UMC Utrecht

NGS panels in clinical diagnostics: Utrecht experience

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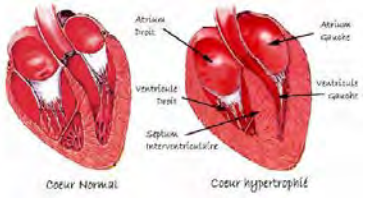
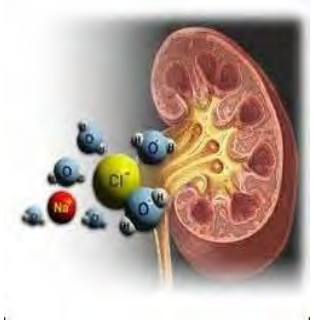
93 Gene panels UMC Utrecht

- Cardiovascular disease (CAR) (5 panels)
- Epilepsy (EPI) (11 panels)
- Hereditary tumors (ONC)(3 panels)
- Metabolic diseases (MET) (6 panels)
- Neuromuscular diseases (NEM) (21 panels)
- Neurological diseases (NEU) (4 panels)
- Renal Disease (NEF)(19 panels)
- Primary immunodeficiencies (PID) (8 panels)
- Erythrocyte membrane disorders
- Erythroderma
- Obesity, syndromal and non-syndromal
- Fraser syndrome
- Amelogenesis imperfecta
- Trombo(cyto)penia





Number of requests per NGS panel

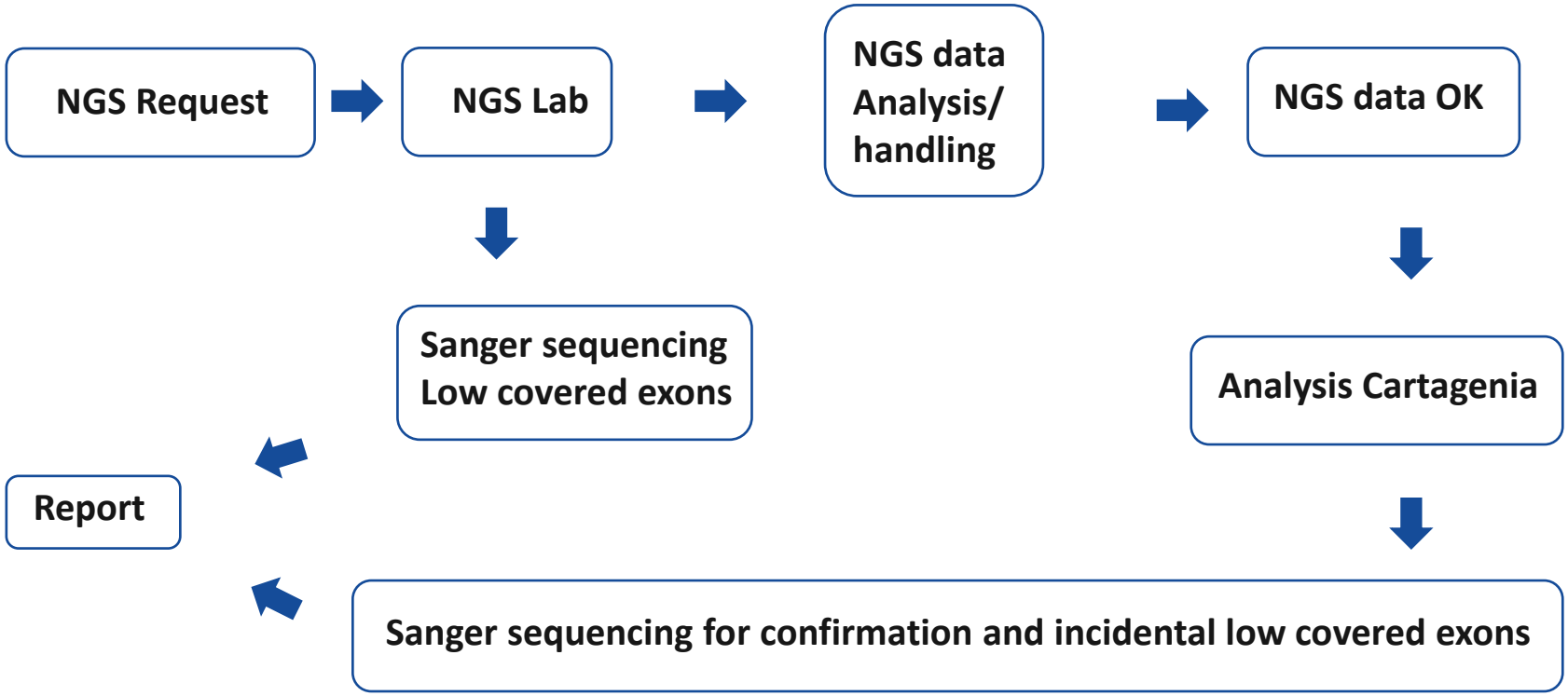
Panel	CAR N=5	EPI N=11	NEF N=19	NEM N=21	OBE N=1	ONC N=3	PID N=8	WES	Other	Total
Monthly	45	75	18	12	62	41	18	90	17	375
Yearly	540	900	216	144	744	492	216	1080	204	4482

Diagnostic yield: 5%- 80%

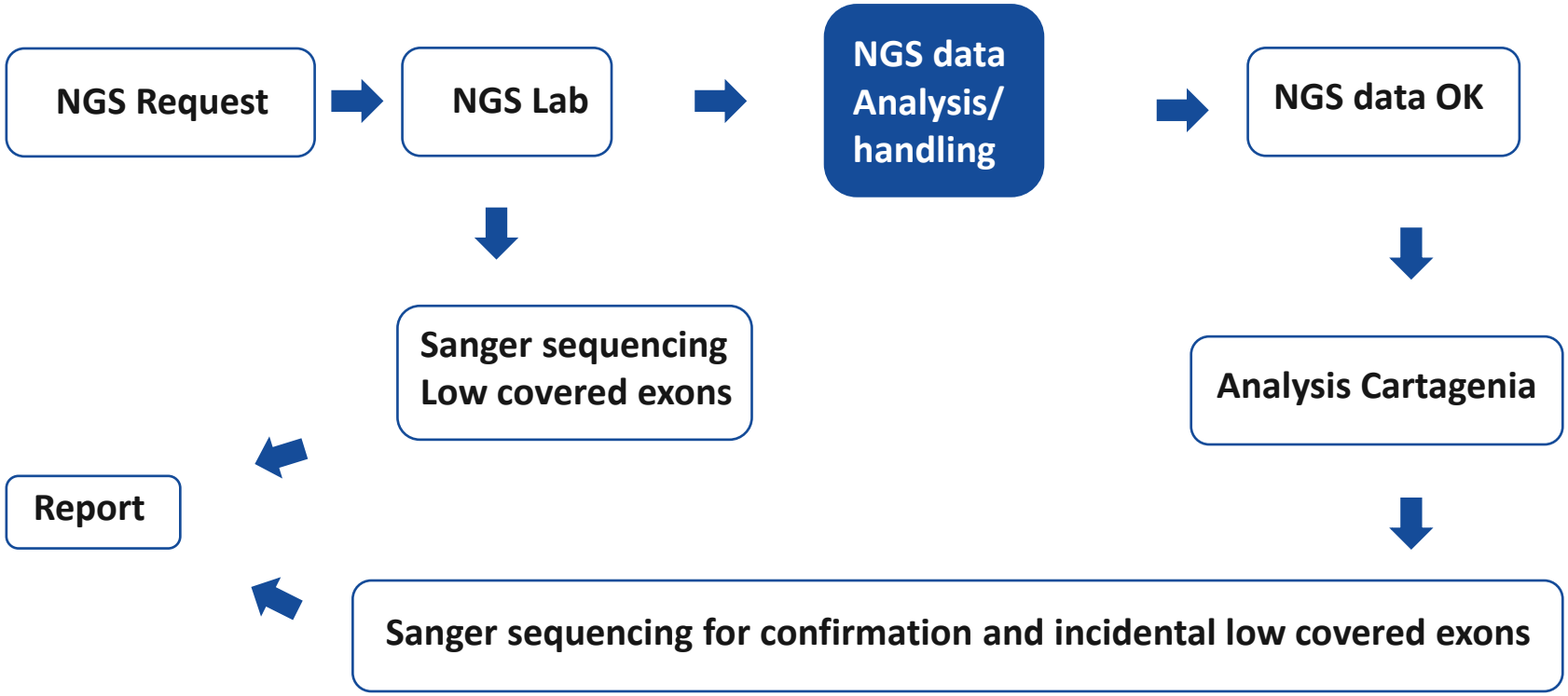
Challenges NGS gene panels

- Lab logistics
  - Targeted sequencing (SureSelect)
  - 100 to 750 genes per design
  - Many samples
  - Flexibility necessary because of many different panels
- Information on coverage/quality
- Variant prioritization/interpretation

NGS workflow



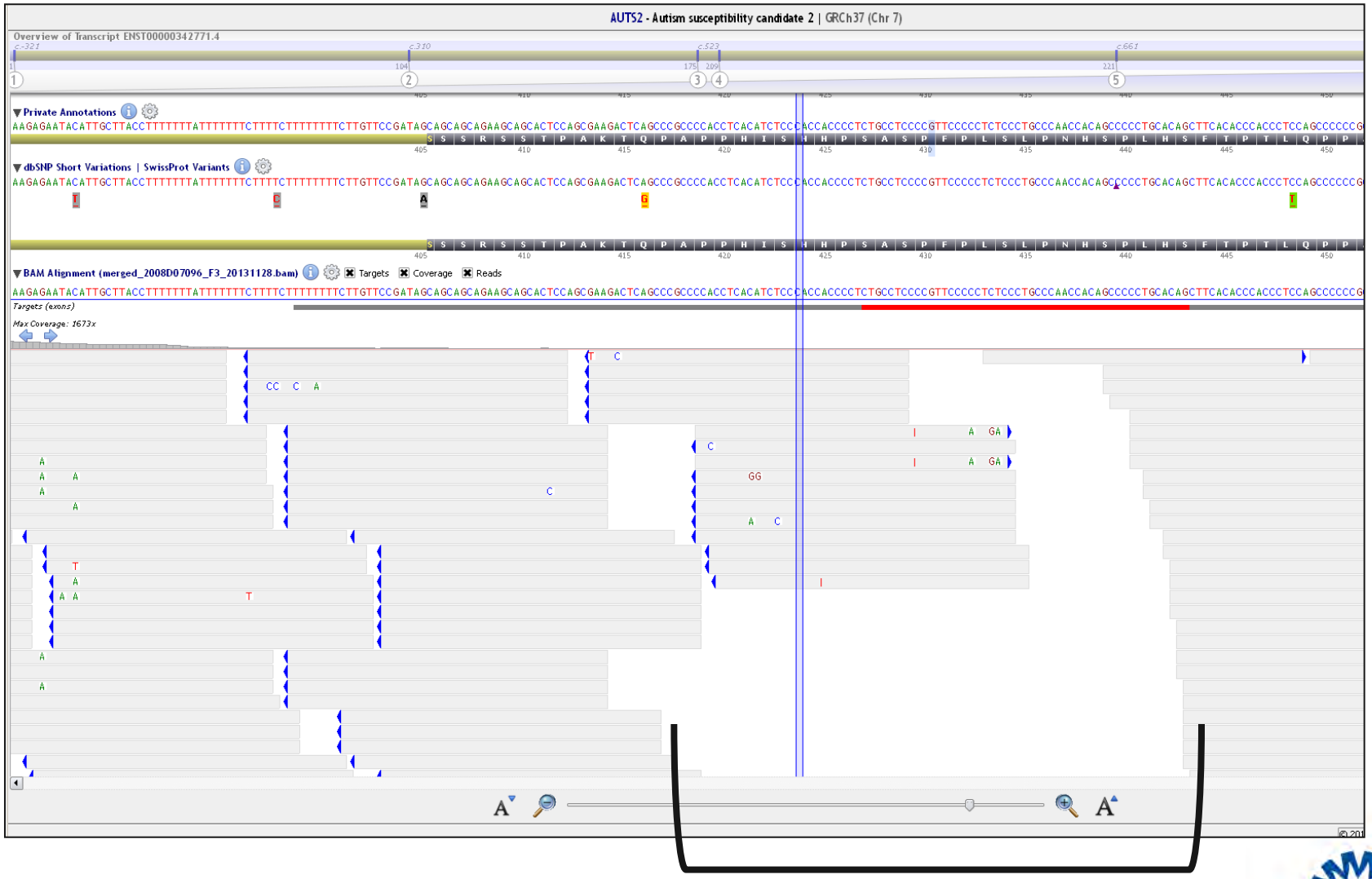
NGS workflow



NGS data analysis/handling

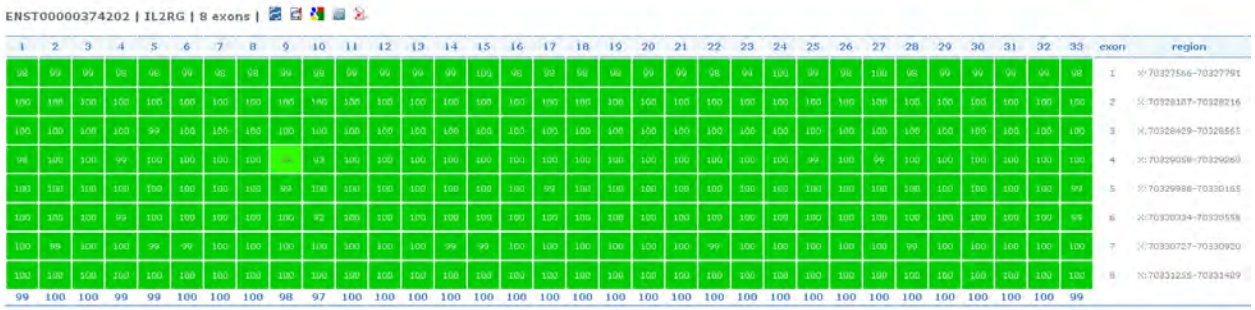
- In-house variant calling pipeline for mapping and variant calling
- Each run includes a control sample for quality control
- SNP concordance >97%
- Coverage complete design median 100x

Coverage



Low/no coverage

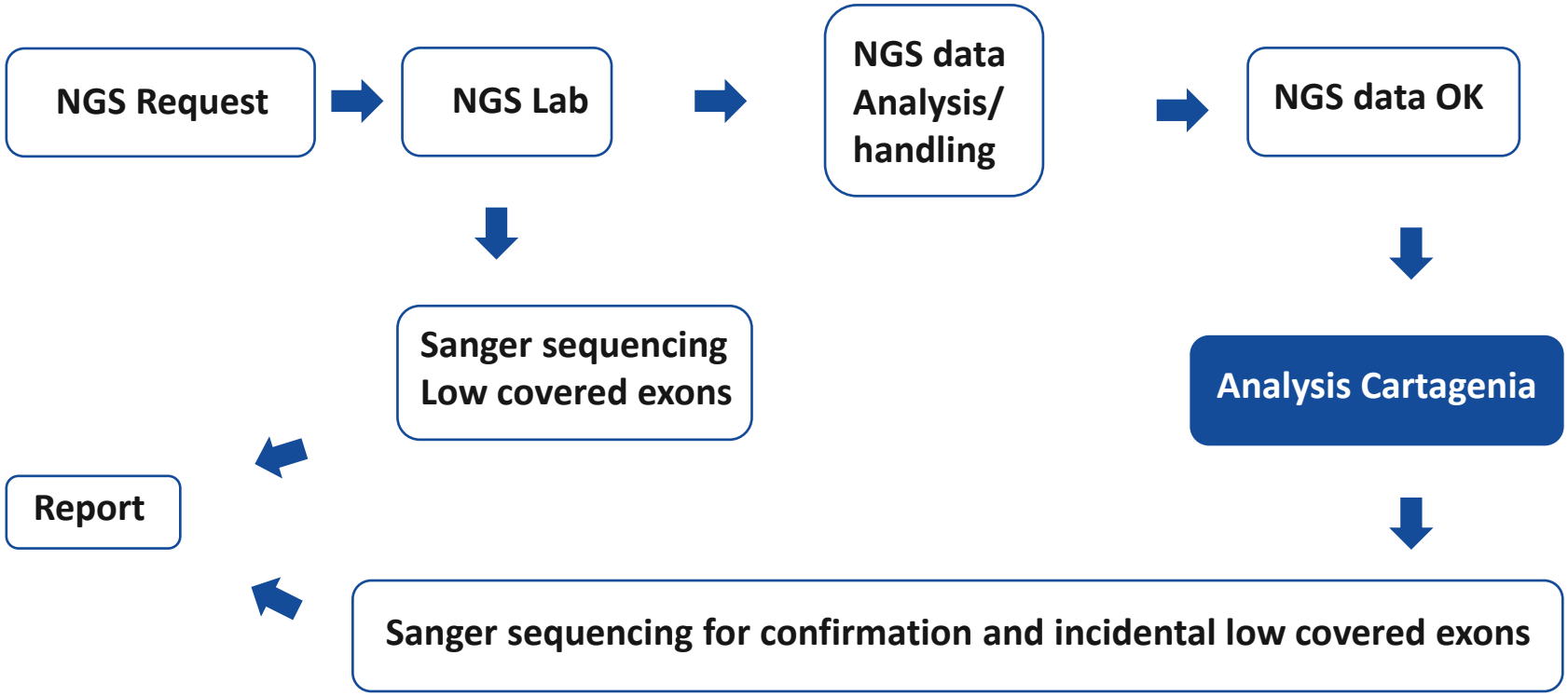
Coverage tool: Coverage per gene/exon



ENST00000360027 | SH2D1A | 4 exons |

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	exon	region
81	92	98	85	85	90	98	96	57	64	100	100	97	95	99	93	97	98	97	99	100	99	98	1	X:123480473-123480649
99	100	100	100	99	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	2	X:123499591-123499694
93	98	95	98	98	99	99	98	98	89	99	98	99	97	98	99	95	99	98	99	99	99	98	3	X:123504006-123504181
0	0	0	0	0	0	41	52	0	0	0	28	6	0	0	59	0	2	1	35	54	1	0	4	X:123505181-123505261
76	82	83	79	79	81	90	91	70	70	85	89	85	82	84	91	82	84	83	90	93	84	84		

NGS workflow



Managed Variant list (MVL)

CARTAGENIA Bench Lab NGS

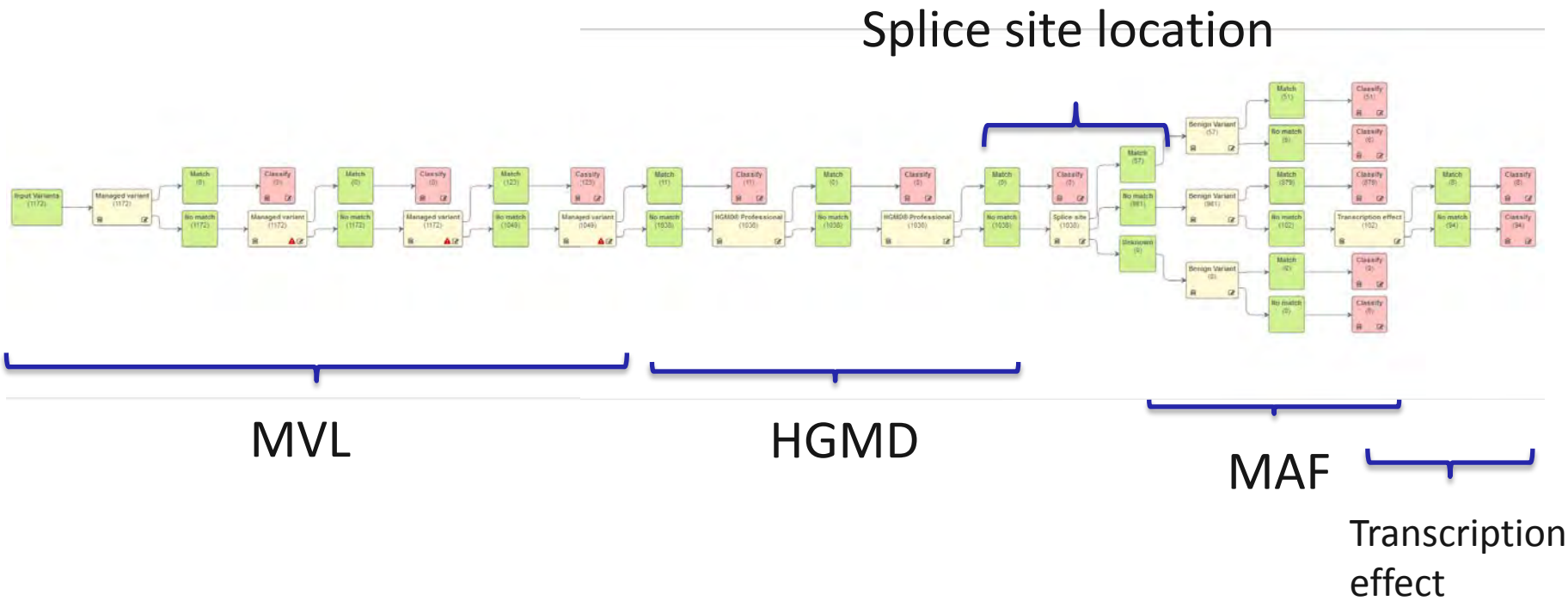
Upload Files | Patients | Analyses | Gene Profiles | MVL | Administration | Search | Help

Managed Variant Lists

Managed Variant Lists Search

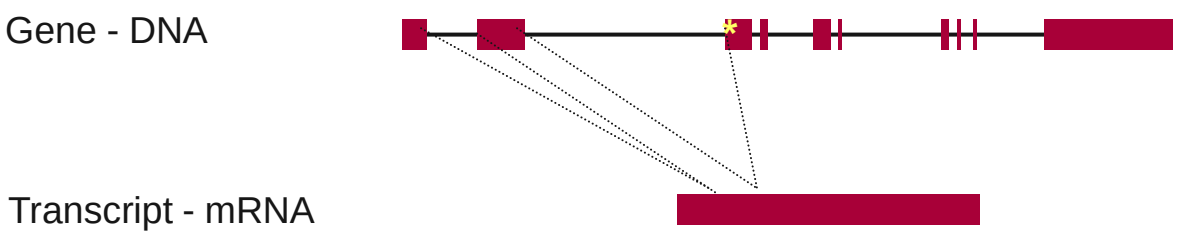
Name	Description	Last updated by	# Variants
DataSharing - Agreement		Cartagenia	707
DataSharing - Discordance		Cartagenia	660
MVL - Artefact	MVL is aangemaakt om de vals positieven varianten uit de analyses te filteren	MKempen	314
MVL - Artefact_Illumina	MVL voor Illumina artefacten	marielle	14
MVL - Benign		MKempen	3908
MVL - de novo	MVL voor centrale opslag van de novo varianten en gekoppelde kliniek	MKempen	61
MVL - Likely benign		MKempen	1776
MVL - Likely pathogenic		MKempen	124
MVL - Pathogenic		marielle	789
MVL - VOUS		MKempen	1093

Filter/Classification tree



Interpretation of DNA variants

- Nonsense mutation
- Splicing mutation



- Insertion, deletion, duplication, inversion

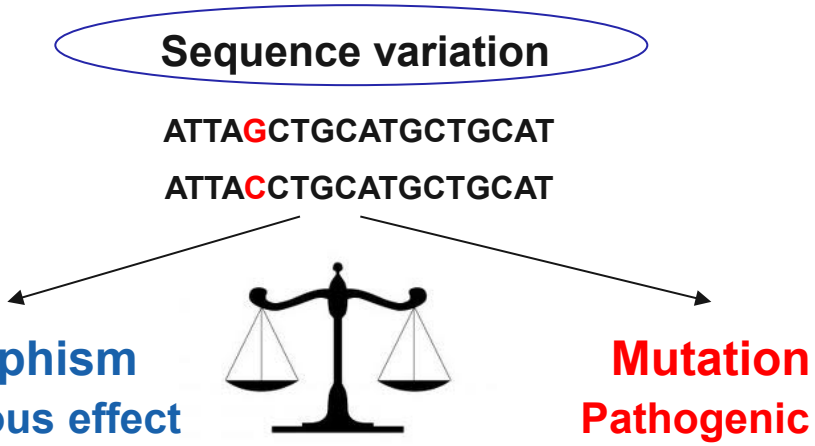
Missense mutations = replacement of 1 AA by another AA

known mutation

confirmed diagnosis

unknown mutation ?

Interpretation of missense variants



Segregation with phenotype	no	yes
Found in the general population	yes	no
Modifies the protein structure	no or mildly	yes
Alters the protein function	no	Yes
Affects conserved residues	rarely	frequently

Rare missense mutations in sporadic cases are difficult to interprete

Primary Immunodeficiency panels

Panel	Requests	(mono) Genetic Diagnosis	Genetic variants reported but no definitive diagnosis
IUIS+	122	19	59
Autoinflammatory	179	22	40
HLH	12	2	4
ALPS	9	2	0
SCID	6	0	1
Antibody def	4	0	0
HIES	14	0	2
CMC	3	2	0
total	349	47 (14%)	106 (30%)

Problem: VUS

- Problem for clinicians
- Increased workload created by the interpretation of NGS generated DNA variants
- Public databases don’t contain all knowledge (yet)
- How to reduce amount of unclassified variants

Frequency?

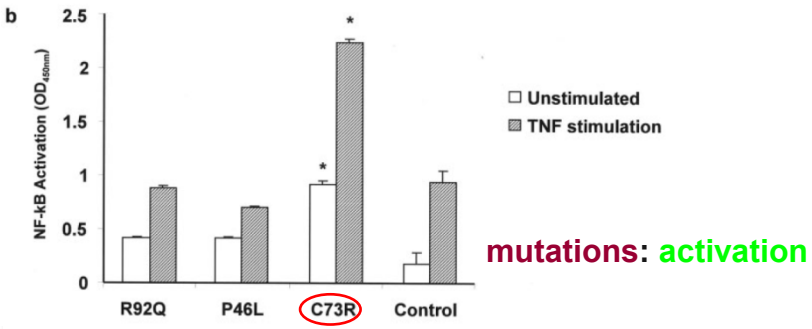
- Filtering tree: Benign >5% in control population N>200

- Benign
  - MAF>0.5% dominant (control population N>400)
  - MAF>1% recessive inheritance (control population N>400)
  - Except for pathogenic founder mutations (literature, databases).

- Founder mutations in recent described disease associated genes?

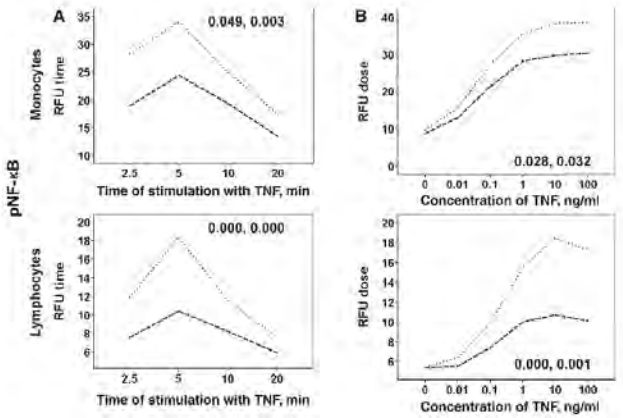
Functional assays?

TNFR1 - NF-κB - PBMC



mutations: activation

Nedjai et al, *Arthritis Rheum*, 2008



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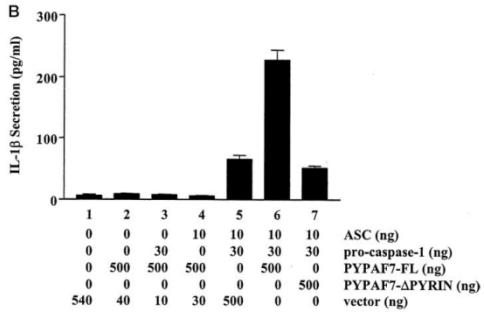
– – – – patients
(C73R, n=2;
C88Y, n=4;
F112I, n=4)

mutations: inhibition

Stjernberg-Salmela et al, *Rheumatology*, 2010

NLRP12 – IL-1β – several systems

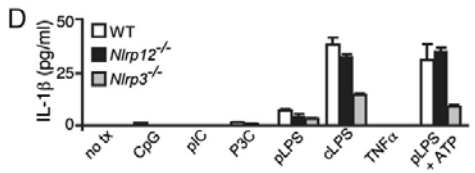
transfected COS-7 cells



WT: activation

Wang et al, *J Biol Chem*, 2002

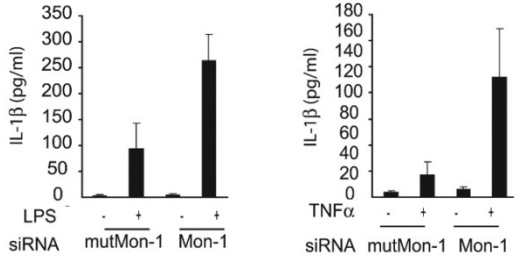
dendritic cells
Nlrp12-/- mice



WT: no effect

Arthur et al, *J Immunol*, 2010

siRNA THP-1 cells



WT: inhibition

Williams et al, *J Biol Chem*, 2005

Functional assays?

- Can be difficult to set up
- Caution in interpretation is required
- In most cases not applicable on a routine basis

DATA-sharing?

- Diagnostic labs have a lot of data
- Share the knowledge about DNA variants
  - Reliable frequency data for Dutch population
  - Share the knowledge/interpretation of detected DNA variants
- Share what with whom?
- LowLands Consortium CNV-Database
  - 8 Dutch Genome Diagnostic Laboratories

Why share variant data?

- Decrease workload created by the interpretation of NGS generated DNA variants
- Reduce number of VUS
- Patient receives less discordant classification for variants in different laboratories

Conclusion

- Targeted panel analysis is replacing Sanger Sequencing single genes
- Logistics and sequencing quality is under control
- Interpretation remains a challange
  - Equilibrium between workload and good clinical practice