QualiGene
EXCELLENCE IN GENETIC TESTING

2023 1st half PT Program Summary

Edited by Chen Gargi-Levi

Ms. Mor Vizel participated in data collection and statistical analysis

26.10.2023

ISRAC
Israel Laboratory Accreditation Authority
ISO/IEC 17043
No. 396

- QualiGene uses from time to time the sample validation services of Israeli Ministry of Health-registered clinical diagnostic laboratories.
- QualiGene is ISO 9001:2015 accredited and in addition, ISO 17043:2010 accredited for the following PT programs: PGT-M, MLPA, DMD, NGS, Y-chromosome microdeletion, QF-PCR and Sanger sequencing.

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Contents

Updates

- Two new proficiency testing programs for specific techniques are now available for registration: **WES-Trio data interpretation** and **Expanded carrier screening**. Please contact us for information and registration.
- We are pleased to introduce our new expert consultant for WES-Trio PT, Dr. Ginat Narkis. Dr. Narkis is an experienced bioinformatician and Genetic Counselor, currently holding positions both at Soroka Hospital, Israel, and at Pronto Diagnostics Ltd.

Disease-Specific PT Program - Summary

Program Design:

DNA samples were sent out to participating laboratories according to the diseases and variants tested by them. In most cases, two samples were provided for each gene/disease for diagnosis by the method routinely employed by each laboratory.

Reporting Requirements:

- A filled-in results form provided by QualiGene
- A final report provided to the referring clinician

Performance Criteria:

- Genotype identification
- The report provided to the referring clinician
- Nomenclature

Consultants:

- Dr. Ruth Shomrat
- Ms. Sigal Tsabari

Disease	Gene	Mutations
Mucolipidosis Type IV (ML4)	MCOLN1	c.-1015_789del
		c.1207C>T
Spinal Muscular Atrophy (SMA)	SMN1	Exons 7-8 del
Tay-Sachs Disease	HEXA	c.509G>A
		c.1274_1277dup
		c.571-2A>G
Joubert Syndrome Type II	TMEM216	c.218G>T
Bloom Syndrome	BLM	c.2207_2212delinsTAGATTC
Walker Warburg Syndrome	FKTN	c.1167dup
Infantile Cerebral Cerebellar Atrophy -ICCA	MED17	c.1112T>C
Familial Mediterranean Fever (FMF)	MEFV	c.1105C>T
Achondroplasia	FGFR3	c.1138G>A
Deafness, Autosomal Recessive 7	TMC1	c.1939T>C
		c.1810C>T
		c.1210T>C
Progressive Cerebello-Cerebral Atrophy Pontocerebellar Hypoplasia type 2E (PCCA2)	VPS53	c.1556+5G>A
		c.2084A>G

Breast and Ovarian Cancer	BRCA2	c.7007G>C
Colon cancer	MUTYH	c.1187G>A
Cockayne type B	ERCC6	c.1034_1035insT
Albinism	OCA2	c.79G>A
		c.1441G>A
		c.79G>A
		c. 1320G>C
		c. 2373_2375del
Albinism	TYR	c.1A>G
		c.1118C>A
MELAS-Mitochondrial disease	MT-TL1	m.3243A>G

Participation:

Twenty-one laboratories participated in the disease-specific PT scheme, two of them participated in the expanded carrier screening program.

Scheme Summary

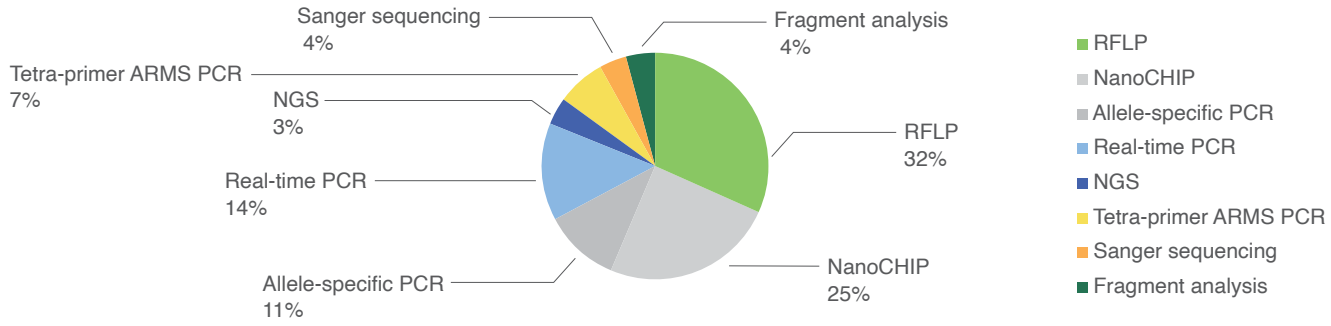
Disease	Number of Participating Laboratories	Testing Methods*
Mucolipidosis Type IV (ML4)	15	RFLP, NanoCHIP, Allele-specific PCR, Real-time PCR, NGS, Tetra-primer ARMS PCR, Sanger sequencing
Spinal Muscular Atrophy (SMA)	14	Real-time PCR, NGS, MLPA, AmplideX® PCR
Tay-Sachs Disease	18	RFLP, NanoCHIP, Allele-specific PCR, Real-time PCR, NGS, Sanger sequencing, Fragment analysis
Joubert Syndrome Type II	9	RFLP, NanoCHIP, Allele-specific PCR, Real-time PCR, NGS, Sanger sequencing
Bloom Syndrome	15	RFLP, NanoCHIP, Allele-specific PCR, Real-time PCR, NGS, Sanger sequencing, Fragment analysis
Walker Warburg Syndrome	8	Allele-specific PCR, Real-time PCR, NGS, Sanger sequencing, Fragment analysis
Infantile Cerebral Cerebellar Atrophy (ICCA)	3	RFLP, Allele-specific PCR, Real-time PCR, NGS
Familial Mediterranean Fever (FMF)	9	RFLP, NanoCHIP, Real-time PCR, Sanger sequencing
Achondroplasia	7	RFLP, Real-time PCR, Sanger sequencing
Deafness, Autosomal Recessive 7	11	RFLP, Allele-specific PCR, Real-time PCR, NGS, Sanger sequencing
Progressive Cerebello-Cerebral Atrophy Pontocerebellar hypoplasia type 2E	14	RFLP, Allele-specific PCR, Real-time PCR, NGS, Sanger sequencing
Breast and Ovarian Cancer	2	Real-Time PCR, NanoCHIP, NGS, Sanger sequencing
Colon cancer	3	NanoCHIP, NGS, Real-time PCR

* Most laboratories use more than one method.

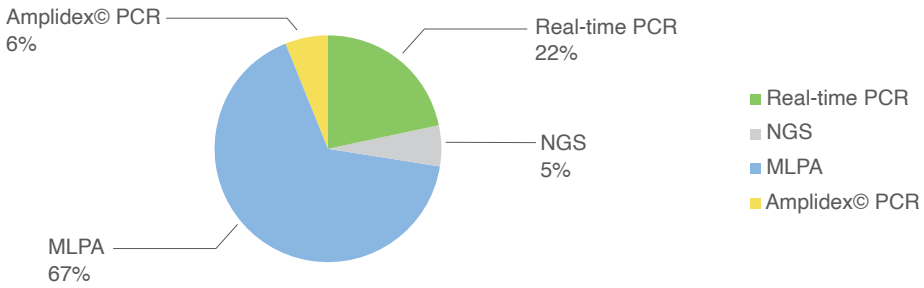
**Diseases tested by more than one laboratory.

Diagnostic technique used for each tested disease:

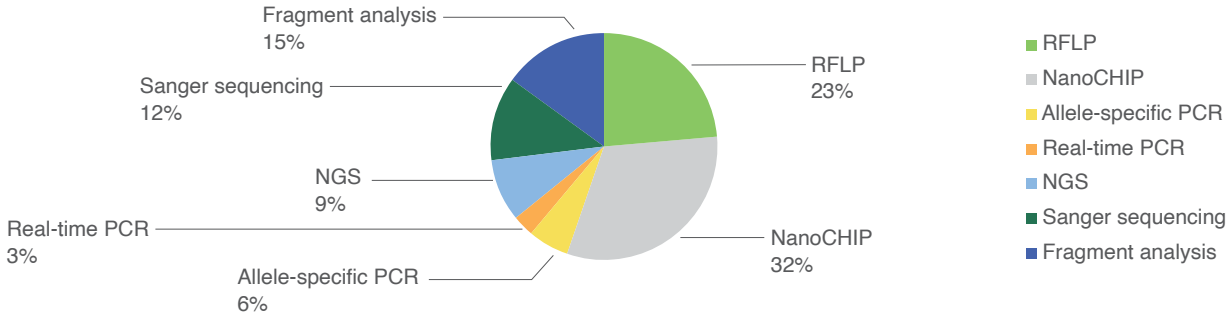
Mucolipidosis Type IV (ML4):



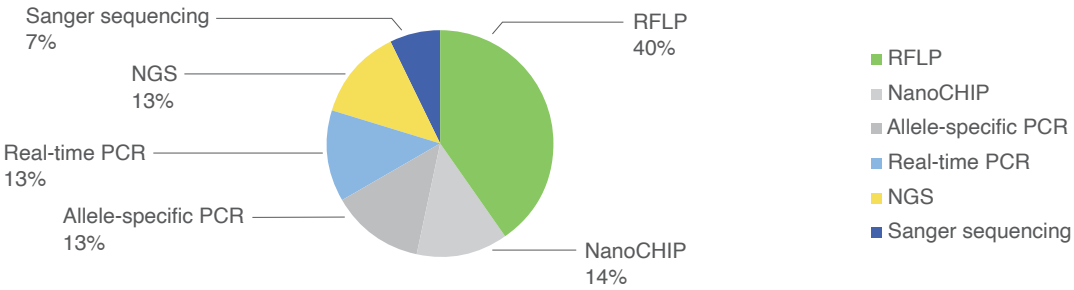
Spinal Muscular Atrophy (SMA):



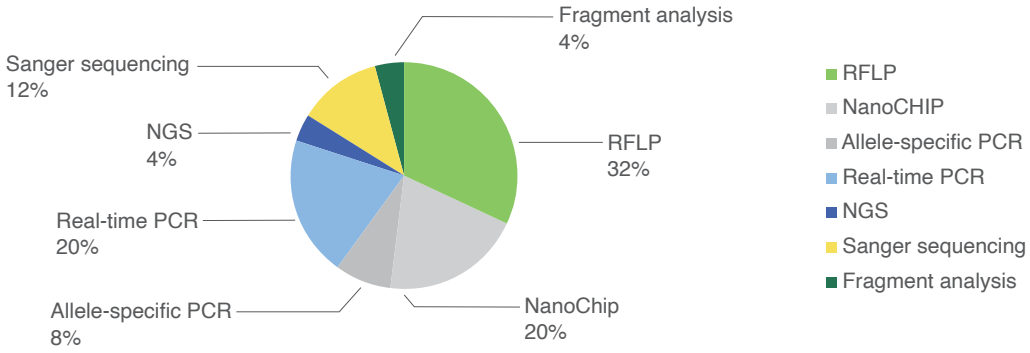
Tay-Sachs Disease:



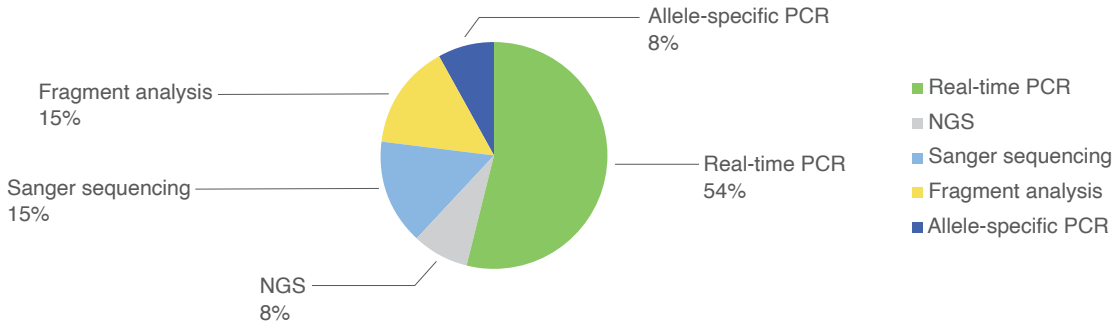
Joubert Syndrome Type II:



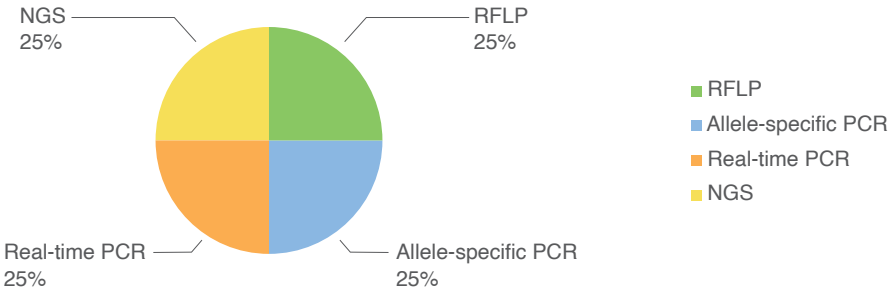
Bloom Syndrome:



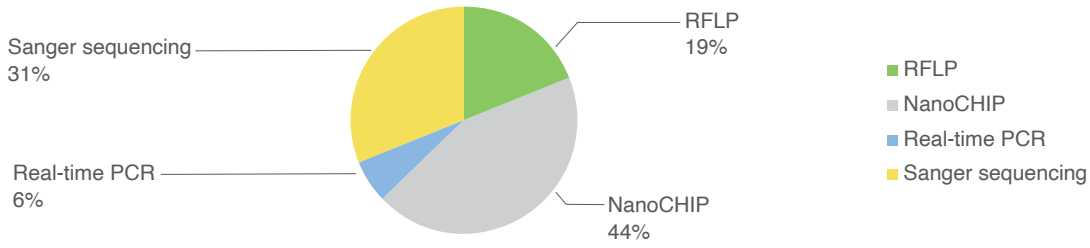
Walker Warburg Syndrome:



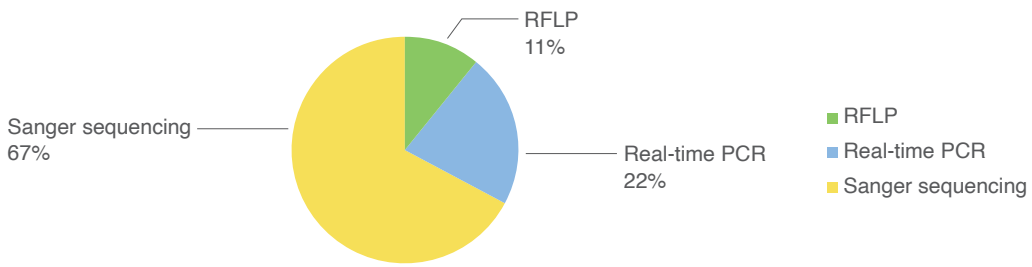
Infantile Cerebral Cerebellar Atrophy (ICCA):



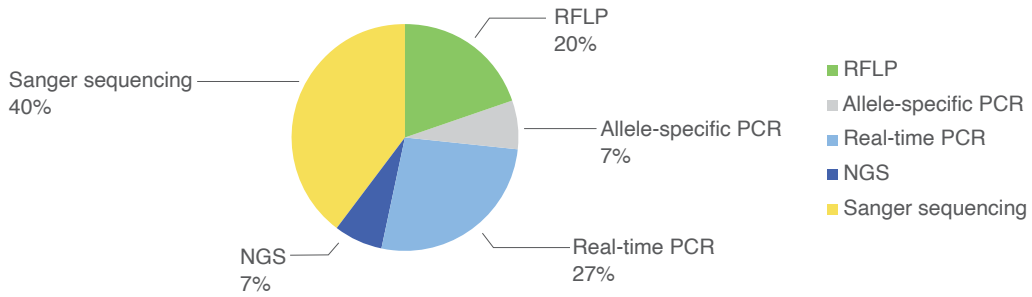
Familial Mediterranean Fever (FMF):



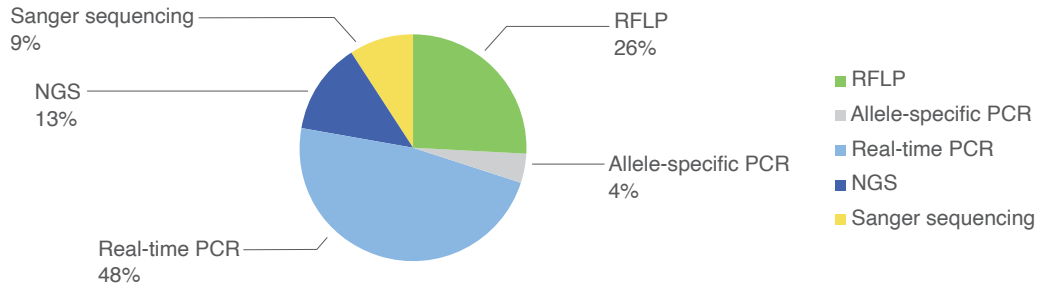
Achondroplasia:



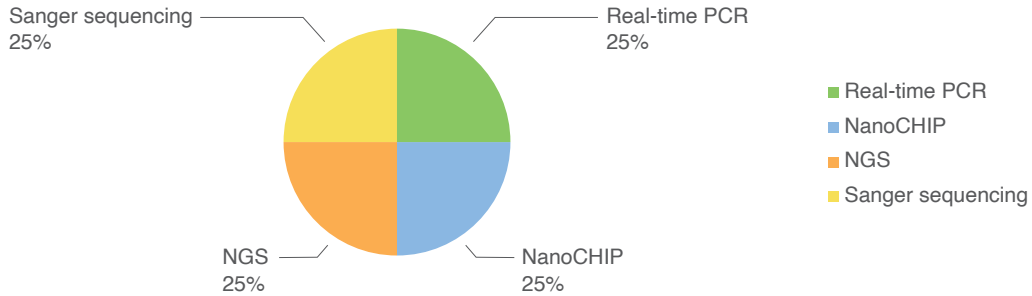
Deafness, Autosomal Recessive 7:



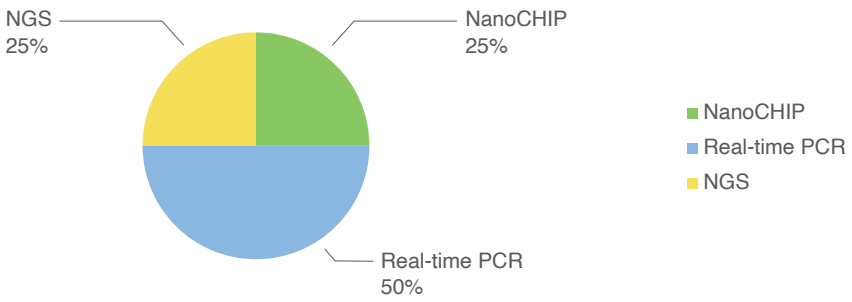
Progressive Cerebello-Cerebral Atrophy Pontocerebellar Hypoplasia type 2E:



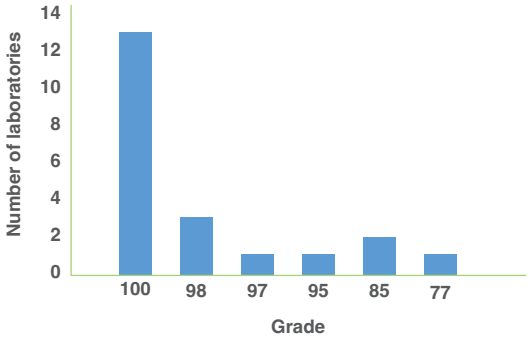
Breast and Ovarian Cancer:



Colon Cancer:



Score Summary



Grade

Number of samples sent	258
Number of diseases tested	24
Genotyping errors	4
Samples mix-ups	0
Errors in nomenclature	3
Deficiencies in the final results report	2

Sanger Sequencing PT Program - Summary

Program Design:

The participating laboratories were provided with two PCR products and sequencing primers for a specific exon in the tested gene. The PCR products were treated and sequenced according to each laboratory's protocol, using the Sanger method.

Reporting Requirements:

- A filled-in results form supplied by QualiGene
- The raw data files for sequencing quality evaluation

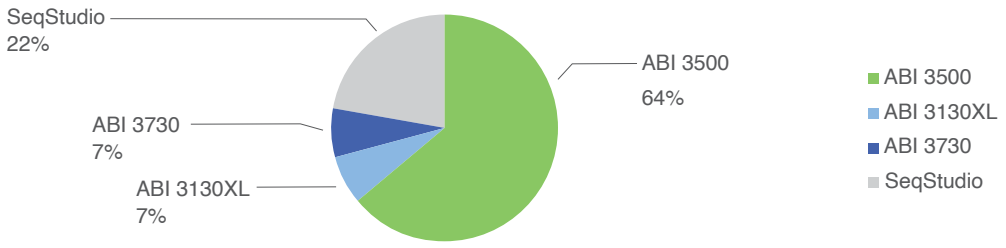
Performance Criteria:

- Genotype identification
- Sequencing quality
- Nomenclature

Consultants:

- Dr. Ruth Shomrat
- Ms. Sigal Tsabari
- Dr. Sarit Levin

CE instruments used by the participating laboratories:



Tested Gene: PKHD1 (NM_138694.3)

Exon number: 32

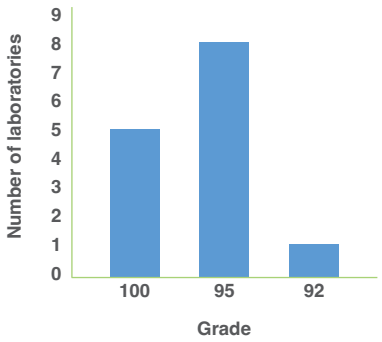
Tested variant: c.3761_3762delinsG

Variant classification summary:

All participance classified the main sequence variant as pathogenic.

Score Summary:

Number of Participating Laboratories: 14



Number of samples sent	28
Number of diseases tested	1
Genotyping errors (main variant)	0
Genotyping errors (additional variants)	9
Samples mix-ups	0
Errors in nomenclature	1
Low quality sequences	0

Prenatal QF-PCR Program – Summary

Program Design:

The participating laboratories were challenged with one DNA sample, carrying a chromosomal aberration, detectable by QF-PCR. Each laboratory was required to test the DNA sample according to its routine protocol and kit.

Reporting Requirements:

- A filled-in results form provided by QualiGene

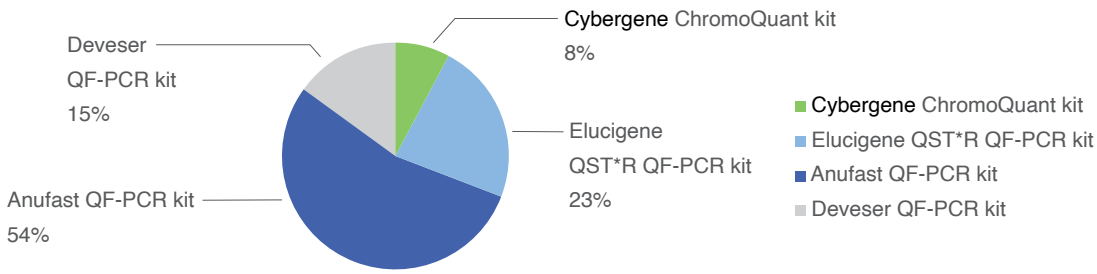
Consultants:

- Dr. Ruth Shomrat
- Ms. Sigal Tsabari

Performance Criteria:

- Correct identification of chromosomal aberration

Laboratory Practice:

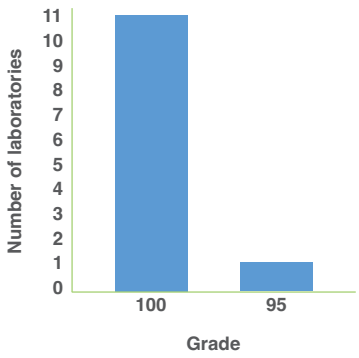


Tested Syndrome:

Down syndrome

Score Summary:

Number of Participating Laboratories: 12



Number of samples sent	12
Number of tested syndromes	1
Genotyping errors	0
Other mistakes	1

MLPA PT Program - Summary

Program Design:

The participating laboratories received one DNA sample, harboring one or more specific variants detectable by MLPA, and an appropriate probemix. The laboratories were required to test the DNA sample according to MLPA SALSA kit protocol, while including in their reaction the test sample duplicate, three controls and a No-DNA sample.

Reporting Requirements:

- A filled-in results form provided by QualiGene
- The FSA files for MLPA reaction quality evaluation

Performance Criteria:

- Genotype identification
- Quality of MLPA reaction
- Electrophoresis parameters

Consultants:

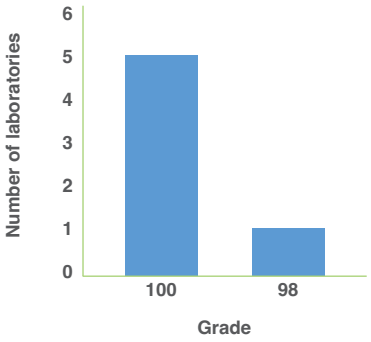
- Ms. Sigal Tsabari

Tested Gene:

CHM (one laboratory tested the MECP2 gene)

Score Summary:

Number of Participating Laboratories: 6



Number of samples sent	6
Diseases tested by each laboratory	1
Deletion / duplication identification errors	0
MLPA reaction quality deficiencies	1
MLPA reaction setup deficiencies	0
Electrophoresis parameters deficiencies	0

CMA PT Program - Summary

Program Design:

The participating laboratories were challenged with one DNA sample, potentially harboring chromosomal gains or losses detectable by CMA, together with a short case report. The laboratories reported all pathogenic abnormalities greater than 200 Kb for losses and 500 Kb for gains using the International System for Human Cytogenetic Nomenclature (ISCN).

Reporting Requirements:

- A filled-in results form provided by QualiGene
- CEL or CYCHP files (optional)

Performance Criteria:

- Correct identification of the gain/loss
- Nomenclature according to the ISCN

Laboratory Practice:

All participating laboratories used the CytoScan® 750K array

Consultants:

- Dr. Reut Matar

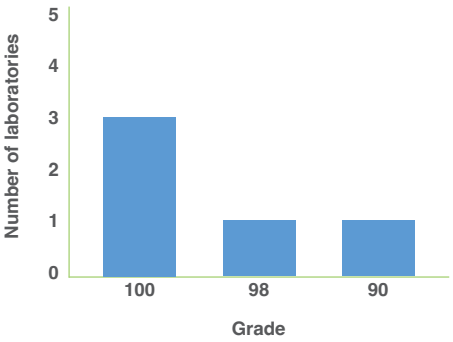
Tested Syndrome:

Potocki-Shaffer Syndrome (partial deletion).

Additional Findings: No additional findings reported.

Score Summary:

Number of Participating Laboratories: 4



Number of samples sent	5
Number of tested syndromes	1
Gain/loss identification deficiencies	0
Nomenclature errors	1
Misidentification of the syndrome	1

DMD PT Program – Summary

Program Design:

Each of the participating laboratories received two DNA samples, potentially harboring one or more deletion or duplication variants in the DMD gene. They were asked to test those DNA samples according to protocols regularly used for this test, including three appropriate controls and a No-DNA sample.

Reporting Requirements:

- A filled-in results form provided by QualiGene
- The FSA files for MLPA reaction quality evaluation
- A final report provided to the referring clinician

Performance Criteria:

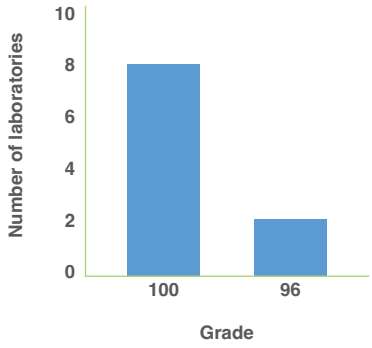
- Genotype identification
- Quality of MLPA reaction
- Electrophoresis parameters
- Final report sent to the referring clinician

Consultants:

- Ms. Sigal Tsabari

Score Summary:

Number of Participating Laboratories: 10



Number of samples sent	20
Deletion \ duplication identification errors	0
MLPA reaction quality deficiencies	2
MLPA reaction set-up deficiencies	0
Electrophoresis parameters deficiencies	1
Deficiencies in the final results report	0

NGS of Single Genes PT - Summary

Program Design:

The participating laboratories were challenged with a single DNA sample, harboring a variant, detectable by NGS (all participating laboratories report which genes they test, and the samples are provided accordingly). Each laboratory was required to detect the mutation, using its own NGS kits, protocols and equipment.

Reporting Requirements:

- A filled-in results form provided by QualiGene
- FASTQ, BAM, VCF and BED files uploaded to a specified server

Performance Criteria:

- Correct identification of the variant/s
- Coverage of the target region
- Sequencing quality
- Nomenclature

Laboratory Practice:

	Laboratory 1	Laboratory 2
Sequencer type	MiSeq	NextSeq500
Library construction kit	Nextera Flex for enrichment - Illumina Cancer Panel	Agilent SureSelect
Reagent kit	V3 reagent kit - 600 cycles	NextSeq 500 Mid Output 300 cycles
Analysis software	Variantyx	Custom In-house pipeline using BWA-MEM aligner, Strelka2 variant caller (Illumina), and SNPPET variant caller (Agilent Technologies Genomics)

Consultants:

- Dr. Gil Stelzer

Tested Genes: DICER1

Tested Mutation: NM_ 177438.3: c.2117-1G>A

Score Summary:

Number of Participating Laboratories: 2

All participating laboratories achieved the maximum grade (100)

Number of samples sent	2
Number of tested diseases	1

Common Mistakes and Challenges in the 1-2023 PTs

- Sanger sequencing PT: all participants should closely observe all sequence variants, especially when a frameshift variant is present, as it can make identification more challenging.