



Single NGS-based Method for Carrier Screening: Streamlined Analysis of Challenging Genomic Regions

VOLKAN BALTACI MD, PhD
Mikrogen Genetic Diagnosis Laboratory - Türkiye

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 ?*

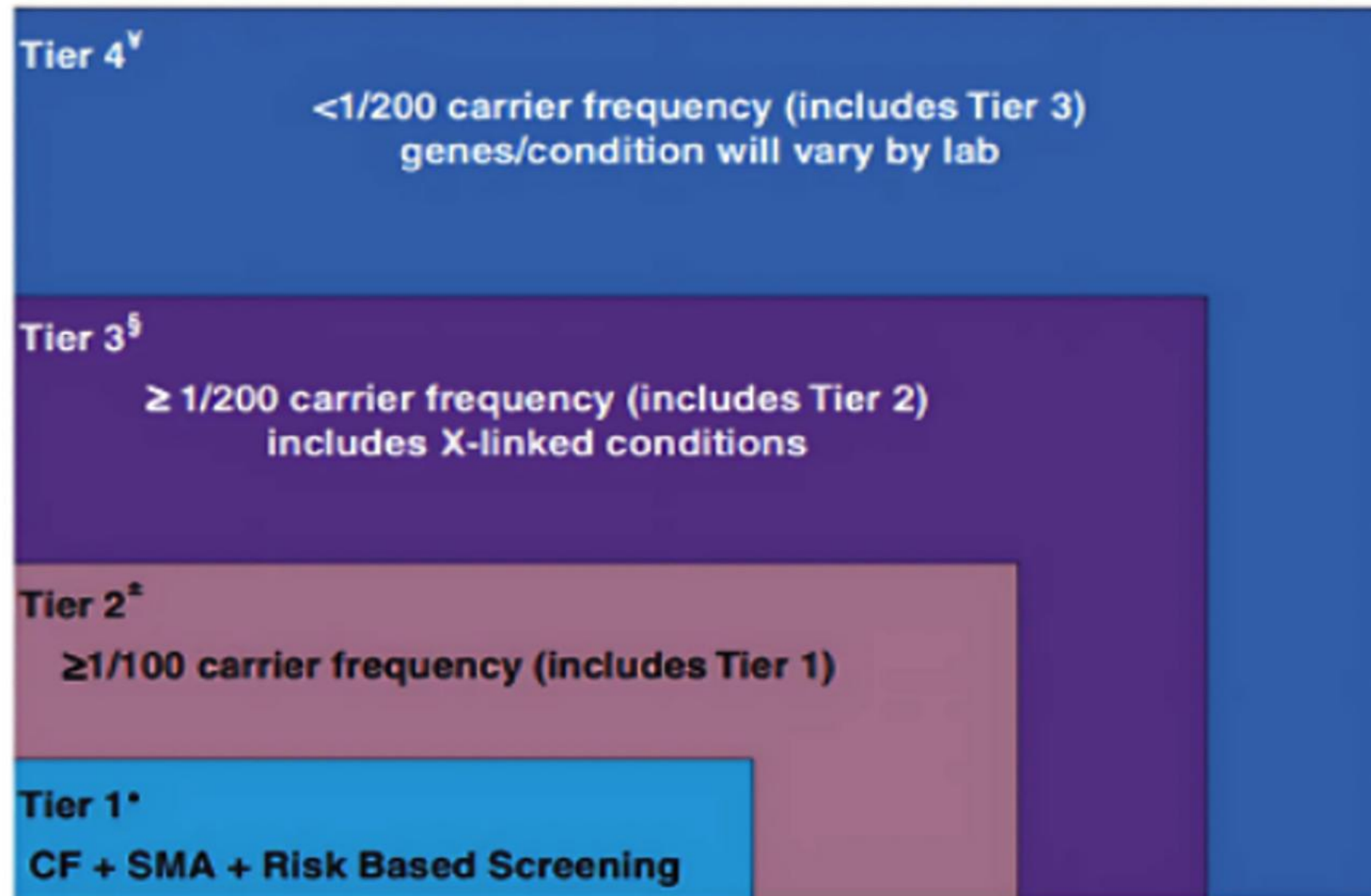
What should we screen ?

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 recommends:

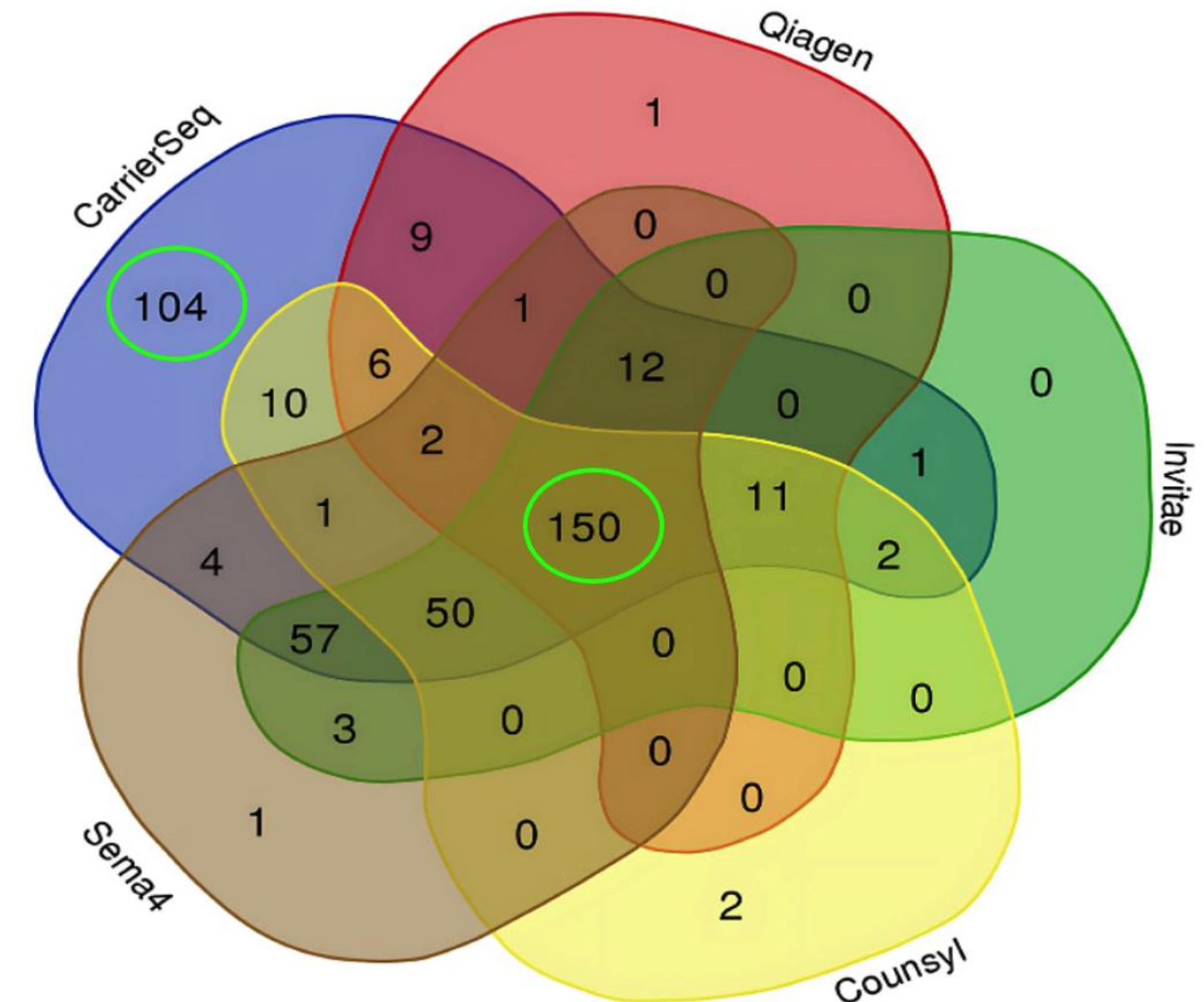


ACMG recommended a “tier system” based on carrier frequency

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





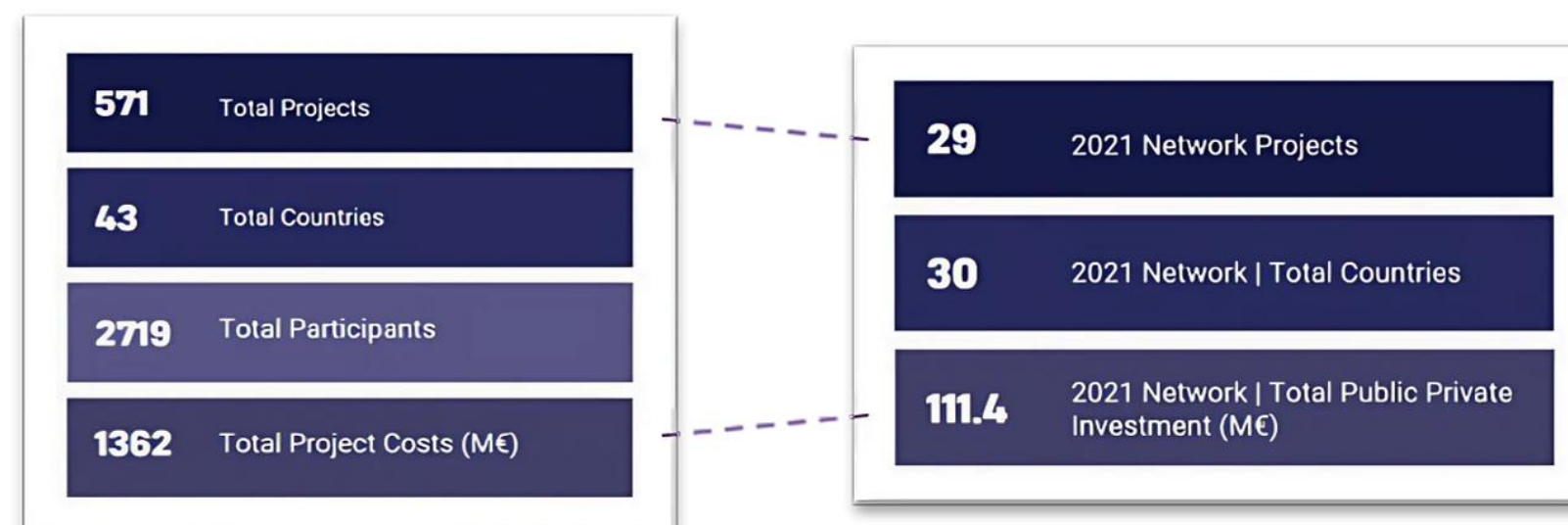
CARRIERCHECK

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



- 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



2.3 M€

CarrierCheck

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



genoox

Selection strategies of “Carriercheck” panel genes

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

43

93 genes were selected from 1600 PGT-M and 39,000 targeted single-gene sequencing cases

(43 genes reimbursed by government for PGT-M)

WES

50 genes were selected from our 2445 WES analysis cases

Among the genes showing copy number changes, 9 genes were selected and included in the panel

CNVs

Selected genes from panels of global companies



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.

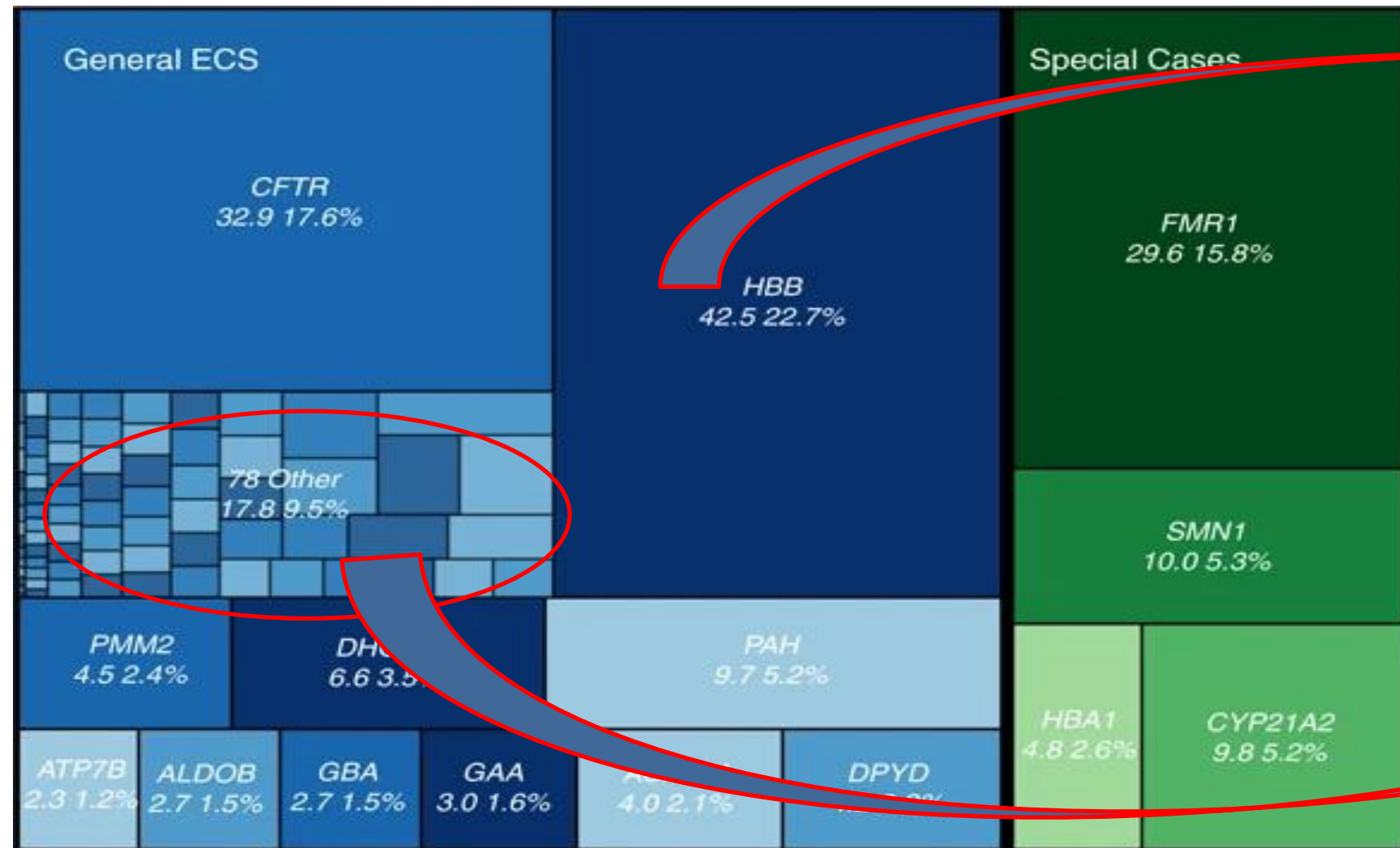
b) The results of 2,445 WES cases performed at Mikrogen Laboratories were evaluated and it was decided to add 50 genes to the panel.

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

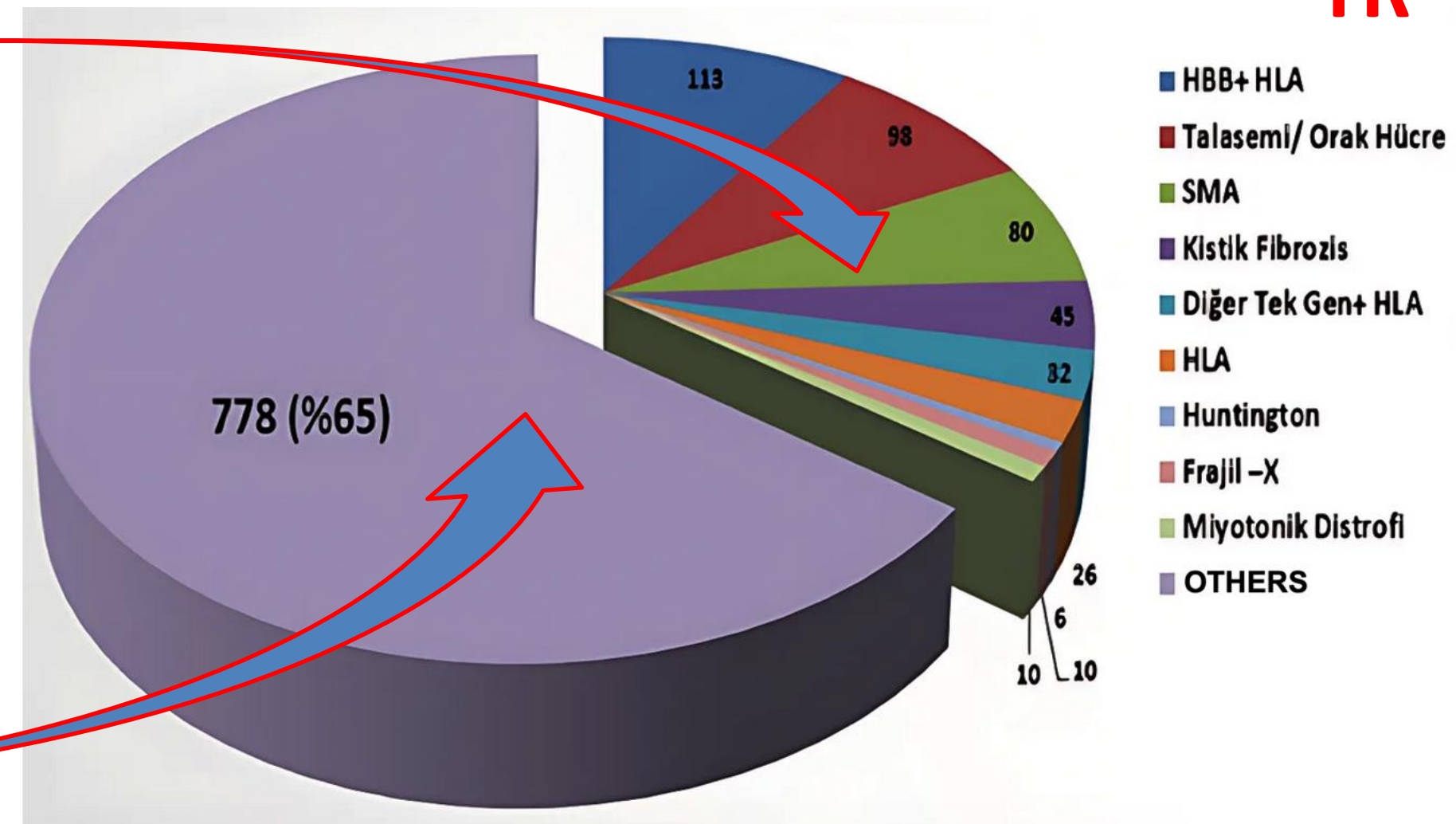


Distribution of monogenic diseases in Europa:

- 15 diseases constitute 90% of all cases
- 78 diseases constitute only 10% of the cases.

(Beauchamp et. al. (2018) 474.644 test)

TR



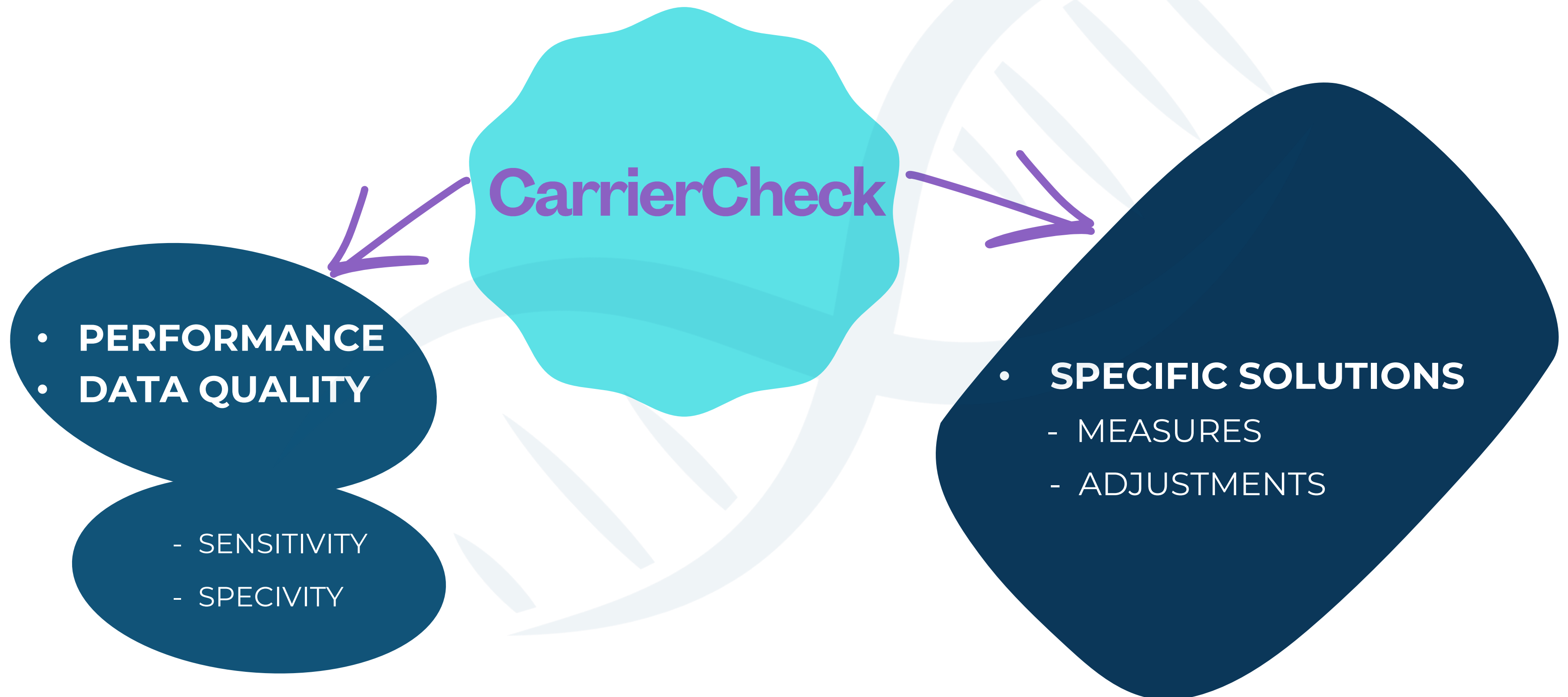
Mikrogen's data consisting of 1198 PGT-M + (2445 WES);

- 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

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

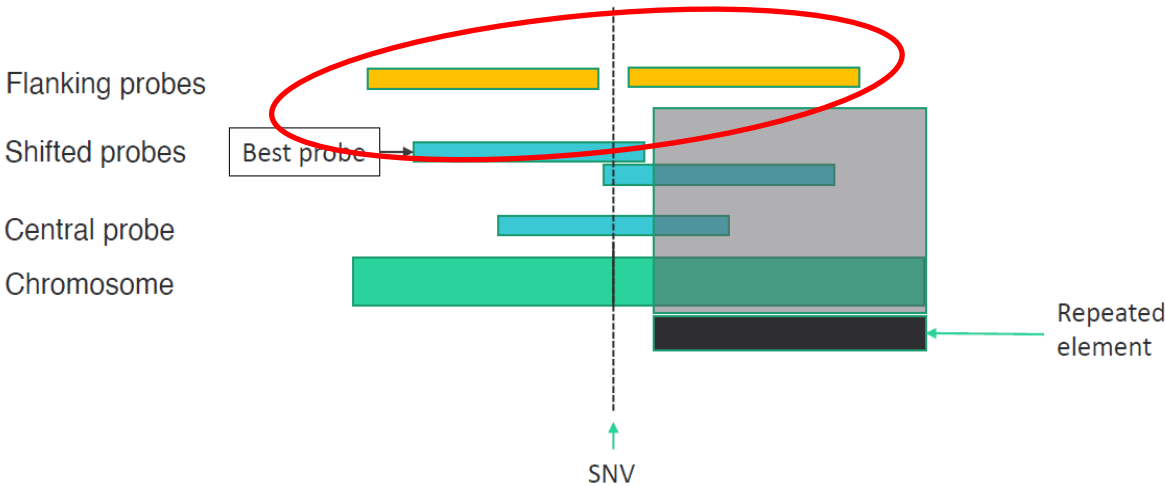
Genome assembly: hg38

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

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

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

1X Tiling



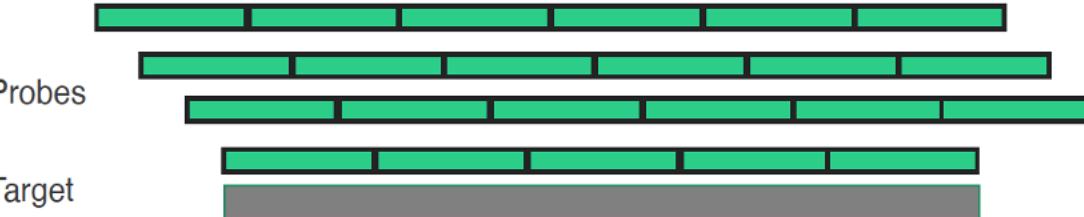
2X Tiling (overlap of two consecutive probes 60nt):



3X Tiling (overlap of two consecutive probes 80nt):



4X Tiling (overlap of two consecutive probes 90nt):



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

Franklin
by genoox

Search any case

SEARCH

KNOWLEDGE BASE

MY CASES

?

Suleyman Aktuna
mikrogen genetic di

All Cases > V350166486_L04_91

V350166486_L04_91

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

Follow Case

Workbench

Variants

Coverage Report

Report Preview

Panel (42 Gene)

☐ Twist Cancer Updated

☐ Tübüler Nefrotik Sendrom DEU

☐ Tübüler Nefrotik Sendrom DEU- Ek Basamak

☐ Uzun QT Sendorumu Paneli (5 Gen)

☐ x-linked syndrome panel DEU

☐ YAĞ ASİTİ OKSİDASYON PANELİ -DEU

☐ Yağ Metabolizması Bozukluğu Paneli İKÇÜ

☐ İnflamatuvar Bağırsak Hast. DEU

☐ İskelet Hastalıkları Paneli İKÇÜ

☐ İZMİR ATATÜK HSP PANEL

☐ İŞİTME BOZUKLUĞU/ SAĞIRLIK PANELİ İKÇÜ

Position

Enter start position

Enter end position

> Gene Properties

Qualified Coverage 1

Show only regions with the following % of covered bases (assay threshold x100):

☐ None (0%)

☐ Low (1%-50%)

☐ Medium (50%-90%)

☐ High (90%-99%)

☒ Full (100%)

☐ Custom

We found 140 genes with the following coverage:

Sort by: Gene

Export

ABCA4 100% Full Coverage	ABCB11 100% Full Coverage	ABCD1 100% Full Coverage	ACADM 100% Full Coverage	ACADVL 100% Full Coverage	ACAT1 100% Full Coverage	ADA 100% Full Coverage	AGA 100% Full Coverage	AGL 100% Full Coverage	AGXT 100% Full Coverage	AIRE 100% Full Coverage	ALDH3A2 100% Full Coverage	ALDOB 100% Full Coverage	ALPL 100% Full Coverage
ARG1 100% Full Coverage	ARSA 100% Full Coverage	ARSB 100% Full Coverage	ASL 100% Full Coverage	ASPA 100% Full Coverage	ASS1 100% Full Coverage	ATM 100% Full Coverage	ATP7B 100% Full Coverage	BBS1 100% Full Coverage	BBS10 100% Full Coverage	BBS4 100% Full Coverage	BCKDHA 100% Full Coverage	BCKDHB 100% Full Coverage	CAPN3 100% Full Coverage
CEP290 100% Full Coverage	CFTR 100% Full Coverage	CLN3 100% Full Coverage	CLN5 100% Full Coverage	CLN8 100% Full Coverage	CLRN1 100% Full Coverage	CNGA3 100% Full Coverage	COL4A3 100% Full Coverage	COL4A4 100% Full Coverage	CPS1 100% Full Coverage	CPT1A 100% Full Coverage	CPT2 100% Full Coverage	CTNS 100% Full Coverage	CYP17A1 100% Full Coverage
CYP21A2 100% Full Coverage	DBT 100% Full Coverage	DHCR7 100% Full Coverage	DLD 100% Full Coverage	DMD 100% Full Coverage	DYSF 100% Full Coverage	EDA 100% Full Coverage	ESCO2 100% Full Coverage	ETFA 100% Full Coverage	ETFDH 100% Full Coverage	F8 100% Full Coverage	F9 100% Full Coverage	FAH 100% Full Coverage	FANCA 100% Full Coverage
FANCC 100% Full Coverage	FKTN 100% Full Coverage	G6PC 100% Full Coverage	GAA 100% Full Coverage	GALC 100% Full Coverage	GALNS 100% Full Coverage	GALT 100% Full Coverage	GCDH 100% Full Coverage	GJB2 100% Full Coverage	GJB6 100% Full Coverage	GLA 100% Full Coverage	GLB1 100% Full Coverage	GLDC 100% Full Coverage	GNPTAB 100% Full Coverage
GRHPR 100% Full Coverage	GUSB 100% Full Coverage	HADHA 100% Full Coverage	HAX1 100% Full Coverage	HBA1 100% Full Coverage	HBB 100% Full Coverage	HEXA 100% Full Coverage	HEXB 100% Full Coverage	HGSNAT 100% Full Coverage	HMGCL 100% Full Coverage	HYAL1 100% Full Coverage	IDS 100% Full Coverage	IDUA 100% Full Coverage	IVD 100% Full Coverage
MAN2B1 100% Full Coverage	MCCC1 100% Full Coverage	MCCC2 100% Full Coverage	MCEE 100% Full Coverage	MCOLN1 100% Full Coverage	MEFV 100% Full Coverage	MLC1 100% Full Coverage	MMAA 100% Full Coverage	MMACHC 100% Full Coverage	MMADHC 100% Full Coverage	MMUT 100% Full Coverage	NAGLU 100% Full Coverage	NAGS 100% Full Coverage	NBN 100% Full Coverage
NDUFS4 100% Full Coverage	NPC1 100% Full Coverage	NPHS1 100% Full Coverage	NPHS2 100% Full Coverage	OXTR 100% Full Coverage	PAH 100% Full Coverage	PCCA 100% Full Coverage	PCCB 100% Full Coverage	PCDH15 100% Full Coverage	PEX1 100% Full Coverage	PEX7 100% Full Coverage	PKHD1 100% Full Coverage	PMM2 100% Full Coverage	PPT1 100% Full Coverage
PRDX1 100% Full Coverage	PRF1 100% Full Coverage	PYGM 100% Full Coverage	RAG1 100% Full Coverage	RPE65 100% Full Coverage	SACS 100% Full Coverage	SAMHD1 100% Full Coverage	SERPIN... 100% Full Coverage	SGCA 100% Full Coverage	SGCB 100% Full Coverage	SGSH 100% Full Coverage	SLC22A5 100% Full Coverage	SLC26A2 100% Full Coverage	SLC26A4 100% Full Coverage
SMPD1 100% Full Coverage	STX11 100% Full Coverage	STXBP2 100% Full Coverage	TCIRG1 100% Full Coverage	TGM1 100% Full Coverage	TH 100% Full Coverage	TMEM216 100% Full Coverage	TPP1 100% Full Coverage	TTPA 100% Full Coverage	TYR 100% Full Coverage	UGT1A1 100% Full Coverage	UNC13D 100% Full Coverage	USH2A 100% Full Coverage	WAS 100% Full Coverage

COVERAGE

Franklin
by genoox

Search any case

SEARCH

KNOWLEDGE BASE

MY CASES

All Cases > V350166486_L04_91

V350166486_L04_91

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

Workbench

Variants

Coverage Report

Report Preview

Panel (42 Gene)

☐ Twist Cancer Updated

☐ Tübüler Nefrotik Sendrom DEU

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☐ İZMİR ATATÜK HSP PANEL

☐ İŞİTME BOZUKLUĞU/ SAĞIRLIK PANELİ İKÇÜ

Position

Enter start position

Enter end position

> Gene Properties

Qualified Coverage 3

Show only regions with the following % of covered bases (assay threshold x100):

☐ None (0%)

☒ Low (1%-50%)

☒ Medium (50%-90%)

☒ High (90%-99%)

☐ Full (100%)

☐ Custom

We found 6 genes with the following coverage:

BTD

95%

High Coverage

CBS

20%

Low Coverage

GBA

98%

High Coverage

HBA2

67%

Med Coverage

NEB

87%

Med Coverage

SMN1

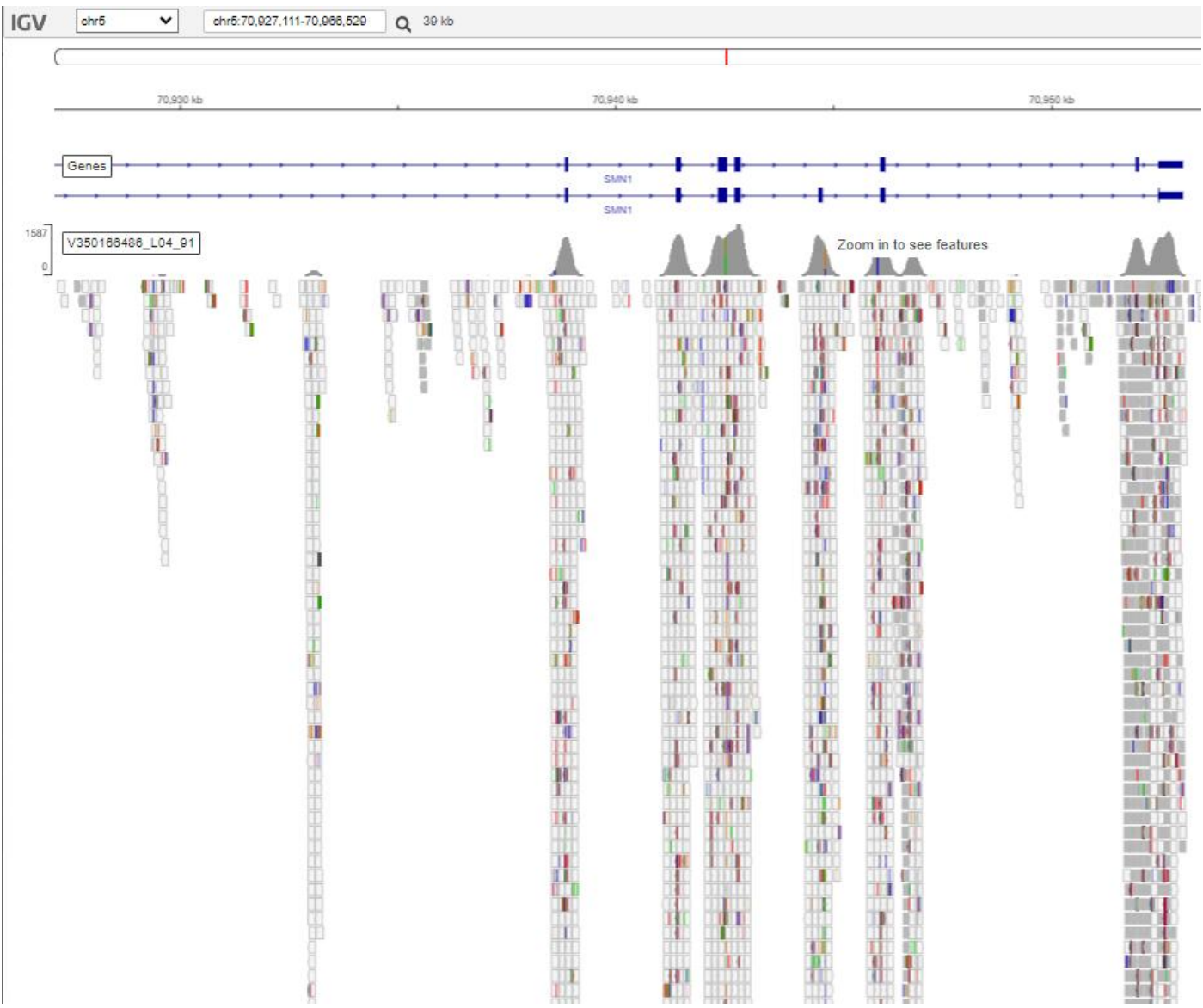
10%

Low 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

161590

Assay: MGI WES | Assignee: Unassigned | Status: Resolved

Analysis Results

1 Clinically relevant variants

Quality Control

All Samples Passed QC

▼ Variants

0 clinically relevant variants detected

▲ Causal Variants (1)

★ Marked as Pathogenic by clinvar

P

SMN1

Deletion | Exons 8 (out of 9 exons)

OCCURRENCE

N/A

INTERNAL

25

SENSITIVITY

HL: N/A

TS: N/A

CONFIDENCE

Failed

INHERITANCE

AR

9 Conditions

Chr 5:70247758-70247829 | 5q13.2 | 71 bp | Heterozygote

V350166486_L04_94

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

Analysis Results

5 Clinically relevant variants

Quality Control

3 Quality Control Warnings

▼ Variants

4 clinically relevant variants detected

▲ Causal Variants (1)

★ Spinal Muscular Atrophy

VUS

SMN1

Deletion | Exons 8 (out of 8 exons)

OCCURRENCE

69

INTERNAL

0

SENSITIVITY

HL: N/A

TS: N/A

CONFIDENCE

High

INHERITANCE

AR

9 Conditions

Chr 5:70951897-70952676 | 5q13.2 | 779 bp

[All Cases](#) > 156779 Carrier

156779 Carrier

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

Analysis Results

2 Clinically relevant variants

Quality Control

1 Quality Control Warnings

▼ Variants

1 clinically relevant variants detected

▲ Causal Variants (1)

★ Spinal Muscular Atrophy

VUS

SMN1

Deletion | Exons 8 (out of 9 exons)

OCCURRENCE

N/A

INTERNAL

2

SENSITIVITY

HL: N/A

TS: N/A

CONFIDENCE

Low

INHERITANCE

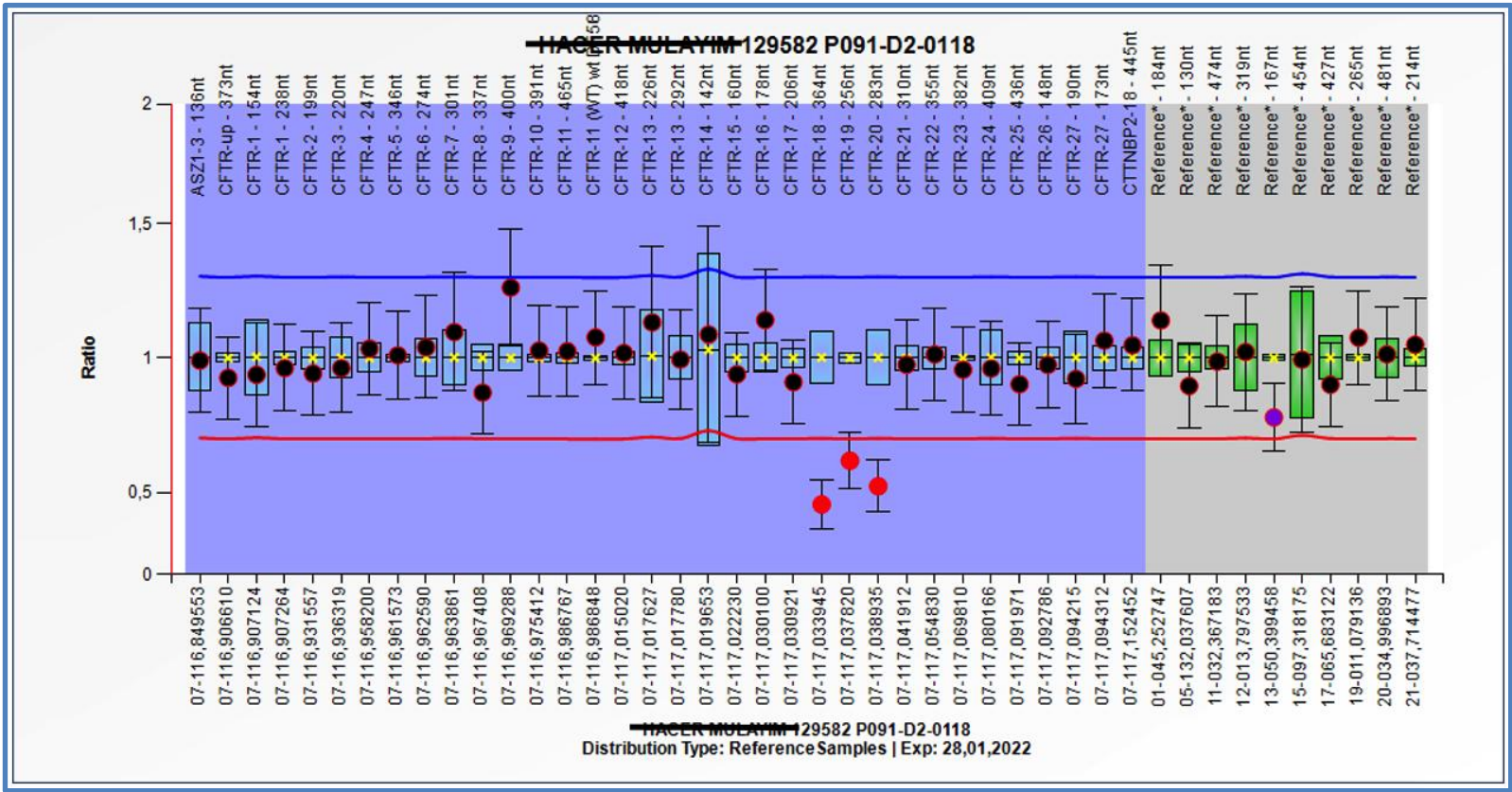
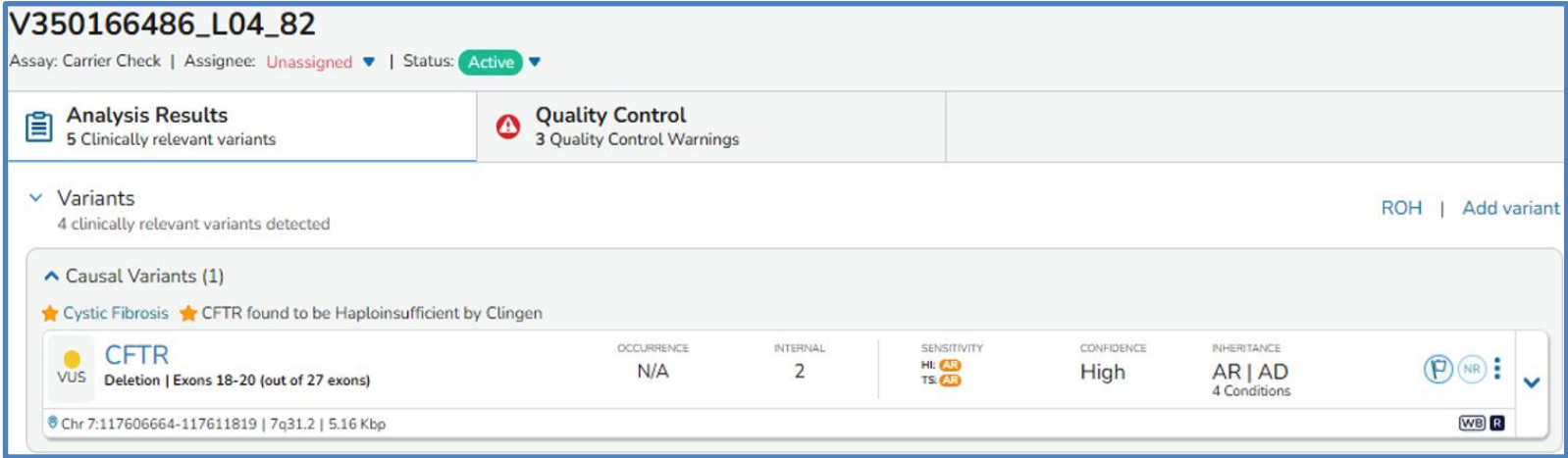
AR

9 Conditions

Chr 5:70951897-70952096 | 5q13.2 | 199 bp

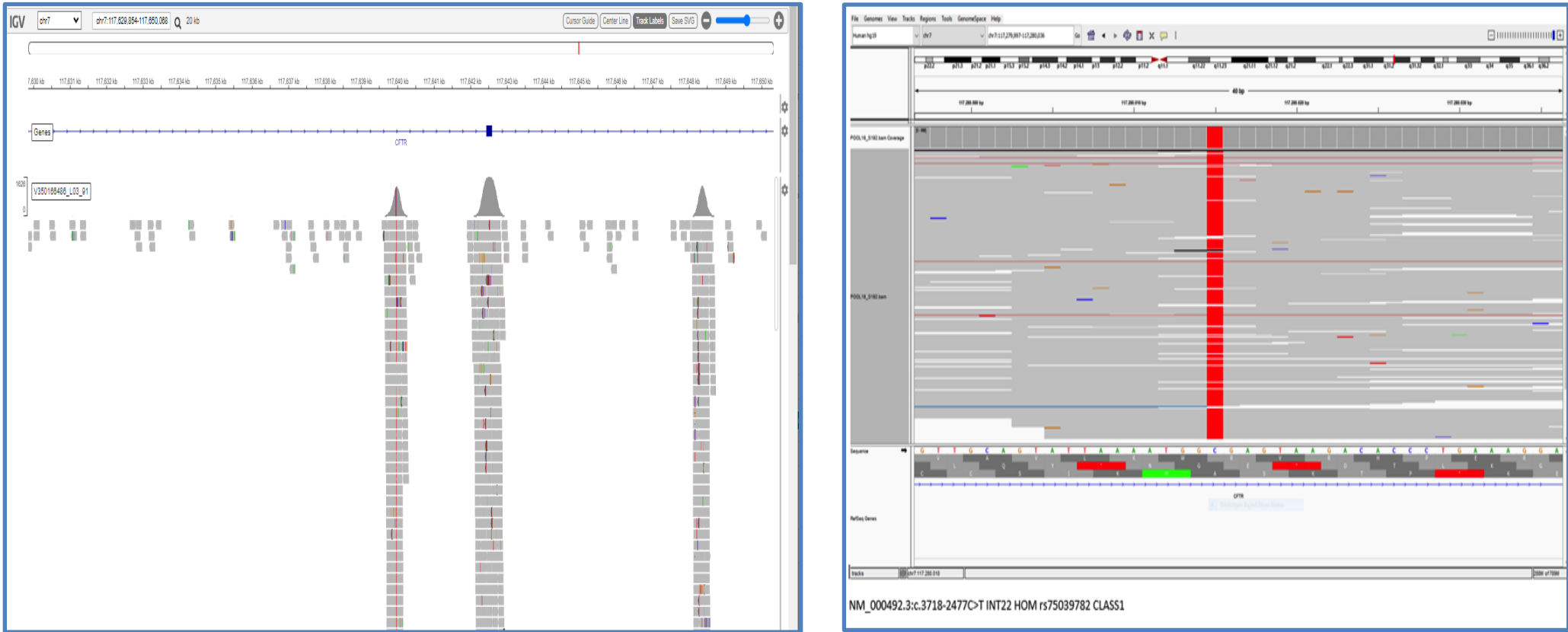
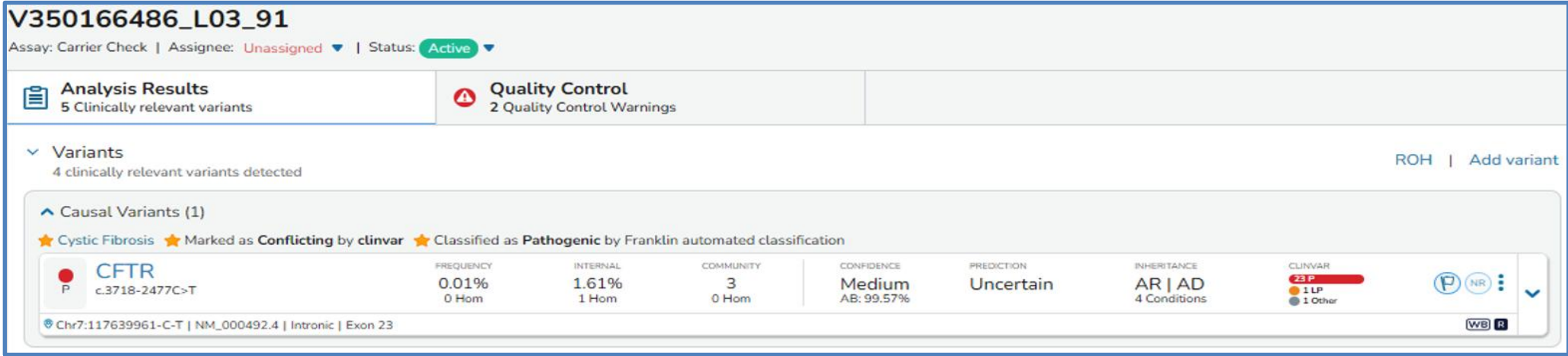
The CNV detection capability of the analysis software was improved by training on a large number of samples with SMN1 exon 7/8 deletions

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

V350166486_L03_89

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

Analysis Results
3 Clinically relevant variants

Quality Control
4 Quality Control Warnings

Variants
2 clinically relevant variants detected

Causal Variants (1)

★ DMD Related Muscular Dystrophies ★ DMD found to be Haploinsufficient by Clingen

	OCCURRENCE	INTERNAL	SENSITIVITY	CONFIDENCE	INHERITANCE
DMD LP Deletion Exons 8-9 (out of 79 exons)	N/A	2	HL: ⚠ TS: N/A	Medium	XLR XL 4 Conditions
Chr X:32697860-32699304 Xp21.1 1.44 Kbp					

Del: DMD

1.4kb | chrX:32.697.860-32.699.304 | Xp21.1 | Exonic | [UCSC](#)

[Franklin ACMG Classification](#) [Variant Assessment](#) [Genes and Regions](#) [Associated Conditions](#) [Publications](#) [My Organization Ass](#)

Clinical
Summary

Region
Viewer

Confidence

Occurrences

Internal
Occurrence

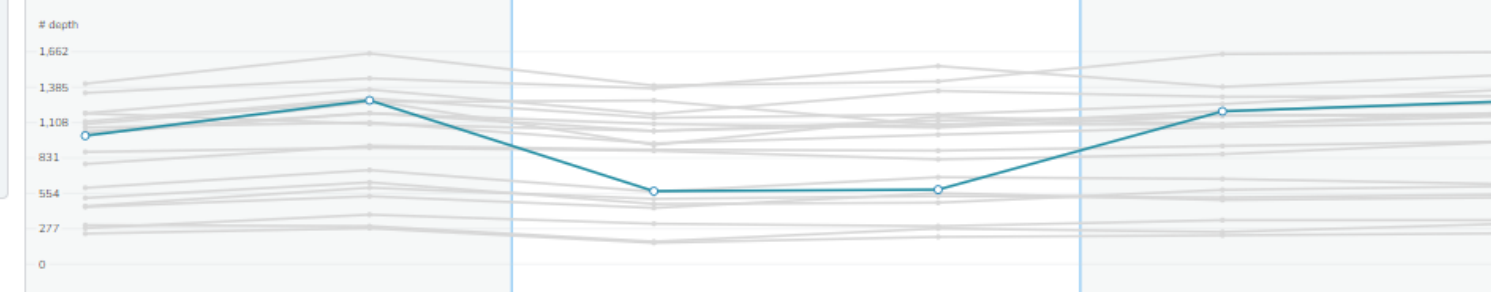
Sequence
Browser

References

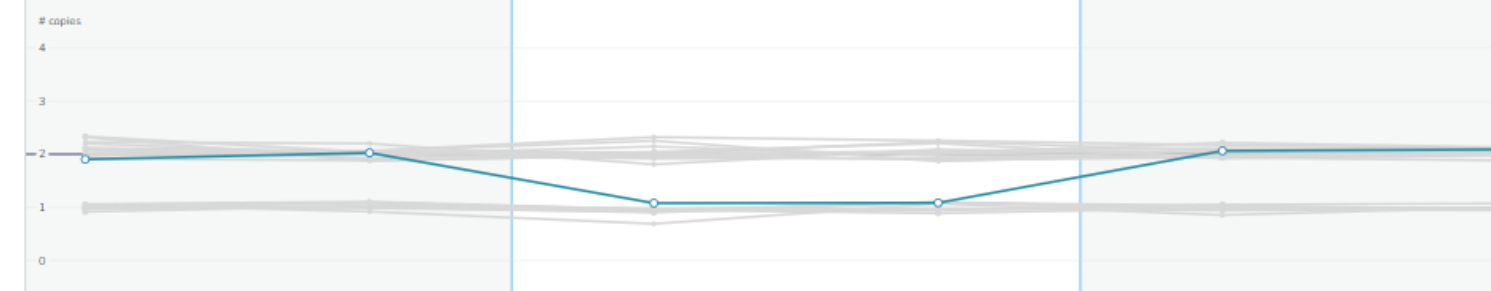
chrX:32644132-32816640

DMD

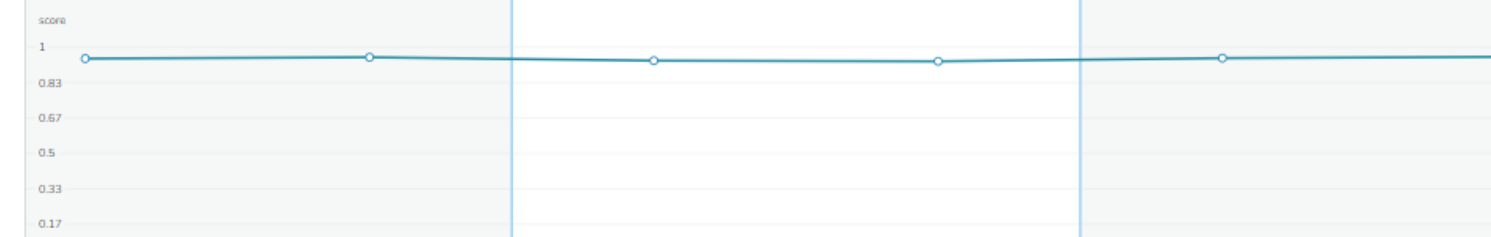
Depth (median 583)



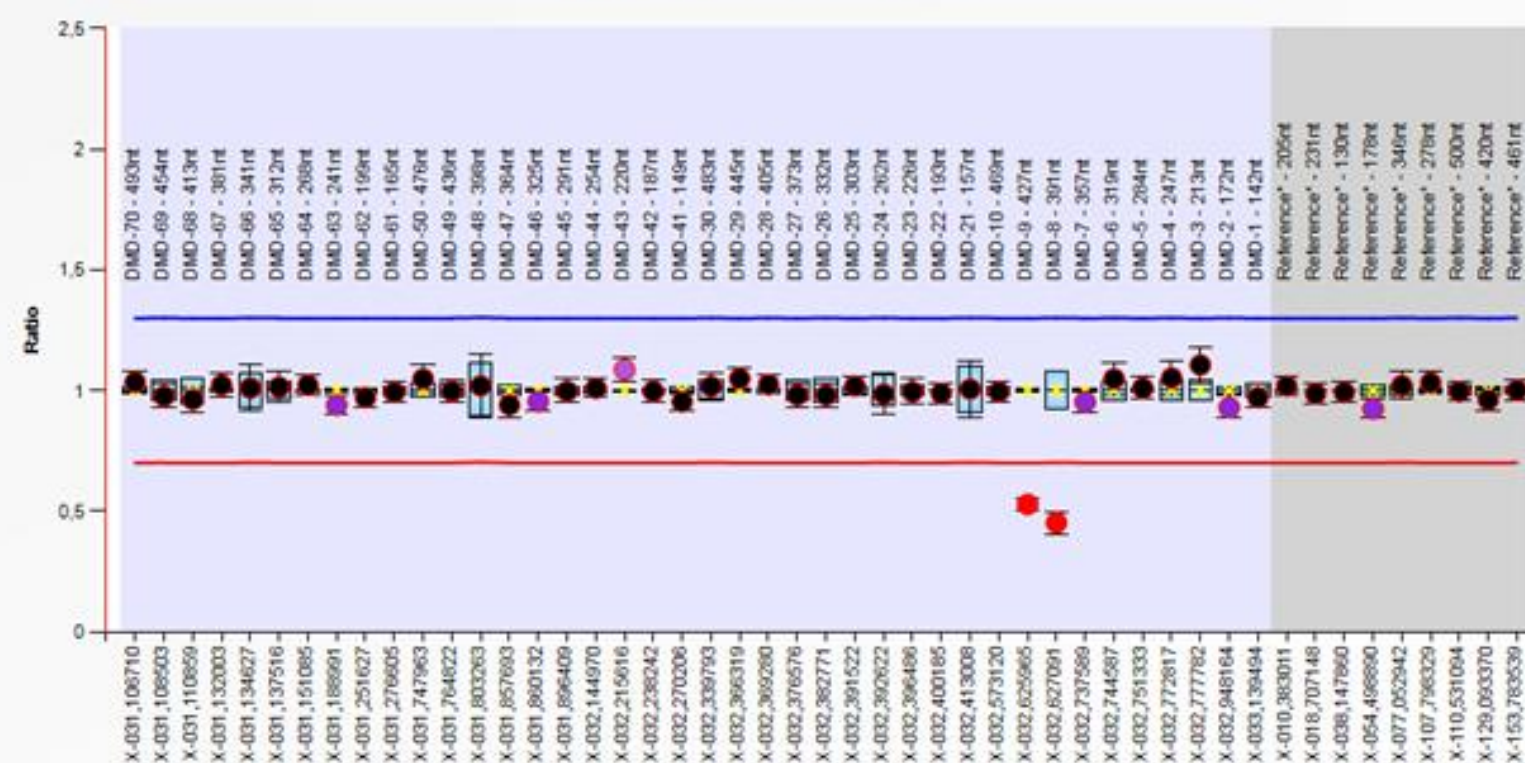
Predicted Copy Number (1.084)



Prediction Score (0.937)



CIGDEM KAYA 82387 P034 B2 0618



CIGDEM KAYA 82387 P034 B2 0618
Distribution Type: Reference Samples | Exp: 09.11.2019 DMD 34

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

VUS

HBA1 | HBA2

Deletion | Exonic

Occurrence

N/A

Internal

6

Sensitivity

HI: AR
TS: N/A

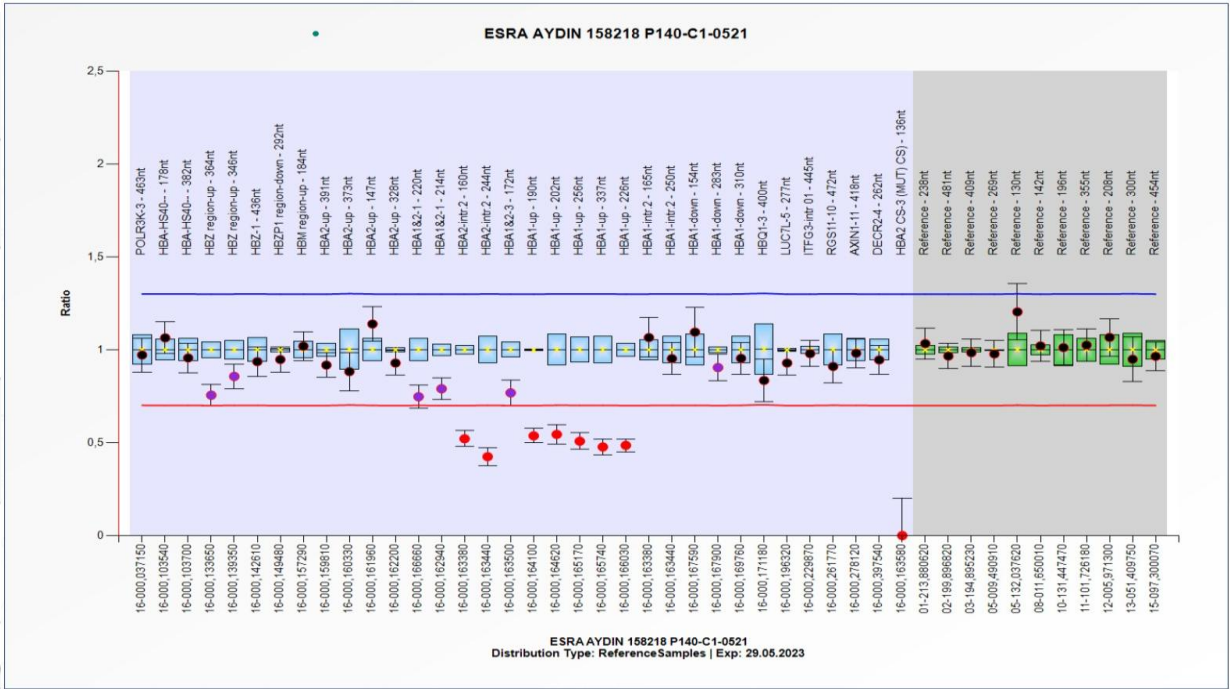
Confidence

Medium

Inheritance

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

VUS

HBA1 | HBA2 | HBQ1

Deletion | Exonic

Occurrence

499

Internal

2

Sensitivity

HI: AR
TS: N/A

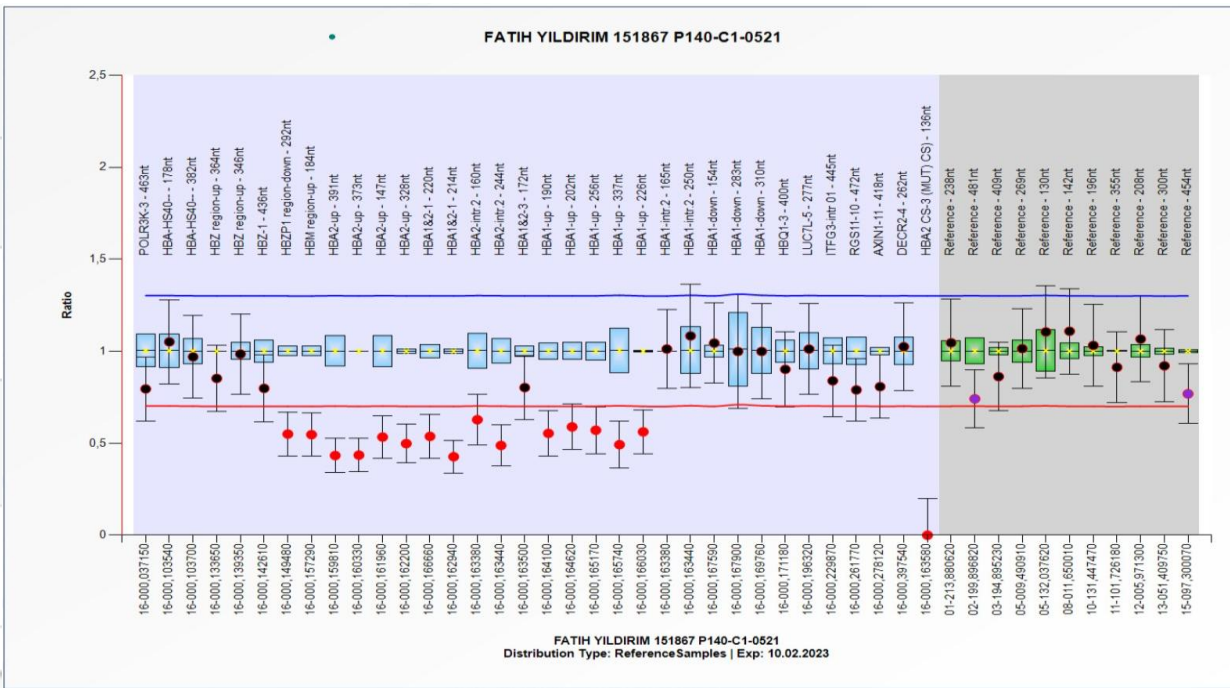
Confidence

High

Inheritance

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)

NATIONALITY	Number of Participants
Turkish	248
Syrian	128
Georgian	16
<i>TOTAL</i>	<i>392</i>

Design of Patient Groups for the Validation

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%
BTBD	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%

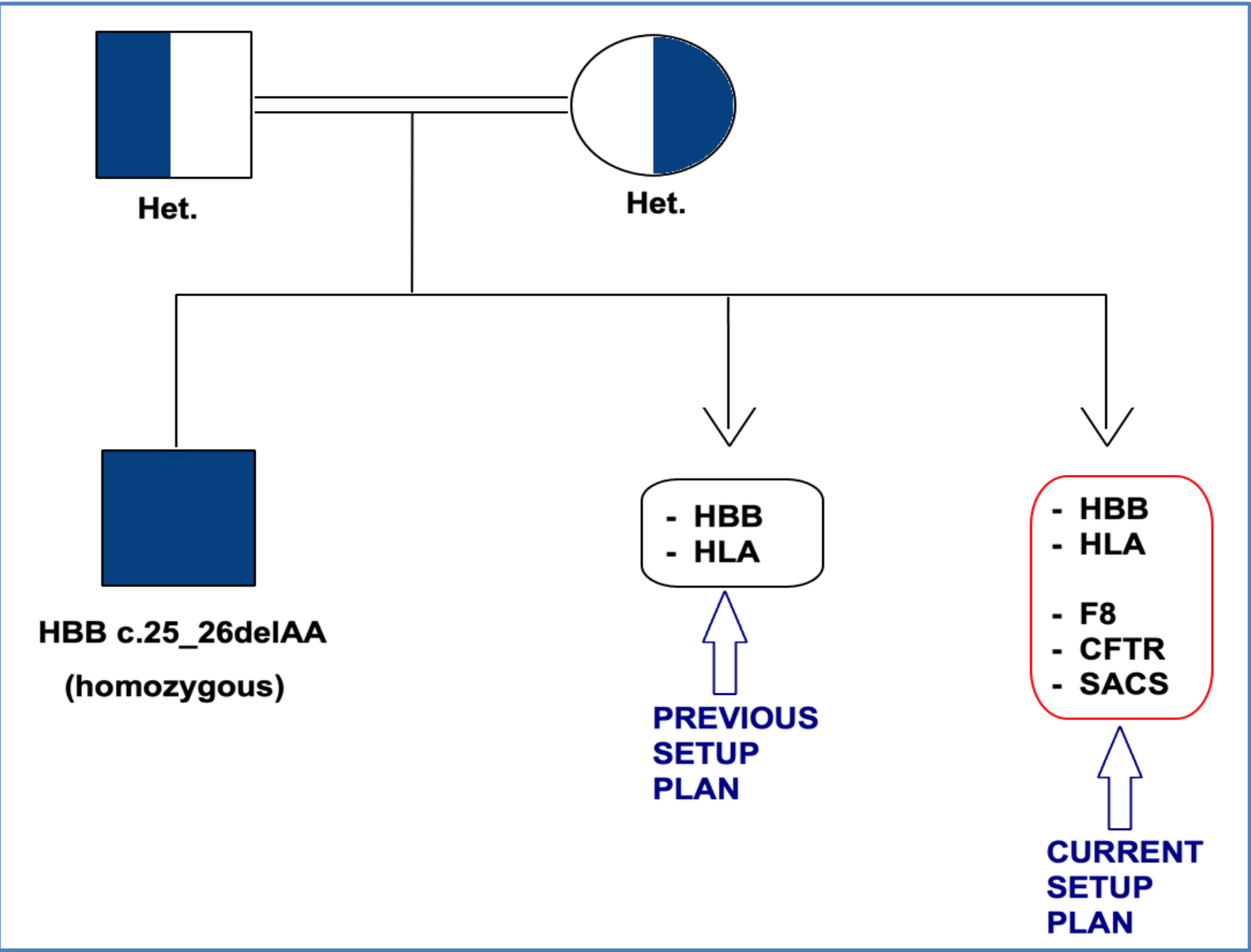
Regarding discordant genes «The rates were high, except the specificity rate of the CYP21A1 gene».

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



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 Referral			
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 9	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”

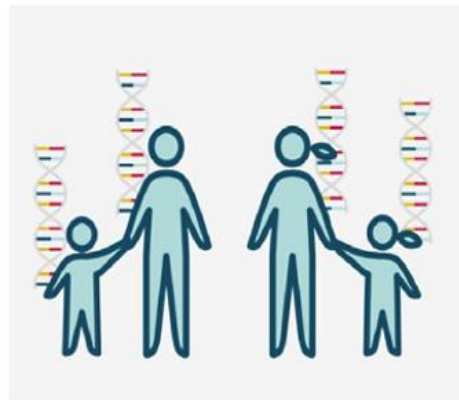
What is the CarrierCheck test?

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 detection 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
- ◆ Additional 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.



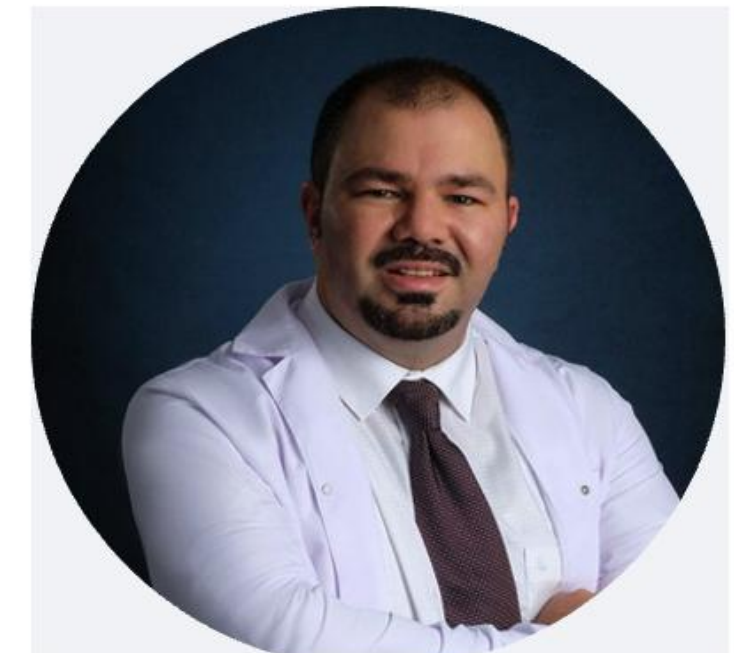
“CarrierCheck enables genetic matching of gamete donors to their recipients”

What is the CarrierCheck test?

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.



Prof Evrim Ünsal PhD.



Dr Süleyman Aktuna PhD

İdari Yönetim Amiri (CEO)
Prof. Dr. Volkan Baltacı, MD

Prof Evrim Unsal, PhD.

Süleyman Aktuna, PhD

Leyla Özer, MD, PhD.

Salih Çiçek, MD, PhD,

Nur Yeğenoğlu, MD

Kurumsal İletişim Direktörü
Orhan Doğan

Tufan Özdamar
Canan Ebru Tüter
Cem Sevindik
Gökhan Şentürk
Renata Argymbayeva
Belgin Tuğçe Bozkurt
Tuğba Işık
Gökberk İnanç Reyhan
K.Tolga Kurul
Barış Demirel
Önder Araz
Mizgin Esmer
Emre Can Öz
Dilara Kaya
Nimet Kaya
Melis Tannıver
M. Büşra Koçak
Gizem Özdemir
Esra Demirbaş
Ali Gücün
Cennet Değirmenci
Cansu Aslan
Berkay Çetinkaya
Hanım Demirel
Gülten Mutluer

Moleküler Genetik Laboratuvar
Yöneticisi

Doç. Dr. Evrim Ünsal
Dr. Öğr. Üyesi Süleyman Aktuna
Dr. Öğr. Üyesi Emre Evin
Uzm. Biyolog Didem Öğütveren
Uzm. Biyolog Golchin Memari
Uzm. Biyolog Cemile Engüzel
Ümmügülsüm Ercan
Uzm. Biyolog Erhan Gönen
Dr. Elza Balakışiyeva
Biyolog Ecem Mercan
Uzm. Biyolog Müge Bilgin
Biyolog Duysev Gülbay
Mol. Biyolog Özgenur Berber
Biyolog Zeynep Şahin
Uzm. Biyolog Arzu
Gizem Kınıcı Uzm.
Biyolog Esra Köksal
Uzm. Biyolog Işıl Küçükkalıpcı
Mol. Biyolog Zeynep Kula

Kalite Yöneticisi
Güler Eskin
Ali Serhan Uyar

Sitogenetik Laboratuvar Yöneticisi

Burak Kasal
Mediha Kasal
Biyolog Adem Öğütveren
Uzm. Biyolog Zeynep Öncü
Uzm. Biyolog Esra Erikel, MSc
Biyolog Dilek Uzun
Biyolog Emre Uslu
Biyolog Nisanur Ersöz
Biyolog Ali Berk Alican
Biyolog Serda Nikbay
Biyolog Ezgi Sarı

İstanbul Laboratuvarı

Uzm. Biyolog Gökçen Kolsal
Biyolog Ata Mert Adanır
Biyolog Mustafa Aksu
Pınar Erdoğan
Zeki Boyraz
Yasin Sert
Lütfiye Boncuk



+90 554 496 14 02



www.mikrogenlab.com



info@mikrogenlab.com



@mikrogenlaboratuvarlari



Mikrogen Genetik Hastalıklar
Tanı Merkezi



100. Yıl, Reşit Galip Cd. No:18, 06680
Çankaya/Ankara



Thank
you