04-054.822
398	**EGFR** probe 05436-L24070		Exon 3			07-055.178
407	**PDGFRA** probe 18755-L24123				Exon 22	04-054.851
415	**MDM2** probe 18255-L22981				Exon 10	12-067.517
421 ¥	**EGFR** probe 21280-L27637		Exon 2			07-055.177
429	**EGFR** probe 02063-L21569		Exon 8			07-055.191
438	**PDGFRA** probe 18756-L24124				Exon 5	04-054.826
445	**TP53** probe 17424-L24621				Exon 11	17-007.514
453 «	**CDK4** probe 18752-L22100				Exon 8	12-056.428
461	Reference probe 11674-L24181	16p11				16-029.914
469	**MIR26A2** probe 18710-L24620				Exon 1	12-056.505
475	**PTEN** probe 17386-L22174			Exon 7		10-089.708

Length (nt)	SALSA MLPA probe	Chromosomal position				
		Reference	EGFR	PTEN	Other targets	Location (HG 18) in kb
481	**MDM2** probe 07178-L24069				Exon 2	12-067.489
490	Reference probe 16456-L24172	18q21				18-045.630
500	Reference probe 17001-L22947	20q11				20-034.954

¥ Changed in version D2 (from lot D2-0217 onwards). Small change in length, no change in sequence detected.
± SNP rs587780544 could influence the signal of the probe at 208 nt and SNP rs121912660 could influence the signal of the probe at 283 nt. In case of apparent deletions, it is recommended to sequence the region targeted by these probes.
‡ The ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by the IARC TP53 Database (http://p53.iarc.fr/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.

Note: Exon numbering used here may differ from literature! Please notify us of any mistakes.

Table 2a. P105 target 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
PDGFRA gene, at 4q12 PDGFRA amplification or gain is detected in 2-18% of gliomas (Alentorn A. et al. 2012. *Neuro Oncol.* 14:1393-403; Bleeker FE. et al. 2012. *J Neurooncol.* 108:11-27; TCGA, 2008. *Nature*. 455:1061-8). The frequency of PDGFRA amplification is shown to increase with tumour grade, and the amplification of PDGFRA is suggested to be a poor prognostic factor in anaplastic gliomas and in IDH1-mutant de novo glioblastoma multiforme (GBMs) (Alentorn A. et al. 2012. *Neuro Oncol.* 14:1393-403; Phillips JJ. et al. 2013. *Brain Pathol.* 23:565-73).					
391	03107-L02038	PDGFRA, ex 3	NM_006206.4; 495-496	GGAGAGTGAAGT-GAGCTGGCAGTA	3.8 kb
438	18756-L24124	PDGFRA, ex 5	NM_006206.4; 1042-1043	ACCTGTGCTGTT-TTTAACAATGAG	25.4 kb
407	18755-L24123	PDGFRA, ex 22	NM_006206.4; 3263-3264	ATCCTGCTGTGG-CACGCATGCGTG	-
EGFR gene, at 7p11.2 EGFR amplification is frequently detected in primary glioblastomas and is associated with poor prognosis (Hurtt MR. et al. 1992. *J Neuropathol Exp Neurol.* 51:84-90). Glioma patients with amplification of wt-EFGR have been suggested to respond well to EGFR kinase inhibitors, especially in combination therapy (Joshi AD. et al. 2012. *PLoS ONE.* 7(10):e44372). **EGFR variant III** (EGFRvIII) is an oncogenic, constitutively active mutant form of EGFR that is commonly expressed in glioblastoma. EGFRvIII is formed by an in-frame genomic deletion of exons 2 to 7 of EGFR, producing a truncated receptor lacking a portion of the extracellular ligand binding domain. The expression of EGFRvIII is suggested to confer worse prognosis than wt-EGFR expression alone (Shinojima N. et al. 2003. *Cancer Res.* 63:6962-70).					
172	06405-L24605	EGFR, ex 1	NM_005228.3; 208-209	CCGTCCAGTATT-GATCGGGAGAGC	123.1 kb
421	21280-L27637	EGFR, ex 2	NM_005228.3; 425-426	TAACTGTGAGGT-GGTCCTTGGGAA	1.0 kb
398	05436-L24070	EGFR, ex 3	NM_005228.3; 518-519	TTATGTCCTCAT-TGCCCTCAACAC	3.4 kb
220	17208-L23059	EGFR, ex 4	NM_005228.3, 802-803	ACCACCTGGGCA-GCTGTAAGTGTC	4.6 kb
161	05438-L24607	EGFR, ex 5	NM_005228.3; 837-838	TGTCCCAATGGG-AGCTGCTGGGGT	1.2 kb
137	06121-L20393	EGFR, ex 6	NM_005228.3; 899-900	CTGTGCCCAGCA-GTGCTCCGGGCG	1.6 kb
184	05440-L04856	EGFR, ex 7	NM_005228.3; 1102-1103	AGGGCAAATACA-GCTTTGGTGCCA	1.8 kb
429	02063-L21569	EGFR, ex 8	NM_005228.3; 1200-1201	AGCTATGAGATG-GAGGAAGACGGC	5.7 kb
256	05959-L24612	EGFR, ex 13	NM_005228.3; 1847-1848	CCGAGGCAGGGA-ATGCGTGGACAA	9.6 kb
346	17210-L24618	EGFR, ex 16	NM_005228.3, 2154-2155	CTTGAAGGCTGT-CCAACGAATGGG	27.6 kb
269	05969-L24610	EGFR, ex 23	NM_005228.3; 3022-3023	AGATCTCCTCCA-TCCTGGAGAAAG	-
CDKN2A gene, at 9p21.3 Deletion or mutation of CDKN2A is detected in ~50% of glioma samples (Beroukhim R. et al. 2007. *PNAS.* 104:20007-12; TCGA, 2008. *Nature*. 455:1061-8). Homozygous loss of CDKN2A is suggested to be a progression-associated genetic marker, especially in paediatric gliomas (Korhunov A. et al. 2010. *J Clin Oncol.* 28:3182-90).					
309	17814-L22631	CDKN2A, ex 4	NM_000077.4; 1106-1107	TTGCGAGCCTCG-CAGCCTCCGGAA	3.0 kb
385	17817-L23295	CDKN2A, ex 3	NM_000077.4; 706-707	TGCGCGCGGCTG-CGGGGGGCACCA	4.1 kb
250	16060-L22417	CDKN2A, ex 2	NM_000077.4; 138-139	GCCTGGAAAGAT-ACCGCGGTCCCT	19.6 kb
142	18753-L24594	CDKN2A, ex 1	NM_058195.3; 76 nt before ex 1	CGCAGGGCTCAG-AGCCGTTCCGAG	-
PTEN gene, at 10q23.31 Deletion or LOH of 10q is the most common genomic alteration found in primary and secondary glioblastomas (Ohgaki H. et al. 2004. *Cancer Res.* 64:6892-9; Beroukhim R. et al. 2007. *PNAS.* 104:20007-12). Co-expression of EGFRvIII and PTEN has been suggested to associate with favourable clinical response to EGFR kinase inhibitors (Mellinghoff IK. et al. 2005. *NJEM.* 353:2012-24), while loss of PTEN expression seems to correlate with resistance to gefitinib (Guillamo JS. et al. 2009. *Clin Cancer Res.* 15:3697-704).					
226	17387-L24118	PTEN, ex 1	NM_000314.6; 967-968	CCTGCAGAAGAA-GCCCCGCCACCA	29.9 kb
190	06729-L06339	PTEN, ex 2	NM_000314.6; 217 nt after ex 2 rev.	TATCACATAAGT-ACCTGATTATGT	31.5 kb
178	17314-L20922	PTEN, ex 3	NM_000314.6; 226 nt after ex 3	TTGATCTGCTTT-AAATGACTTGGC	5.2 kb
208 ±	17391-L21278	PTEN, ex 4	NM_000314.6; 14 nt before ex 4 rev.	AAAAGAAAAGTT-TAAAAGTGATAT	2.2 kb
373	03638-L22839	PTEN, ex 5	NM_000314.6; 1436-1437	GGTGTAATGATA-TGTGCATATTTA	19.1 kb
323	03639-L24076	PTEN, ex 6	NM_000314.6; 1659-1658 rev.	CTTACTGCAAGT-TCCGCCACTGAA	5.7 kb
475	17386-L22174	PTEN, ex 7	NM_000314.6; 1736-1737	ACACGACGGGAA-GACAAGTTCATG	3.1 kb
359	17397-L24616	PTEN, ex 8	NM_000314.6; 1973-1974	AATGACAAGGAA-TATCTAGTACTT	5.4 kb
214	07686-L15591	PTEN, ex 9	NM_000314.6; 3189-3188 rev.	ACAGCATCTGAA-TTTTAGCACTGG	-

Length (nt)	SALSA MLPA probe	Gene/ Exon	Location/ Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
CDK4, **MIR26A2** and **MDM2** at 12q14.1-q15 Amplification of 12q14-q15, which harbours the CDK4 and MDM2 genes, is detected in 14-18% of newly diagnosed glioblastoma patients (TCGA, 2008. *Nature.* 455:1061-8). The MIR26A2 gene (at 12q14.1) is also shown to be amplified in high-grade glioma, and this amplification is correlated with monoallelic PTEN deletion (Huse JT. et al. 2009. *Genes Dev.* 23:1327-37). In addition, MIR26A2 is shown to regulate PTEN expression and, thereby, MIR26A2 amplification provides a significant growth advantage for tumour cells (Kim H. et al. 2010. *PNAS.* 107:2183-8).					
453 «	18752-L22100	CDK4, ex 8	NM_000075.4; 1157-1158	TCTCTGAGGCTA-TGGAGGGTCCTC	2.5 kb
339 «	17815-L22422	CDK4, ex 4	NM_000075.4; 644-645	GGCCTGGCCAGA-ATCTACAGCTAC	73.7 kb
469	18710-L24620	MIR26A2, ex 1	NR_029847.1; 43-42	AGGCCTCACAGA-TGGAAACAGCCT	10985 kb
481	07178-L24069	MDM2, ex 2	NM_002392.5; 360-361	CCTACTGATGGT-GCTGTAACCACC	27.5 kb
415	18255-L22981	MDM2, ex 10	NM_002392.5; 1200-1199 rev.	TCAGGATCTTCT-TCAAATGAATCT	-
NFKBIA gene, at 14q13.2 Deletion of NF-κB inhibitor (NFKBIA) is detected in about 25% of glioblastomas (Bredel M. et al. 2011 *NJEM.* 364:627-37). It is suggested that deletion of NFKBIA and amplification of EGFR show a pattern of mutual exclusivity and that NFKBIA deletion could substitute for EGFR amplification (Bredel M. et al. 2011. *NJEM.* 364:627-37).					
231	18757-L24608	NFKBIA, ex 5	NM_020529.2; 813-812 rev.	CTTCAACAGGAG-TGACACCAGGTC	0.2 kb
277	18759-L24127	NFKBIA, ex 4	NM_020529.2; 711-712	GCATCGTGGAGC-TTTTGGTGTCCT	0.6 kb
157	18758-L24126	NFKBIA, ex 3	NM_020529.2; 450-451	ATTCGTAGACTC-CACTCCACTTGG	-
TP53 gene, at 17p13.1 Mutations or homozygous deletions of TP53 are detected in 35% of newly diagnosed diffuse glioblastomas (TCGA, 2008. *Nature.* 455:1061-8). Clinical and prognostic significance of TP53 deletions is still under evaluation.					
445	17424-L24621	TP53, ex 11	NM_000546.5; 1360-1361	CTCATGTTCAAG-ACAGAAGGGCCT	1.0 kb
353	17422-L24617	TP53, ex 10	NM_000546.5; 1248-1249	TGAGGCCTTGGA-ACTCAAGGATGC	3.1 kb
283 ± ‡	01999-L21074	TP53, ex 7	NM_000546.5; 1041-1042	CTGTCCTGGGAG-AGACCGGCGCAC	1.2 kb
316	17421-L24613	TP53, ex 5	NM_000546.5; 795-796	TATCCGAGTGGA-AGGAAATTTGCG	0.3 kb
263 ‡	02376-L21409	TP53, ex 4b	NM_000546.5; 606-607	CAAGATGTTTTG-CCAACTGGCCAA	0.8 kb
299	17420-L29693	TP53, ex 3	NM_000546.5; 511-510 rev.	TAGCTGCCCTGG-TAGGTTTTCTGG	0.6 kb
196	01996-L09268	TP53, ex 2a	NM_000546.5; 177-178	CTCTTGCAGCAG-CCAGACTGCCTT	10.8 kb
166	01588-L06028	TP53, ex 1	NM_000546.5; 118-119	TCCGGGGACACT-TTGCGTTCGGGC	-

± SNP rs587780544 could influence the signal of the probe at 208 nt and SNP rs121912660 could influence the signal of the probe at 283 nt. In case of apparent deletions, it is recommended to sequence the region targeted by these probes.

‡ The ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by the IARC TP53 Database (http://p53.iarc.fr/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

« 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.

Notes:

- **The exon numbering of the EGFR and MDM2 genes has changed.** From product description version 27 onwards, we have adopted the NCBI exon numbering that is present in the NM_ sequences for this gene. This exon numbering used here may differ from literature!
- Complete probe sequences are available on request: info@mlpa.com.

Table 2b. P105 reference probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Location	Partial sequence (24 nt adjacent to ligation site)	Location (HG 18) in kb
202	04542-L03931	SCN1A	2q24	AACACCACAACT-GGTGACAGGTTT	02-166.567
238	15174-L16949	RYBP	3p13	GAATCTTTCTGA-AATTGCACATGG	03-072.510
131	16316-L22397	RAB7A	3q21	CACAATAGGAGC-TGACTTTCTGAC	03-130.000
126	18709-L21698	IL4	5q31	ATCGACACCTAT-TAATGGGTCTCA	05-132.038
292	11900-L12706	PKHD1	6p12	TGCTCTCTGGAT-TCAAGACTGAAA	06-052.028
366	06760-L24615	CHD7	8q12	GATTTTTACCGT-GTGGTATCCACC	08-061.928
331	08905-L24614	MYBPC3	11p11	CGTGGGAGAGGA-CTCCTGCACAGT	11-047.316
148	14279-L15949	OCA2	15q13	AGGGGGAAAATA-TCTCACCCTTTC	15-025.951
461	11674-L24181	HIRIP3	16p11	GGCGAGCCTCAA-AGGCAGTTGAGG	16-029.914
490	16456-L24172	MYO5B	18q21	TCTGACTCATCA-TCTCCCACTTCC	18-045.630
500	17001-L22947	SAMHD1	20q11	CCCTGTCACCTC-AAGTTTGAGGAT	20-034.954
244	12431-L13432	LARGE	22q12	ATCCACCTGGTA-TGGTCGACGGGG	22-032.003

SALSA MLPA probemix P105-D2 Glioma-2 sample picture

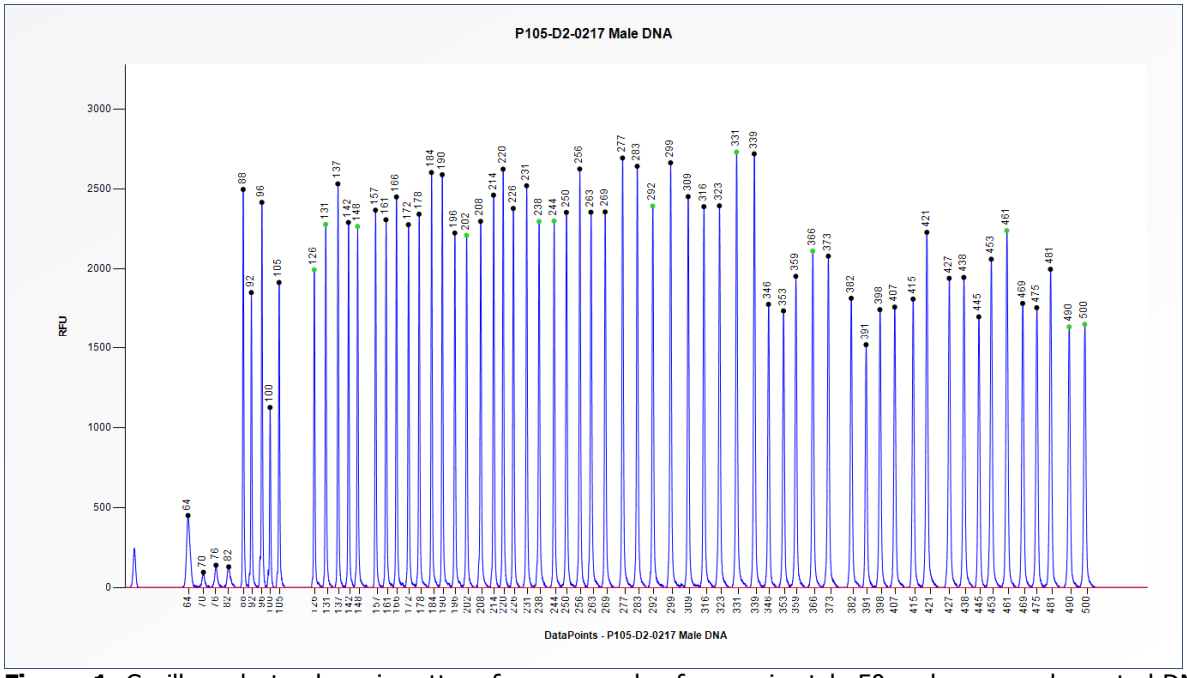

Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P105-D2 Glioma-2 (lot D2-0217).

Implemented Changes – compared to the previous product description version(s).

Version 28 – 12 December 2018 (T08)
- Related probemixes updated - ME012-MGMT-IDH1-IDH2 probemix added on page 2.
- New reference added to 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 sequence and ligation sites for CDK4 probes are updated in Table 2.
- Minor layout changes.

Version 27 – 28 April 2017 (T08)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Exon numbering of the EGFR and MDM2 genes has been changed in page Table 1 and Table 2a.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- New references added on page 1.
- Various minor textual and layout changes.

Version 26 – 08 July 2015 (T07)
- New reference added on page 2.

Version 25 (T06)
- New references added on page 2.

Version 24 (T06)
- Updated link for "Database of Genomic Variants"
- Minor layout changes.

Version 23 (T03)
- Product description adapted to a new product version (version number changed, lot number added, Table 1 and Table 2 modified and new pictures included).
- Several textual changes on page 1.
- New related probemixes added on page 1.
- New references added on page 2.
- Warning added on page 2 about consequences of low tumour cell percentage for MLPA analysis.

Version 22 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 14 (45)
- Volume of the silencer solution has been corrected.
- Various minor textual changes in the tables and on page 1; tables have been numbered; data analysis method has been modified.