Product Description
SALSA® MLPA® Probemix P103-C1 DPYD

To be used with the MLPA General Protocol.

Version C1
For complete product history see page 9.

Catalogue numbers:
- **P103-025R:** SALSA MLPA Probemix P103 DPYD, 25 reactions.
- **P103-050R:** SALSA MLPA Probemix P103 DPYD, 50 reactions.
- **P103-100R:** SALSA MLPA Probemix P103 DPYD, 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 P103 DPYD is a **research use only (RUO)** assay for the detection of deletions or duplications in the *DPYD* gene, which is associated with DPD deficiency and the breakdown of 5-fluorouracil (5-FU) which is one of the most widely used drugs for cancer chemotherapy. Furthermore, this probemix can also be used to detect the presence of the c.1129-5923C>G and the c.1905+1G>A (IVS14+1G>A) mutations and the wild type sequences of the c.1679T>G and c.2846A>T mutations.

DPD deficiency is a clinically heterogeneous disorder. The variability ranges from no symptoms to a convulsive disorder with motor retardation and intellectual disability in homozygous patients (OMIM#274270). The frequency of a homozygous defect in the *DPYD* gene is estimated to be 1:10.000 in most populations. Individuals with enzymatic activities in the heterozygous range can experience acute 5-FU toxicity when they receive chemotherapy with 5-FU. The number of individuals that have only one intact copy of the *DPYD* gene might be as high as 3%. It is estimated that 0.5% of all colorectal cancer patients that receive 5-FU treatment die from adverse drug reactions. See the table below for more information about the predicted effect of the *DPYD* mutations included in this mix on DPD activity.

***DPYD* gene variants** **Nomenclature and predicted consequences**		
***DPYD* variant**	**Alternative name(s)**	**Predicted Consequence**
c.1679T>G	DPYD*13, rs55886062	Abolished DPD activity
c.1905+1G>A	DPYD*2A, IVS14+1G>A, rs3918290	Abolished DPD activity
c.2846A>T	rs67376798	Decreased DPD activity
c.1129-5923C>G	rs75017182	Decreased DPD activity

The *DPYD* gene (23 exons) spans ~843 kb of genomic DNA and is located on 1p21.3, ~97 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK395610/.

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/
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE

Exon numbering
The *DPYD* exon numbering used in this P103-C1 DPYD product description is the exon numbering from the MANE Select transcript NM_000110.4 (derived from the MANE project). The *DPYD* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2. 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 P103-C1 DPYD contains 45 MLPA probes with amplification products between 130 and 490 nucleotides (nt). This includes 33 copy number probes for the *DPYD* gene, at least one probe for all 23 exons. Furthermore, two probes specific for the c.1129-5923C>G and c.1905+1G>A mutations, which will only generate a signal when the mutations is present, and two probes specific for the wild type sequence of the c.1679T>G and c.2846A>T mutations are included. A reduced signal in these wild type probes can point towards the presence of the mutation **or** a deletion of the exon. In addition, eight 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 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 DPD deficiency. 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. Sample ID numbers HG02684, NA21112, and NA07048 from the Coriell Institute have been tested with this P103-C1 probemix at MRC-Holland and can be used as positive control samples to detect a homozygous c.1905+1G>A mutation, a heterozygous c.1905+1G>A mutation, and a heterozygous c.2846A>T mutation, respectively. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Mutation	Expected alteration
HG02684	Coriell Institute	c.1905+1G>A	Homozygous positive
NA21112	Coriell Institute	c.1905+1G>A	Heterozygous positive
NA07048	Coriell Institute	c.2846A>T	Heterozygous positive

SALSA Binning DNA SD073

The SD073 Binning DNA provided with this probemix can be used for binning of all probes including the two mutation-specific probes (355 nt probe 21643-L30258 detecting c.1129-5923C>G mutation and 436 nt probe 06352-L05868 detecting c.1905+1G>A mutation). SD073 Binning DNA is a mixture of genomic DNA from healthy individuals and synthetic DNA that contains the target sequences detected by the above mentioned probes. Inclusion of one reaction with 5 µl SD073 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(s). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD073 Binning DNA product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

Data analysis

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

Interpretation of results

The standard deviation of each individual probe over all the reference samples should be ≤0.10 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 *DPYD* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P103 DPYD.
- 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.

***DPYD* mutation database**

https://databases.lovd.nl/shared/genes/DPYD. 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 *DPYD* exons 7 and 9 but not exon 8) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P103-C1 DPYD

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*DPYD*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 21303-L29709	3p	
136	**DPYD probe** 05316-L04703		**Exon 1**
145	Reference probe 19948-L29905	10q	
154	**DPYD probe** 05318-L04705		**Exon 2**
160	**DPYD probe** 06350-L05865		**Exon 14**
166	**DPYD probe** 05329-L06207		**Exon 12**
172	**DPYD probe** 05319-L05477		**Exon 3**
178	Reference probe 05725-L05164	9q	
183	**DPYD probe** 05334-L30573		**Exon 16**
193	**DPYD probe** 05321-L30493		**Exon 4**
199 ∞	**DPYD probe** 21600-L30728		**c.1679T>G WT**
208	**DPYD probe** 05322-L04709		**Exon 5**
214	**DPYD probe** 05333-L04720		**Exon 16**
221	**DPYD probe** 06345-L06205		**Exon 4**
227	**DPYD probe** 11949-L16801		**Exon 22**
237	**DPYD probe** 05323-L30433		**Exon 6**
243	**DPYD probe** 05335-L30434		**Exon 17**
250	**DPYD probe** 21543-L30494		**Intron 14**
256	**DPYD probe** 05324-L04711		**Exon 7**
265	**DPYD probe** 05336-L04723		**Exon 18**
271	**DPYD probe** 20728-L05861		**Exon 5**
277	**DPYD probe** 21545-L05858		**Upstream**
285	**DPYD probe** 05325-L30442		**Exon 8**
292	Reference probe 09801-L10216	15q	
301	**DPYD probe** 05337-L30435		**Exon 19**
308	**DPYD probe** 05326-L04713		**Exon 9**
318	**DPYD probe** 05339-L04726		**Exon 20**
328	**DPYD probe** 21544-L06206		**Exon 15**
337	**DPYD probe** 05327-L04714		**Exon 10**
346	**DPYD probe** 06355-L05871		**Exon 21**
355 §	**DPYD probe** 21643-L30258		**c.1129-5923C>G**
371	**DPYD probe** 06348-L30580		**Exon 11**
387 ∞	**DPYD probe** 11951-L30155		**c.2846A>T WT**
392	**DPYD probe** 05320-L05474		**Intron 3**
400	**DPYD probe** 05343-L04730		**Exon 23**
409	Reference probe 10063-L10487	8q	
418	**DPYD probe** 05338-L04725		**Exon 19**
427	**DPYD probe** 05330-L04717		**Exon 13**
436 §	**DPYD probe** 06352-L05868		**c.1905+1G>A**
445	**DPYD probe** 05340-L05475		**Exon 20**
453	Reference probe 20385-L27825	11q	
463	Reference probe 11264-L11947	19q	
472	**DPYD probe** 06347-L05862		**Exon 8**
481 ±	**DPYD probe** 06349-L05864		**Exon 12**
490	Reference probe 10218-L10698	7q	

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

§ Mutation-specific probe. This probe will only generate a signal when the c.1129-5923C>G (355 nt) or the c.1905+1G>A (436 nt) mutation is present. The c.1129-5923C>G specific probe has only been tested on artificial DNA **but not on positive human samples!**

∞ Wild type sequence detected. A lowered probe signal can be due to the c.1679T>G (199 nt) or the c.2846A>T (387 nt) mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.
± SNP rs116364703 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. *DPYD* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*DPYD* exon[a]	Ligation site NM_000110.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
277	21545-L05858	Upstream (Exon 1)	145 nt before exon 1	TCCCTTCTGCTT-GCAGGCTGGGGC	0.3 kb
		start codon	*113-115 (Exon 1)*		
136	05316-L04703	Exon 1	114-115	AGGCACTGCCAT-GGCCCCTGTGCT	37.6 kb
154	05318-L04705	Exon 2	226-227	AAATTAGACAAG-AAACATTGGAAA	55.2 kb
172	05319-L05477	Exon 3	321-322	TCTTGGTGAGCG-AGGAGCTCTCCG	0.2 kb
392	05320-L05474	Intron 3 (Exon 3)	145 nt after exon 3	ACTTCCCTTGAG-GCTGTACTTTGG	87.5 kb
193	05321-L30493	Exon 4	369-370	TGCAGATGCCCC-GTGTCAGAAGAG	0.1 kb
221	06345-L06205	Exon 4	24 nt after exon 4	TTAACTCTGCAA-ATGAAAATAACA	18.7 kb
271	20728-L05861	Exon 5	487-486 *reverse*	ACCATTCCACAA-GTCAGACCAAGT	0.1 kb
208	05322-L04709	Exon 5	550-551	TATGCCACTGAA-GAGGGACCCATT	22.1 kb
237	05323-L30433	Exon 6 (7)	694-695	GCTCTTTTTGGT-GCTGGGCCTGCA	7.7 kb
256	05324-L04711	Exon 7 (8)	826-827	CGGCTGCCGTAT-GATGTAGTGAAT	12.6 kb
285	05325-L30442	Exon 8 (9)	928-927 *reverse*	TAGCCTTTTTCT-TTCAAAGTGCTA	0.1 kb
472	06347-L05862	Exon 8 (9)	80 nt after exon 8	AATGACTGCCTT-CAGACATTTTCG	83.9 kb
308	05326-L04713	Exon 9 (10)	1028-1029	GGTTTTATACAT-CCAAAGACTTTT	1.7 kb
337	05327-L04714	Exon 10 (11)	1104-1105	ATTGCCATCGAT-ACGGGGAGTCGT	13.5 kb
355 §	21643-L30258	Intron 10 (11)	5923 nt before exon 11 *reverse*; c.1129-5923C>G	AAAAATTCTCAC-CTTTGATTTTCA	6.1 kb
371	06348-L30580	Exon 11 (12)	1430-1431	GTGCCTTTGGTT-CAGTTCTGAGTG	24.1 kb
166	05329-L06207	Exon 12 (13)	1536-1537	TAGTGAAGCATG-GGTATTTGCAGG	0.1 kb
481 ±	06349-L05864	Exon 12 (13)	1606-1607	GATGGAAAGCAA-GCTTCTTGGTAC	33.7 kb
427	05330-L04717	Exon 13 (14)	1665-1666	CGTTTCTGCCAA-GCCTGAACTACC	0.1 kb
199 ∞	21600-L30728	Exon 13 (14)	1791-1790 *reverse*	AAGCTCTTCGAA-TCATTGATGTGC	65.6 kb
160	06350-L05865	Exon 14 (15)	1865-1866	ACATTGTGACAA-ATGTTTCCCCCA	0.2 kb
436 §	06352-L05868	Exon 14 (15)	1 nt after exon 14; c.1905+1G>A, IVS14+1G>A	TTCCAGACAACA-TAAGTGTGATTT	67.4 kb
250	21543-L30494	Intron 14 (Exon 16)	210 nt before exon 15	GATAGTAAATGA-GAAAATCTCCTA	0.2 kb
328	21544-L06206	Exon 15 (16)	2060-2059 *reverse*	AAGTTCCGTCCA-GTCATTTTTATT	8.8 kb
214	05333-L04720	Exon 16 (17)	8 nt before exon 16	TTTTCCTTTCTT-GTTTAAAGGATT	0.1 kb
183	05334-L30573	Exon 16 (17)	2148-2149	GGGAGAAAGAGG-AATGGGCCTGGC	67.3 kb

Length (nt)	SALSA MLPA probe	*DPYD* exon[a]	Ligation site NM_000110.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
243	05335-L30434	Exon 17 (18)	2184-2185	TCCAGAGCTGGT-GCGGAACATCTG	1.0 kb
265	05336-L04723	Exon 18 (19)	2392-2393	GCAAAGCGAACT-ACATATGGAGGA	70.4 kb
301	05337-L30435	Exon 19 (20)	2484-2485	ATTTCCCATTTT-GGCTACTGGTGG	0.1 kb
418	05338-L04725	Exon 19 (20)	2550-2551	TGCTTCCGTCCT-CCAGGTAGTCAT	41.6 kb
318	05339-L04726	Exon 20 (21)	2587-2588	CAGGATTTCACT-GTGATCGAAGAC	0.1 kb
445	05340-L05475	Exon 20 (21)	2668-2669	TGGGATGGACAG-AGTCCAGCTACT	94.6 kb
346	06355-L05871	Exon 21 (22)	2788-2789	ATCATAGCAGAA-AACAAGATTAGA	16.1 kb
227	11949-L16801	Exon 22 (23)	2898-2897 *reverse*	CAAGGTACTGCA-GTGCTTTTCCTA	0.1 kb
387 ∞	11951-L30155	Exon 22 (23)	2958-2959	GGCTATGATTGA-TGAAGAAATGTG	3.3 kb
400	05343-L04730	Exon 23 (24)	3068-3069	TAACCGACACTT-GTACAGGCTGTA	
		stop codon	*3188-3190 (Exon 23)*		

[a] See section Exon numbering on page 2 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

§ Mutation-specific probe. This probe will only generate a signal when the c.1129-5923C>G (355 nt) or the c.1905+1G>A (436 nt) mutation is present. The c.1129-5923C>G specific probe has only been tested on artificial DNA **but not on positive human samples!**
∞ Wild type sequence detected. A lowered probe signal can be due to the c.1679T>G (199 nt) or the c.2846A>T (387 nt) mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.
± SNP rs116364703 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P103 DPYD

- Gross et al. (2013). Somatic copy number changes in DPYD are associated with lower risk of recurrence in triple-negative breast cancers. *Br J Cancer*. 109:2347-55.
- Henricks et al. (2018). Capecitabine-based treatment of a patient with a novel DPYD genotype and complete dihydropyrimidine dehydrogenase deficiency. *Int J of Cancer*. 142:424-430.
- Van Kuilenburg et al. (2009). Analysis of severely affected patients with dihydropyrimidine dehydrogenase deficiency reveals large intragenic rearrangements of DPYD and a de novo interstitial deletion del(1)(p13.3p21.3). *Hum Genet*. 125:581-590.

- Van Kuilenburg et al. (2015). Frequent intragenic rearrangements of DPYD in colorectal tumours. *The Pharmacogenomics J*. 15:211.
- Van Kuilenburg et al. (2017). Severe fluoropyrimidine toxicity due to novel and rare DPYD missense mutations, deletion and genomic amplification affecting DPD activity and mRNA splicing. *Mol Basis of Disease*. 1863:721-730.
- Paré et al. (2009). Absence of large intragenic rearrangements in the DPYD gene in a large cohort of colorectal cancer patients treated with 5-FU-based chemotherapy. *Br J Clin Pharmacol*. 70:268-72.
- Saarenheimo J et al. (2021). Preemptive screening of DPYD as part of clinical practice: high prevalence of a novel exon 4 deletion in the Finnish population. *Cancer Chemother Pharmacol*. 1-7.

P103 product history	
Version	*Modification*
C1	Four target probes have been removed, and three target probes detecting specific mutations have been added. Seven reference probes have been replaced, and two reference probes have been removed. Several probe lengths have been adjusted.
B2	Two reference probes have been replaced and one has been added. In addition the control fragments have been adjusted (QDX2).
B1	First commercial release.

Implemented changes in the product description
Version C1-06 – 28 March 2025 (04P) - Updated exon numbering (MANE project) of the probes targeting the DPYD gene. *Version C1-05 – 19 November 2024 (04P)* - The term 'mental retardation' is considered outdated and was updated to 'intellectual disability' where appropriate. *Version C1-04 – 30 September 2021 (04P)* - Probe detecting the WT of the c.2846A>T mutation on 387 nt was erroneously marked in Table 2 as being located in exon 24 while the probe is located in exon 23. - Small textual changes. *Version C1-03 – 14 May 2021 (04P)* - Product description rewritten and adapted to a new template. *Version C1-02 — 08 April 2020 (02P)* - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *DPYD* gene updated according to new version of the NM_ reference sequence.

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