Product Description
SALSA® MLPA® Probemix P128-D2 CYP450

To be used with the MLPA General Protocol.

Version D2
As compared to version D1, one probe is adjusted in length, not in sequence detected. For complete product history see page 11.

This SALSA MLPA probemix is for basic research and intended for experienced MLPA users only! This probemix enables you to quantify genes or chromosomal regions in which the occurrence of copy number changes is not yet well-established and the relationship between genotype and phenotype is not yet clear. Since it will not provide you with clear cut answers, interpretation of results can be complicated. MRC Holland recommends thoroughly screening any available literature. Suggestions from specialists for improvement of this product or product description are highly appreciated.

Catalogue numbers:
- **P128-025R:** SALSA MLPA Probemix P128 CYP450, 25 reactions.
- **P128-050R:** SALSA MLPA Probemix P128 CYP450, 50 reactions.
- **P128-100R:** SALSA MLPA Probemix P128 CYP450, 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.

General information
The SALSA MLPA Probemix P128 CYP450 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GSTM1*, *CYP1B1*, *CYP3A4*, *CYP3A5*, *CYP2C19*, *CYP2C9*, *CYP2E1*, *GSTP1*, *CYP1A2*, *CYP1A1*, *CYP2A6*, *CYP2B6*, *GSTT1* and *CYP2D6* genes.

Cytochrome P450 enzymes are important in the biosynthesis and degradation of endogenous compounds such as steroids, lipids and vitamins. These enzymes reduce or alter the pharmacologic activity of many drugs and facilitate their elimination. Differences in drug metabolism in the intestine or liver between patients are major contributors to drug response, including adverse effects.

Glutathione transferases are a family of proteins that play an important role in detoxification by catalyzing the conjugation of many compounds with reduced glutathione.

In the normal population several of the targeted genes differ in copy number. More information per gene is given under Table 2.

The *GSTM1* gene (8 exons), spans ~5.9 kb of genomic DNA and is located on 1p13.3, 110 Mb from the p-telomere. The *CYP1B1* gene (3 exons), spans ~8.6 kb of genomic DNA and is located on 2p22.2, 38 Mb from p-telomere. The *CYP3A4* gene (13 exons), spans ~27 kb of genomic DNA and is located on 7q22.1, 99 Mb from the p-telomere. The *CYP3A5* gene (13 exons), spans ~32 kb of genomic DNA and is located on 7q22.1, 99 Mb from the p-telomere. The *CYP2C19* gene (9 exons), spans ~93 kb of genomic DNA and is located on 10q23.33,

97 Mb from the p-telomere. The *CYP2C9* gene (9 exons), spans ~51 kb of genomic DNA and is located on 10q23.33, 97 Mb from the p-telomere. The *CYP2E1* gene (9 exons), spans ~12 kb of genomic DNA and is located on 10q26.3, 135 Mb from the p-telomere (close to the q-telomere). The *GSTP1* gene (7 exons), spans ~2.8 kb of genomic DNA and is located on 11q13.2, 67 Mb from the p-telomere. The *CYP1A2* gene (7 exons), spans ~7.8 kb of genomic DNA and is located on 15q24.1, 73 Mb from the p-telomere. The *CYP1A1* gene (7 exons), spans ~6.0 kb of genomic DNA and is located on 15q24.1, 73 Mb from the p-telomere. The *CYP2A6* gene (9 exons), spans ~6.9 kb of genomic DNA and is located on 19q13.2, 46 Mb from the p-telomere. The *CYP2B6* gene (9 exons), spans ~27 kb of genomic DNA and is located on on 19q13.2, 46 Mb from the p-telomere. The *GSTT1* gene (5 exon), spans ~8.2 kb of genomic DNA and is located on 22q11.23, 23 Mb from the p-telomere. The *CYP2D6* gene (9 exons), spans ~4.3 kb of genomic DNA and is located on 22q13.2, 41 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1419/.

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 exon numbering used in this P128-D2 CYP450 product description is the exon numbering from:

Gene	NG or LRG sequence
GSTM1	NG_009246.1
CYP1B1	NG_008386.2
CYP3A4	NG_008421.1
CYP3A5	LRG_1431
CYP2C19	LRG_584
CYP2C9	LRG_1195
CYP2E1	NG_008383.1
GSTP1	LRG_723
CYP1A2	LRG_1274
CYP1A1	LRG_1262
CYP2A6	NG_008377.1
CYP2B6	LRG_1267
GSTT1	NM_000853.3
CYP2D6	LRG_303

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 or NG 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 P128-D2 CYP450 contains 52 MLPA probes with amplification products between 128 and 504 nucleotides (nt). At least two probes are present for each of the targeted genes. In addition, 12 reference probes are included that detect autosomal chromosomal locations. 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).

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 reference 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 unrelated individuals who are from families without a history of glutathione transferase abnormalities. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

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/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

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 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term "dosage quotient", used in older product description versions, has been replaced by "final ratio" to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- 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.
- 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 (as described for *DMD* by 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.
- 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

- In most populations, the major cause of genetic defects in the *CYP2D6*, *CYP2C9*, *CYP2C19*, *CYP1B1*, *CYP3A4*, *CYP3A5*, *CYP2E1*, *CYP1A2*, *CYP1A1*, *CYP2A6*, *CYP2B6*, *GSTP1*, *GSTT1* and *GSTM1* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P128 CYP450.
- 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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe

signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

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.

Mutation databases

https://databases.lovd.nl/shared/genes/GSTM1
https://databases.lovd.nl/shared/genes/CYP1B1
https://databases.lovd.nl/shared/genes/CYP3A4
https://databases.lovd.nl/shared/genes/CYP3A5
https://databases.lovd.nl/shared/genes/CYP2C19
https://databases.lovd.nl/shared/genes/CYP2C9
https://databases.lovd.nl/shared/genes/CYP2E1
https://databases.lovd.nl/shared/genes/GSTP1
https://databases.lovd.nl/shared/genes/CYP1A2
https://databases.lovd.nl/shared/genes/CYP1A1
https://databases.lovd.nl/shared/genes/CYP2A6
https://databases.lovd.nl/shared/genes/CYP2B6
https://databases.lovd.nl/shared/genes/GSTT1
https://databases.lovd.nl/shared/genes/CYP2D6

We strongly encourage users to deposit positive results in the Leiden Open Variation Database. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *CYP2E1* exons 5 and 8 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P128-D2 CYP450

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
64-105	Control fragments – see table in probemix content section for more information		
128	Reference probe 00797-L00093	5q	
132	**GSTT1 probe** 20191-L27966		22q11 / **Exon 1**
137	**CYP1B1 probe** 08149-L31276		2p22 / **Exon 1**
142	**CYP3A5 probe** 09535-L16389		7q22 / **Exon 10**
148	**CYP2E1 probe** 07898-L07634		10q26 / **Exon 8**
153	**CYP3A4 probe** 21366-L09940		7q22 / **Exon 6**
160	Reference probe 17436-L21192	16p	
166	Reference probe 04111-L03471	9q	
172	**GSTP1 probe** 06819-L07011		11q13 / **Exon 4**
178	**CYP1B1 probe** 08151-L16393		2p22 / **Exon 3**
185	**CYP2A6 probe** 08031-L07812		19q13 / **Exon 5**
190	**CYP2A6 probe** 09517-L09927		19q13 / **Exon 1**
196	**CYP2D6 probe** 04325-L07013		22q13 / **Exon 1**
202	**CYP2B6 probe** 21367-L31236		19q13 / **Exon 4**
208	**CYP1A2 probe** 07901-L07637		15q24 / **Exon 2**
214	Reference probe 16426-L18879	18q	
221	**CYP2C9 probe** 05848-L07012		10q23 / **Exon 1**
226	**CYP2C9 probe** 09524-L09934		10q23 / **Exon 9**
232	**CYP2D6 probe** 09528-L09938		22q13 / **Exon 6**
238	**CYP2E1 probe** 07897-L07633		10q26 / **Exon 6**
244	**CYP2A6 probe** 09518-L09928		19q13 / **Exon 2**
250	**CYP3A4 probe** 09529-L09939		7q22 / **Exon 1**
256	**CYP1A1 probe** 07362-L06407		15q24 / **Exon 3**
264	**CYP3A5 probe** 09533-L09943		7q22 / **Exon 2**
268	Reference probe 18918-L24513	6q	
274	**CYP1A2 probe** 07903-L07639		15q24 / **Exon 7**
280	Reference probe 06439-L22985	3p	
286	Reference probe 07737-L21372	20q	
297	**CYP2C19 probe** 20260-L27965		10q23 / **Exon 2**
306	Reference probe 03242-L22875	13q	
312	**CYP2C19 probe** 05677-L16394		10q23 / **Exon 9**
325 Δ	**GSTM1 probe** 06814-L16401		1p13 / **Exon 3**
333	**CYP1A1 probe** 17274-L16402		15q24 / **Exon 2**
338	**CYP3A4 probe** 09532-L16403		7q22 / **Exon 13**
346	**CYP2A6 probe** 09519-L16404		19q13 / **Exon 3**
355	**CYP2C9 probe** 05850-L05352		10q23 / **Exon 7**
364	**GSTM1 probe** 07895-L10846		1p13 / **Exon 5**
370	**CYP2B6 probe** 09520-L16750		19q13 / **Exon 2**
377	**CYP2E1 probe** 02394-L01842		10q26 / **Exon 5**
384 ¥	**GSTT1 probe** 04756-L32887		22q11 / **Exon 5**
394	**CYP2D6 probe** 09527-L16406		22q13 / **Exon 5**
400	Reference probe 16933-L19876	4q	
409	**CYP1A2 probe** 07902-L07638		15q24 / **Exon 4**
418	**CYP2C19 probe** 05676-L05221		10q23 / **Exon 6**
427	**CYP1A1 probe** 19510-L26332		15q24 / **Exon 1**
436	**CYP2D6 probe** 04327-L03680		22q13 / downstream
454	Reference probe 16389-L18782	12q	
463	**CYP2C9 probe** 14698-L16349		10q23 / **Exon 8**
476	**GSTP1 probe** 06818-L28022		11q13 / **Exon 3**
486	Reference probe 15337-L13488	14q	
496	**CYP2C9 probe** 14699-L16350		10q23 / **Exon 8**
504	Reference probe 18539-L23848	17q	

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

¥ Changed in version D2. Minor alteration, no change in sequence detected.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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. P128-D2 probes arranged according to chromosomal location

Table 2a. *GSTM1* gene

Length (nt)	SALSA MLPA probe	*GSTM1* exon[a]	Ligation site NM_000561.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*58-60 (Exon 1)*		
325 Δ #	06814-L16401	Exon 3	39 nt after exon 3	GGGGAAAGTGCA-ACGTGTCTCTGA	0.9 kb
364	07895-L10846	Exon 5	309 nt after exon 5	TCTGTCAGCCAG-TTCACATCACCT	
		stop codon	*712-714 (Exon 8)*		

The *GSTM1* gene is extremely variable in copy number (Moyer et al. 2007). Analysis is complicated further by the presence of many SNPs. In Caucasians, no GSTM1 enzyme activity is present in 50% of individuals, primarily due to gene deletions (Buchard et al. 2007).

Table 2b. *CYP1B1* gene

Length (nt)	SALSA MLPA probe	*CYP1B1* exon[a]	Ligation site NM_000104.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*374-376 (Exon 2)*		
137	08149-L31276	Exon 1	229-230	CAAGAGACTCGA-GTGGGAGTTAAA	4.7 kb
178	08151-L16393	Exon 3	1545-1544 *reverse*	CAAAGCTGGAGA-AGCGCATGGCTT	
		stop codon	*2003-2005 (Exon 3)*		

The *CYP1B1* gene is not located in a genomic region known for having frequent germline copy number variants. Germline mutations in *CYP1B1* are involved in primary congenital glaucoma.

Table 2c. *CYP3A4* / *CYP3A5* genes

Length (nt)	SALSA MLPA probe	Gene / exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		CYP3A4	**NM_017460.6**		
		start codon	*104-106 (Exon 1)*		
250 #	09529-L09939	Exon 1	108-109	AGTAGTGATGGC-TCTCATCCCAGA	14.2 kb
153 #	21366-L09940	Exon 6	45 nt before exon 6	CTTCTGGGACTA-GAGTCTGCACAT	11.8 kb
338 #	09532-L16403	Exon 13	1652-1653	TCTTCAAGAAAT-CTGTGCCTGAGA	82.0 kb
		stop codon	*1613-1615 (Exon 13)*		
		CYP3A5	**NM_000777.5**		
		start codon	*101-103 (Exon 1)*		
264 #	09533-L09943	Exon 2	238-239	CCTCTGCCTTTG-TTGGGAAATGTT	15.5 kb
142 #	09535-L16389	Exon 10	1000-1001	GCCCAGTCAATA-ATCTTCATTTTT	
		stop codon	*1607-1609 (Exon 13)*		

The distance between the two genes is ~77 kb. The *CYP3A4* and *CYP3A5* genes are not located in a genomic region known for having frequent germline copy number variants (Lamba et al. 2006).

Table 2d. *CYP2C19* / *CYP2C9* genes

Length (nt)	SALSA MLPA probe	Gene / exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		CYP2C19	**NM_000769.4**		
		start codon	*26-28 (Exon 1)*		
297 #	20260-L27965	Exon 2	203-204	AGCTCTCAAAAA-TCTATGGCCCTG	45.5 kb
418	05676-L05221	Exon 6	891-892	AAACTTGGTAAT-CACTGCAGCTGA	32.3 kb
312 #	05677-L16394	Exon 9	1438-1439	GACACAACTCCT-GTTGTCAATGGA	85.8 kb
		stop codon	*1496-1498 (Exon 9)*		
		CYP2C9	**NM_000771.4**		
		start codon	*26-28 (Exon 1)*		
221 #	05848-L07012	Exon 1	35-36	CAATGGATTCTC-TTGTGGTCCTTG	42.5 kb
355 #	05850-L05352	Exon 7	1015-1014 *reverse*	TTTCTGCCAATC-ACACGTTCAATC	4.8 kb
463 #	14698-L16349	Exon 8	1191-1190 *reverse*	AAGTCAGGGAAA-TTAATATGGTTG	0.1 kb
496 #	14699-L16350	Exon 8	1254-1255	GTTTGACCCTCA-TCACTTTCTGGA	2.8 kb
226	09524-L09934	Exon 9	1421-1422	TTGACCCAAAGA-ACCTTGACACCA	
		stop codon	*1496-1498 (Exon 9)*		

The distance between the genes is ~86 kb. The *CYP2C19* and *CYP2C9* genes are not located in a genomic region known for having frequent germline copy number variants. Defects in the *CYP2C9* gene have effects on the metabolism of the widely used Warfarin drug.

Table 2e. *CYP2E1* gene

Length (nt)	SALSA MLPA probe	***CYP2E1*** exon[a]	Ligation site NM_000773.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*34-36 (Exon 1)*		
377	02394-L01842	Exon 5	797-798	GGAGCACCATCA-ATCTCTGGACCC	1.0 kb
238	07897-L07633	Exon 6	888-889	CGCTTGTACACA-ATGGACGGTATC	4.1 kb
148	07898-L07634	Exon 8	1329-1330	CCATTTTCCACA-GGTGAGAAAGAT	
		stop codon	*1513-1515 (Exon 9)*		

The *CYP2E1* gene shows frequent germline copy number changes. Variation 2896 and 8670 of the database of genomic variants indicate a gain in *CYP2E1* copy number in 7.4% respectively 5% of the individuals.

Table 2f. *GSTP1* gene

Length (nt)	SALSA MLPA probe	***GSTP1*** exon[a]	Ligation site NM_000852.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*33-35 (Exon 1)*		
476	06818-L28022	Exon 3	121-122	CCAGAGCTGGAA-GGAGGAGGTGGT	0.2 kb
172	06819-L07011	Exon 4	234-235	ACCAGTCCAATA-CCATCCTGCGTC	
		stop codon	*663-665 (Exon 7)*		

Germline copy number changes (gains) of the *GSTP1* gene have been described, but are rare.

Table 2g. *CYP1A2* / *CYP1A1* genes

Length (nt)	SALSA MLPA probe	Gene / exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		CYP1A2	**NM_000761.5**		
		stop codon	*1611-1613 (Exon 7)*		
274	07903-L07639	Exon 7	1464-1465	CCAAGTGGGAGA-TCTTCCTCTTCC	3.1 kb
409	07902-L07638	Exon 4	1061-1062	CTCATGTACCTT-GTGACCAAGCCT	1.4 kb
208	07901-L07637	Exon 2	727-728	CCTCGTGAAGAA-CACTCATGAGTT	24.9 kb
		start codon	*63-65 (Exon 2)*		
		CYP1A1	**NM_001319217.2**		
		start codon	*118-120 (Exon 2)*		
427	19510-L26332	Exon 1	24-25	TCCTTGGAACCT-TCCCTGATCCTT	3.1 kb
333	17274-L16402	Exon 2	780-781	GTCAACCTGAAT-AATAATTTCGGG	0.8 kb
256	07362-L06407	Exon 3	1014-1015	GAGAACGCCAAT-GTCCAGCTGTCA	
		stop codon	*1654-1656 (Exon 7)*		

The distance between the genes is ~23 kb. Germline copy number changes of *CYP1A2* and *CYP1A1* are probably quite rare. Entry 9749 of the database of genomic variants mentions one gain of *CYP1A1* in 112 samples and no CNVs that include *CYP1A2*.

Table 2h. *CYP2A6* / *CYP2B6* genes

Length (nt)	SALSA MLPA probe	Gene / exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		CYP2A6	**NM_000762.6**		
		stop codon	*1504-1506 (Exon 9)*		
185 #	08031-L07812	Exon 5	242 nt before exon 5	AGCCTCGTTTAA-ATACCTGAAACC	1.6 kb
346 #	09519-L16404	Exon 3	137 nt before exon 3	TTTGCAGCTCTG-CTGGGCAATGGC	0.8 kb
244 #	09518-L09928	Exon 2	85 nt after exon 2	ACTCTCCTGCCA-ACTGGAGGCTAA	0.7 kb
190 #	09517-L09927	Exon 1	37-38	TGGCCTCAGGGA-TGCTTCTGGTGG	153.8 kb
		start codon	*22-24 (Exon 1)*		
		CYP2B6	**NM_000767.5**		
		start codon	*25-27 (Exon 1)*		
370	09520-L16750	Exon 2	347-348	CGACCCATTCTT-CCGGGGATATGG	2.8 kb
202 #	21367-L31236	Exon 4	585-586	TTTGGAAAACGA-TTCCACTACCAA	
		stop codon	*1498-1500 (Exon 9)*		

The distance between the genes is ~141 kb. Germ line copy number changes of *CYP2A6* are relatively frequent. Entry 10556 of the database of genomic variants mentions two losses and two gains in 112 samples for *CYP2A6*. Entry 3191 mentions one gain and two losses in the 270 HapMap samples.

Table 2i. *GSTT1* gene

Length (nt)	SALSA MLPA probe	***GSTT1*** exon[a]	Ligation site NM_000853.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*81-83 (Exon 1)*		
132	20191-L27966	Exon 1	187-188	CGTGGATCTGAT-TAAAGGTAGGTC	7.6 kb
384	04756-L32887	Exon 5	700-701	GGAGGATCTCTT-CCAGGAGGCCCA	
		stop codon	*801-803 (Exon 5)*		

The *GSTT1* gene is extremely variable in copy number (Moyer et al. 2007). Analysis is complicated by the presence of many SNPs. In Caucasians, no GSTT1 enzyme activity is present in 15% of individuals, primarily due to gene deletions (Buchard et al. 2007).

Table 2j. *CYP2D6* gene

Length (nt)	SALSA MLPA probe	*CYP2D6* exon[a]	Ligation site NM_000106.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*20-22 (Exon 1)*		
196 #	04325-L07013	Exon 1	19-20	AGTGAGGCAGGT-ATGGGGCTAGAA	2.2 kb
394	09527-L16406	Exon 5	200 nt before exon 5	CTGTACCTCCTA-TCCACGTCAGAG	0.8 kb
232	09528-L09938	Exon 6	68 nt after exon 6	CCCATGAACTTT-GCTGGGACACCC	1.8 kb
436 #	04327-L03680	downstream	495 nt after exon 9 *reverse*	CCTGGGCTTCCA-TGGGGCCTTCCC	
		stop codon	*1511-1513 (Exon 9)*		

Germline copy number changes of *CYP2D6* are common due to the presence of a repeated sequence located just before and after the gene. *CYP2D6* gene deletions resulting in a poor metabolizer phenotype are known as *CYP2D6*5* or *CYP2D6(D)*. *CYP2D6* gene duplications resulting in ultrarapid metabolism of certain drugs are also common. Entry 7350 of the database of genomic variants mentions three gains and six losses in 50 samples. The frequency of the *CYP2D6*5* allele has been reported by others to be 0.04 in Caucasians. *CYP2D6* is known to metabolize more than 65 commonly used drugs. Patients are usually divided into four different subpopulations (poor, intermediate, extensive and ultra metabolizers) that define the rate of drug metabolism by *CYP2D6*. 5 to 10 percent of Caucasians have poor ability to metabolize, as do 1 to 2 percent of Southeast Asians while up to 30 percent of people with a North Eastern African background are ultra metabolizers who can carry gene duplications ranging from 3-13 copies of *CYP2D6*.

[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.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
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.

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.

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P128 product history	
Version	*Modification*
D2	One probe is adjusted in length, not in sequence detected.
D1	One probe for *CYP2B6* and one for *CYP3A5* were removed, in addition one reference probe has been replaced and several probe lengths have been adjusted.
C1	Nine reference probes have been replaced and one probe for *CYP2D6* has been removed.
B2	QDX2 control fragments included.
B1	X and Y fragments, two probes for *CYP2C9* have been added. Probes for *CYP2C19* and several reference probes have been replaced
A1	First release.

Implemented changes in the product description

Version D2-01 – 30 August 2022 (04P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *GSTM1*, *CYP1B1*, *CYP3A4*, *CYP2E1*, *GSTP1* and *CYP2A6* genes updated according to new versions of the NM_ reference sequences. The ligation site for probe 04327-L03680 was corrected.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version D1-01 – 16 November 2018 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version.
- Small changes in Table 1 and Table 2.
- RefSeq transcript NM_001319217.2 replaces NM_000499.5 for the *CYP1A1* gene.
- Warning added below Table 1 and Table 2 for the 325 nt GSTM1 probe which can be sensitive for lower ligation temperatures.

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