Product Description
NXtec™ D038-A1 Carrier Plus

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.

Visit the NXtec D038 Carrier Plus product page on our website to find Certificates of Analysis, additional background information, a list of positive samples, a list of related products and the Probe Information File (PIF).

Product Name	**NXtec D038 Carrier Plus**
Version	A1
Catalogue Numbers & Volumes	D038-025R – 40 µl (25 reactions), including SALSA® Sample DNA SD104 D038-050R – 80 µl (50 reactions), including SALSA® Sample DNA SD104 D038-100R – 160 µl (100 reactions), including SALSA® Sample DNA SD104
Ingredients	Synthetic oligonucleotides, Tris-HCl, EDTA, DTT

The probemix is not known to contain any harmful agents. 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). Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard.

Additional Test Components	**Catalogue Numbers**
NXtec Reagent Kit	DRK01-IL DRK05-IL DRK20-IL
NXtec Barcode Plates	NXtec Barcode Plate 1 (Cat No: BP01-IL (from lot 03-012-xxxxxx and higher)) NXtec Barcode Plate 2 (Cat No: BP02-IL (from lot 03-013-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. -010-) will increase with every new barcode plate lot.
Data analysis software Coffalyser digitalMLPA™ **version 2.6.2** or higher	

Storage and Shelf Life

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 upon arrival. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

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 D038-A1 Carrier Plus 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 25 chromosomal regions mentioned in Table 2, which are associated with autosomal recessive or X-linked conditions that are commonly tested for in carrier testing.

In most populations, a frequent cause of genetic defects in the regions included in D038 Carrier Plus are small variants (e.g., SNVs, small InDels), most of which will not be detected by D038 Carrier Plus. **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 576 target and reference probes are included in D038-A1 Carrier Plus, as well as a large set of control probes to determine the quality of the NXtec experiment, the reaction quality and the DNA sample quality.

Probe Type	Number	Explanation	In PIF*
Copy Number	472	Detecting copy number alterations for the genes/regions mentioned in Table 2	CN
Wildtype	32	Copy number probes that can detect the wildtype sequence of a particular mutation	CN/WT
Double Wildtype	4	Copy number probes that can detect the wildtype sequence at two locations simultaneously	CN/DOUBLE WT
Mutation	9	Presence or absence will be detected with Coffalyser digitalMLPA	MUT
SNP	1	Presence or absence will be detected with Coffalyser digitalMLPA	SNP
Reference	58	Dedicated reference probes (indicated by a 'Yes' in the "Reference probe" column of the PIF)	CN

*See the PIF and Table 2 for more details.

To calculate the number of reactions that can be combined into one sequencer run, use 718 probes in your manual calculation (See chapter "Amplicon Quantification by Illumina Sequencers" in the digitalMLPA NXtec Protocol) or use the calculator tool available at support.mrcholland.com.

Reference probes
A selection of 58 probes, distributed across 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
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. NXtec is a product line within the digitalMLPA technology. The principles of digitalMLPA and the protocol for NXtec products are described in the digitalMLPA NXtec Protocol.

digitalMLPA technique validation
Internal validation using at least 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. Samples used for validation should be representative of samples used in daily practice, with regard to source and DNA extraction method, etc.

Required specimens
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)

Samples must also be treated with RNase A prior to use (see box below). SD104 does not require RNase treatment. For more information see the digitalMLPA NXtec Protocol, section "DNA sample 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.

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. More information regarding the selection and use of reference samples can be found in the chapter "Data analysis and reference samples" below and in the digitalMLPA NXtec Protocol.

SALSA® Sample DNA SD104
The selection of suitable reference DNA samples with a normal copy number for all targets included in D038 Carrier Plus can be complicated, as a significant portion of the global population is found to be a carrier of at least one variant. SD104 is a (pooled) female DNA sample with **a normal copy number for all D038-A1 Carrier Plus 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 SD104 samples in each experiment. See section "Data analysis and reference samples" for further details. **This product is for research use only (RUO).**

Positive control 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/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your digitalMLPA experiments. As cell line quality may change over time, the actual copy number variation (CNV) in a sample may differ from that indicated by the manufacturer.

When commercially available positive control samples have been identified by MRC Holland, these are listed on the assay-specific product page on our website. MRC Holland cannot provide positive DNA samples.

Data analysis and reference samples

For data analysis, use the latest version of MRC Holland's free data analysis software Coffalyser digitalMLPA in combination with the appropriate lot-specific product sheet. 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.

Several approaches can be used to analyse D038 Carrier Plus 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 SD104. Regardless of the chosen method, it is strongly recommended to **include at least three SD104 samples in every experiment**. This provides flexibility between methods during data analysis and helps avoid the need to repeat experiments.

Table 1. Data analysis approaches for D038 Carrier Plus – *in order of preference*

Analysis Method	Description	Use of SD104	CDF settings	Notes
1. Dedicated reference samples from own collection	**Preferred method** Select ≥3* samples as reference	Use SD104 to verify normalisation was successful△	- Define selected reference samples as 'reference' - Define test samples as 'test' - Define SD104 replicates as 'test' and indicate as 'pooled DNA'	Dedicated reference samples should not have any aberrations across all probe-targeted regions. In case SD104 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 SD104 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 SD104 to verify normalisation was successful△	- Define all test samples as 'test' - Define SD104 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. SD104 as dedicated reference samples **(fallback method)**	**Only if other methods are not feasible** Select ≥3* SD104 samples as reference	Use SD104 replicates as dedicated reference samples	- Define SD104 replicates as 'reference' and indicate as 'pooled DNA' - Define all test samples as 'test'	Less preferred due to differences in origin and processing between SD104 and test samples (see "Required specimens"). In addition, 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. Furthermore, SD104 is a cell line-derived sample mixture. When used as a reference sample, it may yield slightly elevated chrX probe ratios in test samples. This is likely due to mosaic loss of chromosome X (mLOX) acquired during cell line passaging, resulting in an effective chrX copy number that may be slightly below two in the reference material.

* 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.
△ SD104 should show normal (non-aberrant) results for all probes in D038-A1, when a suitable reference population was used for normalisation.
° The Coffalyser digitalMLPA software will automatically perform one additional round of normalisation to exclude outliers.
CNVs in the *HBA and SMN* region are frequently observed. Probes detecting 4 copies have narrower result intervals and are more likely to receive nRSQ (range) warnings. For additional details on this warning/error, please refer to the Coffalyser digitalMLPA User Manual.

Interpretation of results

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.

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 report, 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 troubleshooting page on our website for more details.

The expected results for standard 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 the 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*, *FMR1*, *F8*).

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

Copy number status **Autosomal sequences / X-chromosome sequences in females (XX)&**	**Results**
Normal	0.80 ≤ ratio ≤ 1.20
Homozygous deletion	ratio = 0±
Heterozygous deletion	0.40 < ratio < 0.65
Heterozygous duplication/gain	1.30 < ratio < 1.65
Heterozygous triplication/Homozygous duplication/gain	1.75 < ratio < 2.15
Ambiguous copy number	All other values
X-chromosome sequences in males (XY)&	**Results**
Normal	0.60 ≤ ratio ≤ 1.40
Hemizygous 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 cut-off values apply for results of non-standard probes in D038-A1 Carrier Plus:

Probe type	Expected results	
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 & *CYP21A2* double wildtype probes*Δ	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* and *SMN1* & *SMN2**#**	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.
* Probes within a region should be interpreted together. For example, CN probes should be considered alongside (double) wildtype probes and CN = 4 probes to help differentiate presence of mutations and establish overall copy number.
Δ Additional information on interpreting *CYP21A2* double wildtype probes can be found in Table 2 and on the product page.
CNVs in the *HBA* and *SMN* region are frequently observed. Probes detecting 4 copies have narrower result intervals and are more likely to receive nRSQ (range) warnings. For additional details on this warning/error, please refer to the Coffalyser digitalMLPA User Manual.

Technique-specific interpretation notes and confirmation of results
See the digitalMLPA NXtec Protocol.

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/.

D038 Carrier Plus specific notes:

- The high homology and close proximity between *GBA1*& *GBA1LP*, *SMN1* & *SMN2*, *CYP21A2* & *CYP21A1P*, *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 one of these probes can be the result of a non-significant single nucleotide sequence change in the related gene.
- Results for *DMD, FMR1, and F8* are shown for both male and female samples, denoted by the addition of (Male) or (Female) to the PDF report panels. For carrier testing purposes, results in male samples can be masked, see the Coffalyser digitalMLPA User Manual for more details.

Technique-specific limitations
See the digitalMLPA NXtec Protocol.

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

Table 2. D038-A1 Carrier Plus probe targets

More information on the location, mutation details and warnings of the probes present in this probemix can be found in the Probe Information File (PIF) available on the product page.

Gene / Region	Chr. band (hg38)	NM sequence (MANE Select)*	# Probes / region	Associated with (autosomal recessive or X-linked disorder)
ACADM	01p31.1	NM_000016.6	14	**Medium-Chain Acyl-Coenzyme A Dehydrogenase (MCAD) Deficiency** OMIM: https://omim.org/entry/201450 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1424/
	• The *ACADM* gene is targeted by 14 CN probes, all exons are covered by at least one CN probe.			
GBA1	01q22	NM_000157.4	13	**Gaucher disease** OMIM: https://omim.org/entry/230800 *This OMIM entry describes Gaucher disease type 1, the most common form.* GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1269/
	• Genotyping *GBA1* poses a significant challenge due to the existence of the highly homologous pseudogene *GBA1LP*. Recombination between *GBA1* and *GBA1LP* can lead to the pseudogene acquiring the wildtype gene sequence, potentially obscuring the presence of pathogenic SNVs in *GBA1*. • Most *GBA1* exons are covered with at least one CN or CN/WT probe. *GBA1* probes are designed to be specific for *GBA1* sequences. • One probe detects the wildtype sequence at c.1448T, which is also part of the RecNcil genotype. However, D038-A1 Carrier Plus does not detect the other two variants that define this genotype. • One probe is expected to be sensitive to the 55 bp deletion. • Follow-up studies are needed to determine the exact cause of aberrant probe signals in *GBA1*.			
XPC	03p25.1	NM_004628.5	16	**Xeroderma Pigmentosum, complementation group C** OMIM: https://omim.org/entry/278720 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1397/
	• The *XPC* gene is targeted by 16 CN probes, covering most exons.			
SMN region	05q13.2	***SMN1*** NM_000344.4 ***SMN2*** NM_017411.4	8	**Spinal Muscular Atrophy (SMA)** OMIM: https://www.omim.org/entry/253300 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1352/
	• The *SMN1* and *SMN2* 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 transcripts (NM_000344.4 (*SMN1*) and NM_017411.4 (*SMN2*)). • Three probes specifically target *SMN1*, including a SNP probe for the c.*3+80T>G polymorphism. Five probes target *SMN1* and *SMN2* simultaneously, the normal copy number for these probes is 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 7 and exon 8 probes show different copy numbers. In these cases, the exon 7 probe is leading as the nucleotide difference targeted by the exon 8 probe is not clinically relevant. • Although rare, individuals with two *SMN1* copies may still be SMA carriers, for example when both *SMN1* copies are located on the same allele (silent carriers). When interpreting *SMN1* copy number using D038 A1 Carrier Plus, it is not possible to distinguish if the *SMN1* copies are on the same (2+0) or different alleles (1+1). Presence of the c.*3+80T>G polymorphism in combination with two copies of *SMN1* is associated with an increased risk that the person tested is a silent SMA carrier who carries two *SMN1* copies on one allele and none on the other (2+0), as described by Luo et al. 2014; Alias et al. 2018. • The *SMN2* copy number can be deduced by subtracting the *SMN1* copy number from the combined copy number of *SMN1* and *SMN2*. The *SMN2* copy number is inversely correlated with disease phenotype severity, however *SMN2* copy number is not relevant for carrier status determination (McAndrew 1997). Please note: This product should not be used to determine *SMN2* copy numbers for the purpose of determining disease prognosis or eligibility for SMA therapeutics.			
CYP21A2	06p21.33	NM_000500.9	16	**Congenital Adrenal Hyperplasia (CAH)** OMIM: https://www.omim.org/entry/201910 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1171/
	• 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*.			

Gene / Region	Chr. band (hg38)	NM sequence (MANE Select)*	# Probes / region	Associated with (autosomal recessive or X-linked disorder)
	• The *CYP21A2* gene is targeted by 10 CN/WT probes, all of which detect the wildtype sequences of a mutation, and one mutation-specific probe. Additionally, five CN probes for the downstream *TNXB* gene are included to facilitate the determination of the extent of a deletion/conversion. • Four of the CN/WT probes are double wildtype probes that target the wildtype *CYP21A2* sequence at two locations simultaneously; one location where recombination with the pseudogene is common, and one where it is rare. Both wildtype alleles must be in cis for these probes to give signal. The double wildtype probes are specific to *CYP21A2*, even when *CYP21A1P* has acquired the wildtype sequence at one of the two targeted locations. If the wildtype sequences for both loci are present in cis on the *CYP21A1P* pseudogene, these probes will also give signal on the pseudogene. A decreased probe read count can be due to a deletion or 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. • 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. A result of ~0.5 for this 'Average I2G' probe, indicates that one allele does not contain the wildtype (A or C) sequence, or a heterozygous CNV is present. 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. • Additional information on interpreting *CYP21A2* double wildtype probes can be found on the product page.			
CFTR	07q31.2	NM_000492.4	42	**Cystic Fibrosis (CF)** OMIM: https://www.omim.org/entry/219700 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1250/
	• The *CFTR* gene is targeted by 42 CN probes, 11 of which are CN/WT probes that detect the wildtype sequence of a mutation, including the common F508del mutation. Each of the 27 exons is covered by at least one probe.			
GALT	09p13.3	NM_000155.4	12	**Galactosemia** OMIM: https://omim.org/entry/230400 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1518/
	• The *GALT* gene is targeted by 12 CN probes, two of which are CN/WT probes that detect the wildtype sequence of a mutation. Most exons are covered by CN probes. • All probes, except for S016286, are within the complex 5.2 kb deletion region.			
FANCC	09q22.32	NM_000136.3	17	**Fanconi Anemia – complementation group C** OMIM: https://omim.org/entry/227645 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1401/
	• The *FANCC* gene is targeted by 17 CN probes, all exons are covered by at least one probe.			
ELP1	09q31.3	NM_003640.5	16	**Familial Dysautonomia** OMIM: https://omim.org/entry/223900 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1180/
	• Probes are distributed across the *ELP1* gene at approximately 10 kb intervals, covering 15 of the 37 exons.			
***HBB* region**	11p15.4	NM_000518.5	27	**Beta-thalassemia** OMIM: https://www.omim.org/entry/613985 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1426/
	• The *HBB* gene is targeted by nine CN probes, one of which is a CN/WT probe that detects the wildtype sequence of a mutation. Additionally, three (intronic) mutation-specific probes are included. • 10 CN 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.			
SMPD1	11p15.4	NM_000543.5	6	**Acid Sphingomyelinase Deficiency (Niemann-Pick Disease)** OMIM: https://omim.org/entry/257200 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1370/
	• The *SMPD1* gene is targeted by six CN probes, covering all exons.			
DHCR7	11q13.4	NM_001360.3	9	**Smith-Lemli-Opitz Syndrome** OMIM: https://omim.org/entry/270400

Gene / Region	Chr. band (hg38)	NM sequence (MANE Select)*	# Probes / region	Associated with (autosomal recessive or X-linked disorder)
				GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1143/
	• The *DHCR7* gene is targeted by eight CN probes covering most exons, and one mutation-specific probe.			
PAH	12q23.2	NM_000277.3	15	**Phenylketonuria** OMIM: https://omim.org/entry/261600 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1504/
	• The *PAH* gene is targeted by 15 CN probes, one of which is a CN/WT probe that detects the wildtype sequence of a mutation. Each of the 13 exons is covered by at least one probe. One CN probe is within the 3.7 kb deletion region upstream of *PAH*, and one CN/WT probe detects the wildtype sequence at a 15 bp deletion in exon 11.			
***DFNB1* region**	13q12.11	***GJB2*** NM_004004.6 ***GJB6*** NM_001110219.3 ***CRYL1*** NM_015974.3	13	**DFNB1 hearing loss** OMIM: https://www.omim.org/entry/220290 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1272/
	• The *GJB2* gene is targeted by nine CN probes, six of which are CN/WT probes that detect the wildtype sequence of a mutation, including the common c.35delG mutation. • The *GJB6* and *CRYL1* genes are each covered by two CN probes, these probes can detect the common 232 kb and 309 kb deletions, see the PIF for more information.			
HEXA	15q23	NM_000520.6	12	**Tay-Sachs disease** OMIM: https://omim.org/entry/272800 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1218
	• The *HEXA* gene is targeted by 12 CN probes, covering most exons.			
BLM	15q26.1	NM_000057.4	21	**Bloom syndrome** OMIM: https://omim.org/entry/210900 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1398/
	• The *BLM* gene is targeted by 21 CN probes, one of which is a CN/WT probe that detects the wildtype sequence of a mutation, the Ashkenazi founder variant c.2207_2212delins (known as the blmAsh allele). Most *BLM* exons are covered by CN probes.			
***HBA* region**	16p13.3	***HBA1*** NM_000558.5 ***HBA2*** NM_000517.6	27	**Alpha-thalassemia** OMIM: https://www.omim.org/entry/604131 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1435/
	• The *HBA1* and *HBA2* genes are targeted by 10 CN probes. An additional seven probes target *HBA1* and *HBA2* simultaneously, the normal copy number for these probes is 4. A mutation-specific probe (S022719) is included for the Constant Spring mutation in *HBA2* – please note this probe cannot distinguish *HBA1* from *HBA2*. • Six 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. • 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. • Several probes (as indicated in the PIF) within the alpha-globin gene cluster are sensitive to higher salt concentrations, and may also be affected by the presence of RNA – see section "Required specimens" for RNase sample treatment instructions. • Additional information on interpreting *HBA* genotypes can be found on the product page.			
PMM2	16p13.2	NM_000303.3	7	**PMM2 Congenital disorder of glycosylation** OMIM: https://omim.org/entry/212065 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1110/
	• The *PMM2* gene is targeted by seven CN probes, covering most exons. • Two CN probes target exon 8, where whole exon and larger deletions have been reported in the literature.			
CLN3	16p12.1	NM_001042432.2	4	**Juvenile Neuronal Ceroid Lipofuscinosis (*CLN3*-related)** OMIM: https://www.omim.org/entry/204200 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1428/
	• The *CLN3* gene is targeted by four CN probes, two of which are within the common 1.02 kb deletion region.			
ASPA	17p13.2	NM_000049.4	7	**Canavan disease** OMIM: https://omim.org/entry/271900 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1234/
	• The *ASPA* gene is targeted by five CN probes, covering most exons and two mutation-specific probes targeting an Ashkenazi founder variant (c.854A>C) and a common European variant (c.914C>A).			

Gene / Region	Chr. band (hg38)	NM sequence (MANE Select)*	# Probes / region	Associated with (autosomal recessive or X-linked disorder)
CTNS	17p13.2	NM_004937.3	5	**Cystinosis** OMIM: https://www.omim.org/entry/606272 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1400/
	• The *CTNS* gene is targeted by five CN probes, four of which are within the common 57 kb deletion region.			
MCOLN1	19p13.2	NM_020533.3	10	**Mucolipidosis IV** OMIM: https://omim.org/entry/252650 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1214/
	• The *MCOLN1* gene is targeted by nine CN probes, covering most exons. Four of these CN probes are located within the 6.4 kb deletion region that spans from the 5'UTR until exon 6. This variant is common in individuals of Ashkenazi Jewish descent. • One mutation probe is included for the c.406-2A>G mutation that is common in persons of Ashkenazi Jewish descent.			
DMD	Xp21.2 – Xp21.1	NM_004006.3	158	**Duchenne & Becker Muscular Dystrophy** OMIM: https://www.omim.org/entry/300377 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1119/
	• The *DMD* gene is targeted by 158 CN probes, two for each exon.			
FMR1	Xq27.3	NM_002024.6	12	**Fragile X syndrome** OMIM: https://omim.org/entry/300624 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1384/
	• The *FMR1* gene is targeted by 12 CN probes, covering most exons. • *FMR1* probes in and around exon 1 are very sensitive to higher salt concentrations. The denaturation of these probes may also be disrupted in case of long CGG repeats in the 5'UTR of *FMR1*. • CN probes for *FMR1* are used to identify duplications/deletions, they cannot detect the majority of Fragile X Syndrome carriers that are caused by CGG repeat expansion in *FMR1*.			
F8	Xq28	NM_000132.4	31	**Haemophilia A** OMIM: https://omim.org/entry/306700 GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1404/
	• The *F8* gene is targeted by 31 CN probes, covering all exons by at least one probe. • Approximately 48% of severe haemophilia A patients have inversions in *F8*, which will not be detected by D038 Carrier Plus.			

***NM sequence**: **From description version 01 onwards, we have adopted the MANE exon numbering.** When MANE is not available, then the exon numbering is based on the NM or NG sequence. The exon numbering and NM_ sequence used have been retrieved on 09/2026. 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.

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D038 Carrier Plus product history	
Version	*Modification*
A1	First release

Implemented changes in the product description
Version A1-01 – 30 October 2026 (06) - Not applicable, new document.

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