Product Description
NXtec™ D028-A1 Carrier Panel 1

To be used with the digitalMLPA NXtec Protocol.

Version A1
Check the version of your product on the probemix label to ensure you are reading the appropriate product description. For complete product history see page 12.

Catalogue numbers
- **D028-025R:** NXtec D028 Carrier Panel 1, 25 reactions
- **D028-050R:** NXtec D028 Carrier Panel 1, 50 reactions
- **D028-100R:** NXtec D028 Carrier Panel 1, 100 reactions

NXtec D028-A1 Carrier Panel 1 (hereafter: D028 Carrier Panel 1) is to be used in combination with:

1. SALSA® Sample DNA SD100 (included in Cat. No: D028-025R, D028-050R and D028-100R)
2. NXtec Reagent Kit (Cat No: DRK01-IL, DRK05-IL, DRK20-IL)
3. Barcode plates:
   NXtec Barcode Plate 1 (Cat No: BP01-IL (from lot 03-009-xxxxxx and higher))
   NXtec Barcode Plate 2 (Cat No: BP02-IL (from lot 03-008-xxxxxx and higher))
   NXtec Barcode Plate 3 (Cat No: BP03-IL (from lot 03-010-xxxxxx and higher))
   NXtec Barcode Plate 4 (Cat No: BP04-IL (from lot 03-011-xxxxxx and higher))

   N.B. The three-digit number between dashes (e.g. -008-) will increase with every new barcode plate lot.

4. Data analysis software Coffalyser digitalMLPA™ 2.5.1 or higher (Cat No: n.a.)

Volumes and ingredients

Volumes			Ingredients
D028-025R	D028-050R	D028-100R	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, Tris-HCl, EDTA, DTT

The probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis
Information regarding quality tests is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the digitalMLPA NXtec 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
NXtec D028-A1 Carrier Panel 1 is a **research use only (RUO)** assay for the detection of deletions, duplications, large gene conversions and the presence/absence of several selected mutations in nine chromosomal regions mentioned in Table 2, which are associated with autosomal recessive or X-linked conditions that are frequently tested for in carrier screening.

A frequent cause of genetic defects in the regions included in D028-A1 Carrier Panel 1 are small (point) mutations, most of which will not be detected by using D028-A1 Carrier Panel 1. **It is therefore strongly recommended to use this digitalMLPA probemix in combination with sequence analysis.**

This probemix is not CE/FDA registered for use in diagnostic procedures. The digitalMLPA technique is covered by US patent 6,955,901 and corresponding patents outside the US and digitalMLPA products are sold under a license of Labcorp on patent US 9,624,533. The purchase of this product includes a license on these patents to use only this amount of product solely for the purchaser's own use.

Probemix content
A total number of 422 probes are included in D028-A1 Carrier Panel 1, this consists of:

- 204 probes detecting copy number alterations in nine chromosomal regions, of which 24 probes are also wildtype specific probes that can detect the wildtype sequence of a particular mutation and 4 probes are double wildtype specific probes that detect the wildtype sequence at two locations simultaneously.
- Five mutation-specific probes, which will only generate probe reads when that mutation is present.
- One SNP probe, which will only generate probe reads when the specific SNP is present.
- 54 reference probes, spread over all (autosomal) chromosomes.
- More than 120 control probes and fragments: these include probes for sample identification and probes for detection of errors or deviations when performing digitalMLPA assays, impurities in and fragmentation of the DNA samples, ligase and polymerase activity and extent of hybridisation.

See the Probe Information File (PIF) and Table 2 for more details.

The total number of probes can be used to calculate the number of reactions that can be combined into one sequencer run. See chapter 'Amplicon Quantification by Illumina Sequencers' in the digitalMLPA NXtec Protocol or the calculator tool available at support.mrcholland.com.

Reference probes
A selection of 54 probes, spread over all (autosomal) chromosomes, are designated as reference probes.

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): http://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive: http://tark.ensembl.org/

digitalMLPA technique
digitalMLPA (Benard-Slagter et al. 2017) combines the robustness and simplicity of the trusted SALSA® MLPA® technology (Schouten et al. 2002) with next-generation sequencing. For NXtec products a specific protocol of the digitalMLPA technique is used. The principles of digitalMLPA and the protocol for NXtec products are described in the digitalMLPA NXtec Protocol (www.mrcholland.com).

digitalMLPA technique validation

Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using this NXtec probemix for the first time, or when pre-analytical steps, DNA extraction method or the instruments used are changed. This validation experiment should result in a standard deviation ≤0.10 for all reference probes.

Required specimens and sample treatment

Extracted DNA, free from impurities known to affect digitalMLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

Ideally, all samples tested should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Samples must also be treated with RNase A prior to use (see box below). SD100 does not require RNase treatment.

RNase sample treatment (essential for *HBA* and *HBB*)

Since *HBA* and *HBB* are heavily expressed in red blood cells, RNase treatment of samples is essential for whole-blood derived samples. Without RNase treatment, *HBA* and *HBB* mRNA can compete with or bind to probes that detect a sequence within the exons. Please note that some automatic DNA purification methods (e.g. Roche MagNA Pure) do not include an RNase treatment.

The following method can be used to treat RNA containing DNA samples:
Mix 3 µl sample and 1 µl 0.5 mg/ml RNase A. Incubate 30 minutes at 37°C. Continue with the 10 minutes 98°C DNA denaturation step of the digitalMLPA NXtec Protocol.

RNase A is extremely stable; it can be diluted in TE and stored at -20°C. We recommend RNase A from Promega (A7973; 4 mg/ml solution), diluted eightfold in TE (1 ml of 4 mg/ml RNase is sufficient for ~8000 samples). Do not use more than the recommended amount.

For more information see the digitalMLPA NXtec Protocol, section DNA sample treatment.

SALSA® Sample DNA SD100

The selection of suitable reference DNA samples with a normal copy number for all targets included in D028 Carrier Panel 1 can be complicated, as a significant portion of the global population is found to be a carrier of at least one variant. SD100 is a (pooled) female DNA sample with a normal copy number for all D028 Carrier Panel 1 targets, as shown in the PIF under the column 'Normal copy number'. To facilitate correct data analysis and interpretation of copy numbers, it is strongly recommended to include at least three SD100 samples in each experiment. See section "Data analysis and reference samples" for further details.

Positive control DNA samples

MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. The Coriell Institute (https://catalog.coriell.org) has a diverse collection of biological resources which may be used as a positive control DNA sample in your digitalMLPA experiments. The quality of cell lines can change, therefore deviations to the indicated copy number variation (CNV) findings might occur. A list of positive control samples that have been tested with D028 Carrier Panel 1 at MRC Holland can be found on the product page.

Data analysis and reference samples

Coffalyser digitalMLPA must be used for data analysis in combination with the appropriate lot-specific product sheet. For both, the latest version should be used. Coffalyser digitalMLPA is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. Normalisation of results should be performed within one experiment. The Coffalyser digitalMLPA User Manual contains technical guidelines and information on data evaluation/normalisation. Please be aware that the Sample Result reports that are compiled only show gene panels that have aberrant results or quality warnings. For an overview of all results, consult the Excel Report.

Several approaches can be used to analyse D028-A1 Carrier Panel 1 data (methods 1, 2 and 3), as outlined in Table 1, listed in **order of preference**. Each method differs in its requirements and suitability depending on the experimental design and sample characteristics. Selecting the appropriate method for data analysis of your experiment is important to ensure **accurate normalisation** and verification of such using SD100. Regardless of the chosen method, it is strongly recommended to include **at least three SD100 samples** in each experiment. This provides flexibility between methods during data analysis and helps avoid the need to repeat experiments.

Table 1. Data analysis approaches for D028-A1 Carrier Panel – *in order of preference*

Analysis Method	Description	Use of SD100	CDF settings	Notes
1. Dedicated reference samples from own collection	**Preferred method** Select ≥3* samples as reference	Use SD100 to verify normalisation was successful△	- Define selected reference samples as 'reference' - Define test samples as 'test' - Define SD100 replicates as 'test' and indicate as 'pooled DNA'	Dedicated reference samples should not have any aberrations across all probe-targeted regions. In case SD100 shows aberrant results, this may indicate a suboptimal choice of dedicated reference samples. Reconsider reference sample selection and reanalyse or use one of the other analysis methods. *Optional: An initial analysis could be run with SD100 defined as 'reference' to identify suitable dedicated reference samples.*
2. Without dedicated reference samples	**Only suitable for large, heterogeneous sample sets** All samples are used for inter-normalisation°	Use SD100 to verify normalisation was successful△	- Define all test samples as 'test' - Define SD100 replicates as 'test' and indicate as 'pooled DNA'	When sufficient DNA samples from unrelated individuals are tested in an experiment, it is unlikely that a substantial number of the samples will have the same copy number change. In this case, using dedicated reference samples is not necessary and all samples can be used for inter-normalisation.
3. SD100 as dedicated reference samples **(fallback method)**	**Only if other methods are not feasible** Select ≥3* SD100 samples as reference	Use SD100 replicates as dedicated reference samples	- Define SD100 replicates as 'reference' and indicate as 'pooled DNA' - Define all test samples as 'test'	Less preferred due to differences in origin and processing between SD100 and test samples (see section sample treatment). Moreover, using multiple replicates of the same sample for inter-normalisation does not reflect true sample-to-sample variability. This could potentially compromise downstream statistical analyses and may result in data analysis warnings or errors.

* When using dedicated reference samples (methods 1 and 3): A sufficient number (≥3) of reference samples should be included in each digitalMLPA experiment for data normalisation. For >21 test samples, add 1 reference sample per 7 additional test samples.

° This includes one additional round of normalisation to exclude outliers.

△ SD100 should show normal (non-aberrant) results for all probes targeted in D028-A1, when a suitable reference population was used for normalisation.

Interpretation of results

The expected results for autosomal probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 0 (homozygous deletion), 3 (heterozygous duplication) or ≥4 (amplification). The same results can be expected for X-chromosome-specific probes in female samples. For the X-chromosome-specific probes in male samples, expected copy numbers are 1 (normal), 0 (deletion) or 2 (duplication). Please note that sex-chromosome aneuploidies are not detected, but could influence the X-chromosome-specific probe results (*DMD*).

The standard deviation of all reference probes in the reference population should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the result of the probes can be used to interpret digitalMLPA results for autosomal probes and X-chromosome-specific probes (*DMD*).

Copy number status: autosomal sequences / X-chromosome sequences in female (XX) samples[&]	**Result**
Normal (2 copies)	0.80 ≤ ratio ≤ 1.20
Homozygous deletion	ratio = 0[±]
Heterozygous deletion	0.40 < ratio < 0.65
Heterozygous duplication	1.30 < ratio < 1.65
Heterozygous triplication / Homozygous duplication	1.75 < ratio < 2.15
Ambiguous copy number	All other values

Copy number status: X-chromosome sequences in male (XY) samples[&]	**Result**
Normal (1 copy)	0.60 ≤ ratio ≤ 1.40
Deletion	ratio = 0[±]
Duplication	1.60 < ratio < 2.25
Ambiguous copy number	All other values

& For inter-normalisation of X-chromosomal probes the sex of the samples in the reference population in the experiment is automatically accounted for. Therefore, a copy number of 2 in female samples and a copy number of 1 in male samples both give a result of ~1, regardless of the sex of the samples in the reference sample population.
± Background signal: Beware that some probes may have minimal background signal, and therefore results close to, but not exactly 0.

The following non-standard probes in D028-A1 Carrier Panel 1 require special consideration for result interpretation:

- Five mutation-specific probes (*CYP21A2*, *HBB* & *HBA2*): **S013205**, **S018382**, **S018564**, **S018554** & **S022719**. Presence or absence will be detected with Coffalyser digitalMLPA.
- One *SMN1* SNP probe: **S016793**. Presence or absence will be detected with Coffalyser digitalMLPA. Presence of this polymorphism in combination with two copies of *SMN1* indicates an increased risk, depending on the ethnic background, that the person tested is a silent SMA carrier who carries two *SMN1* copies on one allele and none on the other.
- A total of 24 wildtype probes targeting various regions are included (**see PIF for probe numbers**). Wildtype probes detect the normal allele (wildtype sequence) of a mutation. A decreased probe read count can be due to a deletion or by the presence of the mutation. An increased probe read count indicates a duplication of the wildtype sequence.
- Four *CYP21A2* double wildtype probes: **S021918**, **S023020**, **S021921** & **S021922**. These probes have two ligation sites and detect the wildtype sequence at two locations simultaneously. A decreased probe read count can be due to a deletion or by the presence of one of the two (or both) mutations. For each double wildtype probe, an accompanying wildtype probe is included to deduce which mutation is present in case of a decreased read count for the double wildtype probe. The four accompanying wildtype probes are **S021919**, **S021924**, **S023540** & **S021923** respectively. **Warning:** The *CYP21A2* double wildtype probes

require relatively long intact target DNA sequences, rendering these probes more sensitive to sample DNA fragmentation and depurination (the extent of which should be similar across all samples tested within one experiment). Therefore, these probes have dedicated reference probes (not reported) which are equally sensitive to DNA fragmentation. When the extent of DNA fragmentation is suspected to compromise the reliability of the double wildtype probe results for a given sample, RPQ (Reference Probe Quality) warnings/errors will be issued by the software for these probes.

- Two probes cover the *CYP21A2* I2G locus, detecting the wildtype alleles A and C. The average result of these two wildtype probes is shown by the '**Average I2G**' probe. The normal copy number of this probe is 2. Only the average result is reported, not the individual I2G-A and I2G-C probe results. The 'Average I2G' probe has some residual background in absence of its specific target sequence.
- Three probes target *HBA1* and *HBA2* simultaneously: **S022720**, **S018438** & **S017219**. The normal copy number for these probes is 4. These probe results should be interpreted together with the neighbouring *HBA1* and *HBA2* specific probes.
- In the excel report, a set of control probes are reported (identified by the probe function = CTRL) that can be used to help troubleshoot the experiment in case of errors or warnings shown in the PDF reports, or the sample quality tab of the excel. The Coffalyser digitalMLPA software checks these control probes against criteria set by MRC Holland for both intra and inter ratios. Only data that meets the quality requirements is suitable for result interpretation. See the Coffalyser digitalMLPA User Manual for more information on analysis, and the Coffalyser digitalMLPA General Protocol for details on troubleshooting.

The following cut-off values apply for results of non-standard probes in D028-A1 Carrier Panel 1:

Probe type	Copy number status	Result
Average I2G	Normal (2 copies)	0.80 ≤ ratio ≤ 1.20
	Heterozygous variant present (or heterozygous CNV)	0.40 < ratio < 0.65
	Homozygous variant present (or homozygous CNV or combination of CNV and heterozygous variant present)	ratio = 0±
Wildtype probes & double wildtype probes*	Normal (2 copies)	0.80 ≤ ratio ≤ 1.20
	Heterozygous variant present (or heterozygous CNV)	0.40 < ratio < 0.65
	Homozygous variant present (or homozygous CNV or combination of CNV and heterozygous variant present)	ratio = 0±
Probes that target both *HBA1* & *HBA2*#	Normal (4 copies)	~1.00 (0.85 ≤ ratio ≤ 1.15)
	No copies	ratio = 0±
	One copy	~0.25
	Two copies	~0.50
	Three copies	~0.75
	Five copies	~1.25
	Six copies	~1.50
	Seven copies	~1.75
	Eight copies	~2.00

± Background signal: Beware that some probes may have minimal background signal, and therefore results close to, but not exactly 0.
* The presence of CN probes close to (double) wildtype probes may help to differentiate between mutation versus deletion.
# Because CNVs in the *HBA* region are frequently observed and CN=4 probes have narrower intervals, these probes are more likely to trigger nRSQ (range) warnings. For additional details on this warning/error, please refer to the Coffalyser digitalMLPA User Manual.

General notes on digitalMLPA interpretation:

- Arranging probes according to chromosomal location facilitates interpretation of the results. 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. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe read count of several consecutive probes, in particular for probes located in or near a GC-rich region. The use of an alternative DNA extraction method or an additional purification step (e.g. with ethanol precipitation or silica column based kits) may resolve such cases. Control probes are present in all digitalMLPA probemixes that provide a warning for incomplete DNA denaturation. Sequence changes (e.g. single nucleotide variants (SNVs)) in the target sequence detected by a probe can also lead to false-positive results.
- False positive duplication results: Contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe read count (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Not all abnormalities detected by digitalMLPA are pathogenic. 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. In some genes, intragenic deletions are known that result in very mild, or no disease (Schwartz et al. 2007). Duplications that include the first or last exon of a gene (e.g. exons 1-3) might in some cases not result in inactivation of that gene copy.

D028-A1 Carrier Panel 1 specific notes:

- The high homology and close proximity between the *SMN1* & *SMN2*, *HBA1* & *HBA2* and *HBG1* & *HBG2* genes leads to frequent sequence exchange (gene conversion) events. The specificity of these probes often relies on a single nucleotide difference. 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.
- Genotyping *CYP21A2* poses a significant challenge due to the existence of the highly homologous and inactive pseudogene *CYP21A1P*. Recombination between *CYP21A2* and *CYP21A1P* can lead to the pseudogene acquiring the wildtype gene sequence, potentially obscuring the presence of pathogenic SNVs in *CYP21A2*. The four double wildtype probes target the wildtype *CYP21A2* sequence at two locations simultaneously: one location where recombination with the pseudogene is common, and one where it is rare. The double wildtype probes are therefore specific to *CYP21A2*, even when *CYP21A1P* has acquired the wildtype sequence at one of the two targeted locations.
- The *SMN1* exon numbering is the traditional exon numbering (exons 1, 2a, 2b, and 3-8) used in most scientific literature. This exon numbering is different from the MANE select transcript (NM_000344.4).
- The PIF contains information on individual probes that is essential for interpretation of results.

Limitations of the procedure

- In most populations, a frequent cause of genetic defects in the regions included in D028 Carrier Panel 1 are small (point) mutations, most of which will not be detected by using D028 Carrier Panel 1. It is therefore strongly recommended to use this digitalMLPA probemix in combination with sequence analysis.
- digitalMLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect most copy number neutral inversions or translocations. Even when digitalMLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- **Warning:** Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results. Sequence changes can reduce the probe read count by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.

Confirmation of results

Copy number changes of multiple consecutive probes detected with D028 Carrier Panel 1 should be verified by another method when possible. MLPA probemixes are available for many genes in D028 Carrier Panel 1. Several of these MLPA probemixes contain probes with a different ligation site that can be used for initial confirmation of results (see section Related products in the product page). Alternatively, copy number changes can be confirmed by another independent technique such as long range PCR, qPCR, array CGH, FISH or Southern blotting.

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 result was obtained.

Please report false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.

Mutation database

We strongly encourage users to deposit positive results in the Leiden Open Variation Database (https://www.lovd.nl/3.0/home) . Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Table 2. D028-A1 Carrier Panel 1 probe targets

Gene / Region	Chr. band (hg38)	NM sequence (MANE Select)*	# Probes / Gene length	Disorder (autosomal recessive or X-linked)
SMN1	5q13.2	NM_000344.4	3 / 27.9 kb	**Spinal Muscular Atrophy (SMA)** OMIM: https://www.omim.org/entry/253300 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1352/
	▪ The *SMN1* gene is covered by two CN probes, and one SNP probe. ▪ The *SMN1* exon numbering is the traditional exon numbering (exons 1, 2a, 2b, and 3-8) used in most scientific literature. This exon numbering is different from the MANE select transcript (NM_000344.4). ▪ The exon 7 difference between the *SMN1*and *SMN2* genes, as targeted by S017635, is the only clinically relevant difference between these two genes. The great majority of SMA carriers can be identified by the presence of a single copy of *SMN1* exon 7. ▪ In 5-10% of all cases, the *SMN1* exon 8 probe (S019281) will show a different copy number compared to the *SMN1* exon 7 probe (S017635). In these cases, the copy number of *SMN1* is only determined by the exon 7 probe. The nucleotide difference targeted by the exon 8 probe is not clinically relevant. ▪ Although rare, individuals with two *SMN1* copies may still be carriers, for example when both *SMN1* copies are located on the same allele (silent carriers). The frequency of this 2+0 silent carrier genotype varies per population (Hendrickson et al. 2009). When interpreting *SMN1* copy number using D028-A1 Carrier Panel 1, it is not possible to distinguish if the *SMN1* copies are on the same (2+0) or different alleles (1+1). D028-A1 Carrier Panel 1 detects one polymorphism (described by Luo et al. 2014; Alias et al. 2018) that is associated with an increased risk of individuals being silent carriers. Detection of the c.*3+80T>G polymorphism (S016793) can aid in identifying a specific *SMN1* haplotype block associated with two copies of *SMN1* on one allele (silent carriers). ▪ The presence of more than two *SMN1* copies in healthy individuals is a relatively frequent phenomenon, especially in those of African descent (Hendrickson et al. 2009; Sangaré et al. 2014). ▪ Determining the *SMN2* copy number is not relevant for SMA carrier testing, therefore no *SMN2* probes are included in D028-A1 Carrier Panel 1.			

Gene / Region	Chr. band (hg38)	NM sequence (MANE Select)*	# Probes / Gene length	Disorder (autosomal recessive or X-linked)
CYP21A2	6p21.33	NM_000500.9	16 / 3.2 kb	**Congenital Adrenal Hyperplasia (CAH)** OMIM: https://www.omim.org/entry/201910 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1171/
	▪ The *CYP21A2* gene is covered by one mutation-specific probe and 10 CN/WT probes, all of which detect the wildtype sequences of a mutation. Four of these are double CN/WT probes that detect the wildtype sequence at two locations simultaneously (see Interpretation of results for more information). Additionally, five probes for the downstream *TNXB* gene are included to facilitate the determination of the extent of a deletion/conversion. ▪ Quite frequently, *CYP21A1P* pseudogene copies have acquired the wildtype *CYP21A2* sequence at various loci. This can obscure the presence of a pathogenic SNV at these sites in the *CYP21A2* gene. See the interpretation of results section for more details. ▪ Two probes cover the *CYP21A2* I2G locus, detecting the wildtype alleles A and C. The average result of these two wildtype probes is shown by the 'Average I2G' probe. The normal copy number of this probe is 2. Only the average result is reported, not the individual I2G-A and I2G-C probe results. The "Average I2G' probe has some residual background in absence of its specific target sequence. ▪ In the great majority of cases, an increased copy number detected by one or more of the *CYP21A2* probes will not be pathogenic. Increased probe ratios for S023020 and S021924 have been frequently observed in Filipino samples, this benign polymorphism could potentially obscure the presence of a *CYP21A2* I173N or V282L mutation.			
CFTR	7q31.2	NM_000492.4	42 / 188.6 kb	**Cystic Fibrosis (CF)** OMIM: https://www.omim.org/entry/219700 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1250/
	▪ The *CFTR* gene is covered by 42 CN probes, of which 11 are CN/WT probes that detect the wildtype sequence of a mutation. Each of the 27 exons is covered by at least one probe. ▪ The CN/WT probe S013035 targets the wildtype sequence at the location of the very frequent F508del mutation. A decreased read count for probe S013035 can also be due to the I507del, or a deletion.			
***HBB* region**	11p15.4	NM_000518.5	27 / 1.6 kb (*HBB* gene)	**Beta-thalassemia** OMIM: https://www.omim.org/entry/613985 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1426/
	▪ The *HBB* gene is covered by 9 CN probes, of which one is a CN/WT probe that detects the wildtype sequence of a mutation. Additionally, three (intronic) mutation-specific probes are included. ▪ Ten probes are included for other beta-globin cluster genes (*HBD*, *HBBP1*, *HBG1*, *HBG2*, and *HBE1*), and five probes targeting hypersensitive sites (HS1-HS5) in the Locus Control Region (LCR) of the beta-globin gene cluster, which are essential for regulation and expression of the beta-globin genes.			
***DFNB1* region**	13q12.11	***GJB2*** NM_004004.6 ***GJB6*** NM_001110219.3 ***CRYL1*** NM_015974.3	12 / 5.5 kb (*GJB2*), 10.4 kb (*GJB6*) and 122.2 kb (*CRYL1*)	***DFNB1* hearing loss** OMIM: https://www.omim.org/entry/220290 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1272/
	▪ The *DFNB1* locus is associated with non-syndromic, autosomal recessive deafness 1. ▪ The *GJB2* gene is covered by eight CN probes, six of which are CN/WT probes that detect the wildtype sequence of a mutation. The *GJB6* and *CRYL1* genes are each covered by two CN probes. ▪ One of the CN/WT probes (S013372) targets the wildtype sequence at the location of the very frequent *GJB2* c.35delG. A decreased probe read count can be due to the c.35delG mutation or a deletion. ▪ There are two common *GJB6* deletion regions: 232 kb (ClinVar ID 5548) and 309 kb (ClinVar ID 5546). Probes S017417 and S013216 are expected to be affected in case of the 232 kb deletion. Probes S017417, S013216 and S024225 are expected to be affected in case of the 309 kb deletion. ▪ A map that depicts the location of the *GJB2*, *GJB6*, and *CRYL1* genes in relation to common deletions can be found in Hoefsloot et al. 2013.			

Gene / Region	Chr. band (hg38)	NM sequence (MANE Select)*	# Probes / Gene length	Disorder (autosomal recessive or X-linked)
***HBA* region**	16p13.3	***HBA1*** NM_000558.5 ***HBA2*** NM_000517.6	22 / 0.8 kb (*HBA1* gene) and 0.8 kb (*HBA2* gene)	**Alpha-thalassemia** OMIM: https://www.omim.org/entry/604131 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1435/
	▪ The *HBA1 and HBA2* genes are covered by 13 CN probes, three of which detect sequences present in both *HBA1* and *HBA2* (normal copy number of 4). A mutation-specific probe (S022719) is included for the Constant Spring mutation in *HBA2*. Note that this probe cannot distinguish *HBA1* from *HBA2*. Thus, presence of probe signal may also be caused by a sequence change in the *HBA1* gene. ▪ Five probes are included for other alpha-globin cluster genes (*HBZ, HBM, HBQ1*), one flanking probe (*LUC7L*) and two probes targeting a hypersensitive site (HS) in the HS40 regulatory element of the alpha-globin gene cluster, which is essential for regulation and expression of the alpha-globin genes. ▪ Multiple CpG islands are located within the alpha-globin gene cluster. Therefore, a low signal for probes targeting this cluster can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially in GC-rich regions. Warnings will be issued by the software for these conditions in the sample reports. ▪ Due to the very high homology and close proximity of the *HBA1* and *HBA2* genes, sequence exchange (gene conversion) frequently occurs. In some healthy individuals the *HBA2* sequence is therefore changed into the *HBA1* sequence or vice versa, without clinical consequences. Two probe pairs target locations with very small sequence differences between *HBA1* and *HBA2*: S017342 & S017341 and S017340 & S017345. When one probe in a probe pair has a ~50% reduced probe signal (result 0.5), while the other has a ~50% increased probe signal (result 1.5), it is possible that this is a benign polymorphism due to sequence exchange between *HBA1* and *HBA2,* rather than a true deletion and duplication. ▪ Presence of three *HBA* genes on one allele (α-triplication) is relatively common, is not associated with an α-thalassaemia phenotype, and should be considered a polymorphism (Goossens et al. 1980). However, co-inheritance of multiple alpha-globin genes and beta-thalassaemia may lead to relatively severe (transfusion dependent) beta-thalassaemia intermedia (Camaschella et al. 1997, Harteveld et al. 2008).			
CLN3	16p12.1	NM_001042432.2	4 / 14.8 kb	**Juvenile Neuronal Ceroid Lipofuscinosis** (*CLN3*-related) OMIM: https://www.omim.org/entry/204200
	▪ The *CLN3* gene is covered by four CN probes. ▪ There is a common *CLN3* deletion region of 1.02 kb (ClinVar ID 3552). Probes S016657 and S016646 are expected to be affected in case of the 1.02 kb deletion.			
CTNS	17p13.2	NM_004937.3	5 / 26.3 kb	**Cystinosis** OMIM: https://www.omim.org/entry/606272 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1400/
	▪ The *CTNS* gene is covered by five CN probes. ▪ There is a common *CTNS* deletion region of 57 kb (ClinVar ID 4445). Probes S018750, S015422, S015415 and S015410 are expected to be affected in case of the 57 kb deletion.			
DMD	Xp21.2 – Xp21.1	NM_004006.3	79 / 2092.3 kb	**Duchenne & Becker Muscular Dystrophy** OMIM: https://www.omim.org/entry/300377 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1119/
	▪ The *DMD* gene is covered by 79 CN probes, one for each exon. ▪ Duplication of the complete *DMD* gene is not expected to result in disease. Duplications that include only the first or last exons of a gene may not result in inactivation of that gene copy. One should be cautious with the prediction of an expected phenotype based on genotype. The http://www.dmd.nl/ website has a tool to predict the effect of exon deletions/duplications on the reading frame. ▪ Results for *DMD* are by default shown for both male and female samples. For carrier testing purposes, results in male samples can be masked, see the Coffalyser digitalMLPA User Manual for more details. ▪ Please note that sex-chromosome aneuploidies are not detected, but could influence the X-chromosome-specific probe results.			

* The exon numbering and NM sequence was retrieved on 01/2026. As changes to the MANE database can occur after release of this product description, exon numbering may not be up-to-date. Exon numbering used here may differ from literature.
More information on the location, mutation details and warnings of the probes present in this probemix can be found in the PIF available on the product page.

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D028 Carrier Panel 1 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-02 – 09 February 2026 (05) - First unrestricted release.

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