Joni Van der Meulen - MDG Ghent University Hospital

molecular diagnostics in cancer using next-generation sequencing technologies

Capita Selecta in Bioinformatics
March 9 2020

UZ GENT
UNIVERSITEIT GENT

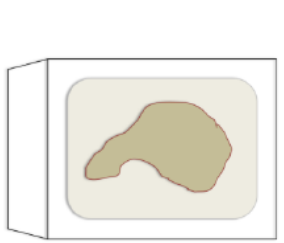
platform molecular diagnostics UZ Gent (MDG)

center for medical genetics (CMGG)

clinical biology

pathology

targeted next-generation sequencing (NGS) to define diagnosis, prognosis and prediction of therapy response



from request till report to the clinici variant interpretation/classification

detection of somatic variants with NGS

prediction of therapy response to molecular drugs

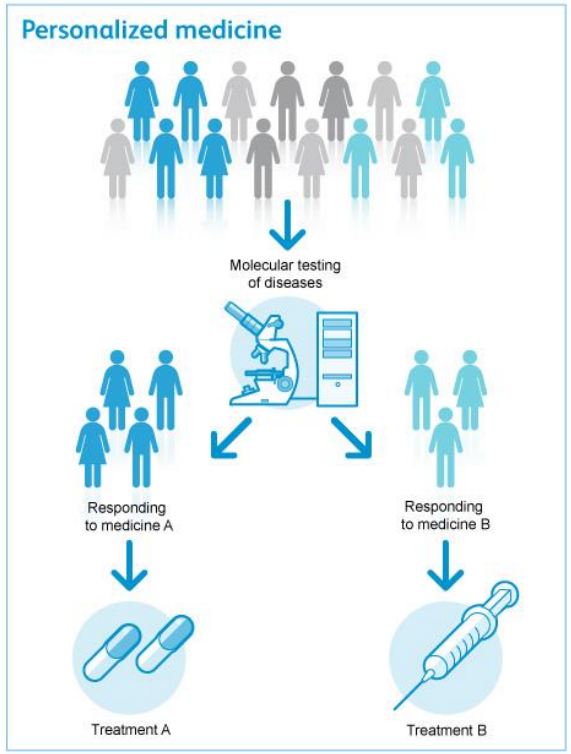
examples. ovarian cancer *BRCA1-BRCA2* variants, ER+ breast cancer *PIK3CA* variants

diagnosis

examples. pancreatic cyst: *GNAS, KRAS, RNF43, VHL, CTNNB1* variants, granulosa tumor: *FOXL2*

prognosis

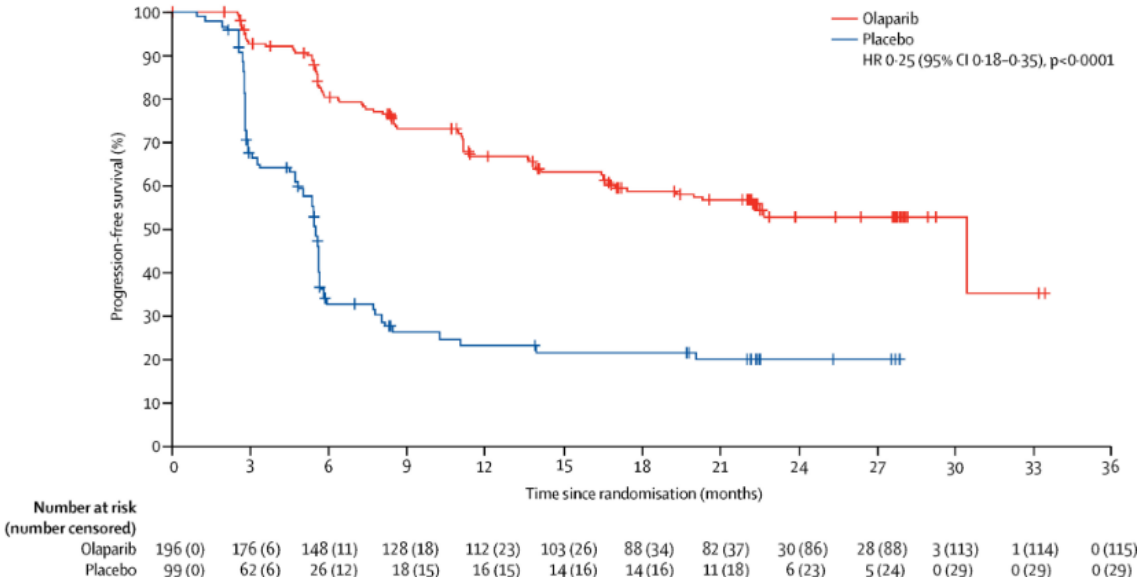
examples. endometrial tumor: *POLE* variants, glioma: *TERT* promoter variants

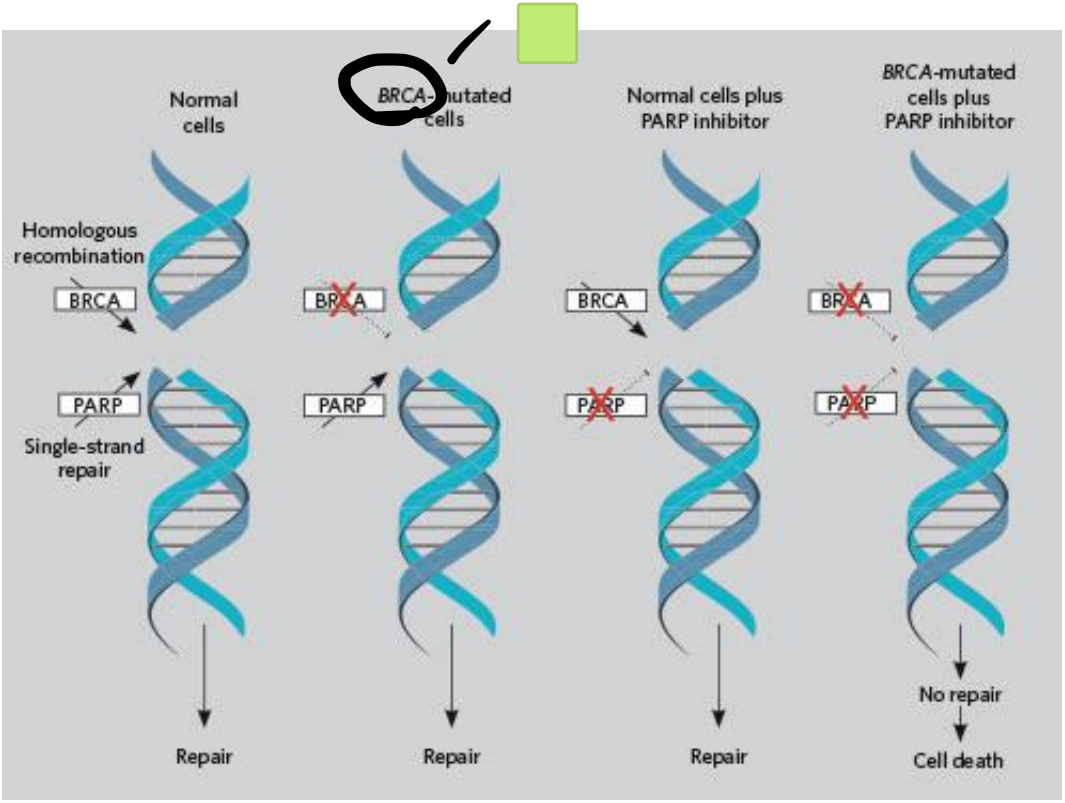


New molecular and diagnostic technologies can be used to match select groups of patients with treatments that may give them the best results

prediction of therapy response to molecular drugs

examples. ovarian cancer *BRCA1-BRCA2* variants, ER+ breast cancer *PIK3CA* variants





Pujade-Lauraine et al. The Lancet Oncology 2017

diagnosis

examples. pancreatic cyst: *GNAS, KRAS, RNF43, VHL, CTNNB1* variants, granulosa tumor: *FOXL2*

Table 3. DNA Analysis of Pancreatic Cyst Fluid Can Identify Patterns of Genetic Alterations that Define Cyst Type

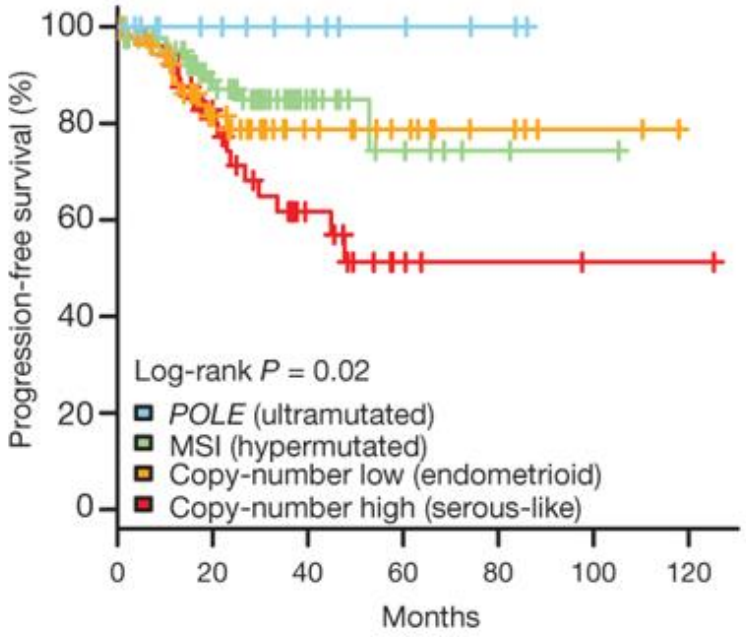
Variable	*KRAS*	*GNAS*	*RNF43*	*CTNNB1*	*VHL*
IPMN	+	+	+		
MCN	+		+		
SPN				+	
SCA					+

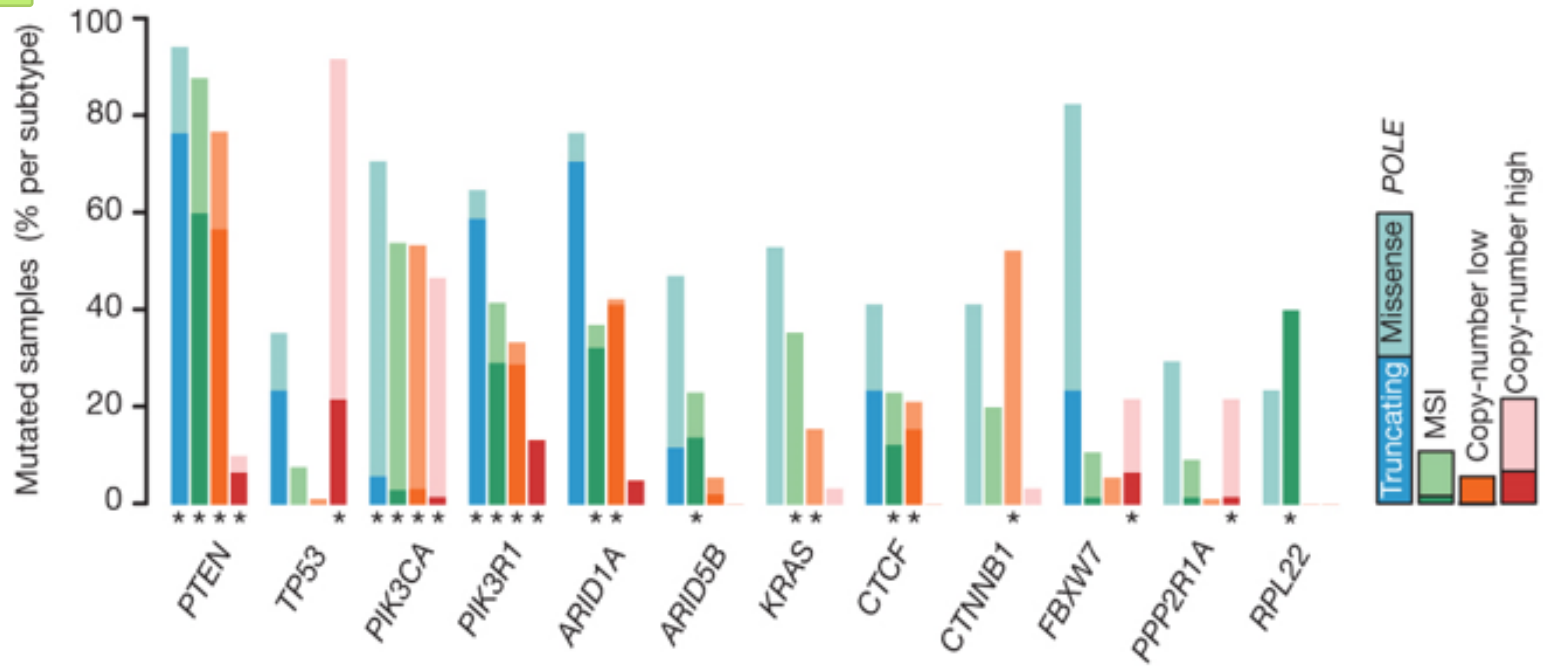
IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; SPN, solid-pseudopapillary neoplasm; SCA, serous cystadenoma.

Lee et al. SpringerPlus 2016, Moris et al. Anticancer Research 2017, Maker et al. J Am Coll Surg 2015

prognosis

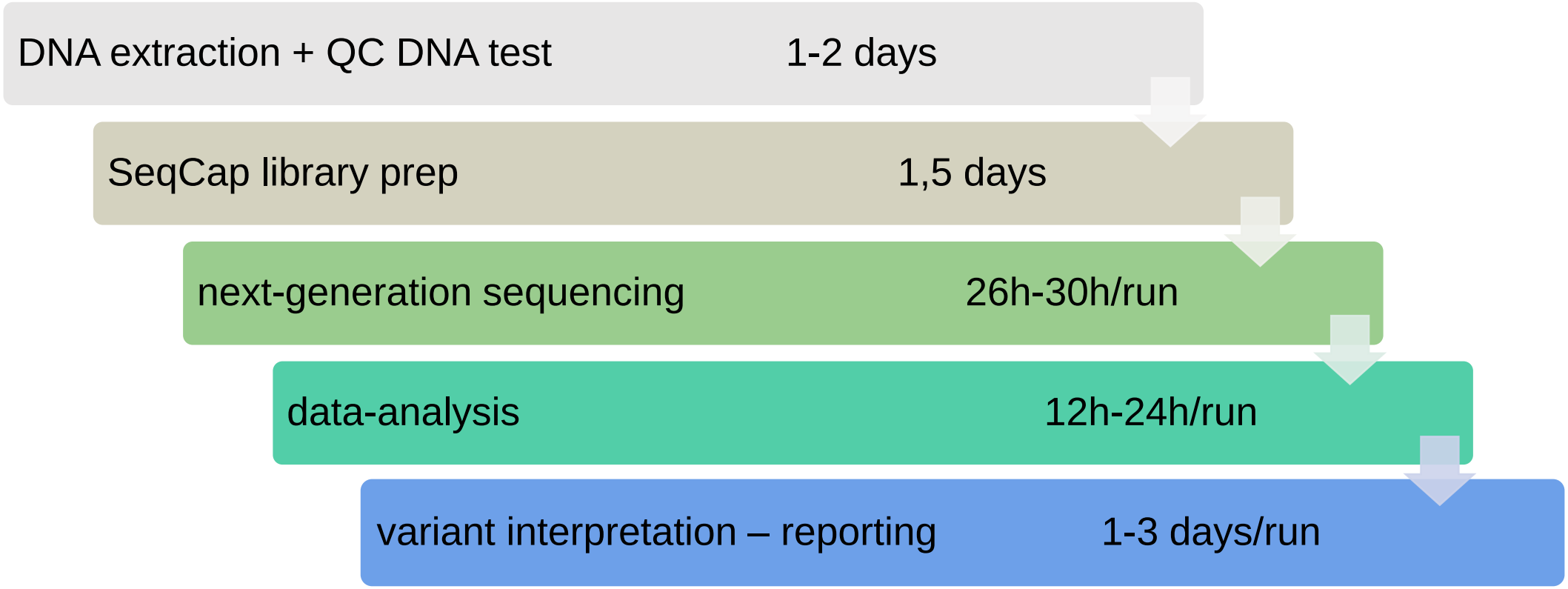
examples. endometrial tumor: *POLE* variants, glioma: *TERT* promoter variants



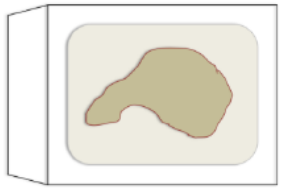


TCGA et al. Nature 2013

next-generation sequencing workflow



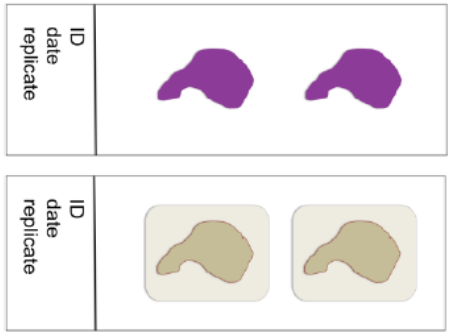
DNA extraction of solid tumors



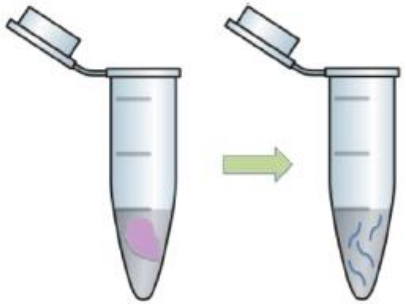
>10% tumor cells

time between biopsy-resection and fixation < 1h

fixation time: 6-72h in 10% neutral buffered formalin

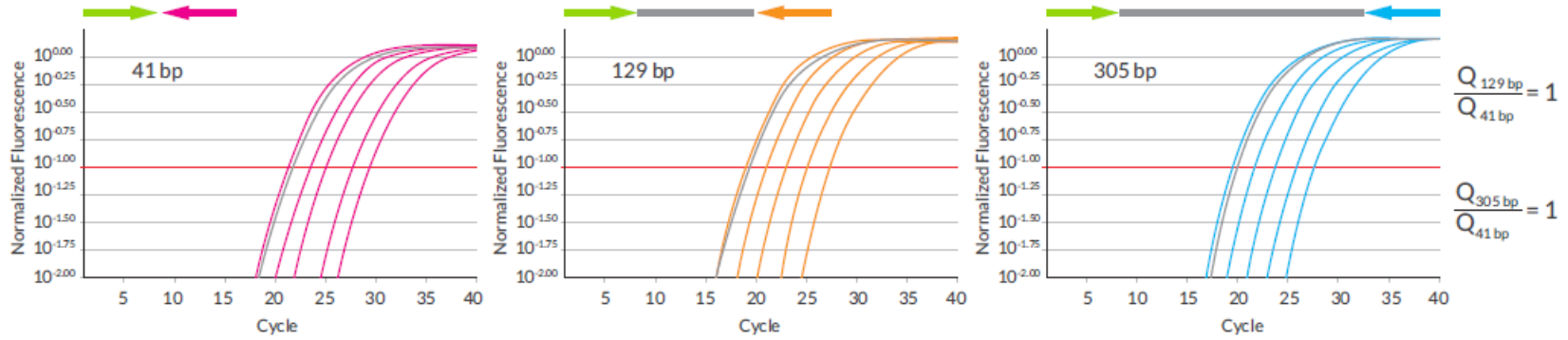


1 H&E slide
5 slides of 10µM

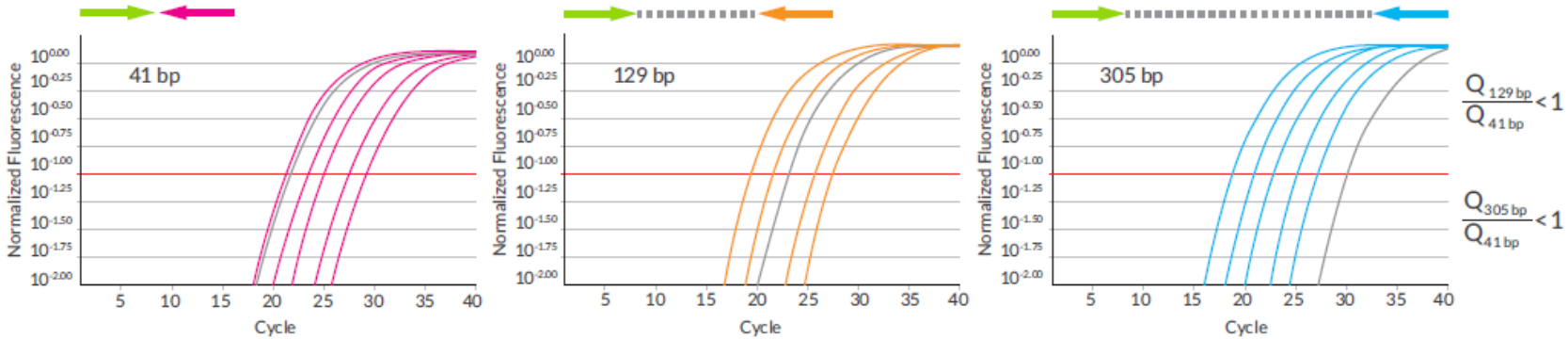


QC DNA test of solid tumors

High-quality hgDNA



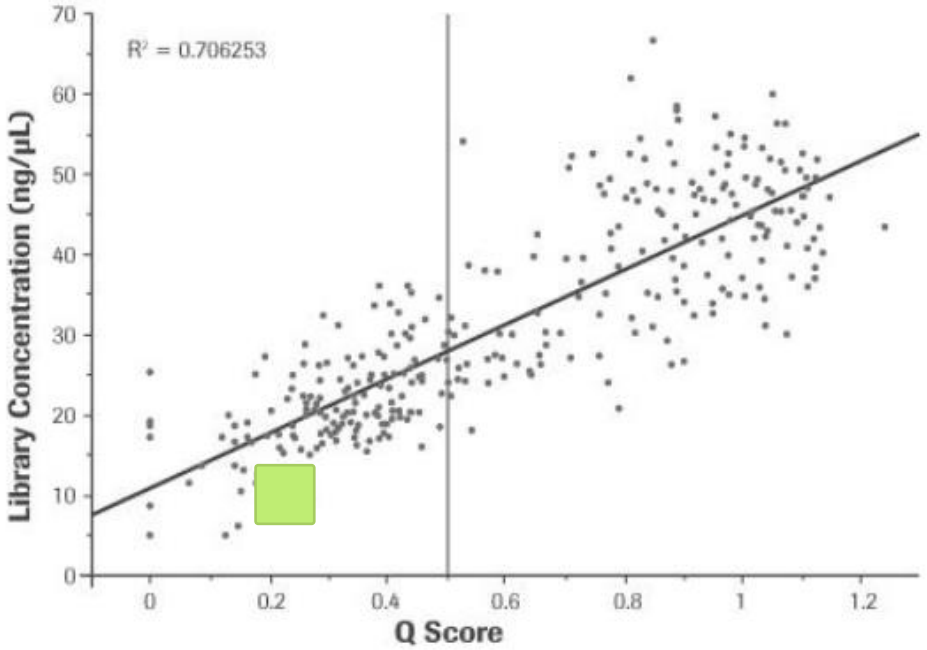
Damaged hgDNA

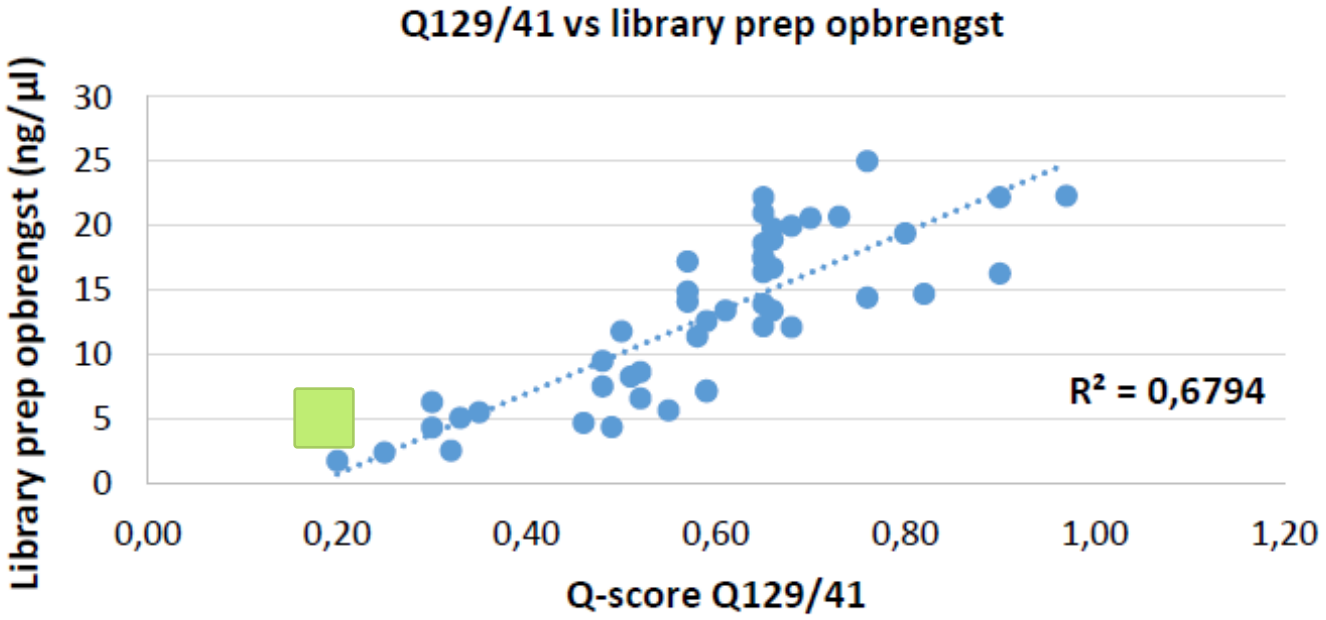


→ determine DNA input for SeqCap NGS library prep based on QC test

QC DNA test of solid tumors

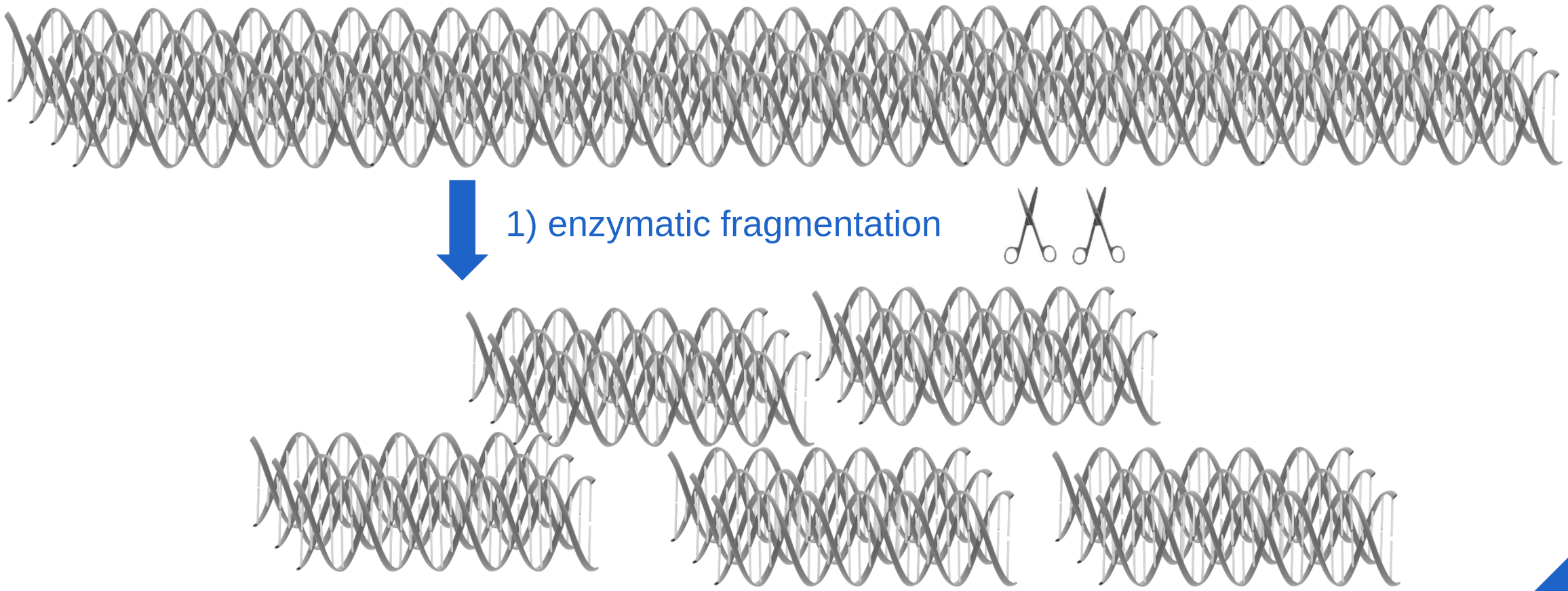
QC scores correlate with SeqCap library prep yields





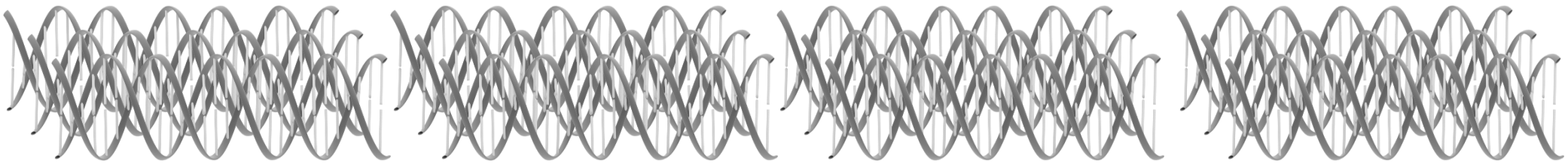
SeqCap library prep – capture-based NGS

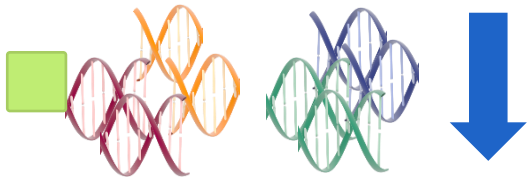
enzymatic fragmentation



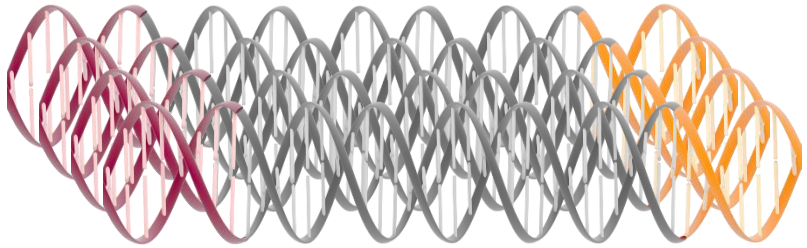
SeqCap library prep – capture-based NGS

ligation of adapters with unique dual indexes (UDI)

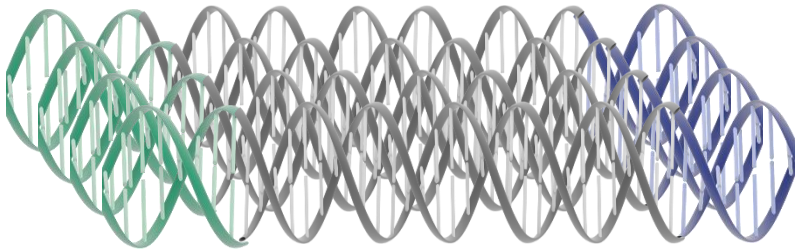




2) ligation of adapters with unique dual indexes (UDI)
*adapters for binding to the flowcell and sequencing of primer sites
* unique dual indexes ≠ for each patient sample



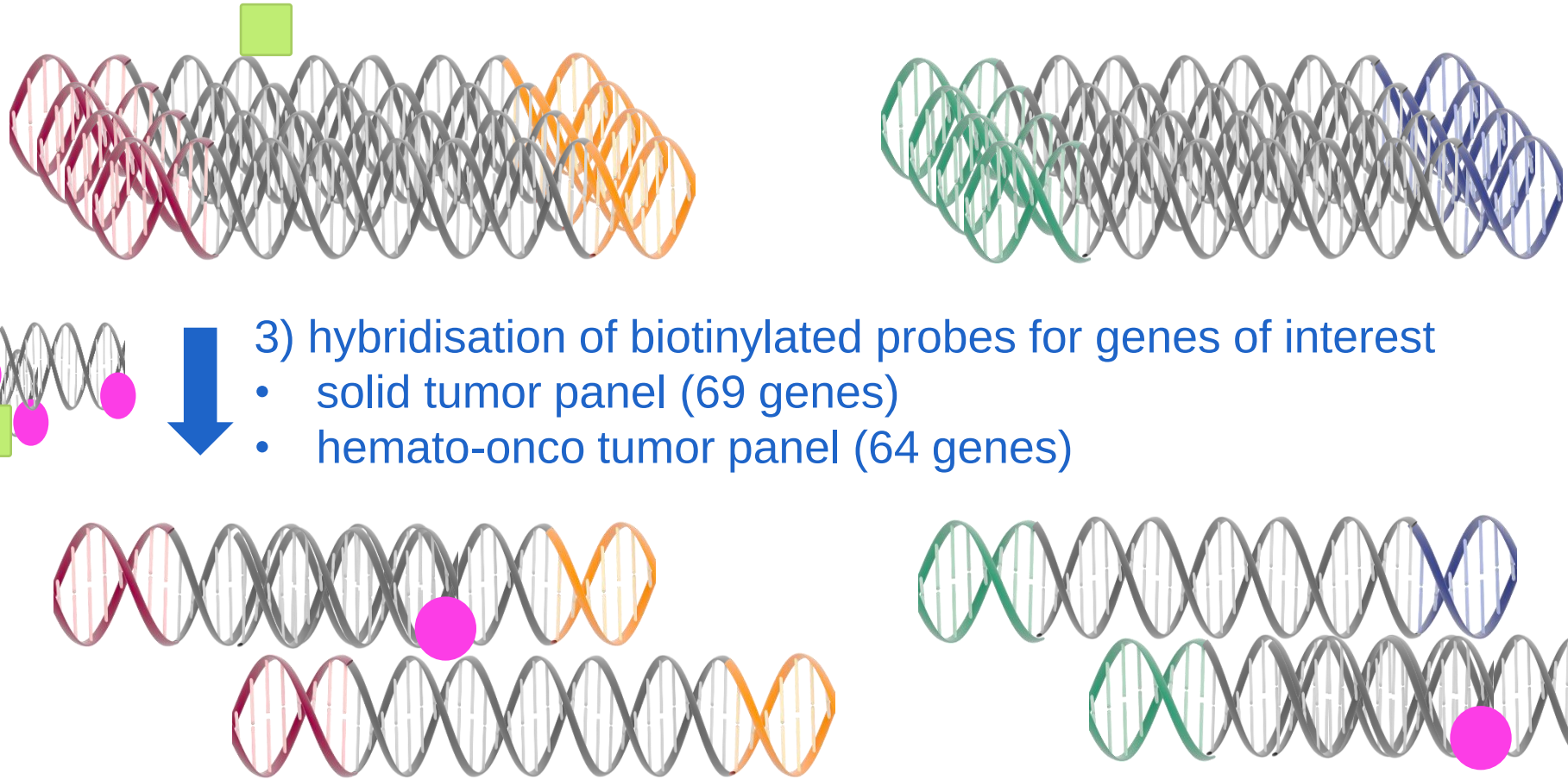
patient sample 1



patient sample 2

SeqCap library prep – capture-based NGS

probe hybridisation



3) hybridisation of biotinylated probes for genes of interest

- solid tumor panel (69 genes)
- hemato-onco tumor panel (64 genes)

SeqCap library prep – capture-based NGS

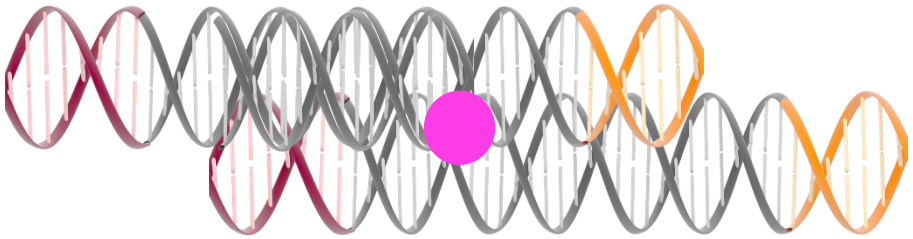
MDG gene panels for solid and hemato-oncological tumors

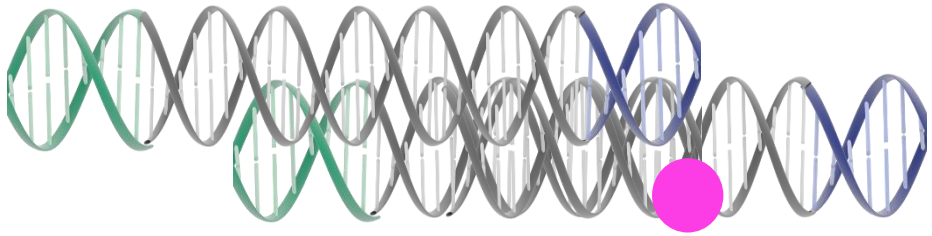
solid tumor panel (69 genes): *AKT1, ALK, APC, AR, BAP1, BRAF, BRCA1, BRCA2, CCND1, CDK4, CDK6, CDKN2A, CDKN2B, CTNNB1, DDR2, DICER1, DPYD, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FBXW7, FGFR1, FGFR2, FGFR3, FOXL2, FRK, GATA3, GNA11, GNAQ, GNAS, H3F3A, H3F3B, HIST1HB3, HIST1H3C, HNF1A, HRAS, IDH1, IDH2, IL6ST, JAK1, JAK2, KIT, KRAS, MAP2K1, MET, NRAS, NTRK1, NTRK3, PDGFRA, PIK3CA, PIK3R1, POLE, PTEN, RB1, RET, RNF43, ROS1, SMAD4, SMARCA4, SMARCB1, SMO, SPOP, STAT3, STK11, TERT, TP53, VHL*

hemato-onco tumor panel (64 genes): *ANKRD26, ASXL1, ATM, BCL2, BCOR, BCORL1, BIRC3, BRAF, BTK, CALR, CBL, CEBPA, CRLF2, CSF3R, CUX1, DDX41, DNMT3A, EGR2, ETNK1, ETV6, EZH2, FBXW7, FLT3, GATA2, HRAS, IDH1, IDH2, IKZF1, IL7R, JAK2, JAK3, KIT, KRAS, MPL, NF1, NFKBIE, NOTCH1, NPM1, NRAS, PAX5, PHF6, PLCG2, POT1, PPM1D, PTPN11, RAD21, RPS15, RRAS, RUNX1, SETPB1, SF1, SF3B1, SH2B3, SMC1A, SMC3, SRSF2, STAG2, STAT5B, TET2, TP53, U2AF1, WT1, XPO1, ZRSR2*

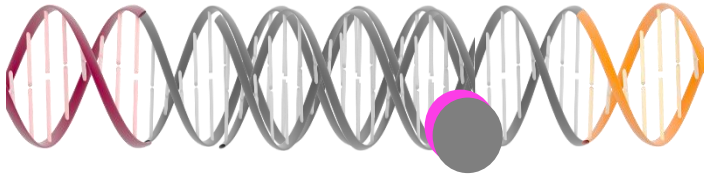
SeqCap library prep – capture-based NGS

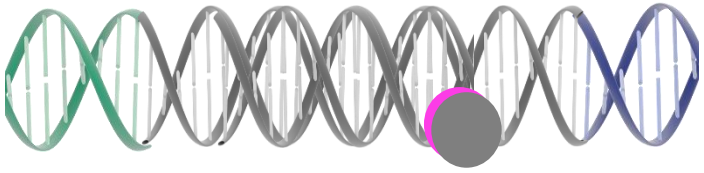
enrichment of DNA fragments with genes of interest





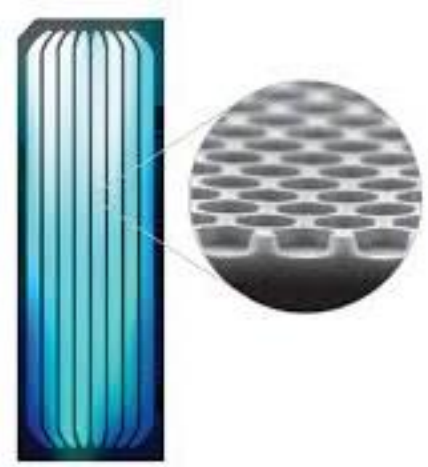
4) purification of biotinylated-probe bound DNA fragments with streptavidin-coated beads + amplification

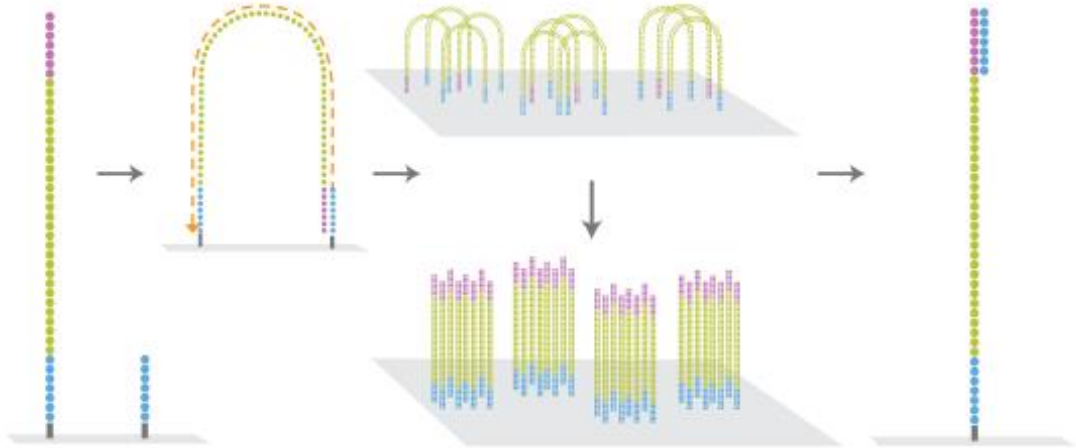


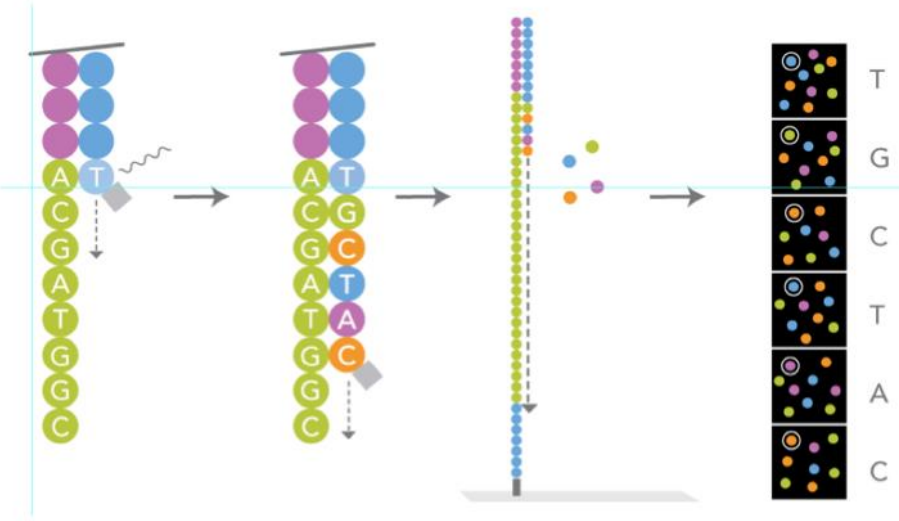


Illumina sequencing of SeqCap library prep

hybridisation, bridge amplification, cluster generation followed by sequencing-by-synthesis







Illumina sequencing van SeqCap library prep

sequencing instruments @CMGG

MiSeq
Illumina
up to 15 Gb

targeted sequencing

NextSeq 500
Illumina
up to 120 Gb

small/polyA/total RNA sequencing

HiSeq 3000
Illumina
up to 750 Gb

shallow whole genome/NIPT

NovaSeq
Illumina
up to 3000 Gb

exome/whole genome sequencing

NGS data-analysis

in-house bcbio datamining workflow

bcbio:

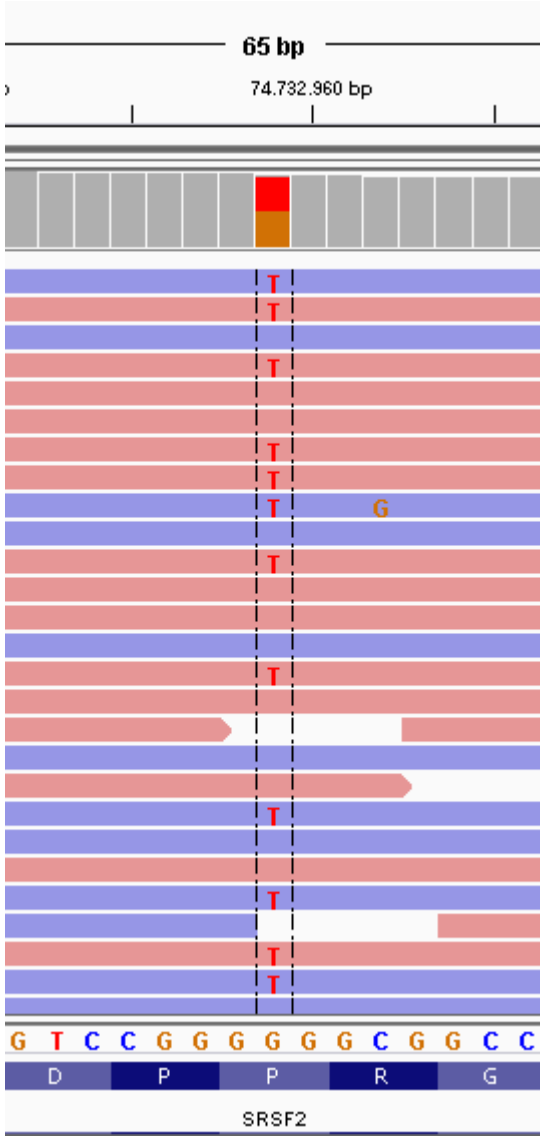
- sequencing adapter trimming
- mapping
- duplicate read marking
- variant calling
- variant annotation: VEP, dbNSFP, dbscSNV

coverage:

- sequencing depth = amount of unique reads for a specific nucleotide in the sequencing data

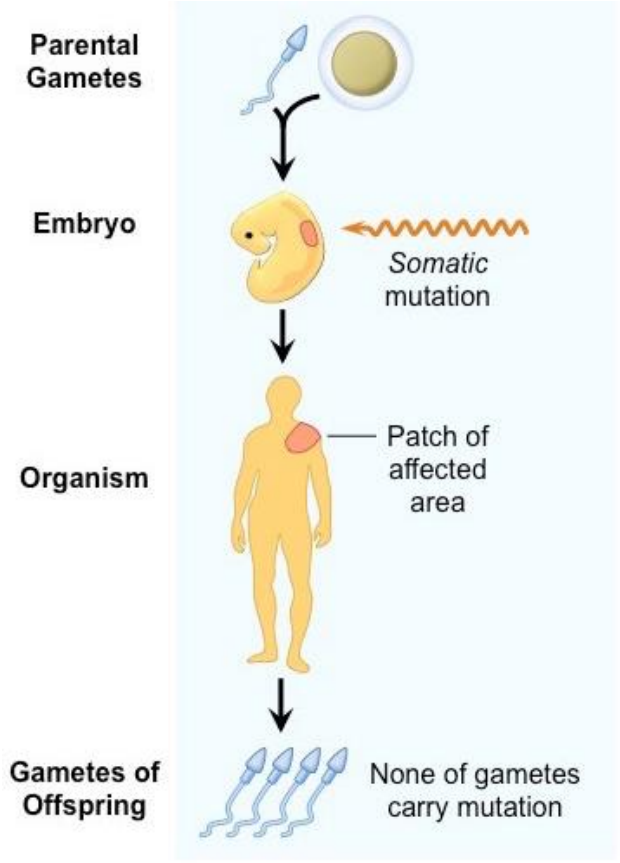
variant calling and reporting:

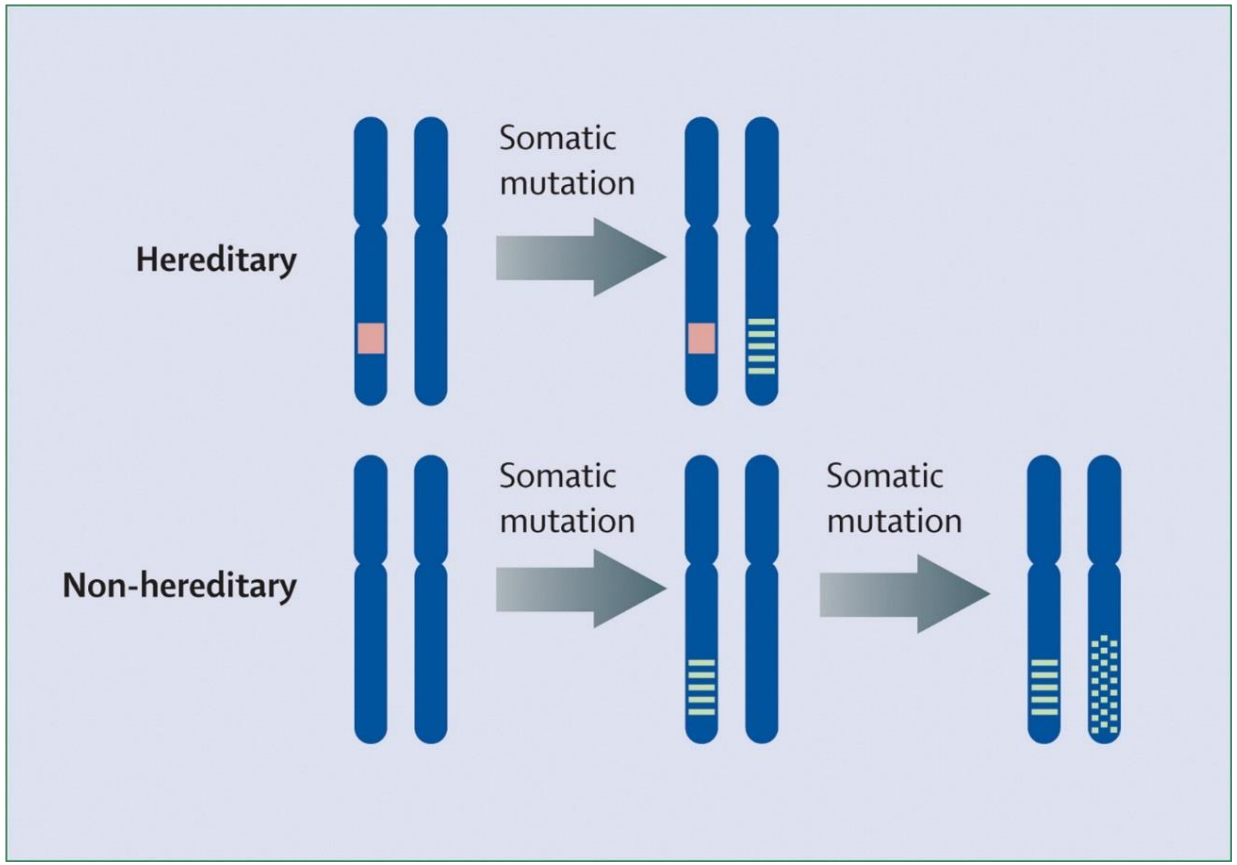
- ≥ 300X coverage & ≥ 5% VAF
- exception: 2-5% VAF known hotspot variants with variant present in >10 reads



SRSF2 c.284C>A (p.(Pro95His))
49% VAF

detection of somatic variants with NGS





Sharma et al. Nat Rev Cancer 2007

detection of somatic variants with NGS

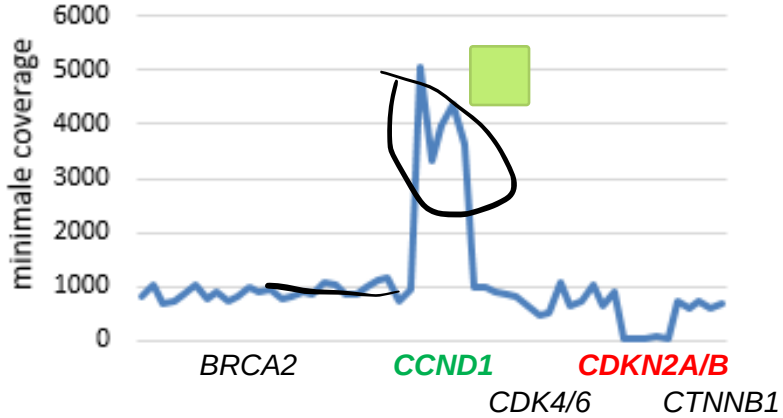
subsitutions (SNVs), deletions, insertions, copy number variants (CNVs) based on coverage

substitutions (SNV)

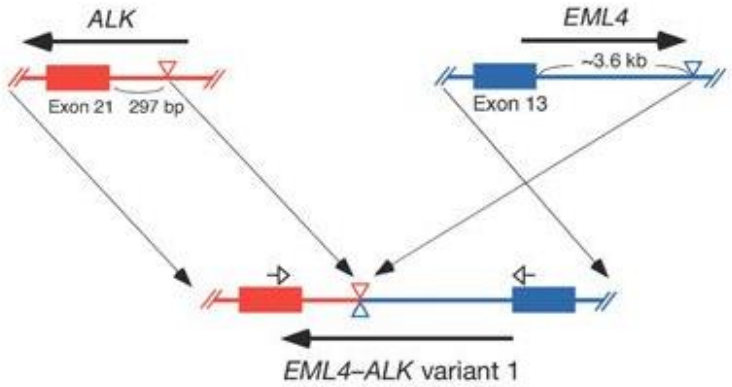
deletions

insertions

copy number variants (CNVs) based on coverage



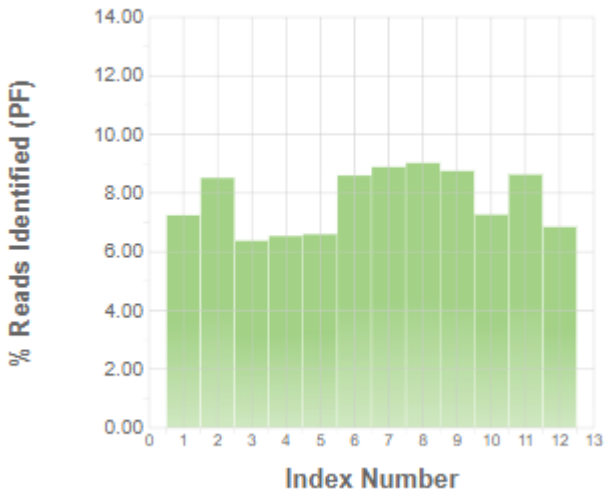
fusions

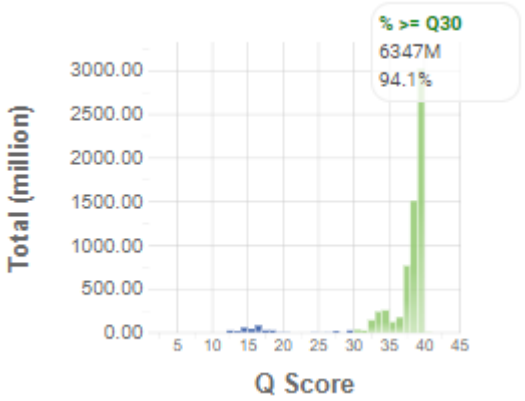


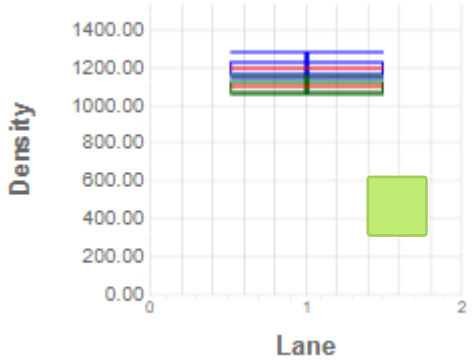
NGS data-analysis

validation of SeqCap SOLID test

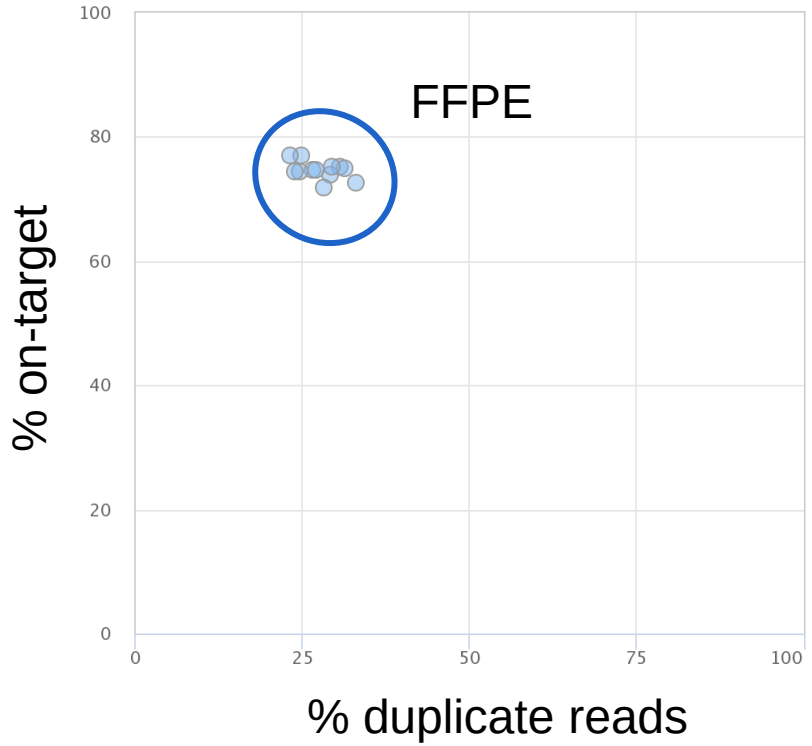
miSeq run with good quality FFPE samples







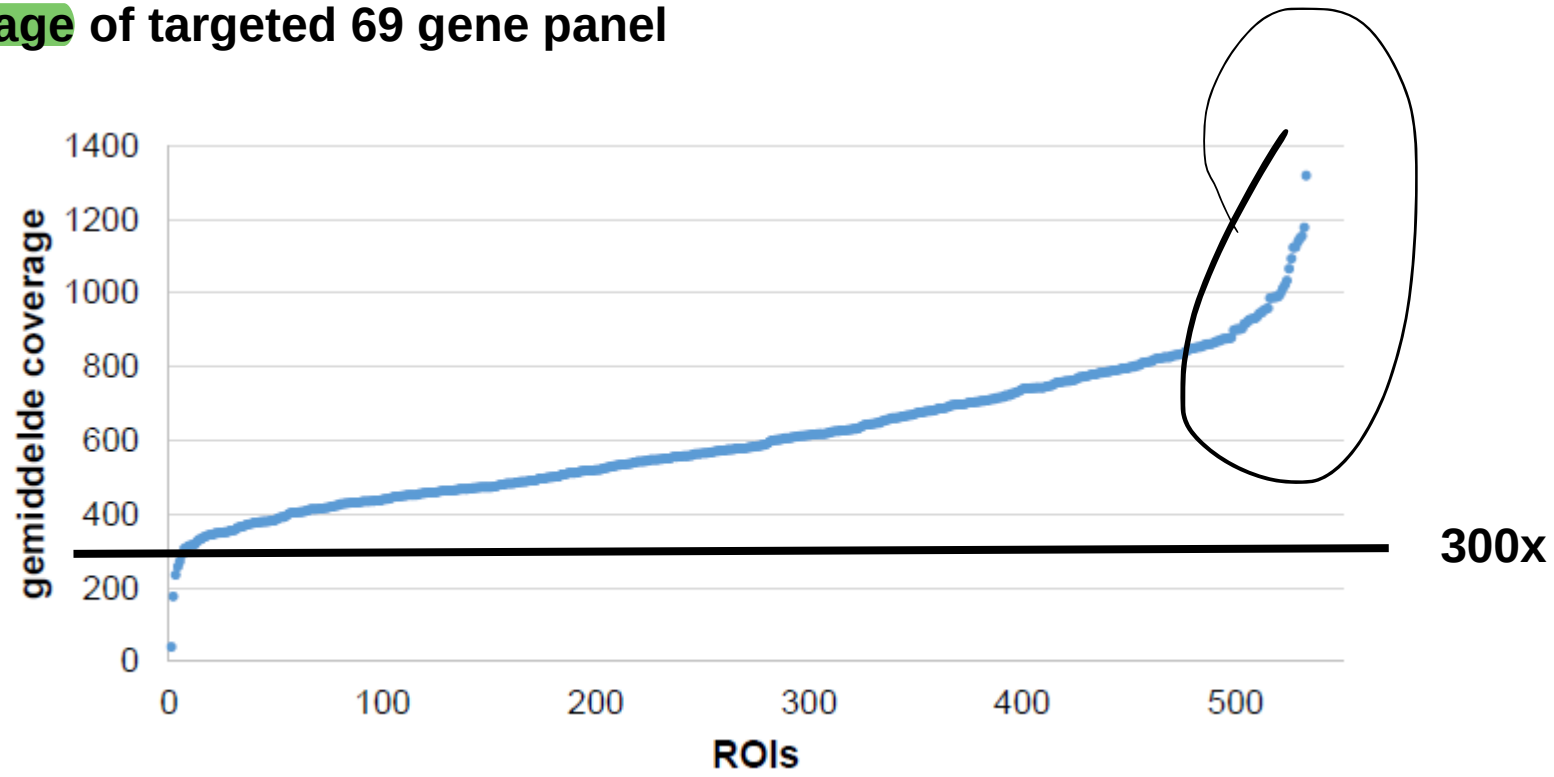
multiQC



NGS data-analysis

validation of SeqCap SOLID test

average coverage of targeted 69 gene panel



NGS data-analysis

validation of SeqCap SOLID test

bcbio:

- sequencing adapter trimming
- mapping
- duplicate read marking
- variant calling: 5 variant callers tested
  - FREEBAYES
  - GATK
  - MUTECT2
  - VARDICT
  - VARSCAN
- variant annotation: VEP, dbNSFP, dbscSNV

NGS data-analysis

validation of SeqCap SOLID test

SNV calling – known SNVs

runM001: 28 known SNVs

- **FREEBAYES**: detects all known SNVs above 10% VAF
- **GATK**: detects all known SNVs above 22-25% VAF
- **MUTECT2**: detects all known SNVs , but 1 incorrect calling of *EGFR* c.2369C>T → *EGFR* c.2369_2370delinsTA
- **VARDICT**: detects all known SNVs
- **VARSCAN**: detects all known SNVs

NGS data-analysis

validation of SeqCap SOLID test

indel calling – known indels

runM001: 12 known indels

- **FREEBAYES**: detects all known indels above 10% VAF
- **GATK**: detects all known indels above 22-25% VAF
- **MUTECT2**: detects all known indels
- **VARDICT**: detects all known indels
- **VARSCAN**: doesn't detect all known indels, *MET* c.3071_3082+11del not detected + detection of indels at lower VAF values

NGS data-analysis

validation of SeqCap SOLID test

SNV calling – novel SNVs

runM001: 12 novel SNVs, except in *POLE* mutant samples, based on VARDICT

- **FREEBAYES**: detects all novel SNVs above 10% VAF
- **GATK**: detects all novel SNVs above 22-25% VAF
- **MUTECT2**: doesn't detect all novel SNVs, 2 *HNF1A* SNVs not called – possible germline variants?
- **VARSCAN**: detects all novel SNVs

NGS data-analysis

validation of SeqCap SOLID test

indel calling – novel indels

runM001: 5 novel indels, except in *POLE* mutant samples, based on VARDICT

- **FREEBAYES**: detects all novel indels above 10% VAF
- **GATK**: detects all novel indels above 22-25% VAF
- **MUTECT2**: detects all novel indels
- **VARSCAN**: doesn't detect all novel indels, *TP53* c.568_574delinsTT not called correctly, *TP53* c.598_620del low VAF not detected + detection of indels at lower VAF values

NGS data-analysis

validation of SeqCap SOLID test

intrarun variability

		VAF intrarun			intrarun		
D1804623	**variant**	**intra1**	**intra2**	**intra3**	**gem VAF**	**st dev**	**CV**
EGFR	c.2235_2249del p.(Glu746_Ala750del)	28%	30%	27%	28%	1,53	5%
PIK3CA	c.1633G>A p.(Glu545Lys)	19%	23%	23%	22%	2,31	11%
TP53	c.660T>A p.(Tyr220Ter)	48%	51%	50%	50%	1,53	3%
TP53	c.869G>A p.(Arg290His)	19%	21%	23%	21%	2,00	10%
APC	c.4348C>T p.(Arg1450Ter)	46%	46%	45%	46%	0,58	1%
D1801351	**variant**	**intra1**	**intra2**	**intra3**	**gem VAF**	**st dev**	**CV**
KIT	c.2467T>G p.(Tyr823Asp)	53%	51%	53%	52%	1,15	2%
KIT	c.1656_1661del p.(Met552_Glu554delinsIle)	48%	53%	50%	50%	2,52	5%
MET	c.4070C>A p.(Ala1357Glu)	68%	68%	69%	68%	0,58	1%
RB1	deletie van 9 exons	del	del	del			

NGS data-analysis

validation of SeqCap SOLID test

interrun variability

		VAF interrun			interrun		
D1806432	**variant**	**inter1**	**inter2**	**inter3**	**gem VAF**	**st dev**	**CV**
EGFR	c.2235_2249del p.(Glu746_Ala750del)	25%	27%	26%	26%	0,82	3%
EGFR	c.2369C>T p.(Thr790Met)	7%	7%	6%	7%	0,47	7%
TP53	c.788del p.(Asn263IlefsTer82)	25%	26%	16%	22%	4,5	20%
D1808171	**variant**	**inter1**	**inter2**	**inter3**	**gem VAF**	**st dev**	**CV**
KIT	c.1651_1662del p.(Pro551_Glu554del)	42%	50%	44%	45%	3,4	7%
KIT	c.2467T>G p.(Tyr823Asp)	52%	49%	49%	50%	1,41	3%
KIT	c.1936T>A p.(Tyr646Asn)	21%	19%	18%	19%	1,25	6%
CDKN2A	deletie van 3 exons	del	del	del	-	-	-

NGS data-analysis

validation of SeqCap SOLID test

limit of detection (LOD)

Tabel 5. Varianten aanwezig in Quantitative Multiplex Reference Standard FFPE (HORIZON) staal

chromosoom	gen	variant	exon	theoretische allelische frequentie (%) (TAF)
7q34	*BRAF*	V600E	exon 15	10,50
4q11-q12	*cKIT*	D816V	exon 18	10,00
7p12	*EGFR*	L858R	exon 21	3,00
7p12	*EGFR*	G719S	exon 18	24,50
12p12.1	*KRAS*	G13D	exon 2	15,00
12p12.1	*KRAS*	G12D	exon 2	6,00
1p13.2	*NRAS*	Q61K	exon 3	12,50
3q26.3	*PIK3CA*	H1047R	exon 21	17,50
3q26.3	*PIK3CA*	E545K	exon 10	9,00
2p23	*ALK*	P1543S	exon 29	33,00
13q12.3	*BRCA2*	A1689fs	exon 12	33,00
4q31.3	*FBXW7*	G667fs	exon 12	33,50
7q31	*MET*	V237fs	exon 2	6,5

NGS data-analysis

validation of SeqCap SOLID test

limit of detection (LOD)

Tabel 6. Theoretische allel frequentie (TAF) versus variant allel frequentie (VAF) van de NGS analyse

Variant	LOD1		LOD2		LOD3		LOD4		LOD5		LOD6		LOD7	
	TAF	VAF	TAF	VAF	TAF	VAF	TAF	VAF	TAF	VAF	TAF	VAF	TAF	VAF
BRAF V600E	10,5	12,6	5,3	10	2,6	6,9	1,3	3,8	0,66	2,2	0,33	-	0,16	-
KIT D816V	10	12,2	5	7,15	2,5	5,7	1,25	2,5	0,63	-	0,31	-	0,16	-
EGFR L858R	3	5,7	1,5	3	0,75	-	0,38	-	0,19	-	0,09	-	0,05	-
EGFR G719S	24,5	21	12	15,7	6,1	8,7	3	4,5	1,5	3,5	0,77	-	0,38	-
KRAS G13D	15	13,5	7,5	10,2	3,75	5,7	1,88	3	0,94	-	0,47	-	0,23	-
KRAS G12D	6	6,5	3	4,5	1,5	2,6	0,75	-	0,38	-	0,19	-	0,09	-
NRAS Q61K	12,5	10,1	6	7,8	3	4,6	1,6	3,2	0,8	-	0,4	-	0,2	-
PIK3CA H1047R	17,5	19,3	8,8	12,3	4,4	8,1	2,2	4,8	1,1	2,3	0,55	-	0,27	-
PIK3CA E545K	9	14,9	4,5	9,5	2,25	4,8	1,1	2,6	0,56	-	0,28	-	0,14	-
ALK P1543S	33	27,8	16,5	18,1	8,2	10,2	4,1	6,9	2	2,9	1	-	0,5	-
BRCA2 A1689fs	33	28,3	16,5	19,8	8,2	14,2	4,1	6,1	2	3,1	1	-	0,5	-
FBXW7 G667fs	33,5	28,6	16,7	19,6	8,4	12,6	4,2	6,2	2,1	3,8	1	2,2	0,5	-
MET V237fs	6,5	5,1	3,25	3,8	1,6	3,6	0,8	3,2	0,4	-	0,2	-	0,1	-

variant interpretation of NGS data

biological classification of somatic variants: 5 classes

cancers

MDPI

Project Report

Standardization of Somatic Variant Classifications in Solid and Haematological Tumours by a Two-Level Approach of Biological and Clinical Classes: An Initiative of the Belgian ComPerMed Expert Panel

Guy Froyen [1,*,†], Marie Le Mercier [2,†], Els Lierman [3,†], Karl Vandepoele [4,†], Friedel Nollet [5,†], Elke Boone [6,†], Joni Van der Meulen [7,†], Koen Jacobs [8], Suzan Lambin [9], Sara Vander Borght [10], Els Van Valckenborgh [11], Aline Antoniou [12] and Aline Hébrant [11]

MolecularDiagnostics.be + Scienscano: guidelines for harmonisation of variant classification/annotation/reporting

classification based on ACMG/AMP standards & guidelines (Richards et al. Genet Med 2015)

- pathogenic — example. *BRAF* c.1799T>A p.(Val600Glu)
- likely pathogenic — example. *PIK3CA* c.1357G>C p.(Glu453Gln)
- VUS — example. *ALK* c.3513C>G p.(Ile1171Met)
- likely benign — example. *ALK* c.4796C>A p.(Pro1599His)
- benign — example. *TP53* c.215C>G p.(Pro72Arg)

reporting of pathogenic, likely pathogenic and VUS variants

sciensano

Molecular Diagnostics be

variant interpretation of NGS data

biological classification of somatic variants: 5 classes

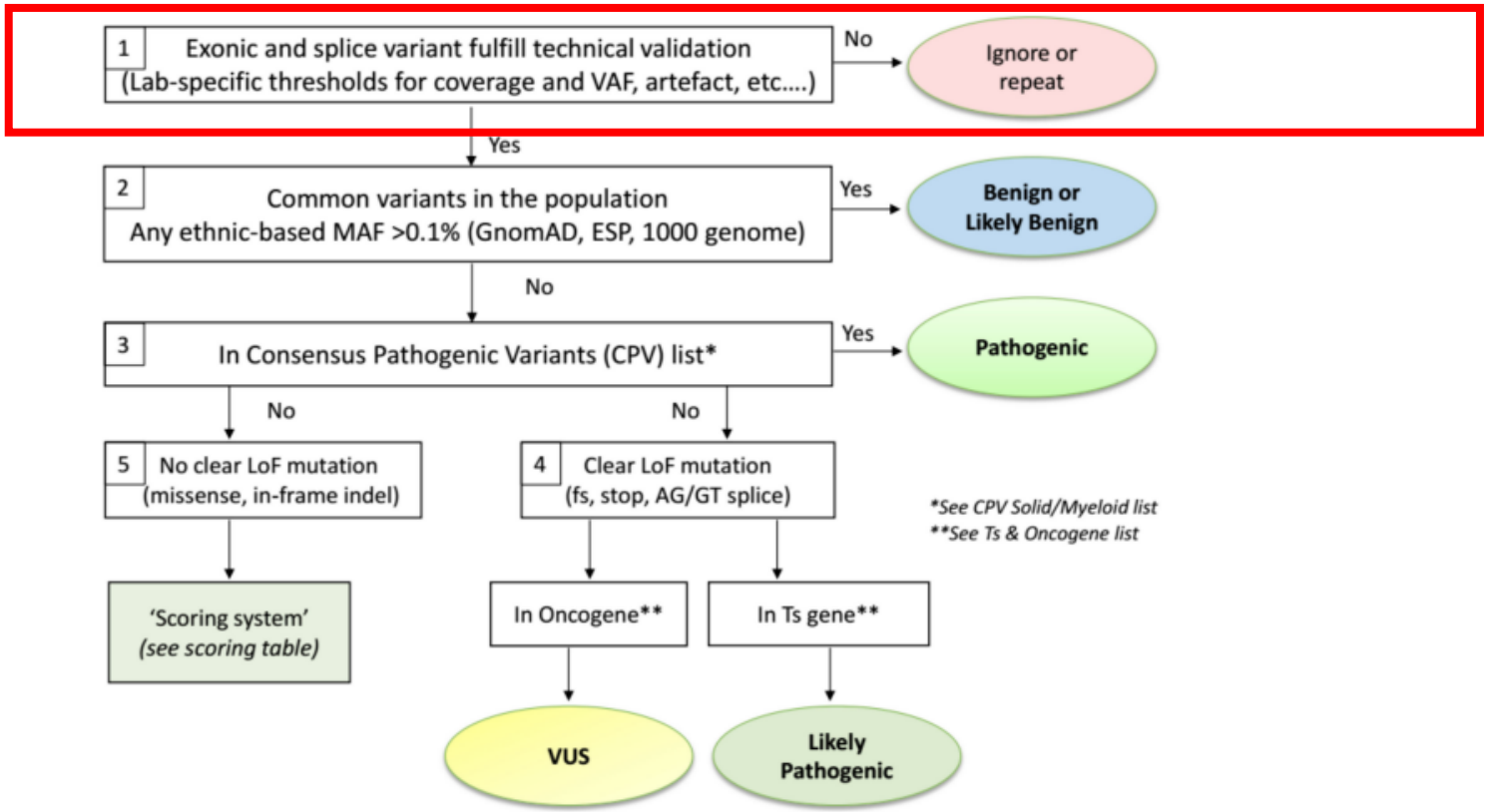
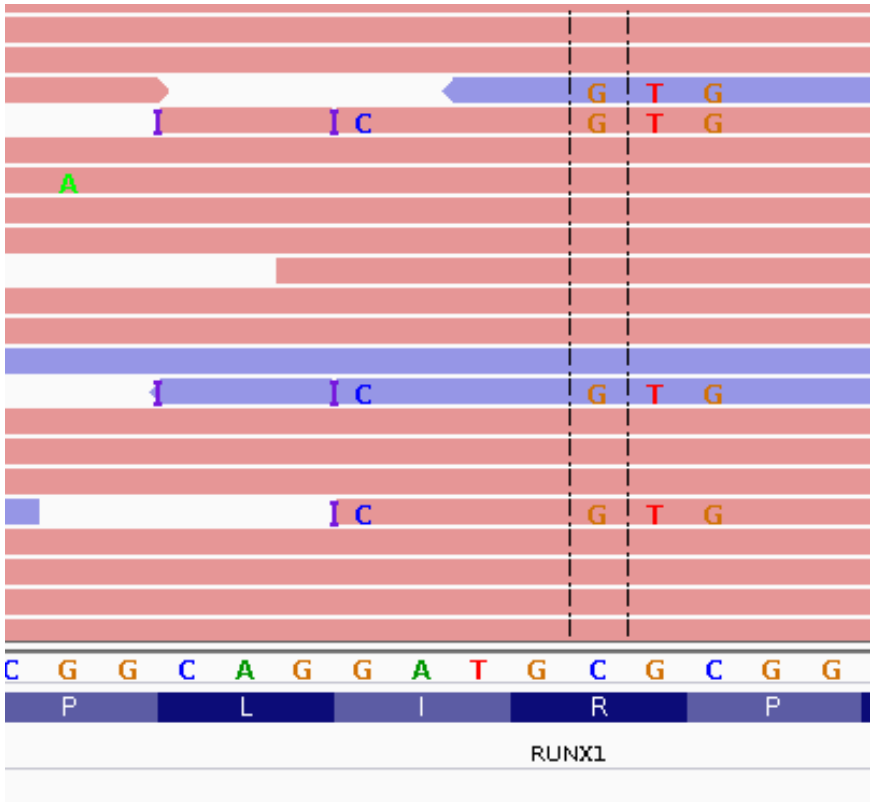


Figure 1. ComPerMed workflow for the biological classification of somatic variants.

variant interpretation of NGS data

biological classification of somatic variants: 5 classes



mapping error

example. *RUNX1* c.1278_1280delinsCAC 5% VAF
and *RUNX1* c.1284delinsGGAGAA 5% VAF

variant interpretation of NGS data

biological classification of somatic variants: 5 classes

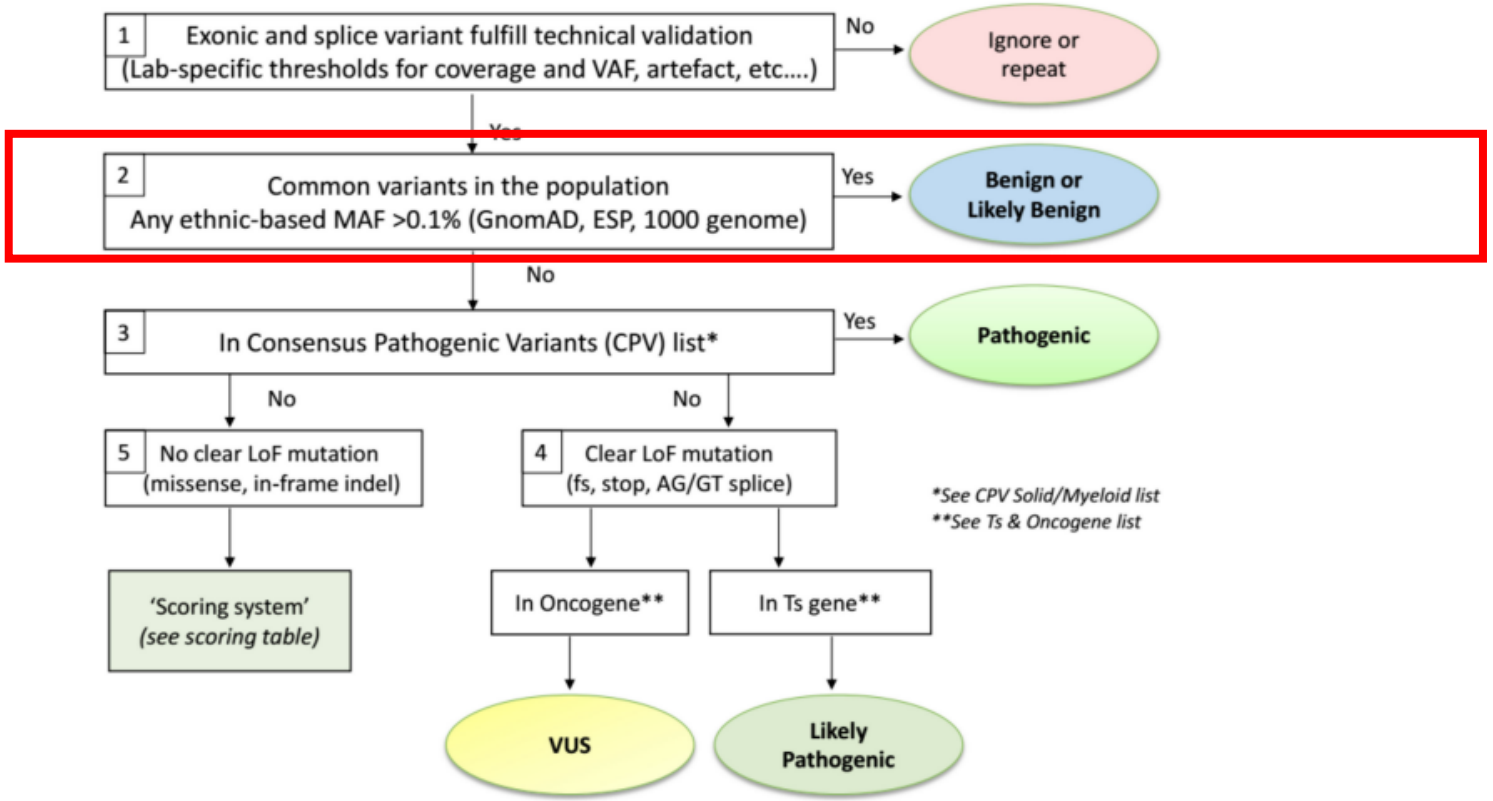


Figure 1. ComPerMed workflow for the biological classification of somatic variants.

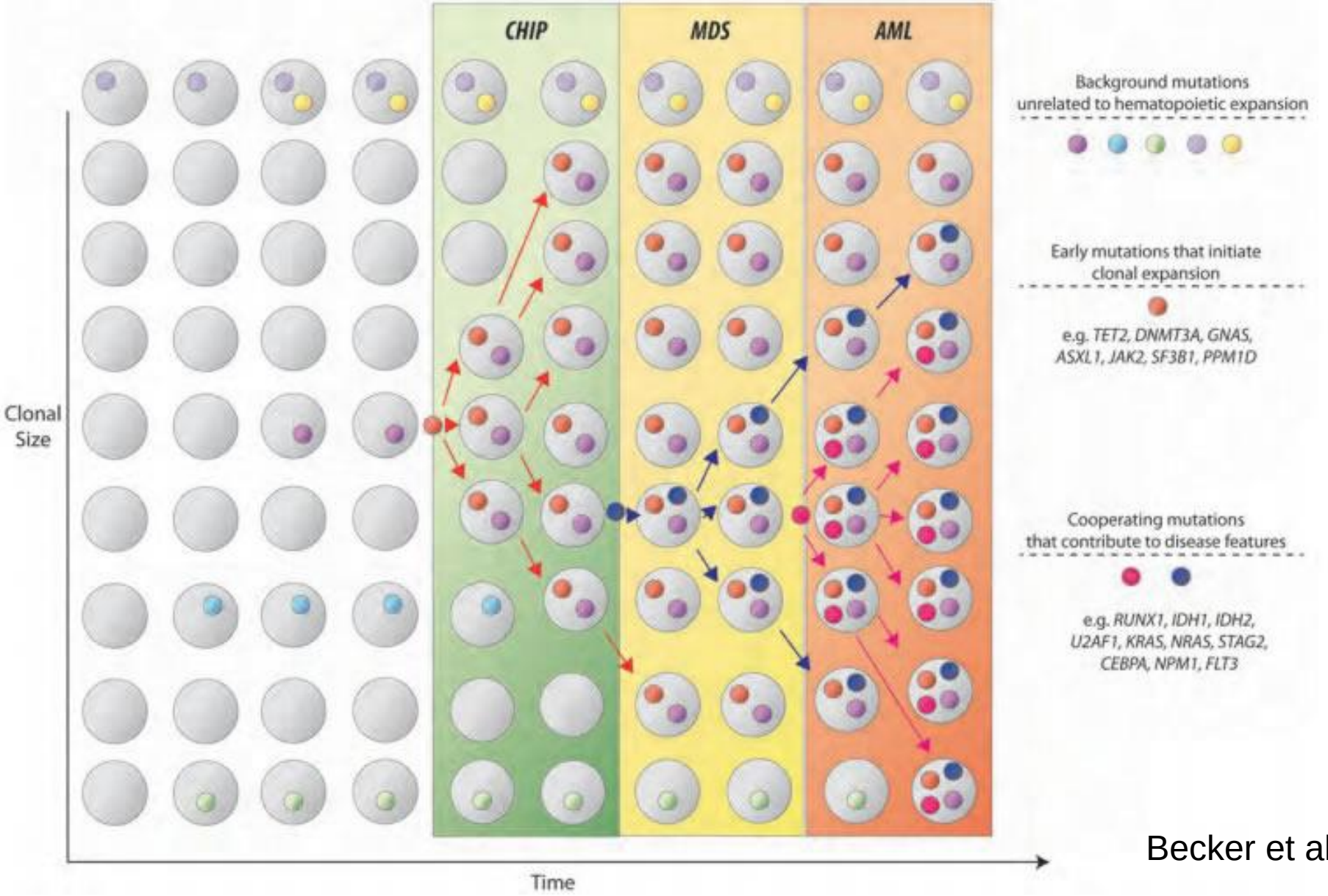
variant interpretation of NGS data

biological classification of somatic variants: 5 classes

germline variants

- present in databases of genome sequencing projects? bv. gnomAD: 125 748 WES and 15 708 WGS, no clinical data present
- some variants are region-specific => Belgian database is coming
- rare germline varianten (bv. *CEBPA, RUNX1, TP53, MET…)* can play a role in the disease
- age-related clonal hematopoiesis of indeterminate potential (CHIP): 93% *DNMT3A* of *TET2* mutations in healthy > 65 years old individuals (>10% of the elderly), and also *ASXL1, JAK2, …*

A Model of Clonal Expansion and Clonal Evolution from Normal Hematopoeisis to Myelodysplasia and Myeloid Leukemia



Becker et al. ASH 2016

variant interpretation of NGS data

biological classification of somatic variants: 5 classes

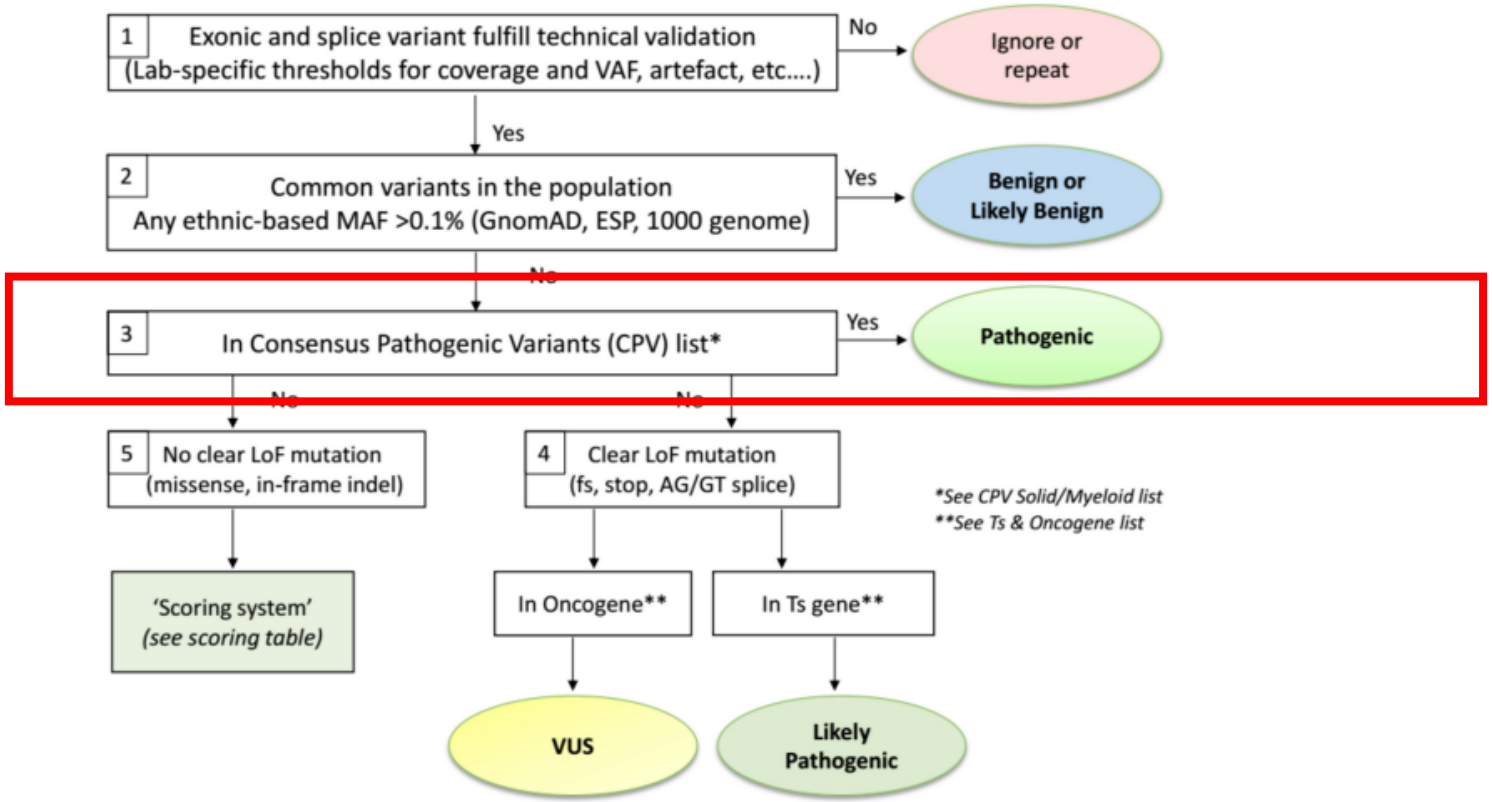


Figure 1. ComPerMed workflow for the biological classification of somatic variants.

Table 1. Consensus Pathogenic Variant (CPV) list of the ComPerMed genes selected for screening in solid tumours.

Gene	Transcript ID	Hs1	Hs2	Hs3	Hs4	Hs5	Hs6	Hs7	Hs8	Hs9	Hs10	Hs11	Hs12
ALK	NM_004304.4	F1174L	R1275Q										
BRAF	NM_004333.5	G469A/E/R/V	D594G/M	T599-K601 if-del/ins	V600E/K/M/R	K601E							
BRCA1	NM_007294.3	all clear LoF variants (nonsense, frameshift, splice site)											
BRCA2	NM_000059.3	all clear LoF variants (nonsense, frameshift, splice site)											
EGFR	NM_005228.4	G719A/C/S	ex19if-del/ins	ex20 if-ins	T790M	C797S	L858R	L861Q					
ESR1	NM_000125.3	K303R	E380Q	V392I	S463P	V533M	V534E	P535H	L536H/P/Q/R	Y537C/N/S	D538G		
GNAS	NM_000516.5	R201C/H											
H3F3A	NM_002107.4	K28M	G35R/W										
HRAS	NM_005343.3	G12C/D/S/V	G13C/D/R/S/V	Q61H/K/L/R									
IDH1	NM_005896.3	R132C/G/H/L/S											
IDH2	NM_002168.3	R140L/Q/W	R172K/M/S										
KIT	NM_000222.2	ex8 D419 if-del	ex9 S501-F504 if-ins	ex11 K550-V560 if-indel	ex11 W557G/R	ex11 V559A/D	ex11 V560D	ex11 L576P	ex13 K642E	ex13 V654A	ex14 T670I	ex17 D816H/V/Y	ex17 N822K
KRAS	NM_004985.4	G12A/C/D/F/R/S/V	G13C/D/R/S/V	A59T	Q61H/K/L/R	K117N	A146T						
MET	NM_001127500.3	ex14 skipping											
NRAS	NM_002524.4	G12A/C/D/R/S/V	G13C/D/R/S/V	A59T	Q61H/K/L/R	K117N	A146T						
PDGFRA	NM_006206.5	S566_E577 if-del	D842V	D842_I843 if-del	V561D								

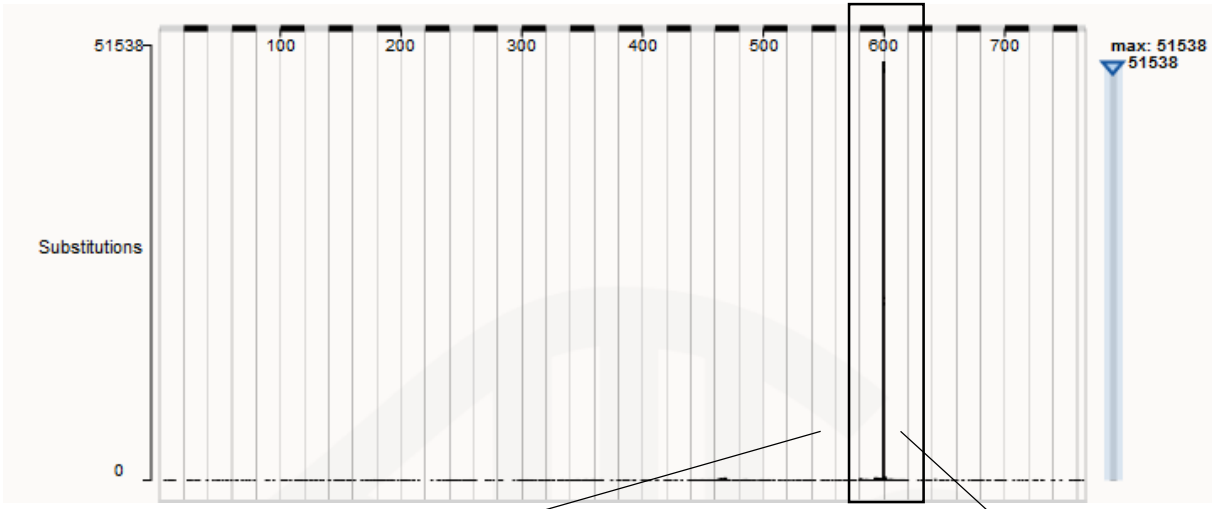
Hs: Hotspot; if-del: inframe deletion; if-ins: inframe insertion; _: denotes the exact positions of that change; -: denotes a region in which the change has to be located; LoF: Loss of Function.

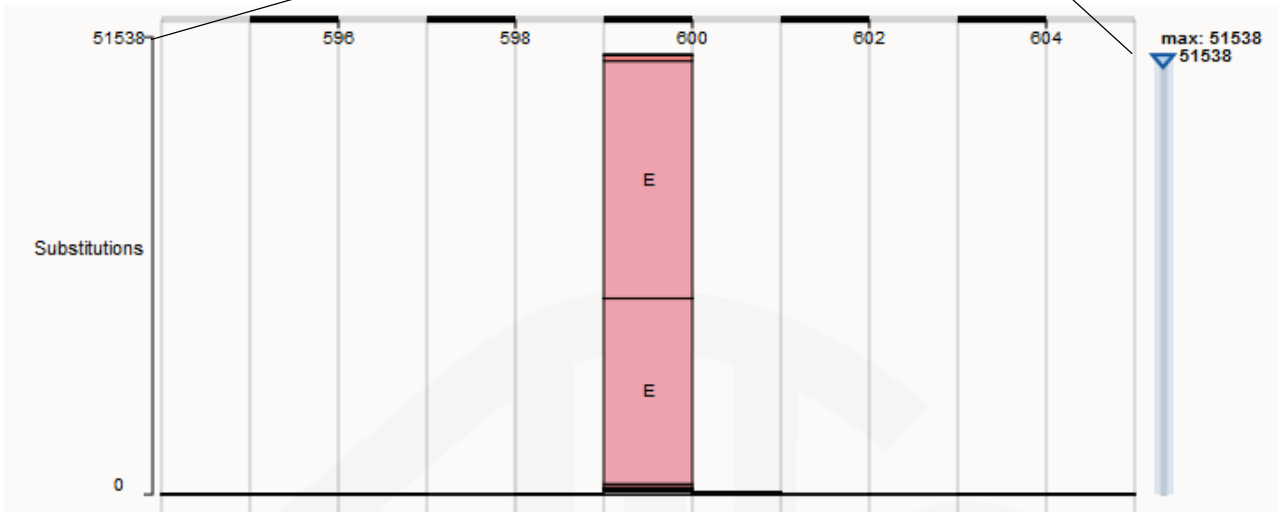
Table 2. Consensus Pathogenic Variant (CPV) list of the ComPerMed genes selected for screening in myeloid tumours.

Gene	Transcript ID	Hs1	Hs2	Hs3	Hs4	Hs5	Hs6
ASXL1	NM_015338.5	none					
CALR	NM_004343.3	ex9of-del	ex9of-ins				
CEBPA	NM_004364.3	none					
CSF3R	NM_156039.3	T618I					
DNMT3A	NM_175629.2	R882C/H					
EZH2	NM_004456.4	Y646F/H/N/S					
FLT3	NM_004119.2	ex14if-dup	D835A/E/H/V/Y				
IDH1	NM_005896.3	R132C/G/H/L/S					
IDH2	NM_002168.3	R140L/Q/W	R172K/M/S				
JAK2	NM_004972.3	ex12 if-del/if-dup	V617F				
KIT	NM_000222.2	*see CPV Solid list*					
MPL	NM_005373.2	S505N	W515any ms				
NPM1	NM_002520.6	ex11of-ins					
RUNX1	NM_001754.4	none					
SETBP1	NM_015559.3	D868N	G870S				
SF3B1	NM_012433.3	E622D	R625C/H	H662Q	K666N/R/T	K700E	G742D
SRSF2	NM_003016.4	P95H/L/R	P95_R102del				
TET2	NM_001127208.2	none					
TP53	NM_000546.5	R175H	Y220C	G245S	R248Q/W	R273C/H	R282W
U2AF1	NM_006758.2	S34F/Y	Q157P/R				
WT1	NM_024426.5	none					

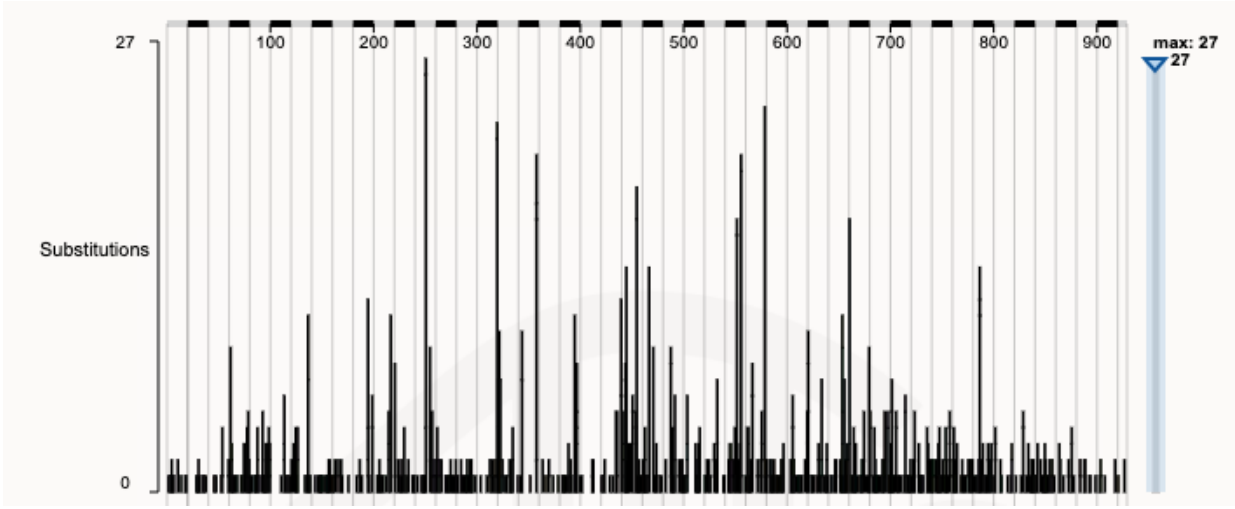
Hs: Hotspot; if-del: inframe deletion; if-dup: inframe duplication; of-del: out of frame deletion; of-ins: out of frame insertion; any ms: any missense variant; none: no consensus pathogenic variants present.

BRAF: hotspot c.1799T>A p.(Val600Glu) (V600E)





RB1: no hotspot



variant interpretation of NGS data

biological classification of somatic variants: 5 classes

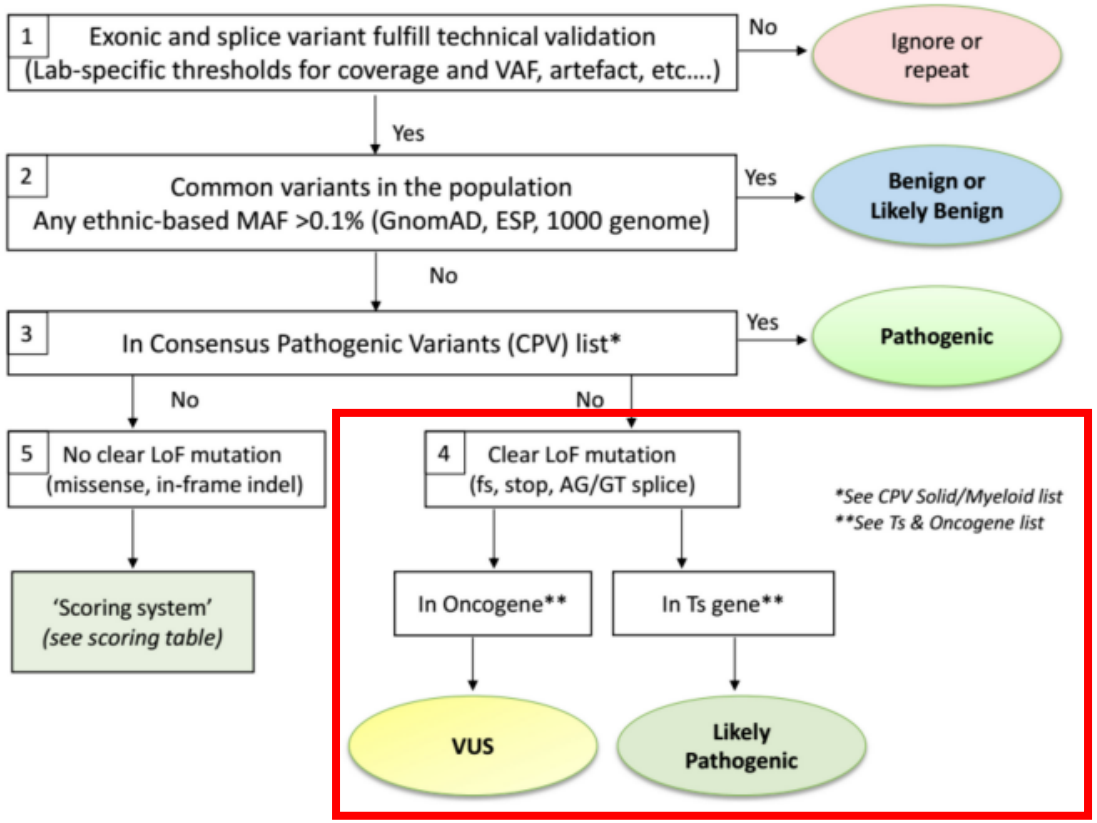


Figure 1. ComPerMed workflow for the biological classification of somatic variants.

variant interpretation of NGS data

biological classification of somatic variants: 5 classes

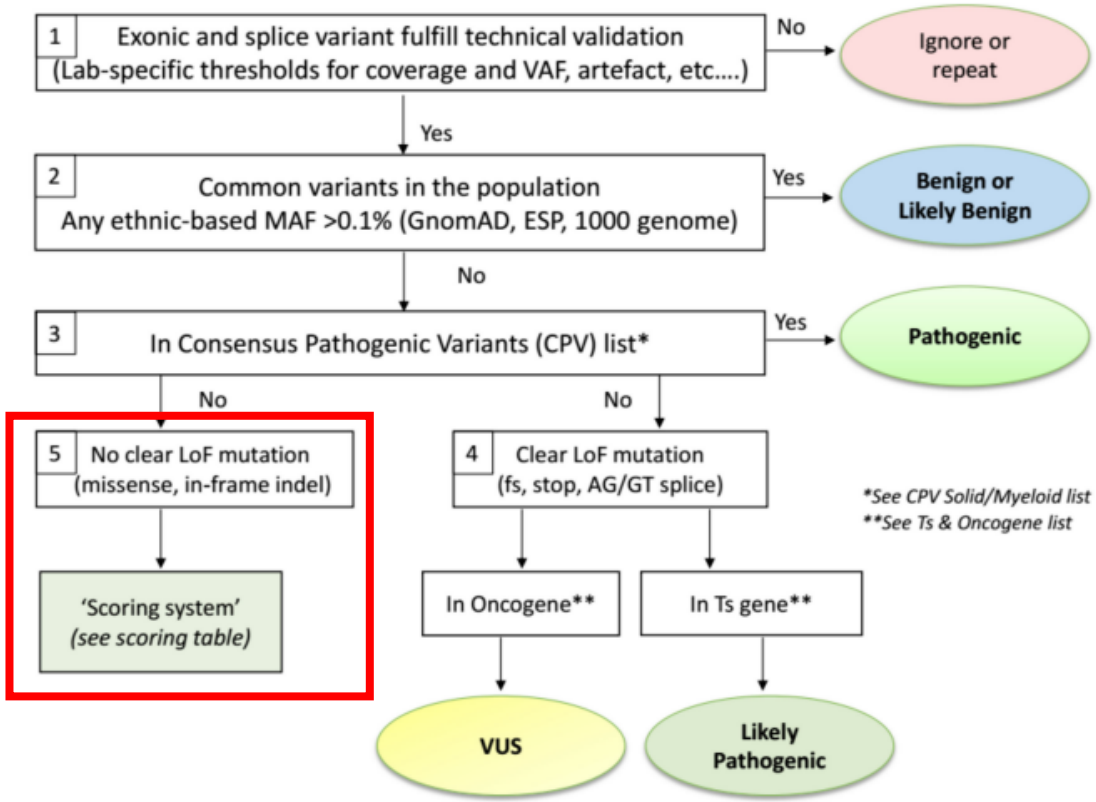


Figure 1. ComPerMed workflow for the biological classification of somatic variants.

variant interpretation of NGS data

biological classification of somatic variants: 5 classes

Table 3. Scoring Table for the biological variant classification of non-loss-of-function (LoF) variants.

Parameter	Score +2	Score +1	Score +0.5	Score 0	Score −1
Total # of entries of that particular AA change at that position in COSMIC	Solid: ≥50 Hemato: ≥10	50 > x > 10 10 > x > 5	/ /	≤10 ≤5	/ /
In silico prediction tools SIFT and MutationTaster	/	/	Both damaging and deleterious	Other	/
Harmful in functional studies (PubMed, JAX-CKB, MDA, MCG)	/	/	Yes	Not reported	No
Described in at least one genomic db (CIVIC, ClinVar, OncoKb, VarSome)	/	/	As (Likely) Pathogenic	Not described/unknown	As (Likely) Benign

Variants with a score ≥2 will be classified as "Likely Pathogenic". Variants with a score <2 are classified as "VUS".

variant interpretation of NGS data

biological classification of somatic variants: 5 classes

COSMIC

- manually curated database, based on publications
- contains also some germline variants
- problem with reference sequences

oncoKB

pct.mdanderson.org

ckb.jax.org

mycancergenome

PubMed

CIVIC

ClinVar (mainly germline variants)

TP53 databases

- IARC TP53
- Seshat

BRCA1/BRCA2 databases

- BRCAexchange.org
- LOVD
- ARUP

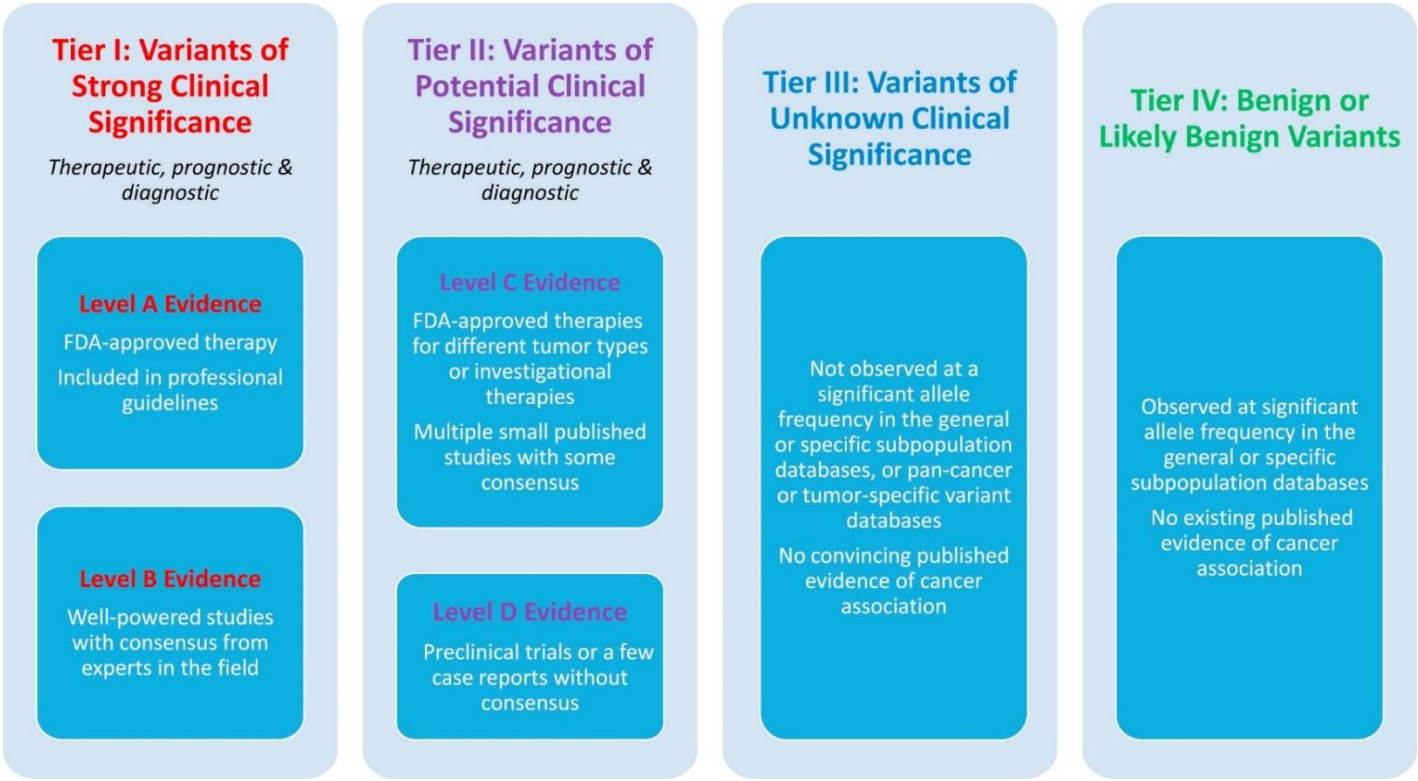
variant interpretation of NGS data

CMGGMC database variants & biological classification

Gen	c-notatie HGVS	g-notatie HGVS	p-notatie HGVS	Build	Categorie	Goedg.
NRAS	c.351G>T	chr1:g.114709668C>A	p.Lys117Asn	GRCh38	Pathogene variant	✓
NRAS	c.183A>T	chr1:g.114713907T>A	p.Gln61His	GRCh38	Pathogene variant	✓
NRAS	c.183A>C	chr1:g.114713907T>G	p.Gln61His	GRCh38	Pathogene variant	✓
NRAS	c.182A>T	chr1:g.114713908T>A	p.Gln61Leu	GRCh38	Pathogene variant	✓
NRAS	c.182A>G	chr1:g.114713908T>C	p.Gln61Arg	GRCh38	Pathogene variant	✓
NRAS	c.182A>C	chr1:g.114713908T>G	p.Gln61Pro	GRCh38	Pathogene variant	✓
NRAS	c.181C>G	chr1:g.114713909G>C	p.Gln61Glu	GRCh38	Pathogene variant	✓
NRAS	c.181C>A	chr1:g.114713909G>T	p.Gln61Lys	GRCh38	Pathogene variant	✓
NRAS	c.175G>A	chr1:g.114713915C>T	p.Ala59Thr	GRCh38	Vermoedelijke pathogene variant	✓
NRAS	c.38_39delinsAA	chr1:g.114716122_114716123deli...	p.Gly13Glu	GRCh38	Pathogene variant	✓

variant interpretation of NGS data

clinical classification



→ recommended: reporting of variants tiers I – III, NOT tier IV

Li et al. J Mol Diagn 2017

NGS report

solid tumors

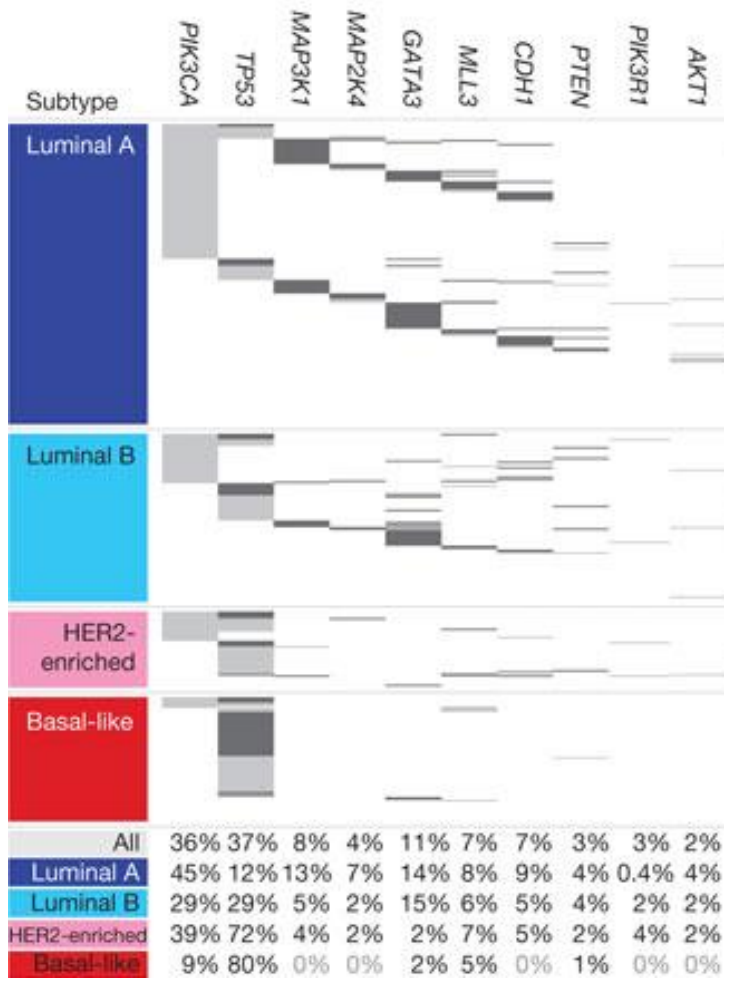
- pathogenic, probably pathogenic variants and variants of unknown significance (VUS) detected in **all 69 genes** are reported in **all solid tumor types**
  - *BRCA2* pathogenic variant in melanoma: precision2 clinical trials with olaparib @UZGent, germline mutation analysis recommended @CMGG
  - *DPYD* pathogenic variant in a colorectal tumor: germline mutation analysis recommended @CMGG, toxicity for 5-FU & capecitabine chemotherapy
  - *FGFR2* pathogenic variant in an endometrial tumor: FGFRi clinical basket trials @UZGent

casus 1

patient with metastatic ER+ HER2- breast cancer, 57 years old – tumour sample with 40% TC

- *PIK3CA* c.3140A>G p.(His1047Arg) 24% VAF: pathogenic variant
- *TP53* c.833C>T p.(Pro278Leu) 41% VAF: pathogenic variant
- *BAP1* c.1039C>T p.(His347Tyr) 50% VAF: VUS

- compassionate use programma / clinical study: alpelisib (PIK3CA inhibitor) in combination with fulvestrant/letrozole for *PIK3CA* mutated ER+ HER2- breast cancer patients



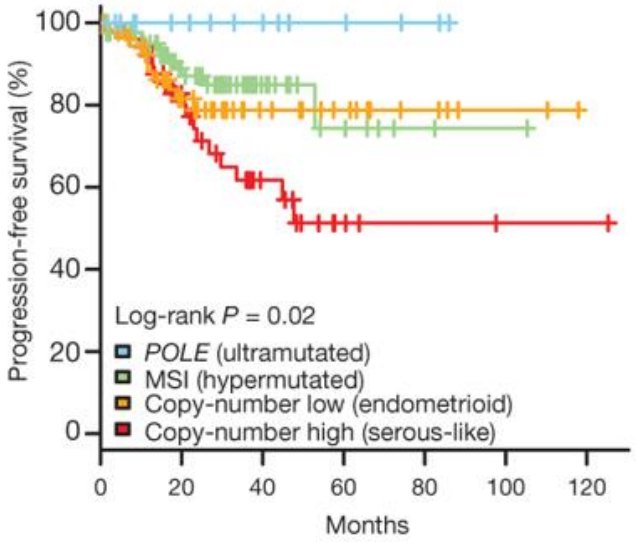
Koboldt et al. Nature 2012

casus 2

patient with endometrioid endometrial cancer, 51 years old – tumour sample with 80% TC

- *POLE* c.857C>G p.(Pro286Arg) 40% VAF: pathogenic variant
- *BRCA2* c.7795G>T p.(Glu2599Ter) 42% VAF: pathogenic variant
- *CTNNB1* c.104T>G p.(Ile35Ser) 45% VAF: pathogenic variant
- *PTEN* c.19G>T p.(Glu7Ter) 41% VAF: likely pathogenic variant
- *PTEN* c.895G>T p.(Glu299Ter) 40% VAF: likely pathogenic variant
- *PIK3R1* c.1042C>T p.(Arg348Ter) 80% VAF: likely pathogenic variant
- *ESR1* c.1610A>C p.(Tyr537Ser) 41% VAF: pathogenic variant

  + 22 VUS variants
- *POLE* mutated endometrial cancer group are associated with good prognosis



TCGA et al. Nature 2013

casus 3

patient with colorectal cancer, 68 years old – tumour sample with 50% TC

- *BRAF* c.1799T>A p.(Val600Glu) 30% VAF: pathogenic variant → bad prognosis, poor response to anti-EGFR monoclonal antibodies, novel combination therapues for *BRAF* c.1799T>A p.(Val600Glu) (V600E) mutant mCRC patients

Phase II, Open-label, Single Arm, Multicenter Study of Encorafenib, Binimetinib Plus Cetuximab in Subjects With Previously Untreated BRAF V600E -Mutant Metastatic Colorectal Cancer

Condition: Colorectal cancer, BRAF V600E mutation

Status: Active **Phase:** Phase II **Type:** Drug, Precision medicine **ID:** ANCHOR-CRC, W00090 GE 2 01

- 8 variants: *TP53, APC, RNF43, PTEN, PIK3R1, BAP1*

→ MMR deficiency (MSI high) → likely sensitive to immunotherapy

detection of somatic variants with NGS

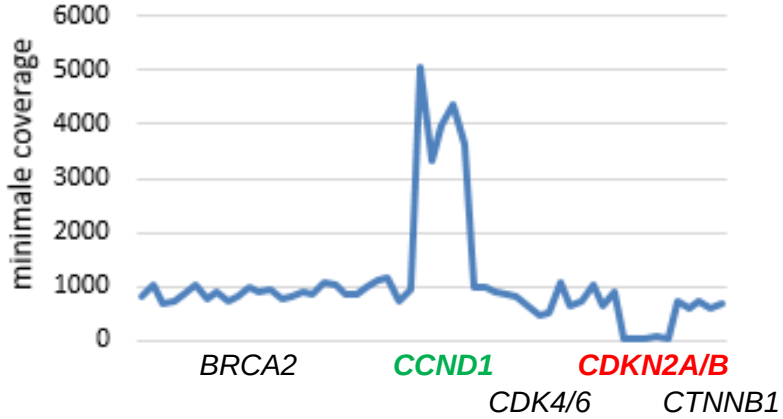
subsitutions (SNVs), deletions, insertions, copy number variants (CNVs) based on coverage

substitutions (SNV)

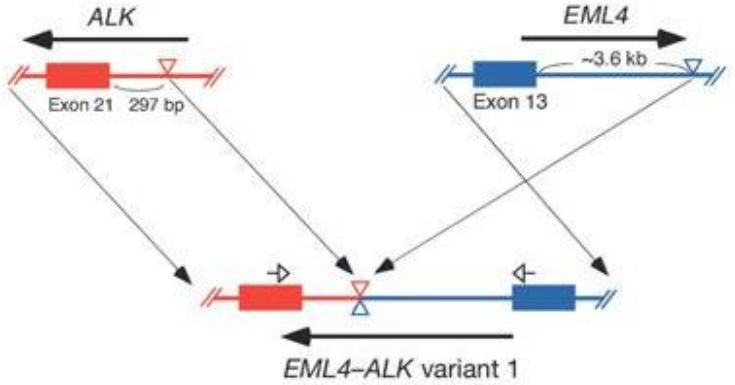
deletions

insertions

copy number variants (CNVs) based on coverage

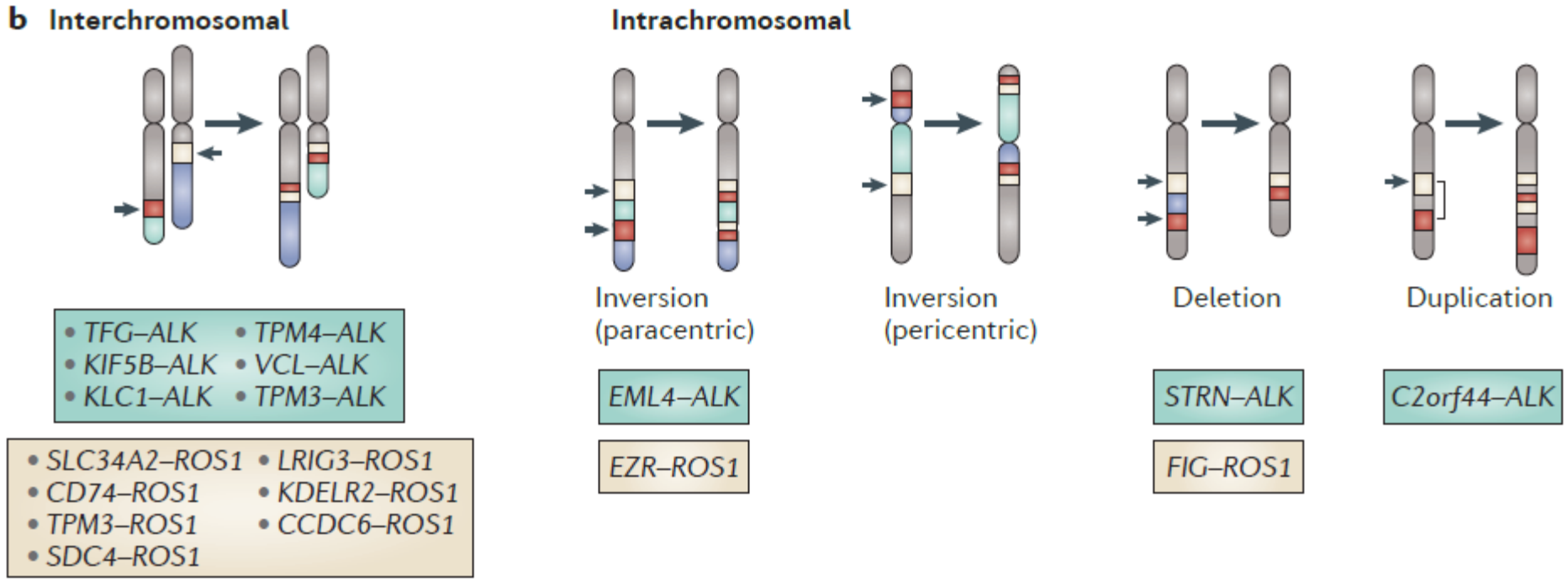


fusions



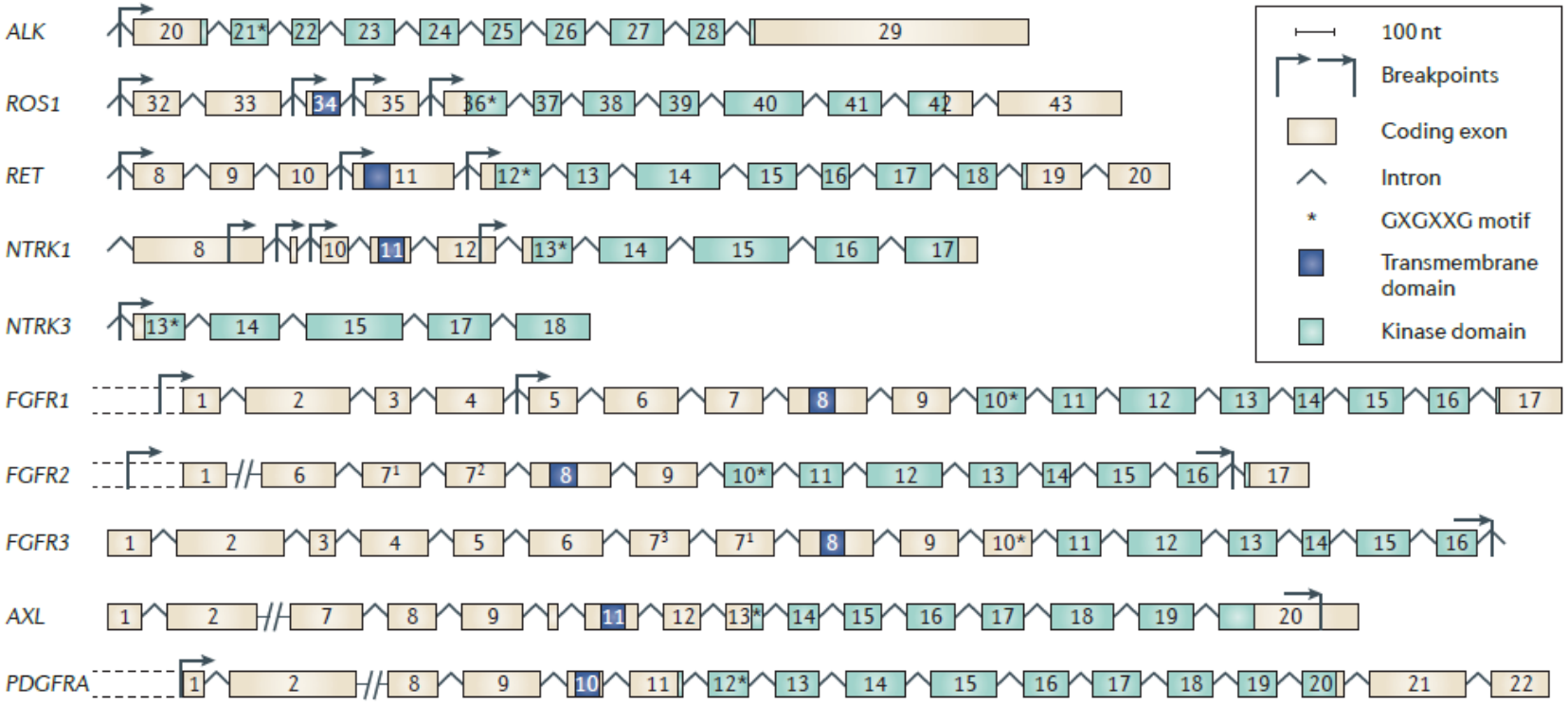
RNA sequencing for fusion detection

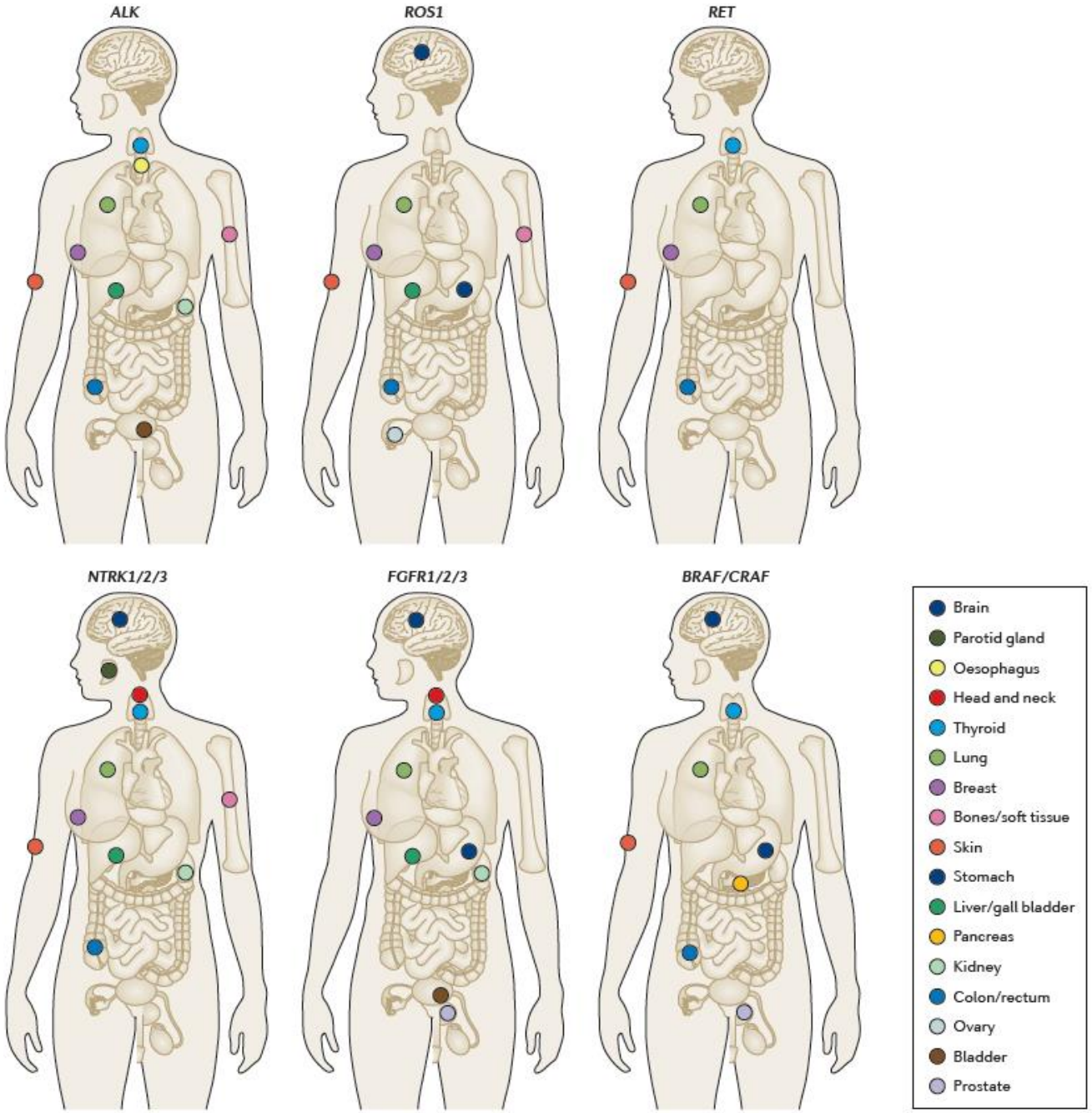
interchromosomal & intrachromosomal rearrangements



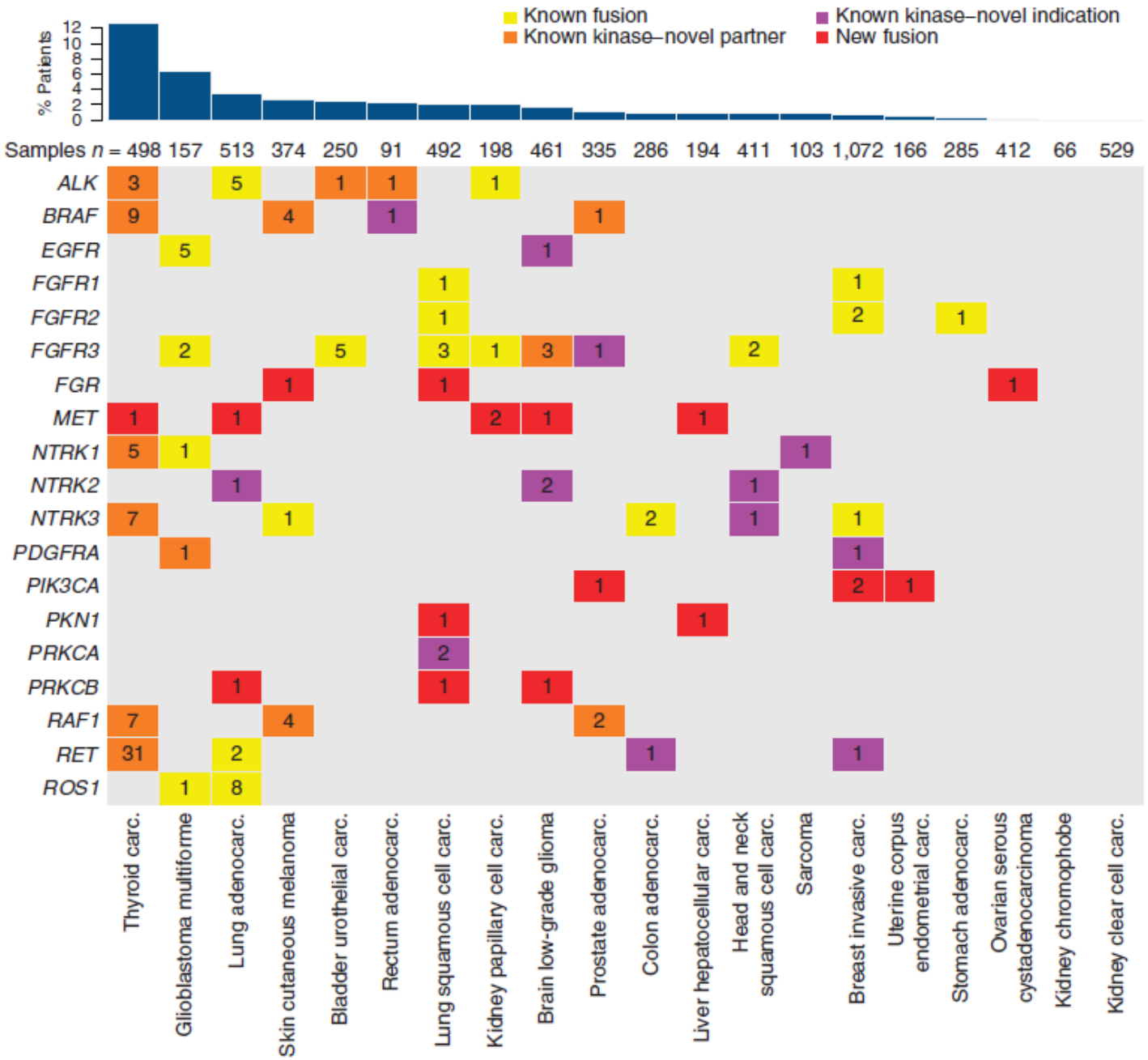
RNA sequencing for fusion detection

targetable tyrosine kinase fusions





Schram et al. Nat Rev Clin Oncol 2017



Stransky et al. Nat Comm 2014

RNA sequencing for fusion detection

fusions characteristic for specific sarcoma subtypes: diagnosis & prognosis

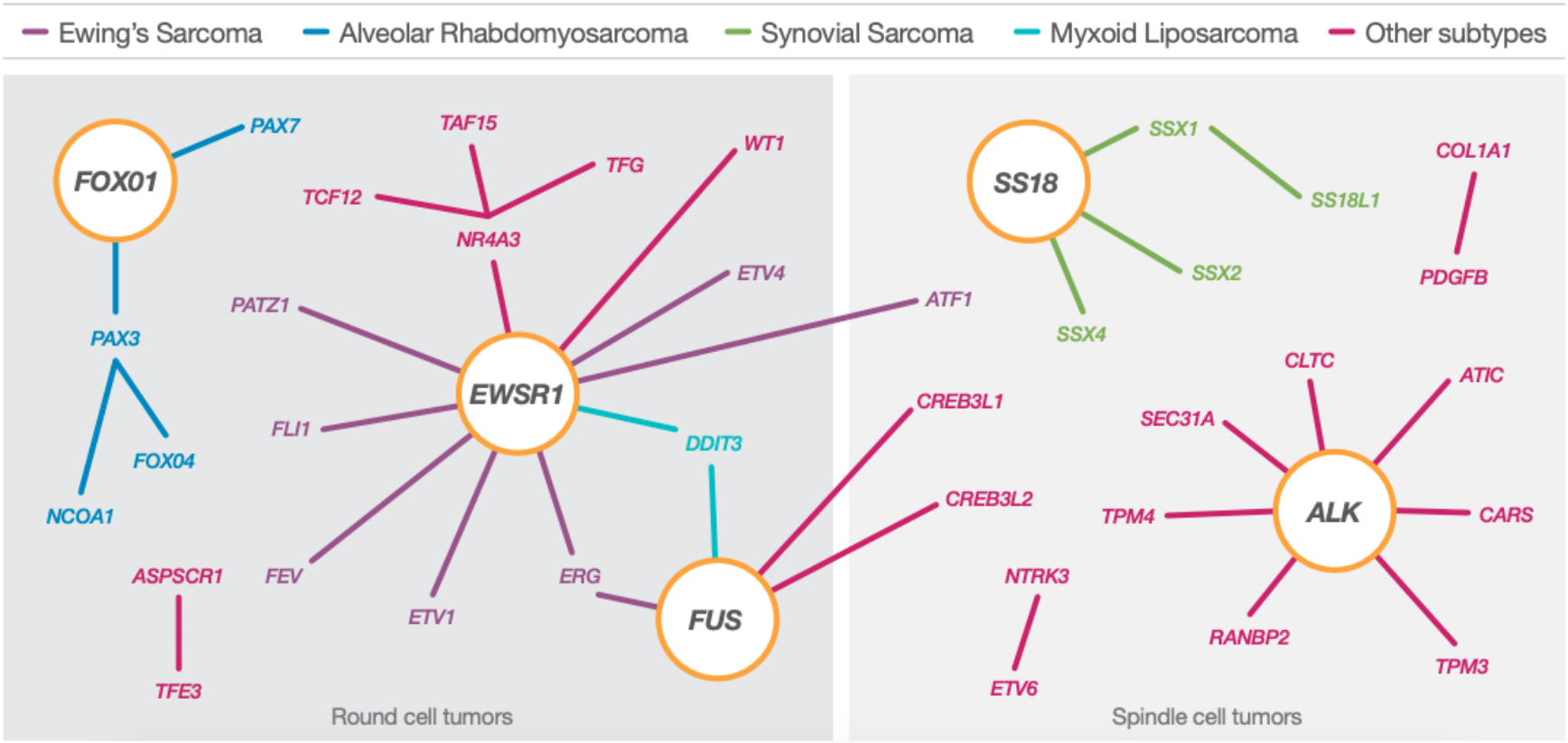


Table 4 | **Gene fusions in malignant solid tumours**

Tumour type	Gene fusion(s)
Sarcomas	
Alveolar soft part sarcoma	*ASPSCR1–TFE3**
Angiomatoid fibrous histiocytoma	*EWSR1–ATF1*, FUS–ATF1**
Bone sarcoma, undifferentiated	*EWSR1–POU5F1*
Chondrosarcoma, myxoid	*EWSR1–NR4A3*, TAF15–NR4A3*, TCF12–NR4A3, TFG–NR4A3*
Clear cell sarcoma	*EWSR1–ATF1**
Dermatofibrosarcoma protuberans	*COL1A1–PDGFB**
Desmoplastic small round-cell tumour	*EWSR1–WT1*, EWSR1–ERG*
Endometrial stromal sarcoma	*JAZF1–PHF1*, JAZF1–SUZ12*, EPC1–PHF1*
Ewing sarcoma or primitive neuroectodermal tumour	*EWSR1–ERG*, EWSR1–ETV1*, EWSR1–ETV4*, EWSR1–FLI1*, EWSR1–FEV*, FUS–ERG*, EWSR1–ZNF278*
Ewing-like soft tissue sarcoma	*CIC–DUX4**
Fibromyxoid sarcoma, low grade	*FUS–CREB3L2*, FUS–CREB3L1*
Fibrosarcoma, infantile	*ETV6–NTRK3**
Inflammatory myofibroblastic tumour	*CARS–ALK*, CLTC–ALK*, RANBP2–ALK*, TPM3–ALK*, ATIC–ALK, SEC31L1–ALK, TPM4–ALK*
Liposarcoma, myxoid	*EWSR1–DDIT3*, FUS–DDIT3**
Rhabdomyosarcoma, alveolar	*PAX3–FOXO1A*, PAX7–FOXO1A*, PAX3–MLLT7, PAX3–NCOA1*
Rhabdomyosarcoma, pleomorphic	*PAX3–FOXO1A*
Synovial sarcoma	*SS18–SSX1*, SS18–SSX2*, SS18–SSX4*, SS18L1–SSX1*
Carcinomas	
Aggressive midline carcinoma	*BRD4–NUT**
Breast carcinoma	*ETV6–NTRK3*, ODZ4–NRG1*, TBL1XR1–RGS17*
Kidney carcinoma	*ALPHA–TFEB*, ASPSCR1–TFE3*, PRCC–TFE3*, CLTC–TFE3, NONO–TFE3, SFPQ–TFE3*
Mucoepidermoid carcinoma	*MECT1–MAML2**
Prostate carcinoma	*TMPRSS2–ERG, TMPRSS2–ETV1, TMPRSS2–ETV4, RPS10–HPR*
Thyroid carcinoma	*AKAP9–BRAF*, PAX8–PPARG*, RET–CCDC6*, RET–GOLGA5*, RET–KTN1*, RET–NCOA4*, RET–PCM1*, RET–PRKAR1A*, RET–RAB6IP2*, RET–RFG9*, RET–TRIM24*, RET–TRIM33*, TFG–NTRK1*, TPM3–NTRK1*, TPM3–TPR*
Other	
Astrocytoma	*GOPC–ROS1*
Mesoblastic nephroma	*ETV6–NTRK3**

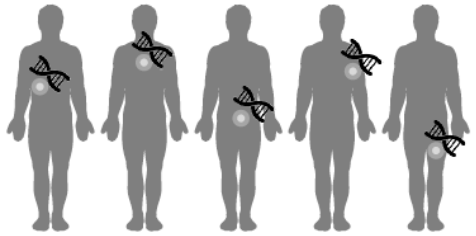
*Recurrent gene fusions.

Mitelman et al. Nat Rev Cancer 2007

RNA sequencing for fusion detection

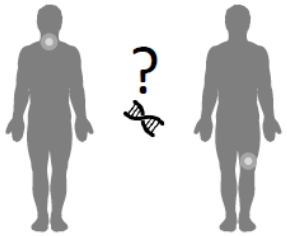
research project lab Vandesompele – APD – MDG: 2 patients cohorts

cohort I (n=16)



different cancer types
with known abberations

cohort II (n=17)



ARMS URCS

illumina®

FFPE samples: TruSight
RNA Pan-Cancer Panel

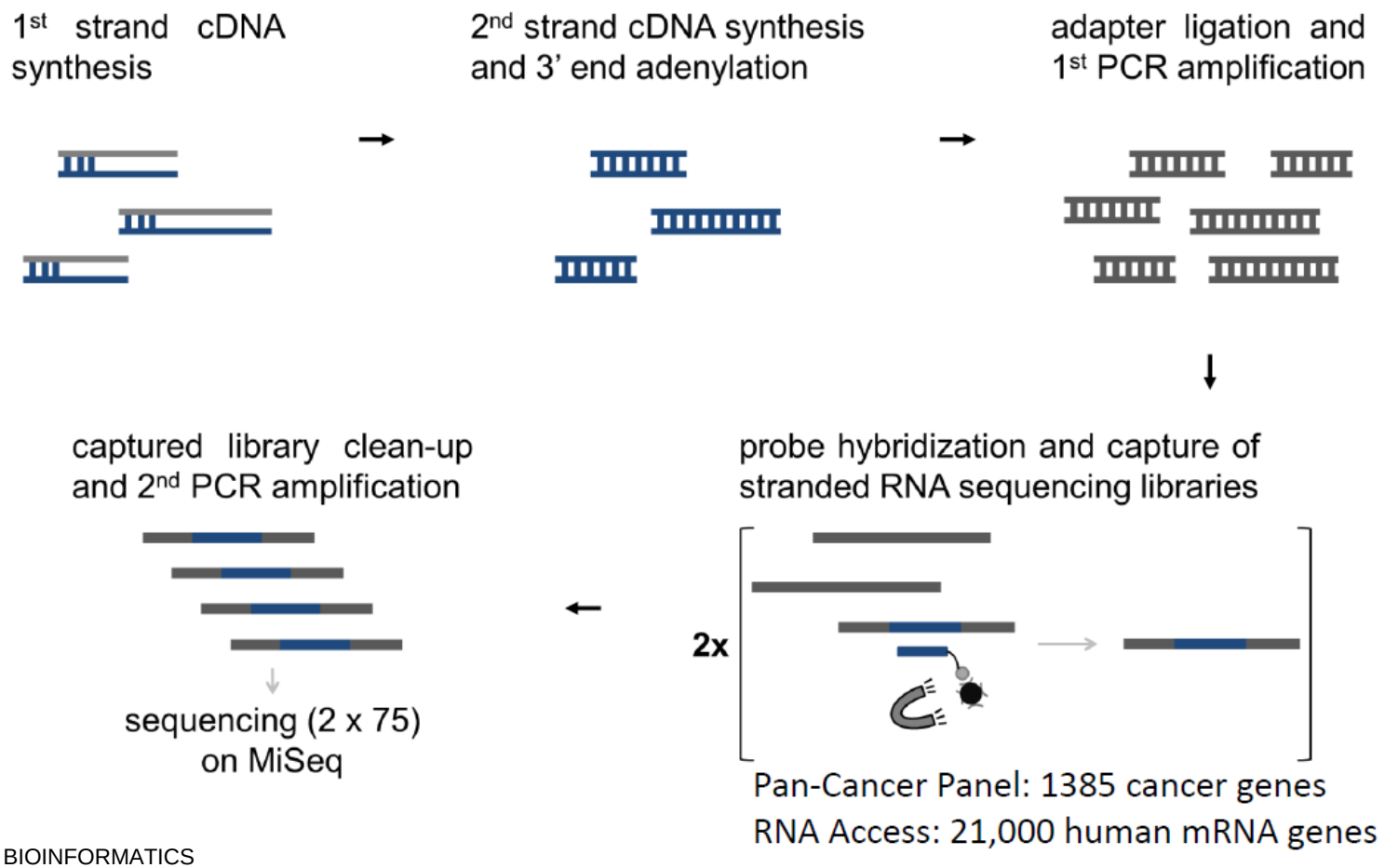
FFPE samples: TruSeq
RNA Access Panel

biogazelle

calling sequencing variants and fusion genes

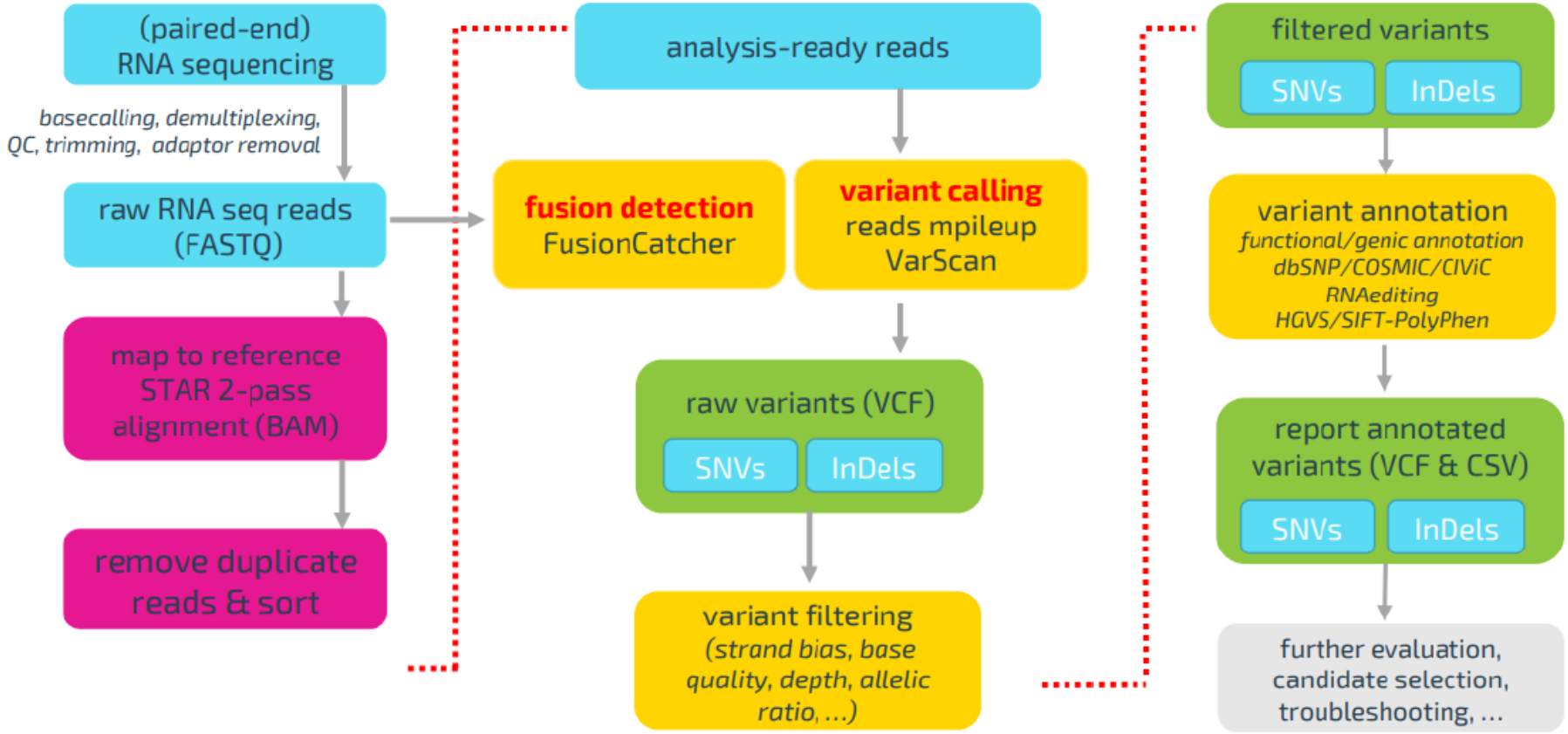
RNA sequencing for fusion detection

research project lab Vandesompele – APD – MDG: TruSight technologie (Illumina)



RNA sequencing for fusion detection

research project lab Vandesompele – APD – MDG: RNA fusion & SNV analysis – pipeline Biogazelle



RNA sequencing for fusion detection

research project lab Vandesompele – APD – MDG: results

cohort I (n=16)

confirmation of known abberations

6 fusion genes
7 SNVs
3 indel variants

cohort II (n=17)

ARMS

p18 - PAX3 FOXO1 -

p25 - PAX3 FOXO1 -

URCS

p26 - EWSR1 ERG -

p29 - EWSR1 FLI1 -

p27 - EWSR1 NFATC2 -

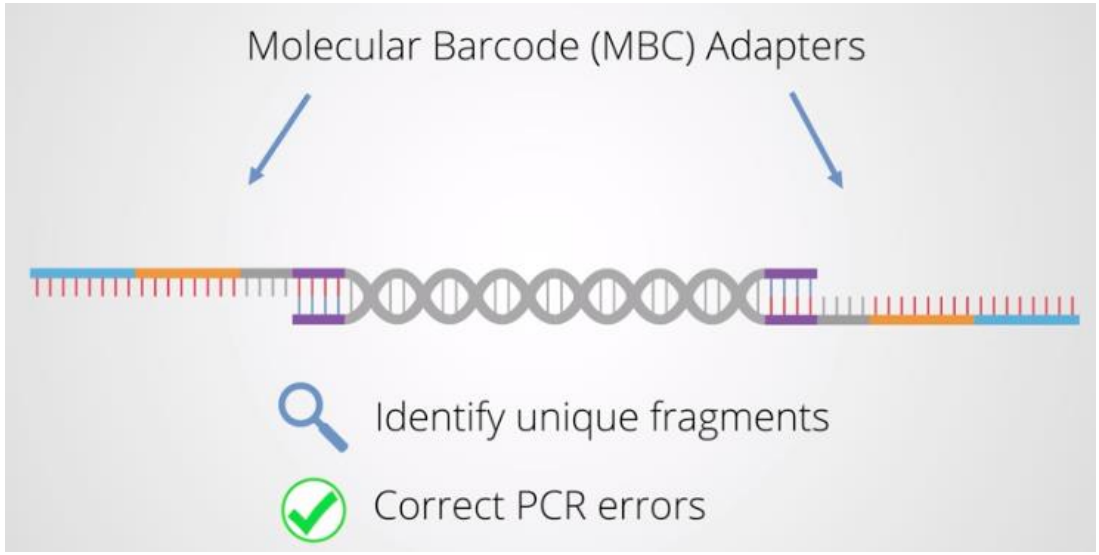
p28 - EWSR1 NFATC2 -

0% of tumor cells independently validated

unclear or only in 10-12% of the tumor cells

RNA sequencing for fusion detection

RNA fusion detection Archer testing for diagnostic routine



- Sarcoma panel
- Solid tumor panel with actionable fusions
- Hemato panel

https://www.youtube.com/watch?v=cHjKsgbmIsY - action=share

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