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Single NGS-based Method for Carrier Screening:

Streamlined Analysis of Challenging Genomic Regions

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Disclosure

– Mikrogen Reproductive Genetics Company

– GenART IVF & Reproductive Biotech Co

– ZEUS Bioscience Co

CARRIER SCREENING TESTS

These tests are performed to detect whether healthy individuals are carriers of any genetic disease

Who should be offered carrier screening ?

ACOG 2017

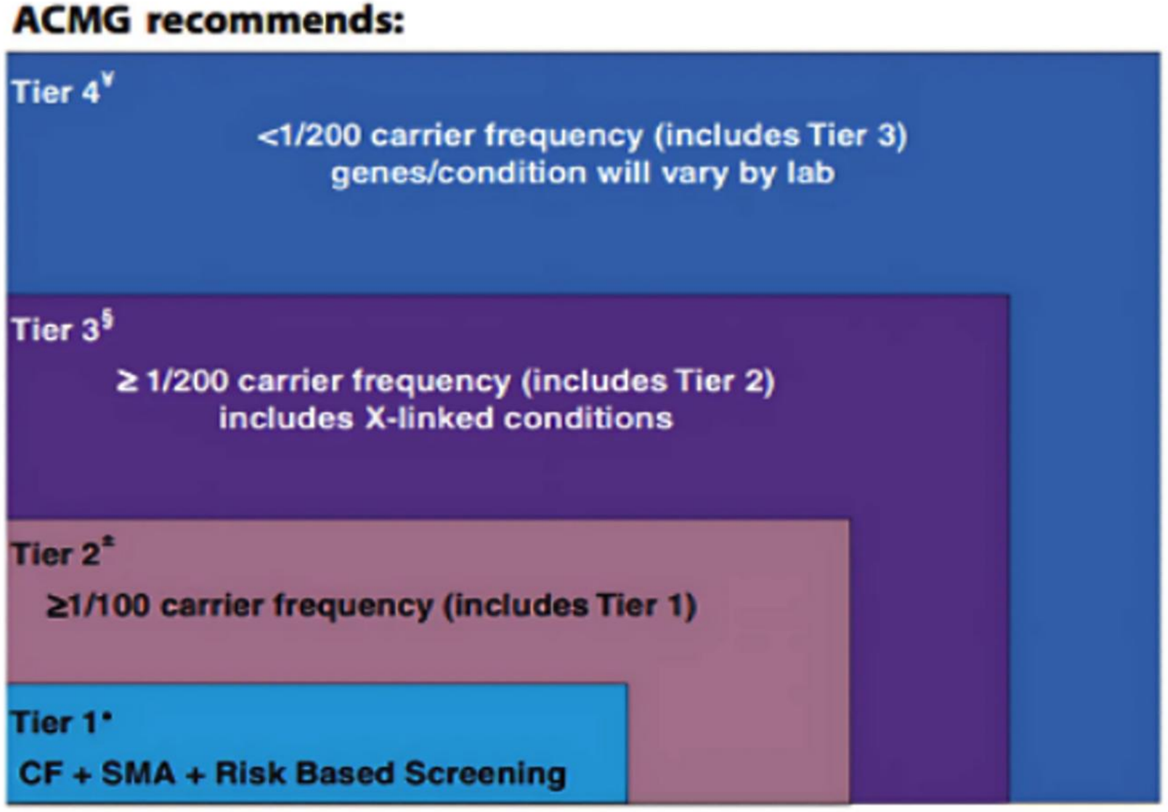
- *Consanguineous marriage*
- *Ethnic Population*
- *IVF cycles*
- *During pregnancy*
- *Patient decision/Patient anxiety*
- *Donor programs*
- *Pan Ethnic All individuals ?*

What should we screen ?

ACMG 2022

- *Autosomal recessive or X-linked disease*
- *Severe childhood disorders (ESHG)*
- *Population-specific disorders*
- *Late onset disorders ?*
- *Autosomal Dominant genes ?*
- *Cancer genes ?*

Which genes should be screened ?

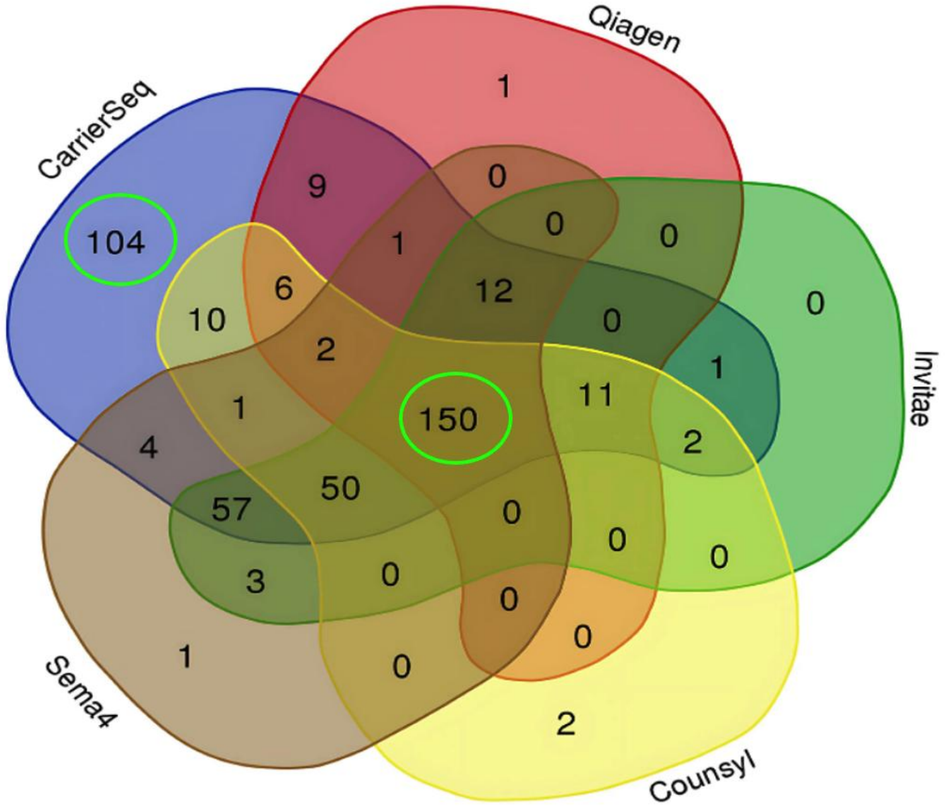


ACMG recommended a "tier system" based on carrier frequency

ACMG 2022

Panels of Global Companies

- **Many panels contain tier 3 genes of ACMG recommendation**
- **Most have similar gene content, only few have significantly different gene content**



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CARRIERCHECK

This is "end to end solution project from wet lab to bioinformatic"

eureka
innovation beyond borders

- Eureka was established in **1985** as an agreement between **18 countries and the European Commission** to foster competitiveness and market integration and to encourage R&D cooperation.
- Tailored programmes to best support international industry-led R&D.
- **Network projects** is a cooperation programme
- We applied to a bilateral call for projects between two or more Eureka countries
- The project idea **must represent cooperation** in the form of a specific project.
- The project **must be directed at researching or developing a product, process or service.**

2020-2021

571	Total Projects
43	Total Countries
2719	Total Participants
1362	Total Project Costs (M€)

29	2021 Network Projects
30	2021 Network | Total Countries
111.4	2021 Network | Total Public Private Investment (M€)

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GENETİK HASTALIKLAR DEĞERLENDİRME MERKEZİ

genoox

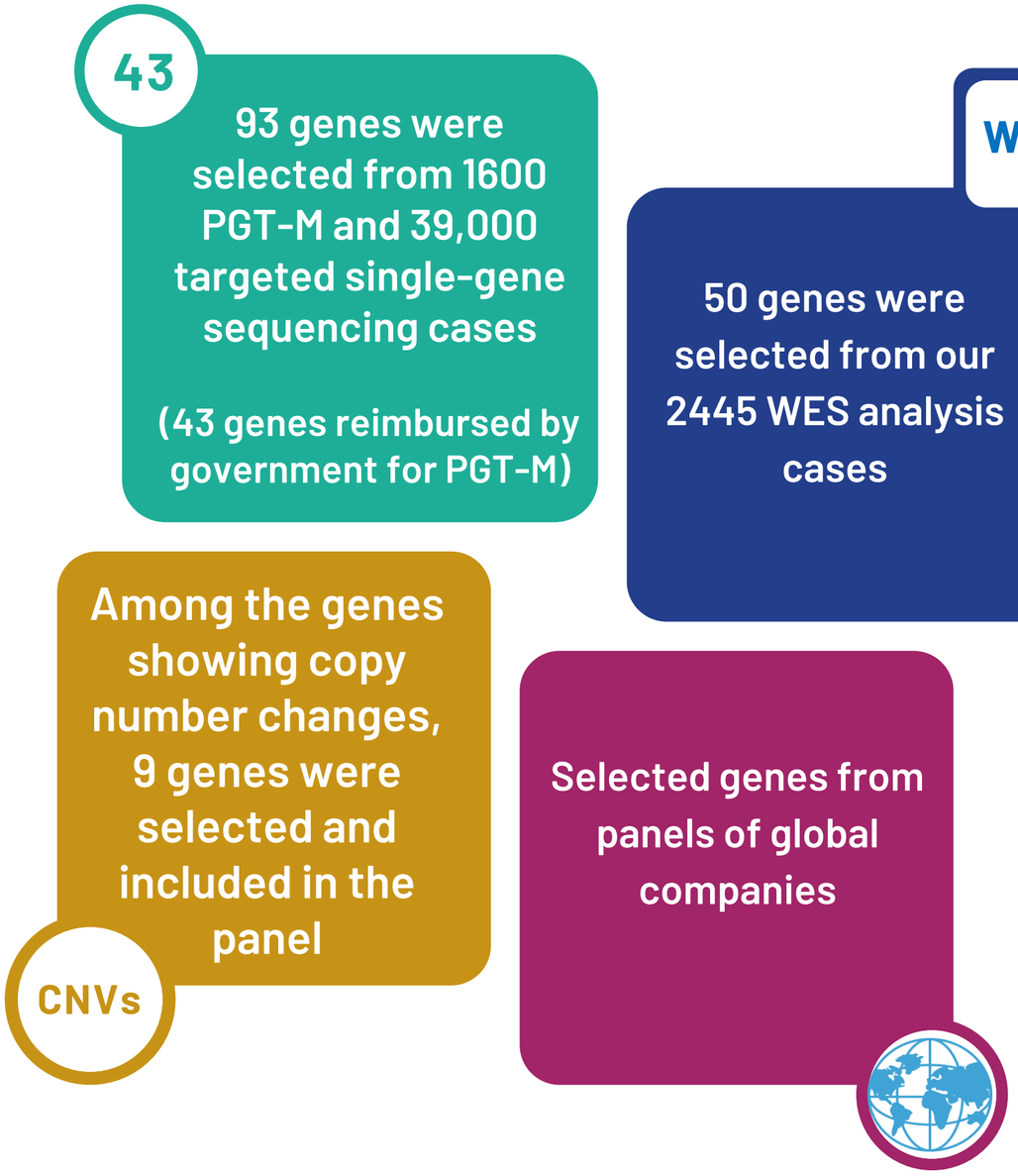
2.3 M€

CarrierCheck

End-to-end Solution to Inherited Disease Screening from Wet-Lab to Bioinformatics

Selection strategies of “Carriercheck” panel genes

Mikrogen's in-house data was used for the selection of panel genes



a) The most frequently requested diseases for PGT-M (1,600 cases) and targeted single-gene sequencing (38,939 cases) tests performed at Mikrogen Laboratories since 2014 were analyzed. Based on this analysis, the 29 most common genetic disorders in Turkish and Middle Eastern populations were selected. Additionally, 43 genes covered under the PGT-M reimbursement program by the Ministry of Health of the Republic of Turkey were also included in our panel.

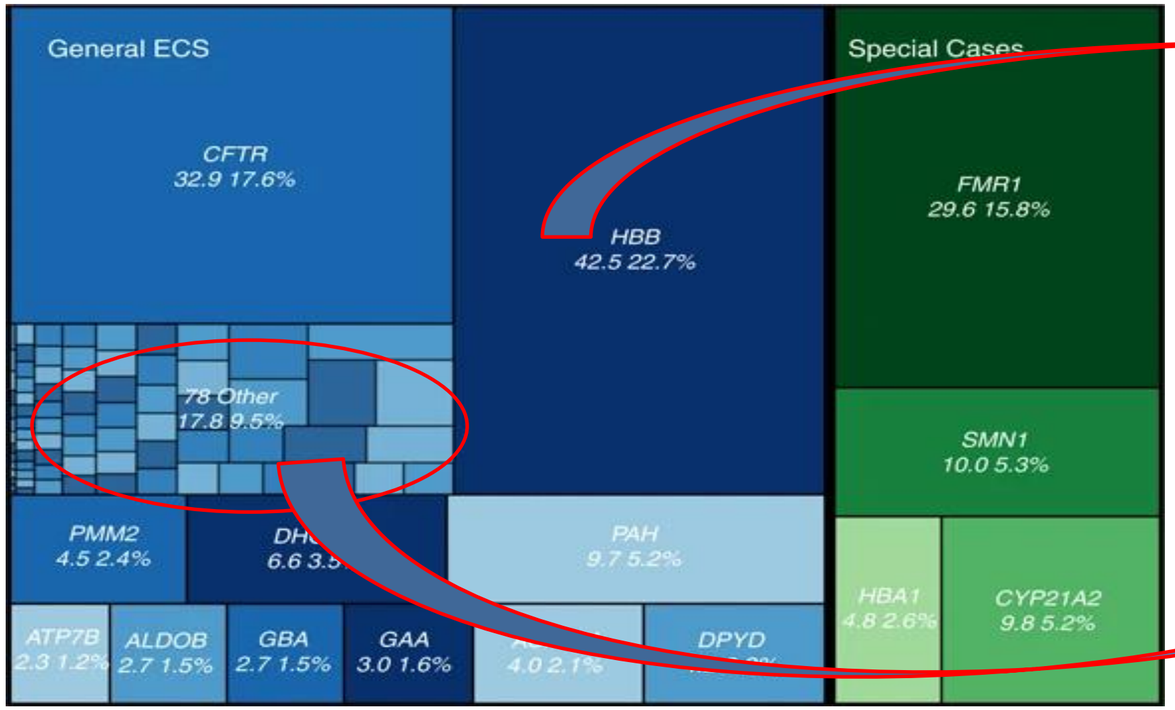
b) The results of 2,445 WES cases performed at Mikrogen Laboratories were evaluated and it was decided to add 50 genes to the panel.

c) To identify the most commonly screened genes, the gene content of carrier screening panels from global companies was analyzed. As a result, 70 genes shared across all panels were added to our panel. el.

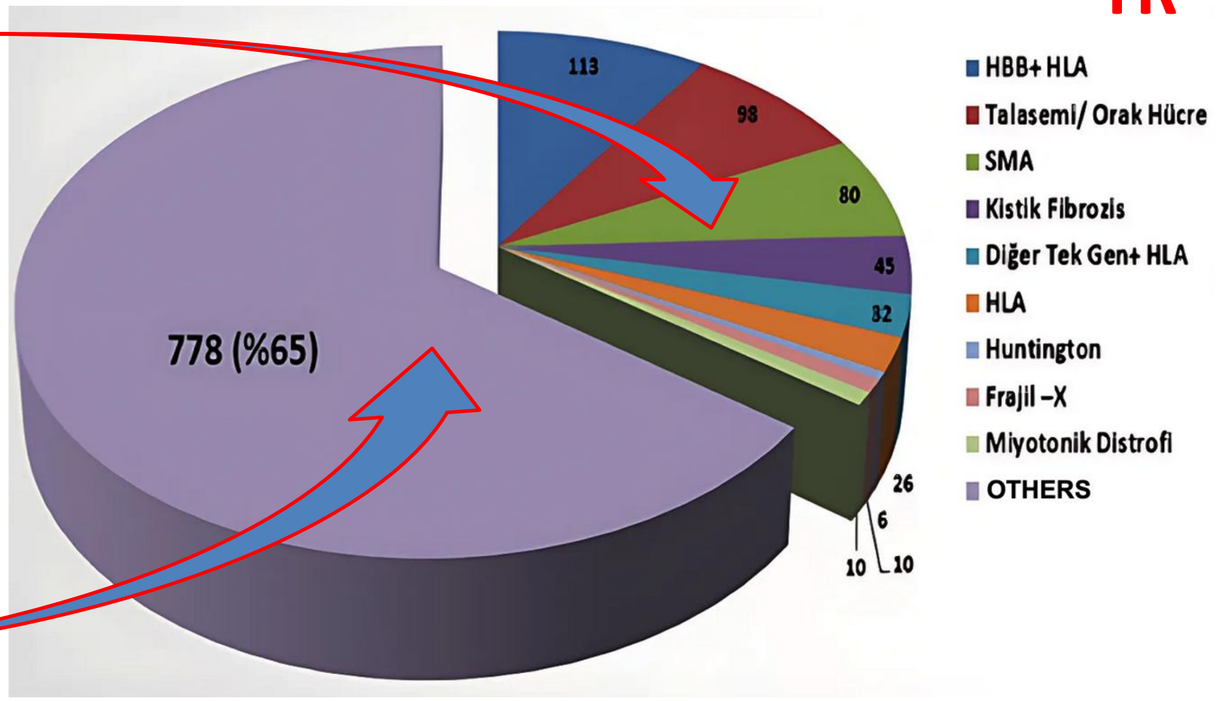
d) Based on Mikrogen's experience and a literature review, 9 genes with frequently observed copy number variations (CNVs) were added to the panel

The most critical issue in the screening panel is the selection of “population-specific genes”

EU



TR

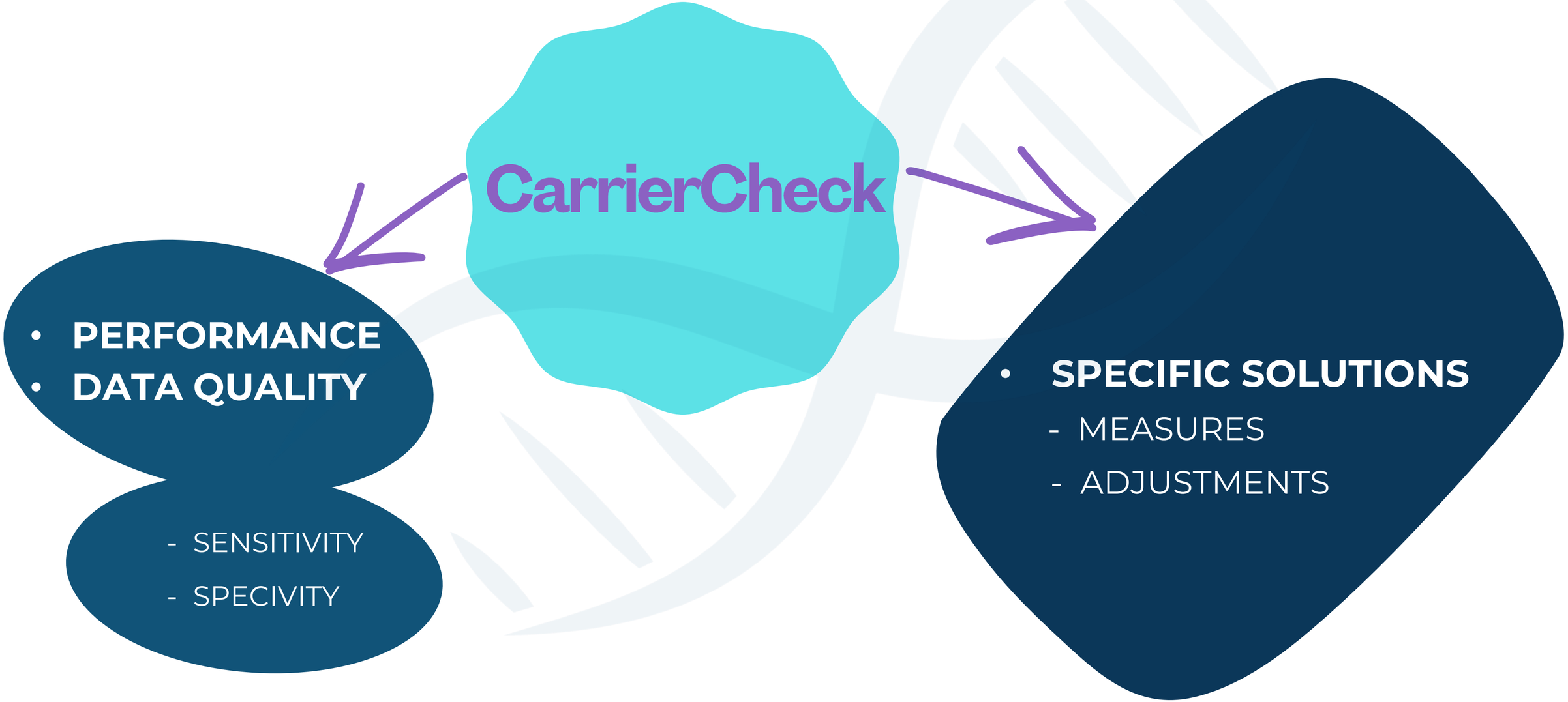


Distribution of monogenic diseases in Europa:
-15 diseases constitute 90% of all cases
-78 diseases constitute only 10% of the cases.
(Beauchamp et. al. (2018) 474.644 test)

Mikrogen’s data consisting of 1198 PGT-M + (2445 WES);
-15 diseases constitute 35% of all cases
- The remaining monogenic diseases make up 65%
(Baltaci et al, un published data)

Thats why our panel had to focus on this group as well.

The 2nd critical issue regarding the panel is whether it provides specific solutions against the pitfalls of the panel genes.



Specific solutions of Carriercheck for wet lab, analysis and reporting stages

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WET LAB Stage Solutions

- High specificity and sensitivity for target genes
- Uniform coverage for target genes
- CNV, SNV information
- Detection of hotspot intronic and intergenic variants
- Ability to detect new variations in the target genes

ANALYSIS Stage Solutions

- Exon level CNV detection in common genes such as CFTR and DMD.
- Detection of SMN1 gene deletions
- A reliable analysis of genes showing sequence homology such as GBA and CYP21A2

REPORTING Stage Solutions

- Reporting of only AR and XL variants
- Reporting of variations of the genes that manifest both AR and AD inheritance
- Reporting of Class 1 and 2 variants
- Reporting of clinically relevant Class 3 variants if family history is present
- Class 3 variants were reported if the other partner was a class 1/2 carrier for the same gene.

Specific Solutions and Special Algorithms for Probe Designs -1

Design Instructions

Redesign of the panel TE-96519675 adding 100% coverage in the F8 introns 1 and 22 with 4X tiling.

The panel was designed to provide 100% coverage for some critical gene regions such as exons 1 and 22 of the F8 gene.

Invitrotek_CarryCheck_TE-94050664_hg38

Data received by Twist Bioscience

- 146 gene symbols
- 10 CNV regions
- 34 SNVs provided as genomic coordinates

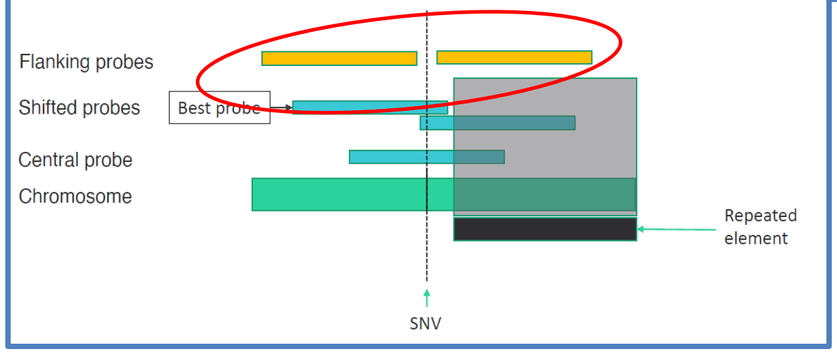
Genome assembly: hg38

Targeted CNV and SNV analyses enabled the detection of deletions in the SMN1 and HbA genes.

Design Instructions

Probes were carefully designed to minimize the overlap with repeated elements of the genome.

For those variants that could not be covered directly, flanking probes were designed with a maximum distance of 100bp from the variant.



Flanking probes were designed for variants that were not covered properly

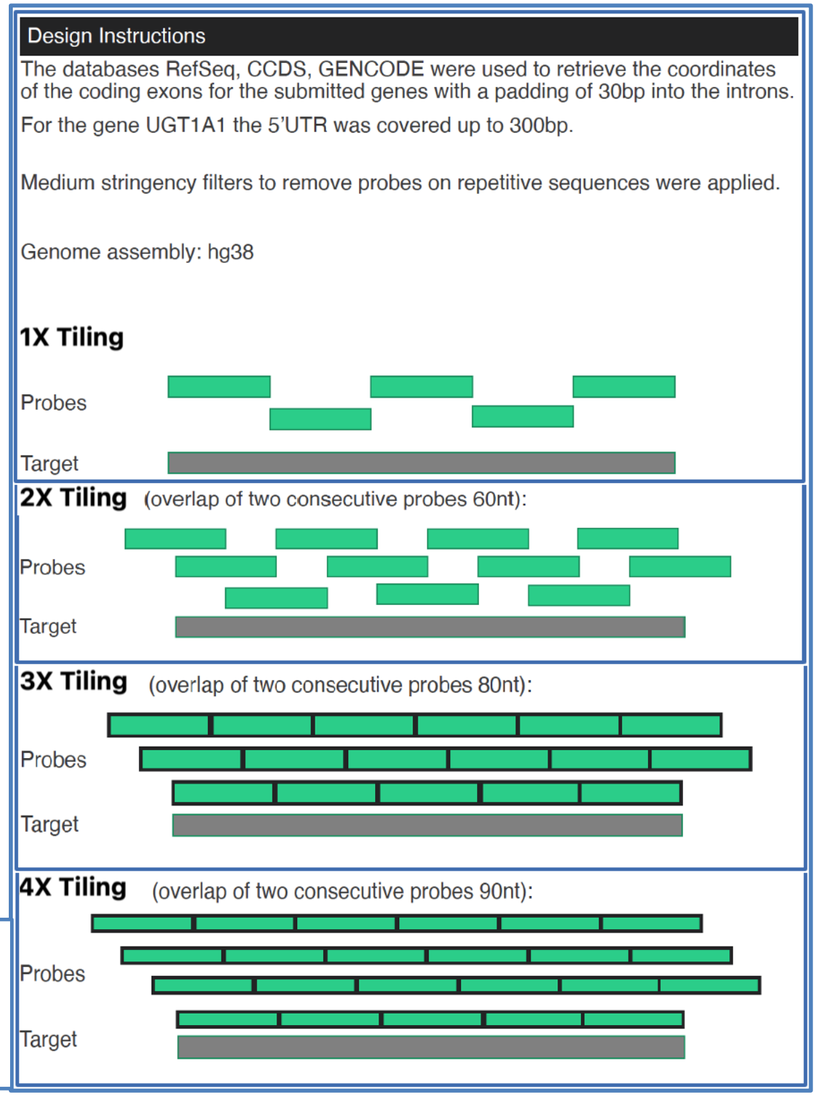
Design Instructions

The databases RefSeq, CCDS, GENCODE were used to retrieve the coordinates of the coding exons for the submitted genes with a padding of 30bp into the introns.

For the gene UGT1A1 the 5'UTR was covered up to 300bp.

Medium stringency filters to remove probes on repetitive sequences were applied.

Genome assembly: hg38



The probes were designed to minimize overlap with repeated segments of the genome.

Specific Solutions and Special Algorithms for Probe Designs - 2

These Hotspot Deep Intronic Variants Integrated into the Panel

CFTR:

c.870-1113_870-1110del (rs397508809)
c.3718-2477C>T (rs75039782)
c.3717+40A>G (rs397508595)
c.3140-26A>G (rs76151804)
c.3874-4522A>G (rs895394181)
c.1680-886A>G (rs397508266)
c.1680-877G>T (rs397508261)
c.1680-883A>G (rs1554388867)
c.1585-9412A>G (rs397508229)
c.2989-313A>T (rs1584821306)

CEP290:

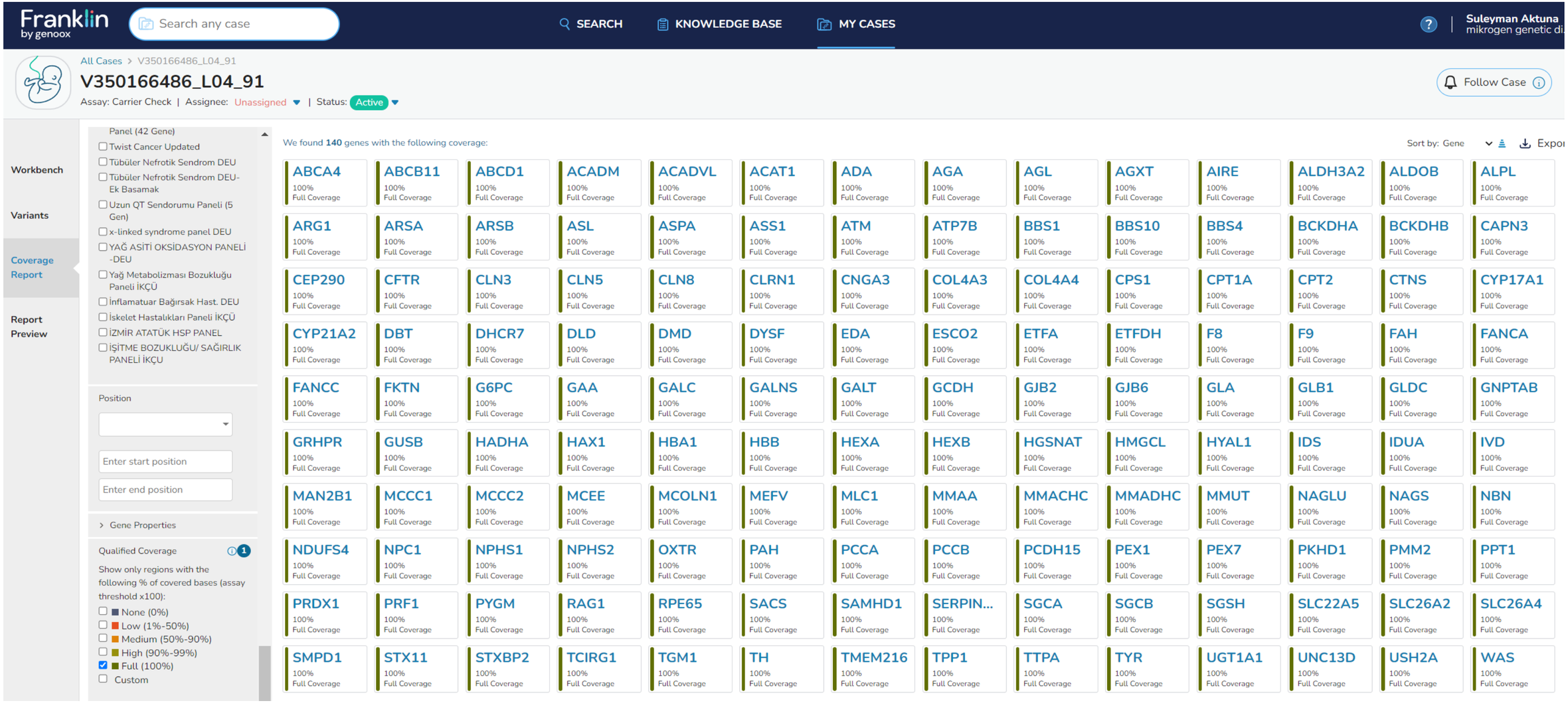
c.1523-412C>T (rs1381940328)
c.2991+1655A>G (rs281865192)

DMD:

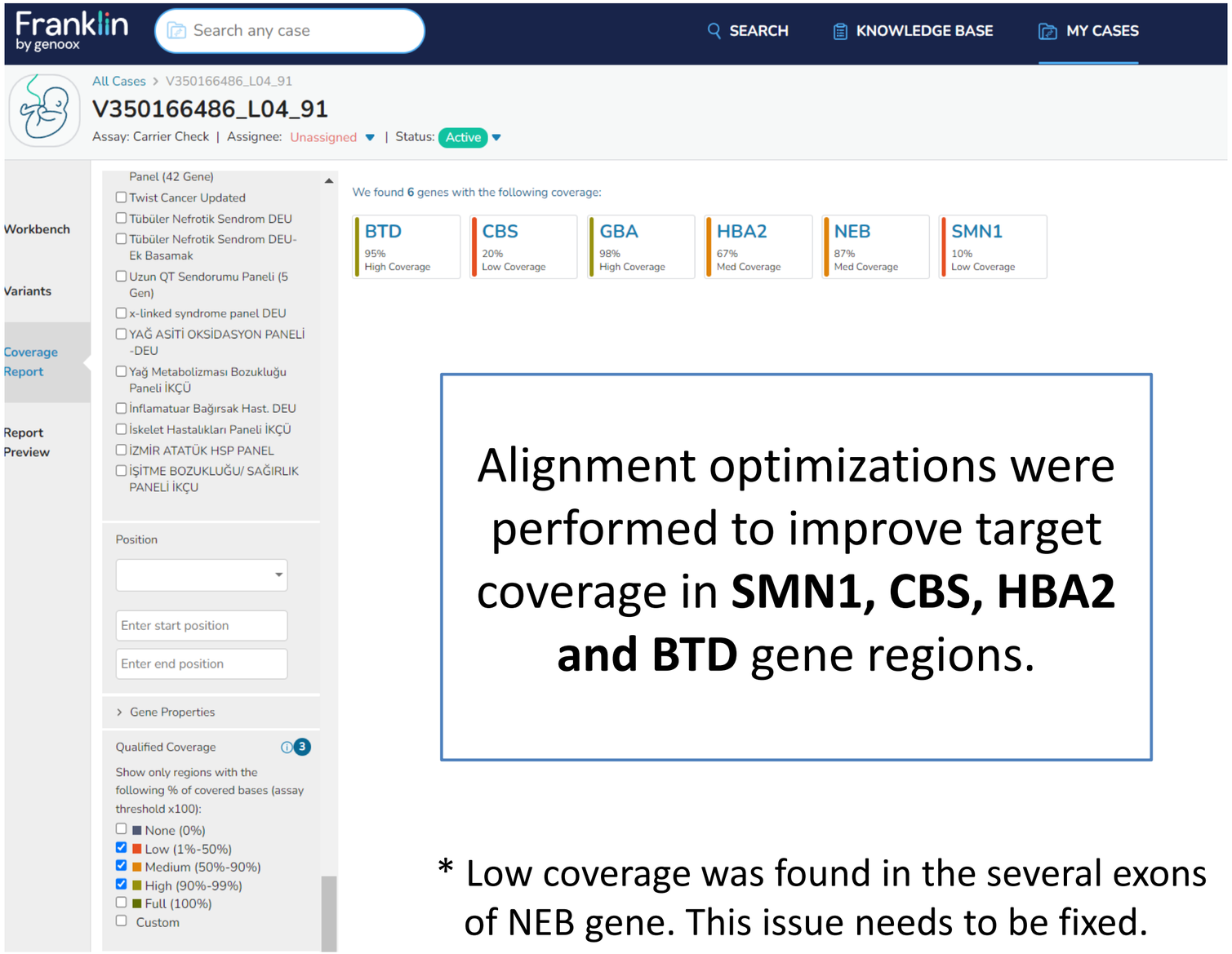
c.31+36947G>A (rs886042106)
c.93+5590T>A (rs1557211730)
c.265-463A>G (rs1603441629)
c.650-39498A>G (rs1556980528)
c.961-5831C>T (rs398124099)
c.1812+601A>G (RCV000850291)
c.2169-12884G>T (RCV001780951.1)
c.3432+2036A>G (rs182575709)
c.30del (rs753288164)
c.5326-215T>G (RCV001780944.1)
c.6614+3310G>T (rs797045526)
c.6913-4037T>G (RCV001780955.1)
c.8217+18052A>G (rs886042109)
c.9085-15519G>T (rs398124091)
c.9225-647A>G (rs398124091)
c.9225-285A>G (rs587776747)
c.9225-160A>G (RCV001780964.1)
c.9361+117A>G (RCV001780938.1)
c.9974+175T>A (rs1602451773)

COVERAGE

CarrierCheck achieved high coverage of up to 500X in each patient sample for all genes



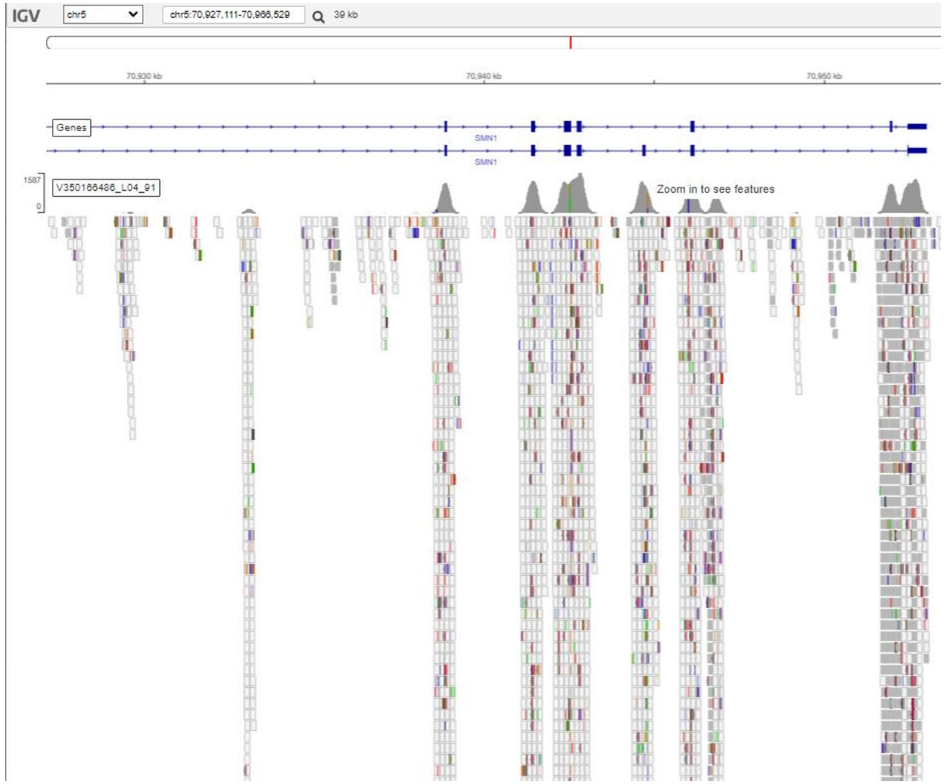
COVERAGE



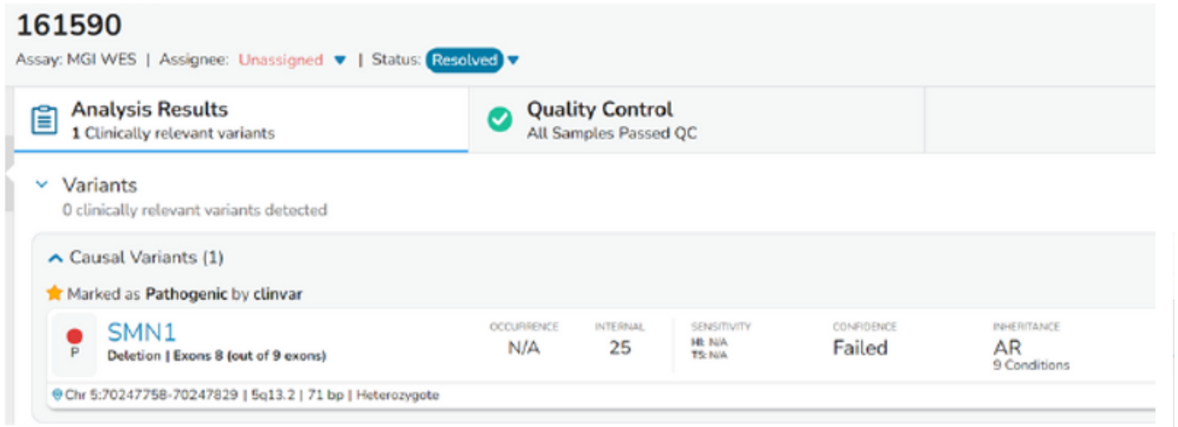
Alignment optimizations were performed to improve target coverage in **SMN1, CBS, HBA2 and BTD** gene regions.

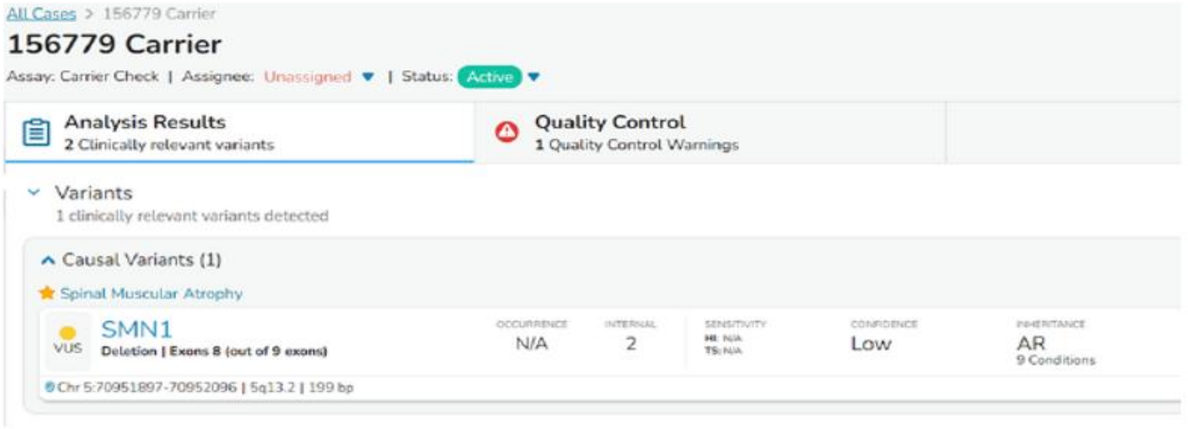
* Low coverage was found in the several exons of NEB gene. This issue needs to be fixed.

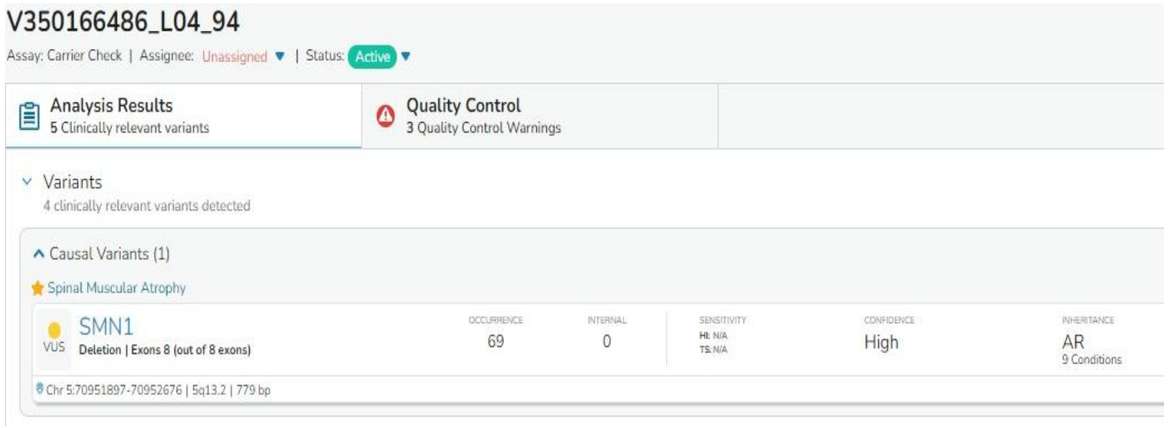
Detection of SMN1 point mutations was also achieved.



Detection Capability of SMN1 Gene Deletions

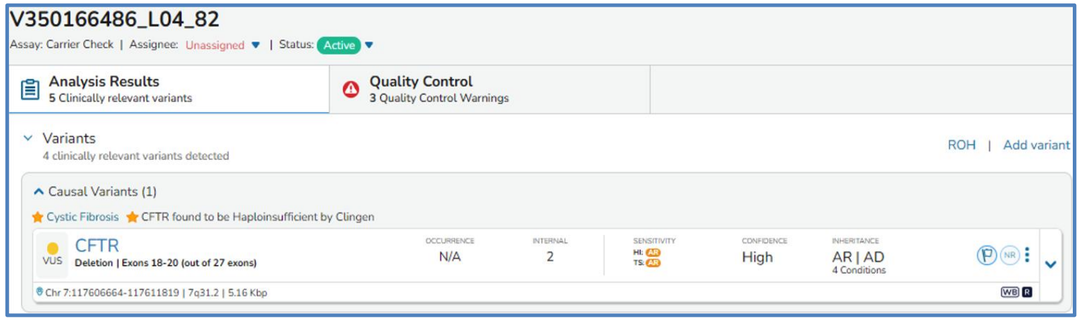


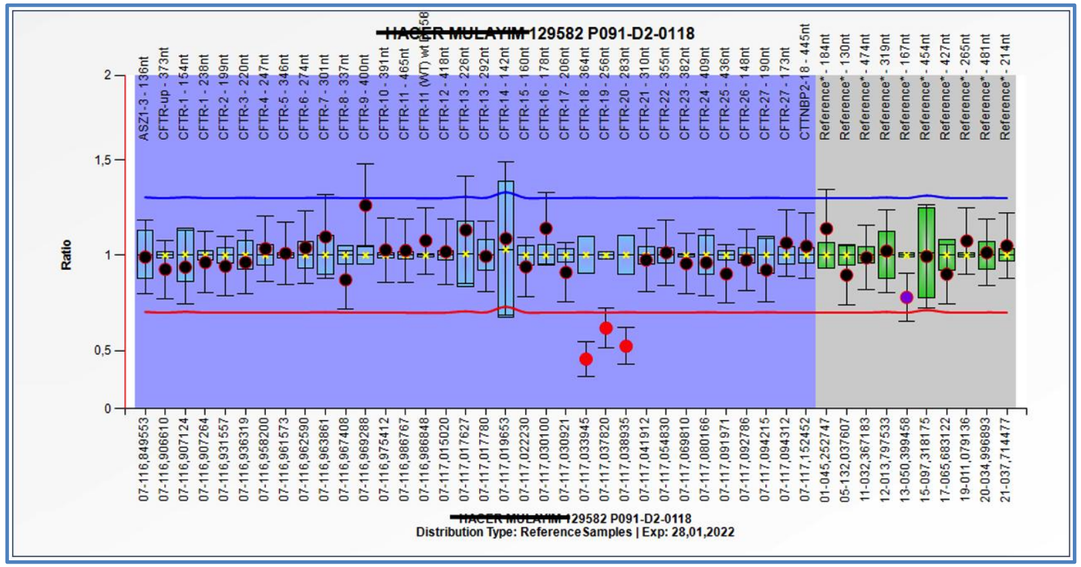




The CNV detection capability of the analysis software was improved by training on a large number of samples with SMN1 exon 7/8 deletions

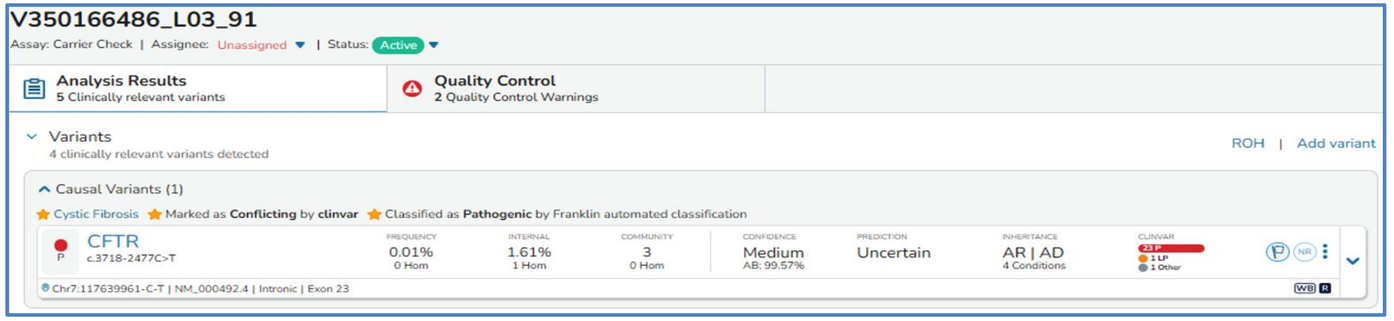
Identification of CNVs in the CFTR Gene

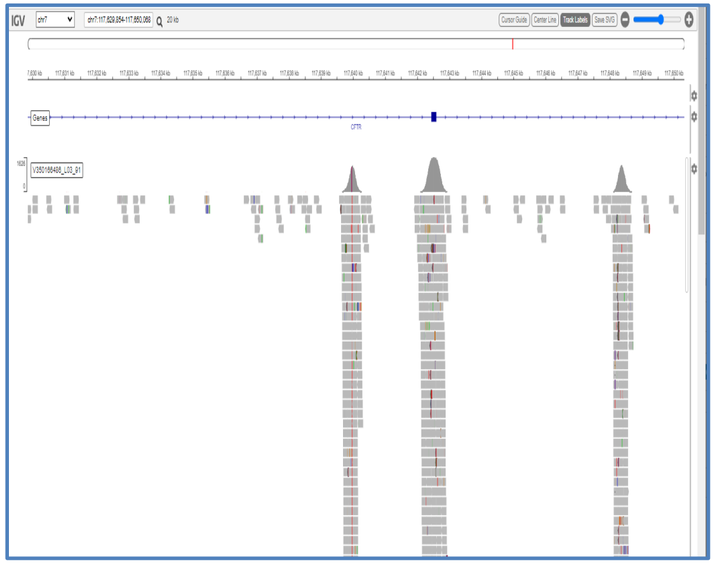




The analysis software detects CFTR gene deletions

Identification of Deep Intronic Variants in the CFTR Gene

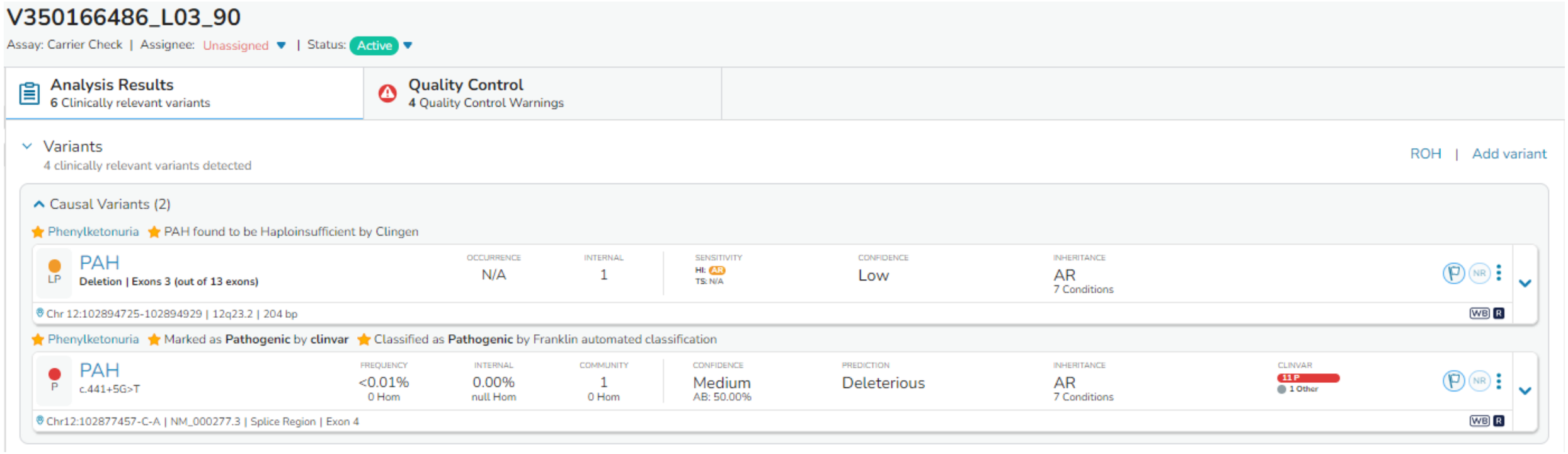




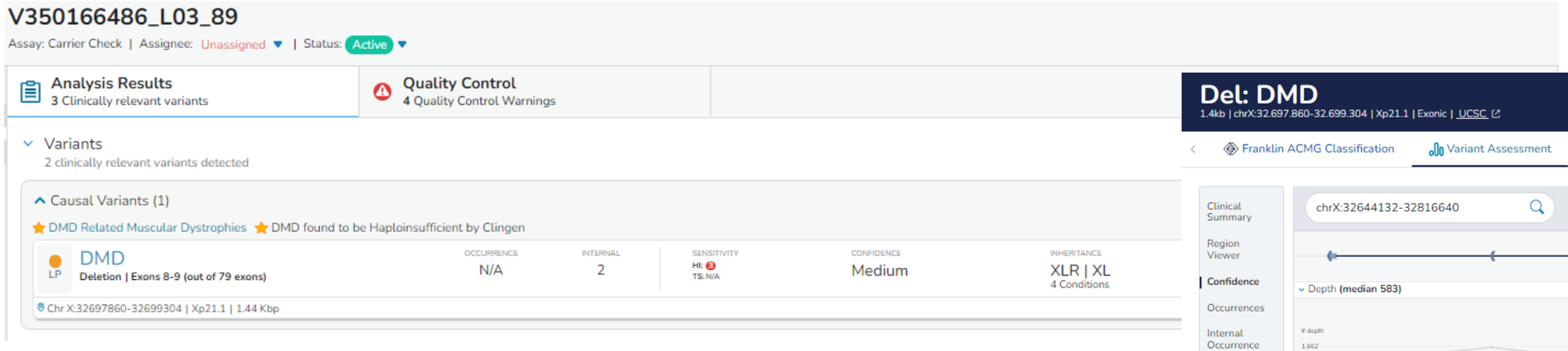
IGV View

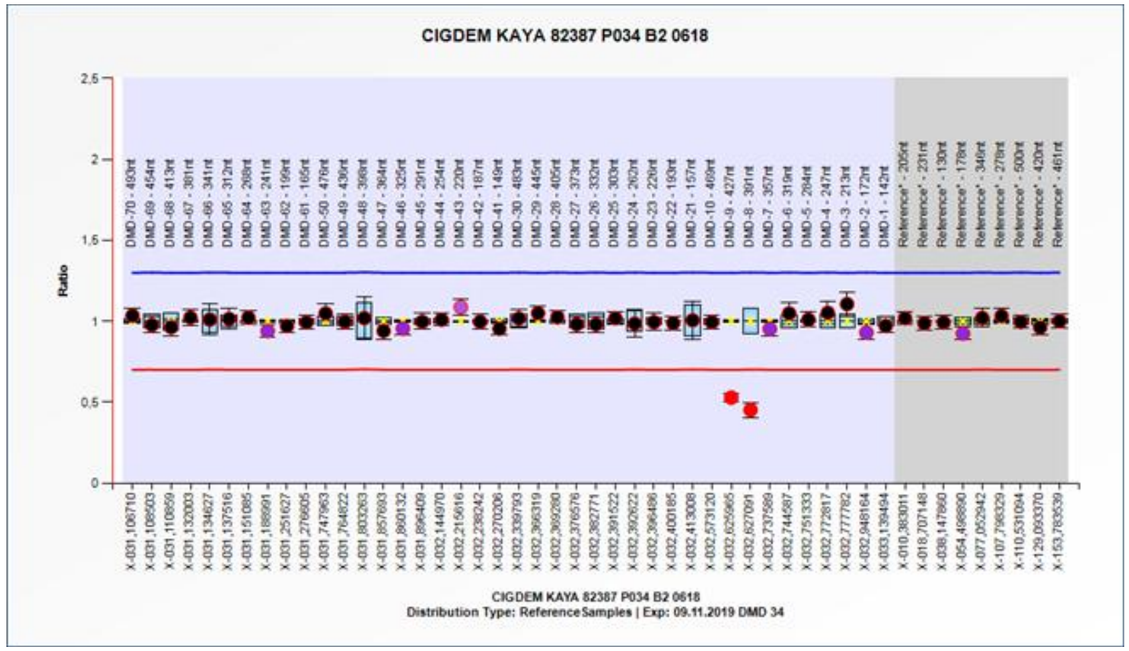
The deep intronic variants of CFTR gene are also detected

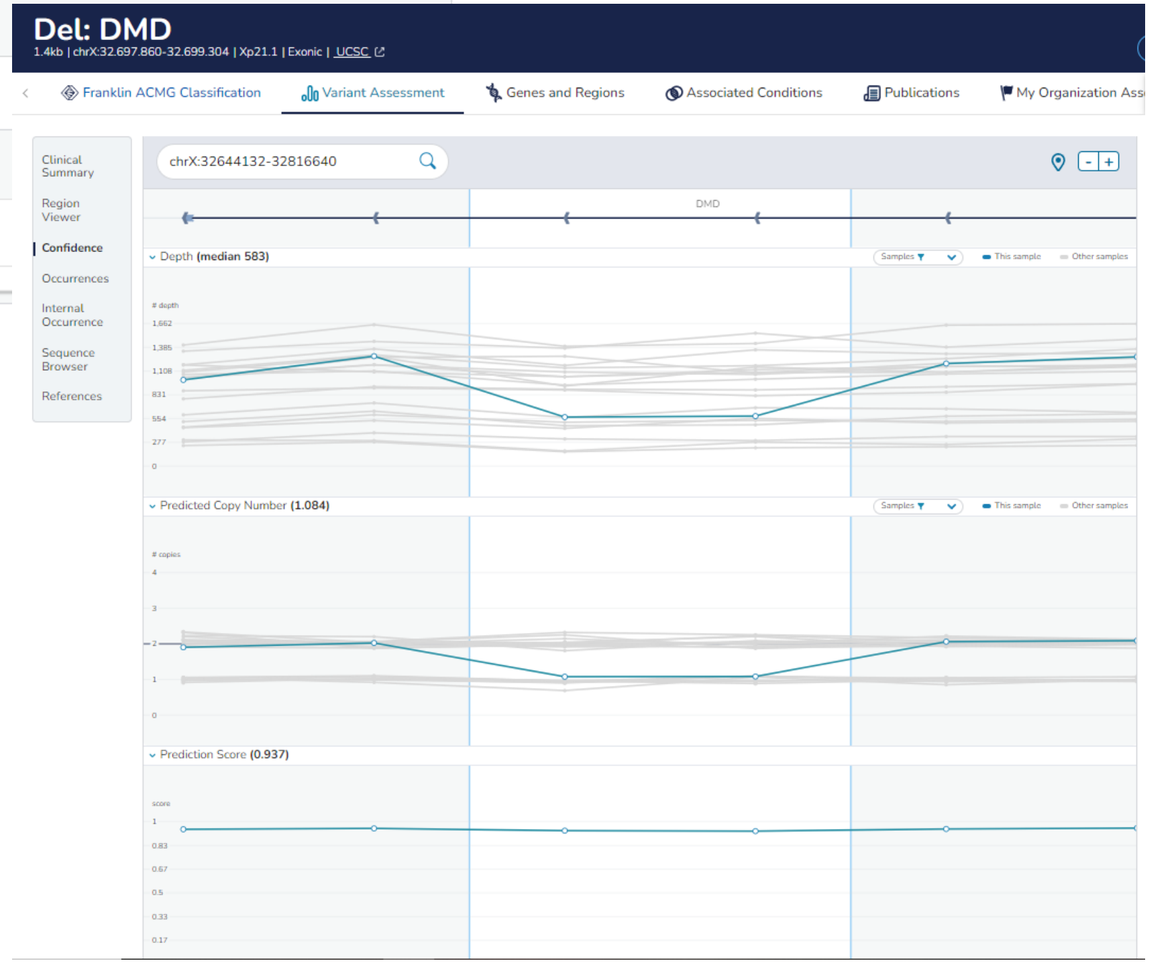
CarrierCheck has the capability of detecting both point mutations and CNV-type mutations in the PAH gene



CarrierCheck can detect the single-exon level deletions and duplications in the DMD gene







CarrierCheck is capable of detecting deletion and duplication-type mutations in the HBA gene

158218 Carrier

Assay: Carrier Check | Assignee: Unassigned | Status: Resolved

Analysis Results
5 Clinically relevant variants

Quality Control
3 Quality Control Warnings

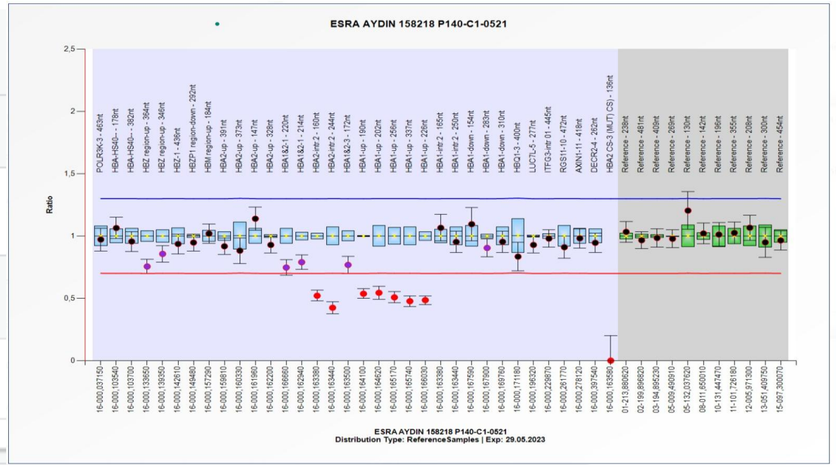
Variants
4 clinically relevant variants detected

Causal Variants (1)

Alpha (α) Thalassemia HBA2 found to be Haploinsufficient by Clingen

		OCCURRENCE	INTERNAL	SENSITIVITY	CONFIDENCE	INHERITANCE
VUS	HBA1 | HBA2 Deletion | Exonic	N/A	6	HI: AR TS: N/A	Medium	AR | AD 17 Conditions

Chr 16:173385-173709 | 16p13.3 | 324 bp



V350166486_L04_96

Assay: Carrier Check | Assignee: Unassigned | Status: Active

Analysis Results
6 Clinically relevant variants

Quality Control
1 Quality Control Warnings

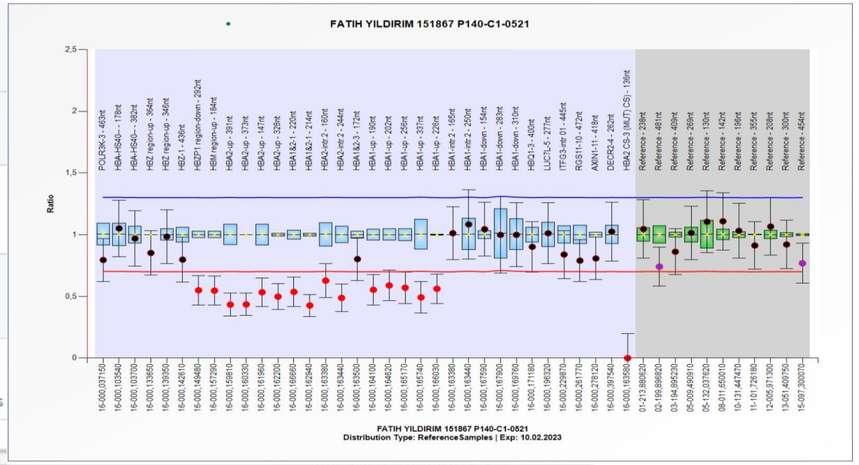
Variants
5 clinically relevant variants detected

Causal Variants (1)

Alpha (α) Thalassemia HBA2 found to be Haploinsufficient by Clingen Marked as **Pathogenic** by **clinvar**

		OCCURRENCE	INTERNAL	SENSITIVITY	CONFIDENCE	INHERITANCE
VUS	HBA1 | HBA2 | HBQ1 Deletion | Exonic	499	2	HI: AR TS: N/A	High	AR | AD 17 Conditions

Chr 16:173385-176676 | 16p13.3 | 3.29 Kbp



(The size of the deletions detected by Carriercheck were not concordant with the MLPA results)

Design of Patient Groups for the Validation

NATIONALITY	Number of Participants
Turkish	248
Syrian	128
Georgian	16
TOTAL	*392*

Patient Groups

Group 1 consisted of 96 randomized cases. Their genomic DNA was used to optimize the panel and check the QC scores.

Group 2 consisted of 288 cases. All of them were heterozygous carriers for at least one of the panel genes.

Samples from this group were used to validation of the panel.

Group 3; It was composed of 392 consanguineous spouses. Carrier status of them had not been tested before. 128 of them were Syrian, 16 of Georgian, refugee couples and 248 were Turkish origin couples.

Samples from this group were used to test the "**efficiency**" and "**sensitivity**" of the panel.

Inclusion Criterias:

1. The couples who are heterozygous mutation carriers of one of the genes which are described in our carrier panel
2. Consanguineous couples
3. Couples consenting to participate in the study

Exclusion Criterias:

1. The couples who are the carriers of other monogenic diseases which are not described in our carrier panel
2. The carriers of chromosomal balanced rearrangements
3. Nonconsanguineous couples Couples who are not consenting to participate in the study

Allelic (Carrier) Frequency Distribution in Panel Gene Set (Group-3)

A carrier frequency exceeding 5% was observed for 10 genes.

Gene	Variant Detected	Carrier Frequency	Gene	Variant Detected	Carrier Frequency	Gene	Variant Detected	Carrier Frequency	Gene	Variant Detected	Carrier Frequency
MEFV	78	19,9%	ALPL	5	1,3%	NBN	2	0,5%	GLDC	1	0,3%
CFTR	54	13,8%	TYR	5	1,3%	PEX1	2	0,5%	HADHA	1	0,3%
ABCA4	43	11,0%	PCCB	5	1,3%	PKHD1	2	0,5%	ABCB11	0	0,0%
SMN1	42	10,7%	ACAT1	4	1,0%	HEXB	2	0,5%	ABCD1	0	0,0%
CYP21A2	35	8,9%	ATM	4	1,0%	NPHS1	2	0,5%	ADA	0	0,0%
HBA1/2	32	8,2%	CAPN3	4	1,0%	PPT1	2	0,5%	AGA	0	0,0%
PAH	29	7,4%	GALT	4	1,0%	ARSB	1	0,3%	AGL	0	0,0%
HBB	25	6,4%	IDUA	4	1,0%	BBS10	1	0,3%	ALDH3A2	0	0,0%
BTD	23	5,9%	FANCA	4	1,0%	BBS4	1	0,3%	ARG1	0	0,0%
GJB2	23	5,9%	GCDH	4	1,0%	CEP290	1	0,3%	ASL	0	0,0%
USH2A	16	4,1%	CNGA3	4	1,0%	CLN3	1	0,3%	CLN8	0	0,0%
SLC26A4	12	3,1%	ACADM	3	0,8%	CLN5	1	0,3%	CPT2	0	0,0%
ATP7B	12	3,1%	ARSA	3	0,8%	CPT1A	1	0,3%	DBT	0	0,0%
PYGM	11	2,8%	CPS1	3	0,8%	CTNS	1	0,3%	DYSF	0	0,0%
NEB	9	2,3%	DMD	3	0,8%	CYP17A1	1	0,3%	EDA	0	0,0%
NPHS2	9	2,3%	MCCC1	3	0,8%	DLD	1	0,3%	ETFA	0	0,0%
MCCC2	9	2,3%	MCOLN1	3	0,8%	ESCO2	1	0,3%	GJB6	0	0,0%
PRF1	9	2,3%	PMM2	3	0,8%	ETFDH	1	0,3%	GNPTAB	0	0,0%
MMACHC	9	2,3%	SMPD1	3	0,8%	F9	1	0,3%	GRHPR	0	0,0%
GAA	8	2,0%	F8	3	0,8%	G6PC	1	0,3%	GUSB	0	0,0%
GBA	8	2,0%	PCCA	3	0,8%	GALNS	1	0,3%	HAX1	0	0,0%
HEXA	8	2,0%	AGXT	2	0,5%	GLA	1	0,3%	HGSNAT	0	0,0%
ACADVL	8	2,0%	AIRE	2	0,5%	IDS	1	0,3%	HMGCL	0	0,0%
MMUT	8	2,0%	ASPA	2	0,5%	MCEE	1	0,3%	MAN2B1	0	0,0%
UGT1A1	8	2,0%	BCKDHA	2	0,5%	NAGS	1	0,3%	MLC1	0	0,0%
SERPINA1	7	1,8%	CBS	2	0,5%	PEX7	1	0,3%	MMAA	0	0,0%
SLC22A5	7	1,8%	CLRN1	2	0,5%	RAG1	1	0,3%	MMADHC	0	0,0%
ALDOB	6	1,5%	COL4A3	2	0,5%	SAMHD1	1	0,3%	NAGLU	0	0,0%
SACS	6	1,5%	FAH	2	0,5%	SGCA	1	0,3%	NDUFS4	0	0,0%
ASS1	5	1,3%	FANCC	2	0,5%	SGCB	1	0,3%	OXTR	0	0,0%
BBS1	5	1,3%	FKTN	2	0,5%	SGSH	1	0,3%	PRDX1	0	0,0%
COL4A4	5	1,3%	GALC	2	0,5%	SLC26A2	1	0,3%	RPE65	0	0,0%
DHCR7	5	1,3%	GLB1	2	0,5%	TH	1	0,3%	STX11	0	0,0%
NPC1	5	1,3%	HYAL1	2	0,5%	UNC13D	1	0,3%	STXBP2	0	0,0%
PCDH15	5	1,3%	IVD	2	0,5%	BCKDHB	1	0,3%	TCIRG1	0	0,0%

A snapshot validation of the retrospective DNA samples with known mutations

Validation of CarrierCheck		
179 retrospective DNA with known mutations were sequenced for validation. At least one variant in each gene in the panel were screened.		
	SNP	**CNV**
Number of Variation Tested	**202**	**47**
Concordance (%)	**98,5%**	**91,5%**

Regarding discordant genes «The rates were high, except the specificity rate of the CYP21A1 gene».

Disconcordant Samples	Variation	Type of Variation
SMN1	c.347T>C / p.Ile116Thr c.283G>C/p.Gly95Arg c.597del/p.Met200CysfsTer13	SNP
HBB	Heterozygous Exon 3 deletion	CNV
HBA	Heterozygous MED2 duplication	CNV
CYP21A2	Deletion (del8nt+I172N,V273E,M239K,F306+T) Duplication (del8nt+I172N,V273E,M239K,F306+T)	CNV

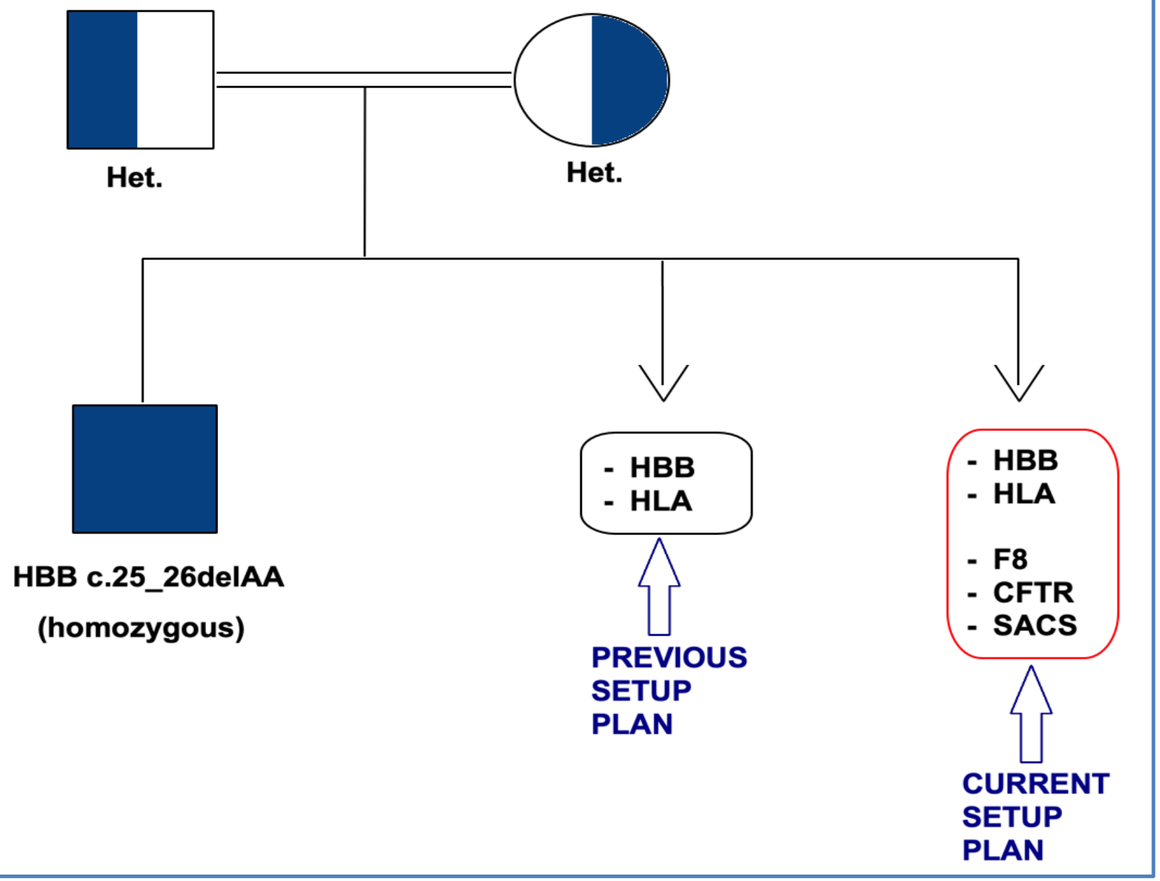
	MLPA + CarrierCheck Samples	MLPA-Detected Carriers	CarrierCheck -Detected Carriers	Sensitivity	Specificity
SMN1	398	44	45	100,0%	99,7%
HBA1/2	62	51	49	96,1%	100,0%
CYP21A2	6	2	0	0	100,0%

	SangerSeq+ CarrierCheck Samples	Sanger Seq-Detected Carriers	CarrierCheck -Detected Carriers	Sensitivity	Specificity
CYP21A2	71	27	47	100%	48,8%

A dramatic case example of Carriercheck

(The family requested healthy and HLA-compatible sibling)

Pedigree of the family with the Thalassemia Major Boy,2.5



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REPRODUCTIVE GENETICS COMPANY

PATIENT'S NAME	PATIENT NUMBER
	*********

Date of Birth / Gender		Date of Sample Arrival	**
Protocol No / Lab No		Lab. Acceptance Date	
Material	Blood with EDTA	Expert Approval Date	
Request Date			
Reason for Referal			
Referring Institution Clinician	*** MEDICAL CENTER		

MOLECULAR GENETICS TEST REPORT

CarrierCheck Trio Analysis

Result:

Gene (Transcript)	Location	Nucleotide (protein) dbSNP	Index Child	Mother	Father	Variant Classification	Disease OMIM# Inheritance
HBB NM_000518.5	Exon 1	c.25_26delAA p.Lys9Valfs*14	Homozygous	Heterozygous	Heterozygous	Pathogenic	Beta Thalassemia OMIM: 6134985 Autosomal Recessive
F8 NM_000132.4	Exon 25	c.6821T>C p.Met2274Thr	Hemizygous	Heterozygous	NORMAL	Pathogenic	Hemophilia A OMIM: 306700 X-Linked Recessive
CFTR NM_000492.4	Exon 8	c.1000C>T p.Arg334Trp rs121909011	Heterozygous	NORMAL	Heterozygous	Pathogenic	Cystic Fibrosis OMIM: 219700 Autosomal Recessive
	Exon	c.3205G>A p.Gly1069Arg rs200321110	Heterozygous	Heterozygous	NORMAL	Pathogenic	
SACS NM_014363.6	Exon 10	c.8373G>T p.Lys2791Asn rs762948322	Heterozygous	Heterozygous	NORMAL	VUS	Charlevoix-Saguenay Type Spastic Ataxia OMIM: 270550 Autosomal Recessive
	Exon 10	c.11786A>G p.His3929Arg	NORMAL	NORMAL	Heterozygous	VUS	

NGS Based CarrierCheck Targeted Screening Panels

CarrierCheck Expanded (462 genes) Comprehensive Carrier Screening Test

Who is a carrier?

A carrier is an individual who has a mutation in one of the alleles of a gene associated with a genetic disease.

"CarrierCheck enables genetic matching of gamete donors to their recipients"

What is the CarrierCheck test?

Expanded preconceptional carrier test enables the detection of couples at risk for single gene diseases. It allows couples to make the right reproductive decision and reduces the risk of having a affected child. It specifically screens autosomal recessive and X-linked recessive inherited disorders. Next-Generation Sequencing (NGS) empowered by powerful bioinformatic tools enable simultaneous screening of hundreds of diseases with a single universal method.

"Carrier Tests can aid to prevent genetic disorders"

Why CarrierCheck test?

- Comprehensive screening capacity with expanded gene panel - 462 genes
- High variant detection sensitivity,
- Simultaneous detecion of CNVs, SNPs with a single NGS based test
- Special analysis algorithms for efficient diagnosis of challenging gene regions –pseudogenes and homologous genes - SMN1, HBA1/2, CYP21A2, DMD, CFTR, GBA
- Additional MLPA tests for detection of deletion/duplication in SMN1/2 and HBA1/2
- Addtional TP-PCR for triplet nucleotide repeat detection in FMR1 gene.
- Exon level CNV detection for genes related to critical diseases - DMD, CFTR
- Fast and reliable results with exclusive analysis tool developed by Franklin by Genoox.

CarrierCheck (146 genes) Carrier Screening Test

Who is a carrier?

A carrier is an individual who has a mutation in one of the alleles of a gene associated with a genetic disease.

"CarrierCheck enables genetic matching of gamete donors to their recipients"

What is the CarrierCheck test?

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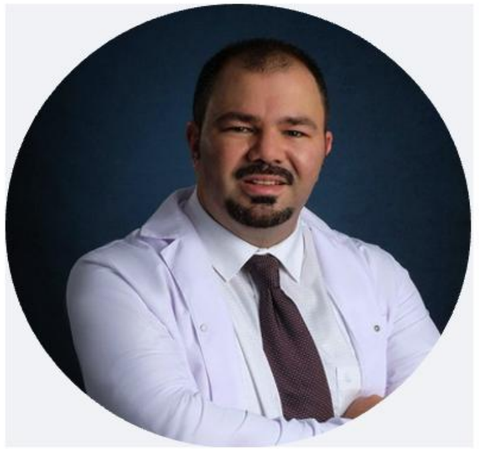
Prof Evrim Ünsal PhD.



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Thank you