ΕΦΑΡΜΟΓΗ NGS ΣΤΗ ΔΙΑΓΝΩΣΗ ΤΩΝ ΜΥΟΣΚΕΛΕΤΙΚΩΝ ΟΓΚΩΝ

Αγγελική Σαέττα, Καθηγήτρια, Α’ Εργαστήριο Παθολογικής Ανατομικής

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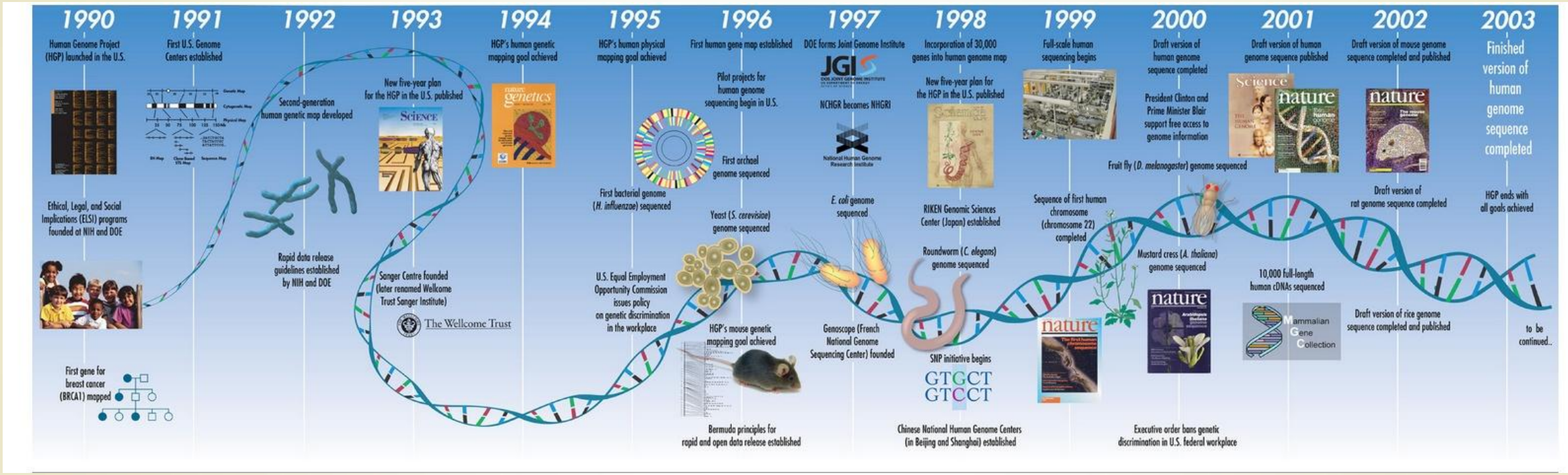
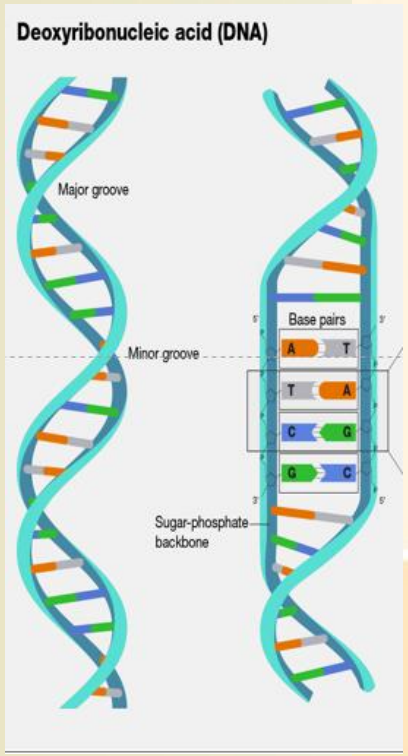
Δομή διάλεξης

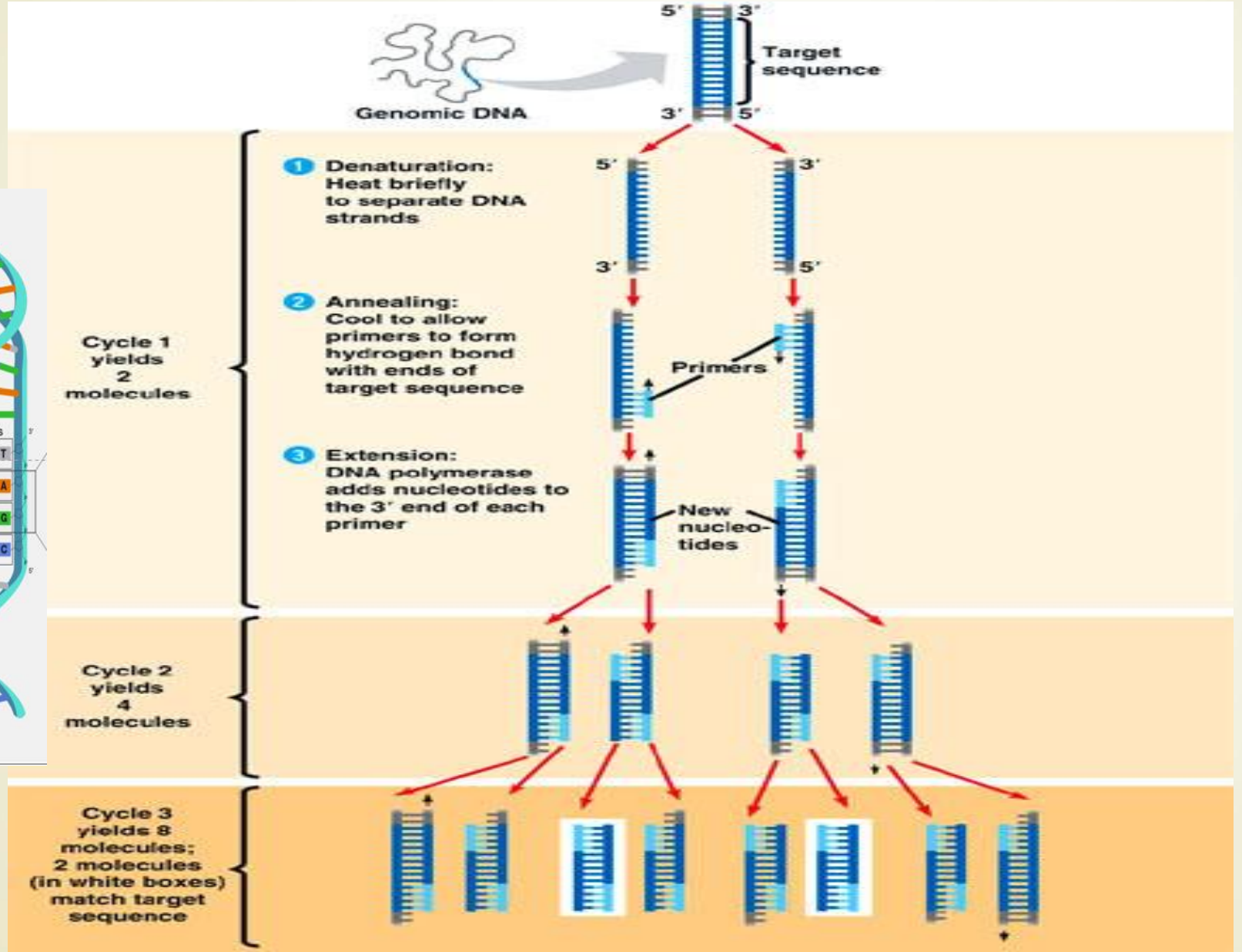
- Εισαγωγή NGS
- Πειραματική διαδικασία NGS
- Ανάλυση σαρκωμάτων με NGS

Ανάλυση του ανθρώπινου γονιδιώματος



Αλυσιδωτή αντίδραση πολυμεράσης
Polymerase Chain Reaction (PCR)





1) DNA (περιοχή του που θέλουμε να ενισχύσουμε)

2) Εκκινητές

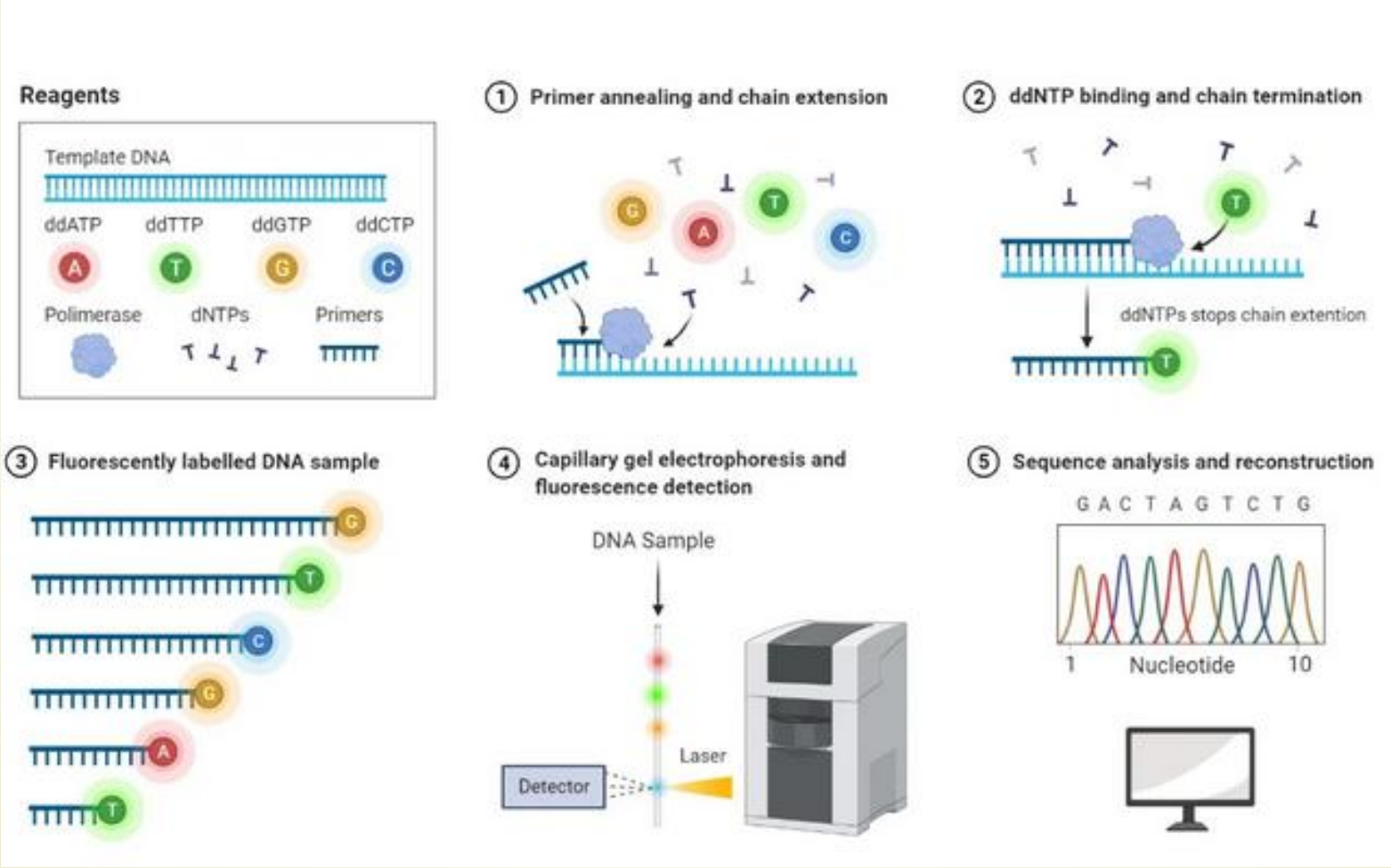
3) Mix:

- Taq πολυμεράση
- dNTPs
- Ρυθμιστικό διάλυμα Tris-HCl
- $MgCl_2$

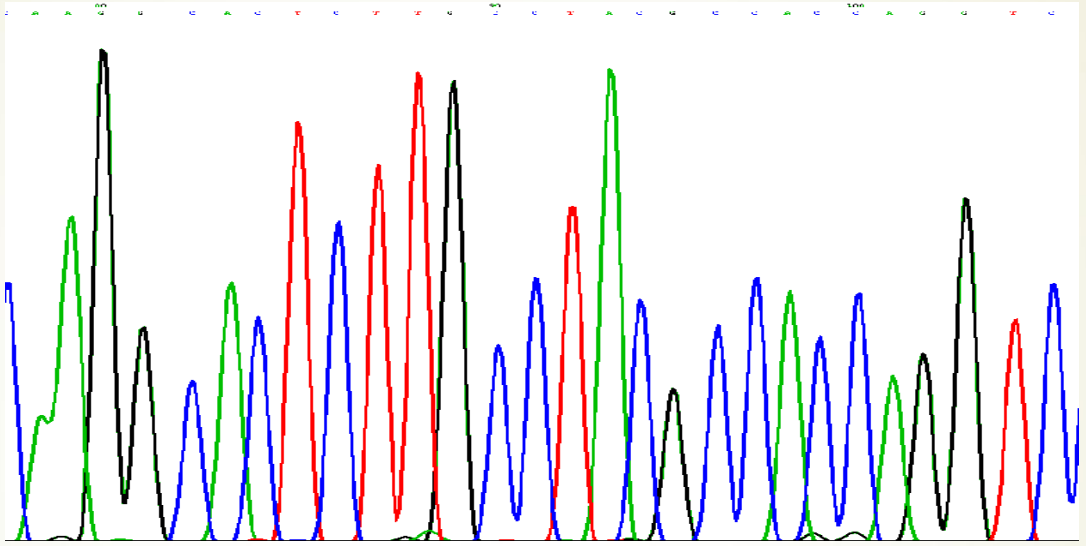
Αλληλούχιση κατά Sanger

Εφαρμογές στη μοριακή διαγνωστική: Ανίχνευση σημειακών μεταλλαγών, ελλείψεις / διπλασιασμοί / ενθέσεις μικρού αριθμού βάσεων

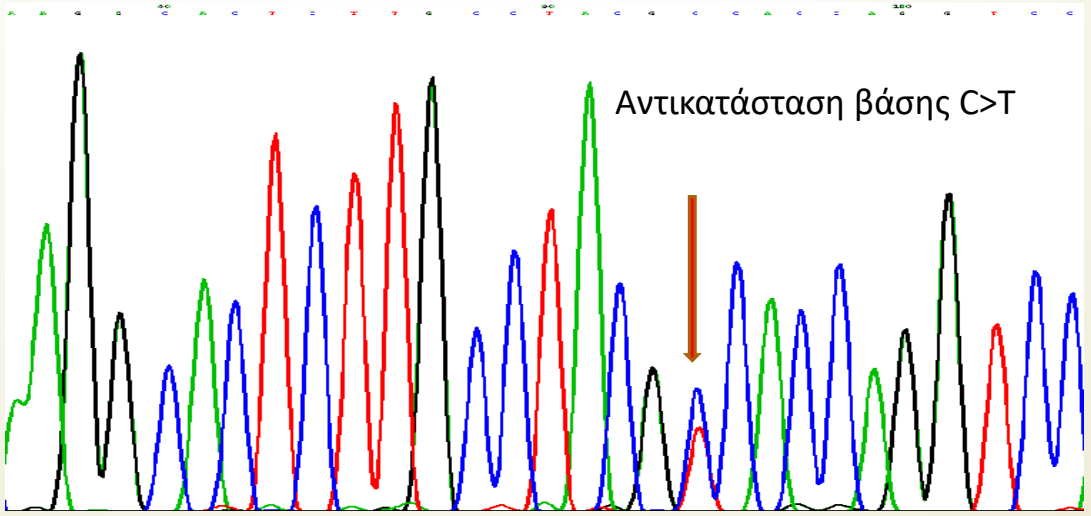
Αρχή της μεθόδου: χρήση διδεοξυ-νουκλεοτιδίων που σταματούν τη σύνθεση της αλληλουχίας καθώς δεν μπορεί να σχηματιστεί φωσφοδιεστερικός δεσμός



Αλληλούχιση κατά Sanger



Φυσιολογική αλληλουχία

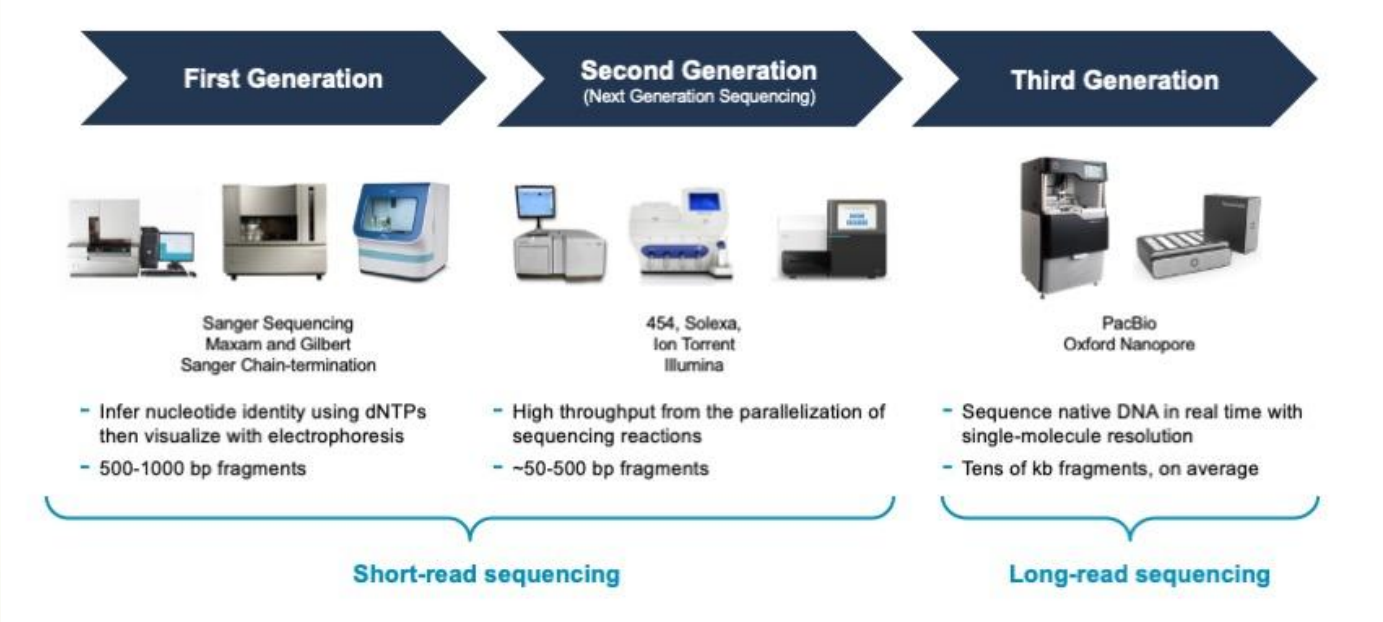


Μεταλλαγμένη αλληλουχία

Αδενίνη: πράσινο χρώμα
Κυτοσίνη: μπλε χρώμα
Θυμίνη: Κόκκινο χρώμα
Γουανίνη: Μαύρο χρώμα

3 γενιές μηχανημάτων αλληλούχισης

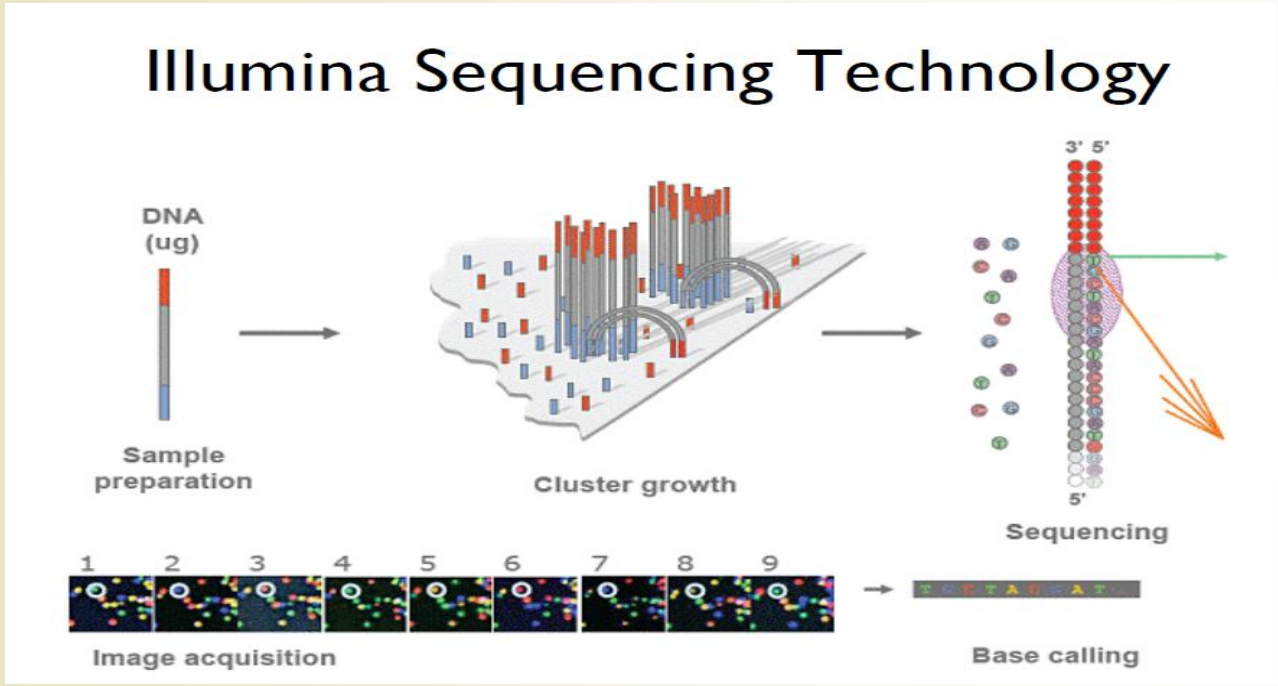
- 1η γενιά
  - Sanger Sequencing
- 2η γενιά
  - Next generation sequencing
- 3η γενιά
  - Third generation sequencing



Αλληλούχιση Νέας Γενιάς
(Next-Generation Sequencing)

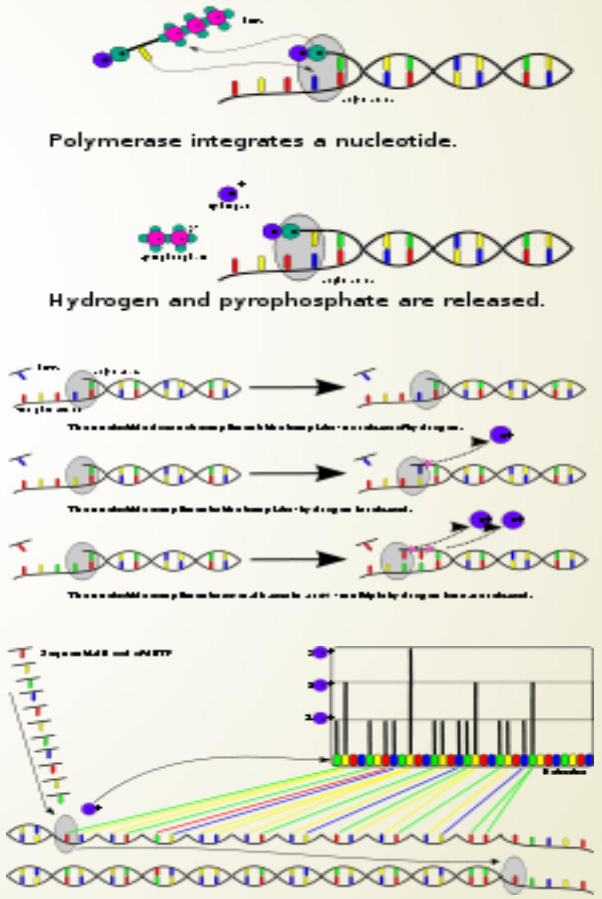
- Διαφορετικές τεχνολογίες αλληλούχισης
- Μαζικό παράλληλο διάβασμα αλληλουχιών DNA
- Υψηλή ποιότητα αλληλούχισης

Διαφορετικές Χημείες NGS



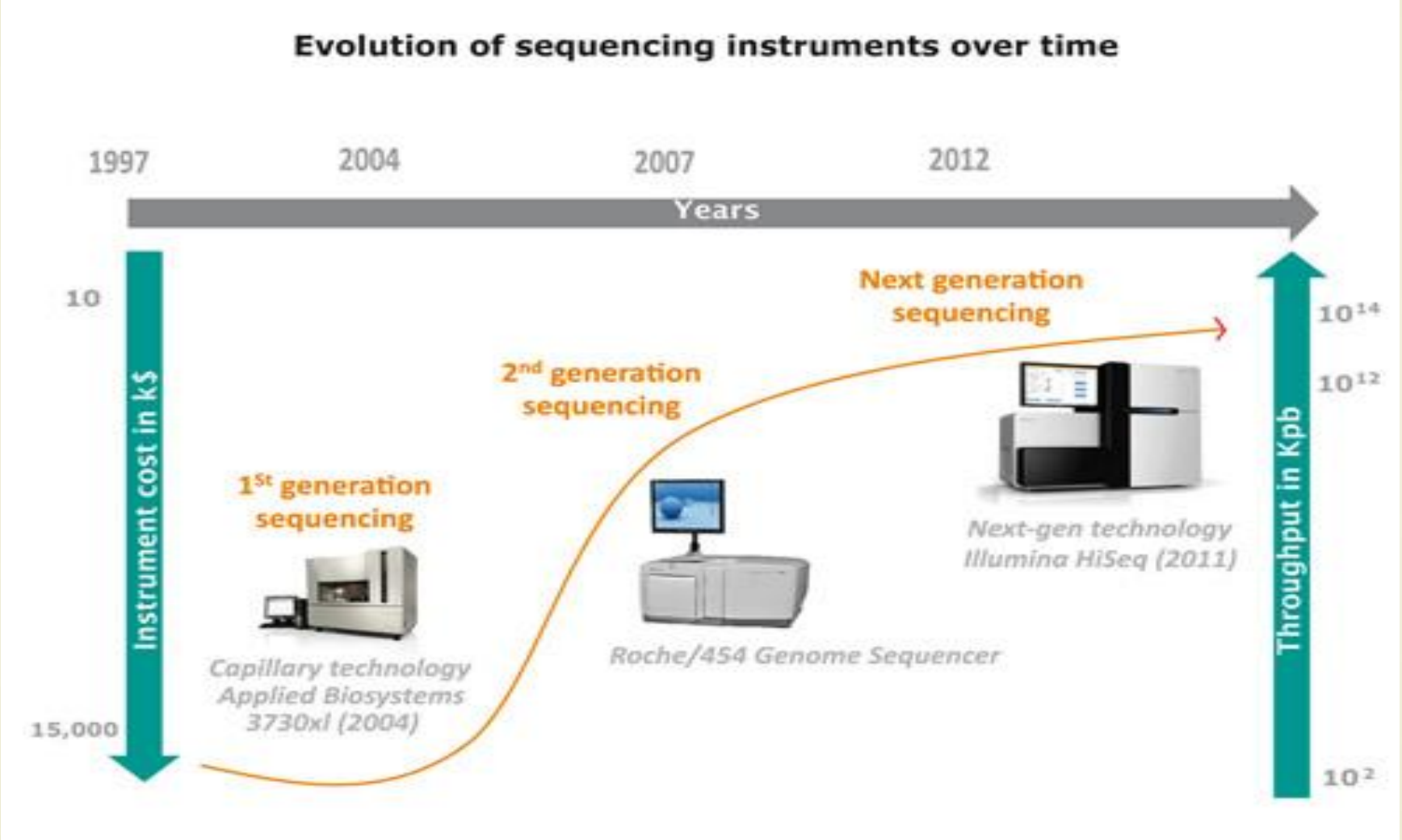
Φθορίζοντα σήματα

Ion semiconductor chemistry/
Ion Torrent



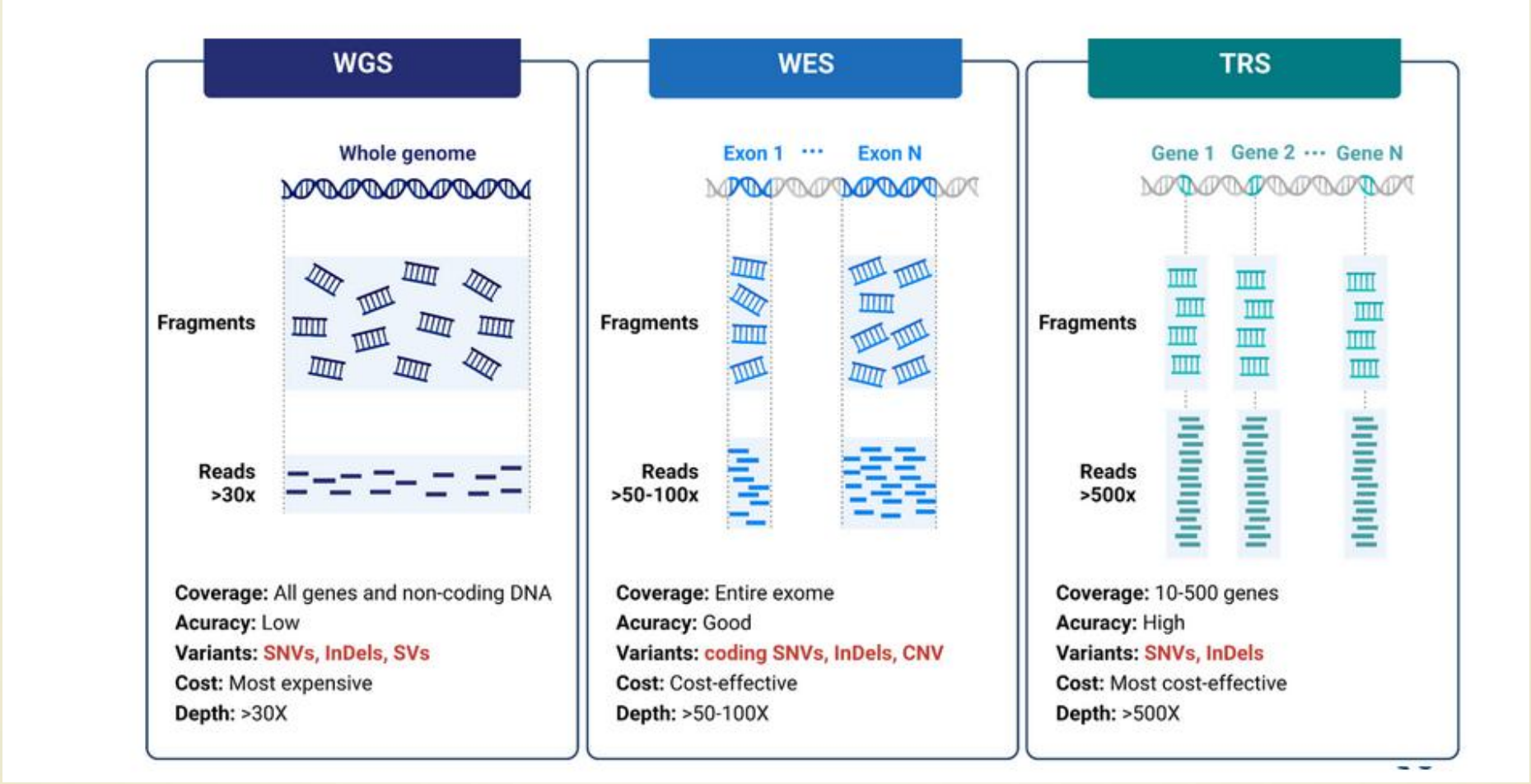
Απελευθέρωση H+, αλλαγή του pH

Κόστος ανάλυσης NGS



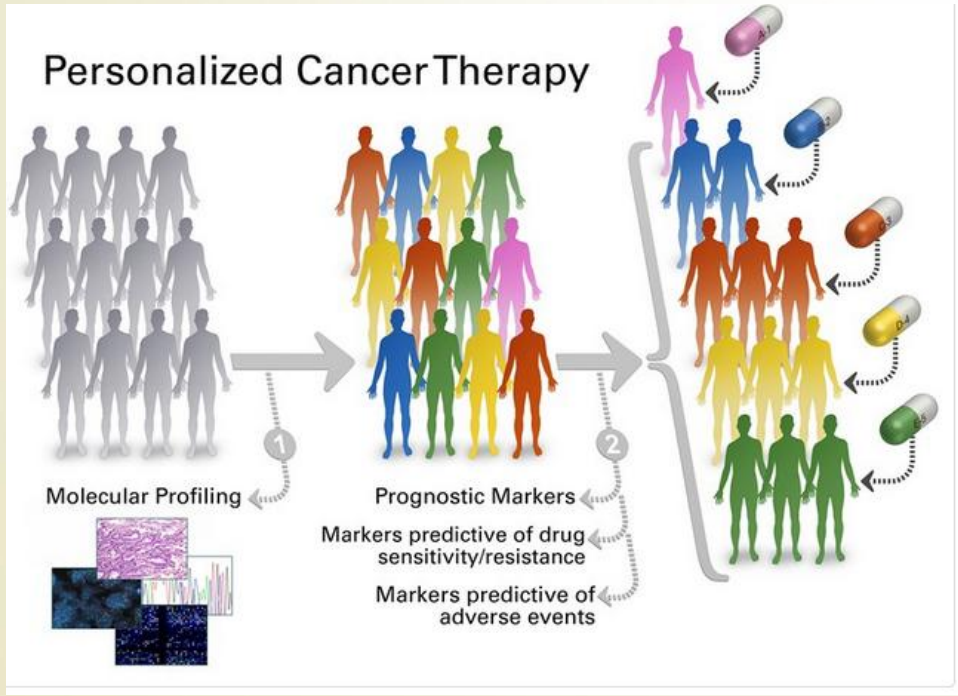
Είδη ανάλυση αλληλούχισης NGS

Whole Genome Sequencing **Whole Exome Sequencing** **Target Region Sequencing**



Εφαρμογές NGS στην Ογκολογία και Μοριακή Παθολογική Ανατομική

- Στην ογκολογία για τη στοχευμένη και εξατομικευμένη ιατρική
- Στην έρευνα: για την ανεύρεση νέων βιοδεικτών, γονιδίων στόχων και γονιδίων ανθεκτικότητας



Εφαρμογή NGS στα Σαρκώματα

NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)

Soft Tissue Sarcoma

Version 5.2024 — March 10, 2025

NCCN.org

NCCN recognizes the importance of clinical trials and encourages participation when applicable and available. Trials should be designed to maximize inclusiveness and broad representative enrollment.

NCCN Guidelines for Patients® available at www.nccn.org/patients

National Comprehensive Cancer Network®

NCCN Guidelines Version 5.2024
Soft Tissue Sarcoma

NCCN Guidelines Index
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Discussion

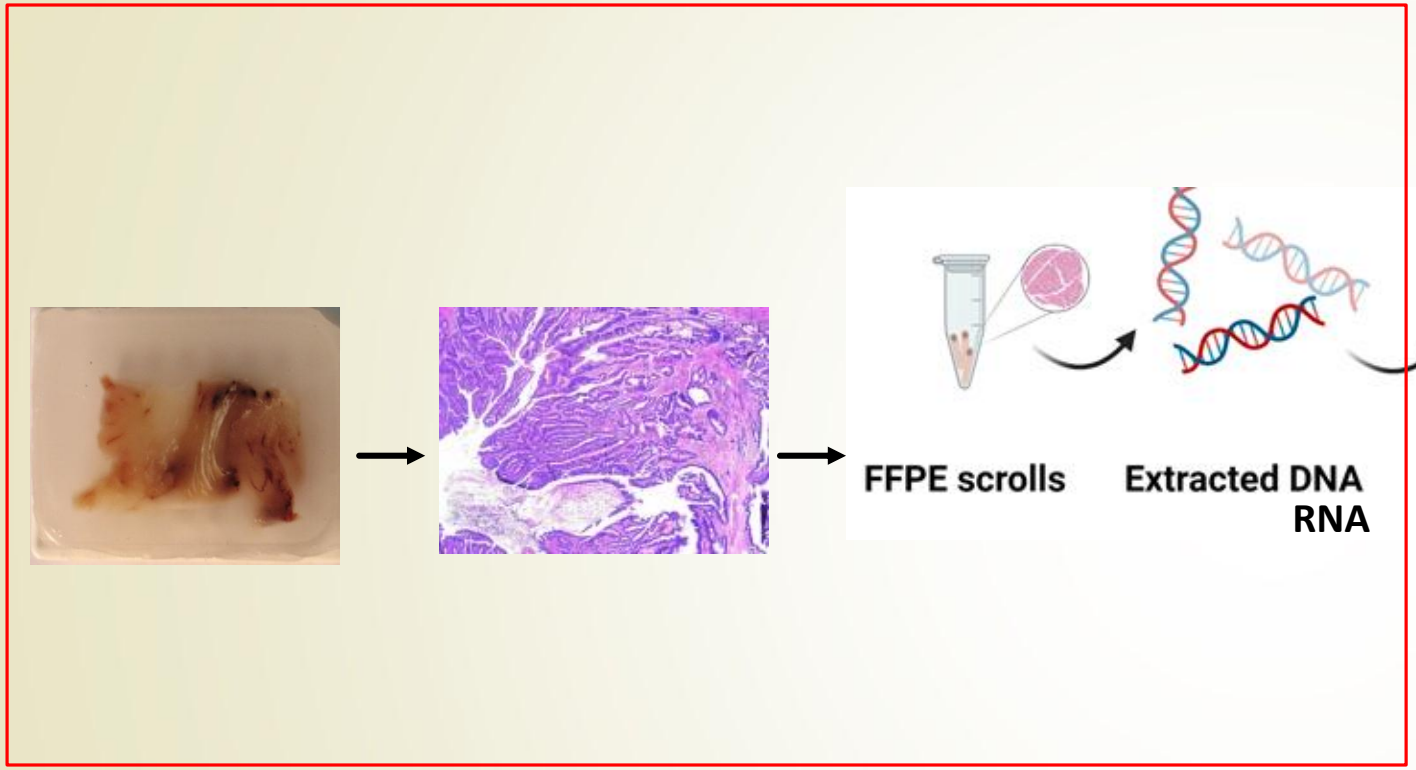
PRINCIPLES OF ANCILLARY TECHNIQUES USEFUL IN THE DIAGNOSIS OF SARCOMAS

Morphologic diagnosis based on microscopic examination of histologic sections remains the gold standard for sarcoma diagnosis. However, several ancillary techniques are useful in support of morphologic diagnosis, including IHC, classical cytogenetics, electron microscopy, and molecular genetic testing. Molecular genetic testing has emerged as an ancillary testing approach since many sarcoma types harbor characteristic genetic aberrations, including single base pair substitutions, deletions and amplifications, and translocations. Molecular testing utilizes multiple techniques such as fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR)-based methods, or next-generation sequencing (NGS)-based methods (including DNA and RNA sequencing).[1] The selection of the "best" technique depends on the individual tumor and clinical needs. NGS may be beneficial; the timing of when to perform NGS and for which patients must be evaluated individually. NGS findings can: determine patient eligibility for clinical trials, identify actionable mutations that may not have been targeted previously, and select patients who may benefit from immunotherapy. Thus, NGS may be appropriate for patients who may qualify for and who are interested in enrolling in a clinical trial or for patients with disease that is refractory or has progressed on standard therapies. NGS also may be helpful in certain histologies where NGS is likely to provide clinically actionable information. NGS should not replace expert pathology review, as NGS only rarely results in a diagnosis change following expert review. Technically successful NGS on bone biopsies requires use of decalcification agents, such as ethylenediaminetetraacetic acid (EDTA), that do not interfere with genomic testing. Each type of molecular testing is associated with test limitations and sources of false-negative results; if negative results are received when a molecular aberration clinically was expected, discussion with the testing lab is highly recommended as testing by another technique may be indicated. Selected recurrent genetic aberrations in sarcoma[2,3] are listed on the next page.

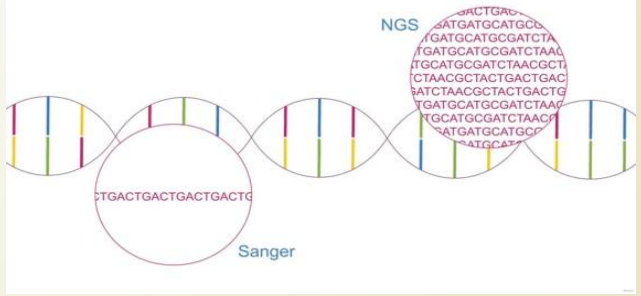
DNA vs RNA

- DNA
  - Μεταλλαγές (ελλείψεις, προσθήκες, σημειακές κτλ)
  - Αριθμός γονιδιακών αντιγράφων (amplification)
- RNA
  - Διαμεταθέσεις
    - Γονίδια σύντηξης (FUSIONS)

Ανάλυση Βιοδεικτών με τη χρήση NGS



NGS



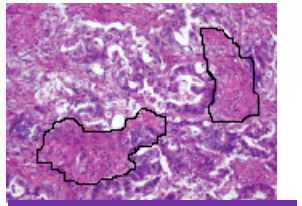
ΑΝΑΛΥΣΗ ΙΣΤΟΥ FFPE

From Cancer Genomics to Precision Oncology—Tissue's Still an Issue

Robert L. Cohen[1] and Jeff Settleman[2,*]
[1]Calico Life Sciences, Suite 1200, 601 Gateway Boulevard, South San Francisco, CA 94080, USA
[2]Discovery Oncology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080, USA
*Correspondence: settleman.jeffrey@gene.com
http://dx.doi.org/10.1016/j.cell.2014.05.027

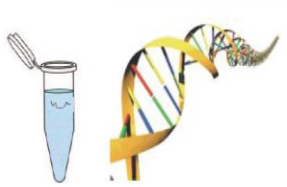
Επάρκεια ιστού

Επαρκής ιστός για τις μοριακές αναλύσεις



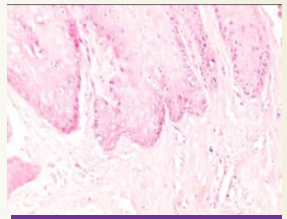
% καρκινικών κυττάρων

Επιλογή κατάλληλης περιοχής προς ανάλυση (macro-dissection)



Συγκέντρωση DNA/RNA

Επαρκή ποσότητα DNA/ RNA για τις μοριακές αναλύσεις
Συγκέντρωση ng/μl
Ratio 260/280nm



Ποιότητα ιστού

Η ποιότητα του ιστού καθορίζει το αποτέλεσμα της μοριακής εξέτασης

FFPE = formalin-fixed, paraffin-embedded

ΠΡΟΑΝΑΛΥΤΙΚΗ ΔΙΑΔΙΚΑΣΙΑ-ΠΑΡΑΓΟΝΤΕΣ ΠΟΥ ΕΠΗΡΕΑΖΟΥΝ ΤΗΝ ΠΟΙΟΤΗΤΑ DNA/RNA

- **Πριν τη μονιμοποίηση του ιστού**

- Χρόνος και συνθήκες πριν τη μονιμοποίηση

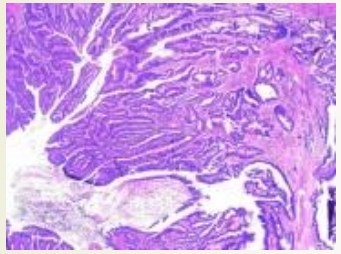
- **Κατά τη μονιμοποίηση**

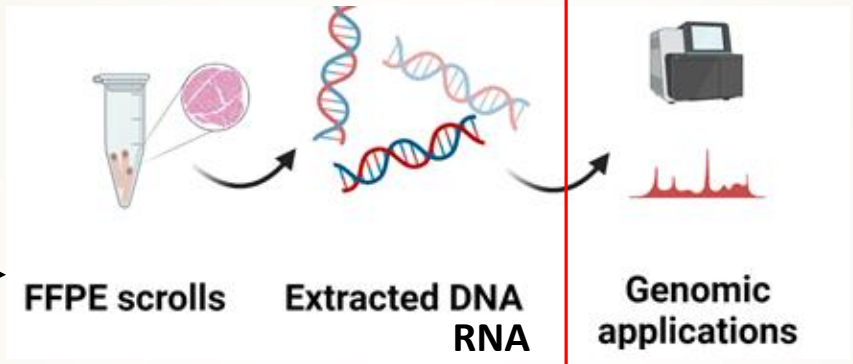
- Μηχανισμός δράσης μονιμοποιητικού μέσου
- Η διάρκεια παραμονής στο μονιμοποιητικό μέσο
- Εμβάπτιση του ιστού σε αφαλατωτικό υγρό μετά τη μονιμοποίηση του
- Η επίδραση παραφίνης (θερμοκρασία τήξης)

- **Μετά τη μονιμοποίηση του υλικού FFPE**

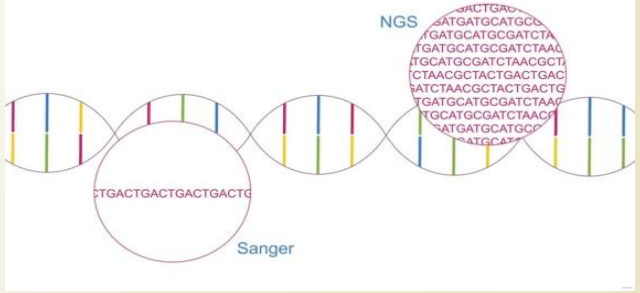
- Διάρκεια αποθήκευσης
- Συνθήκες

Ανάλυση Βιοδεικτών με τη χρήση NGS

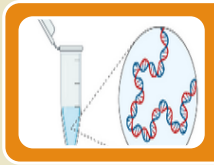




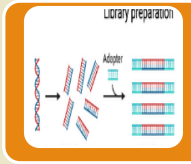
NGS



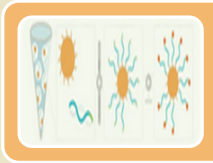
Ροή εργασίας NGS σε δείγματα DNA με τη χρήση πλατφόρμας Ion torrent



Απομόνωση DNA από δείγμα ιστού FFPE

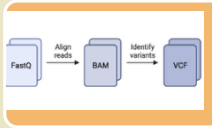


Προετοιμασία βιβλιοθηκών DNA, PCR με τους εκκινητές του panel

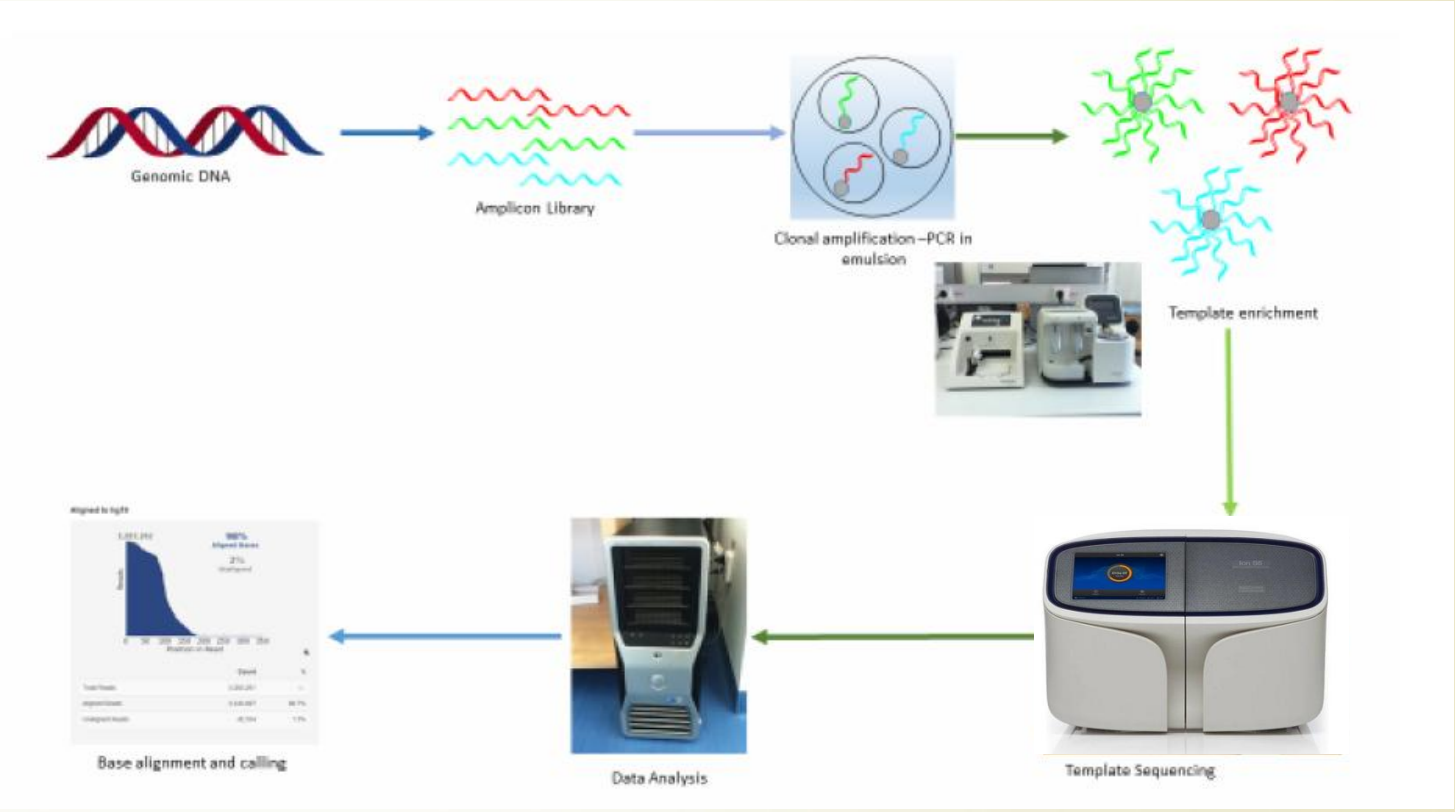


Ενίσχυση βιβλιοθηκών με Emulsion PCR

Αλληλούχιση



Βιοπληροφορική ανάλυση



Lucan et al., 2016

Panels γονιδίων

Εμπορικά διαθέσιμα (IVD ή χωρίς)

FDA approved FoundationOne CDx, MSK-IMPACT,Oncomine Dx Target Test for lung cancer,Illumina Extended RAS Panel for colon cancer, Foundation Focus CDx BRCA LOH

Custom made: σχεδιασμένα σύμφωνα με τις ανάγκες του εργαστηρίου

Table 1. Ion AmpliSeq Colon and Lung Cancer Research Panel v2.

Sample type	FFPE samples
Application	Somatic mutation detection
Genes	*KRAS, EGFR, BRAF, PIK3CA, AKT1, ERBB2, PTEN, NRAS, STK11, MAP2K1, ALK, DDR2, CTNNB1, MET, TP53, SMAD4, FBX7, FGFR3, NOTCH1, ERBB4, FGFR1,* and *FGFR2*

The Ion AmpliSeq Cancer Hotspot Panel v2 targets 50 genes

ABL1	*EGFR*	*GNAS*	*KRAS*	*PTPN11*
AKT1	*ERBB2*	*GNAQ*	*MET*	*RB1*
ALK	*ERBB4*	*HNF1A*	*MLH1*	*RET*
APC	*EZH2*	*HRAS*	*MPL*	*SMAD4*
ATM	*FBXW7*	*IDH1*	*NOTCH1*	*SMARCB1*
BRAF	*FGFR1*	*JAK2*	*NPM1*	*SMO*
CDH1	*FGFR2*	*JAK3*	*NRAS*	*SRC*
CDKN2A	*FGFR3*	*IDH2*	*PDGFRA*	*STK11*
CSF1R	*FLT3*	*KDR*	*PIK3CA*	*TP53*
CTNNB1	*GNA11*	*KIT*	*PTEN*	*VHL*

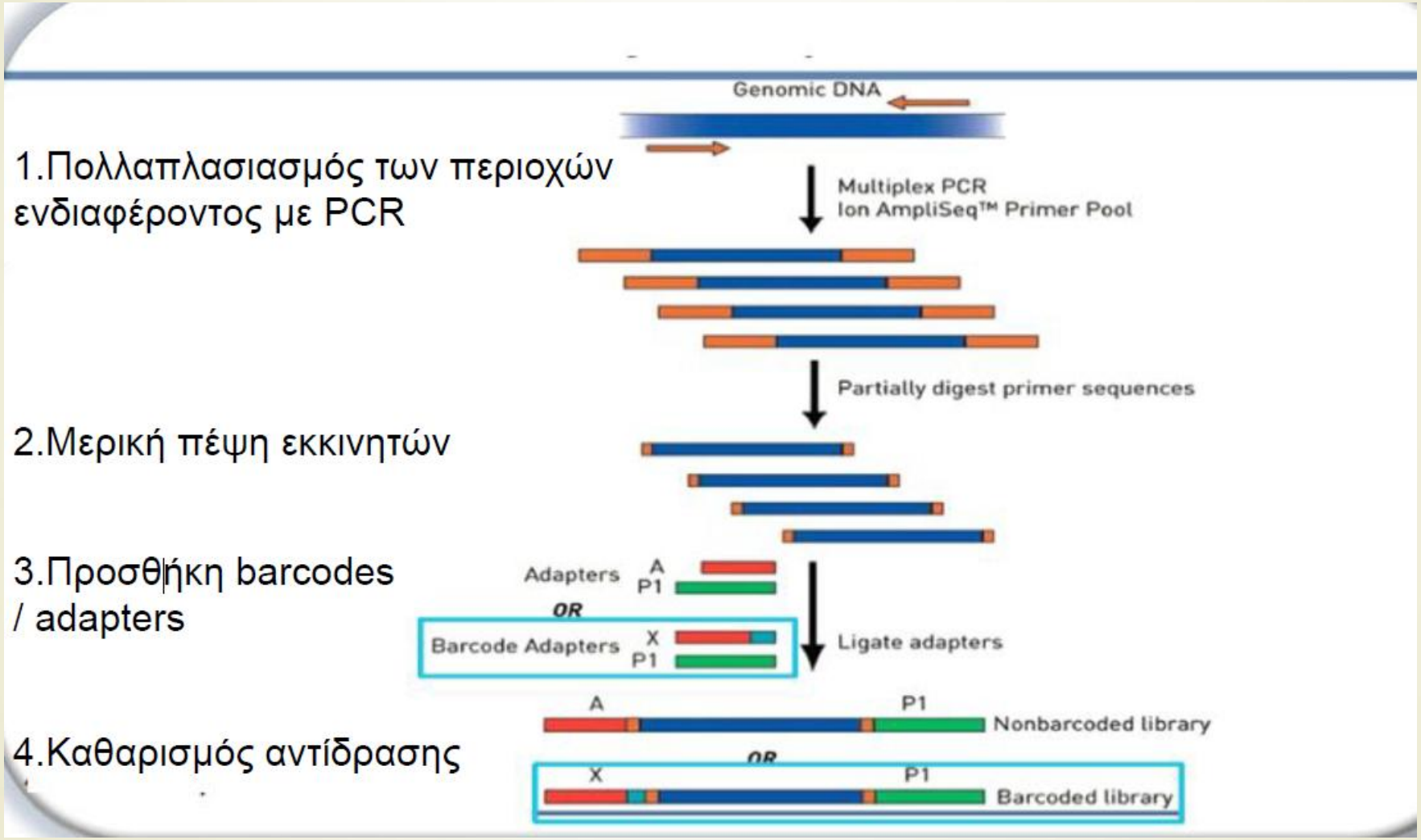
Προετοιμασία βιβλιοθήκων

1.Πολλαπλασιασμός των περιοχών ενδιαφέροντος με PCR

2.Μερική πέψη εκκινητών

3.Προσθήκη barcodes / adapters

4.Καθαρισμός αντίδρασης



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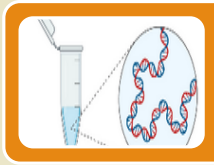
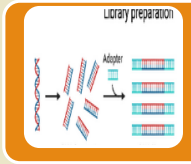


Απομόνωση DNA από δείγμα ιστού FFPE



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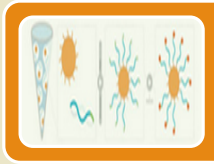
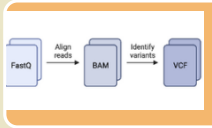
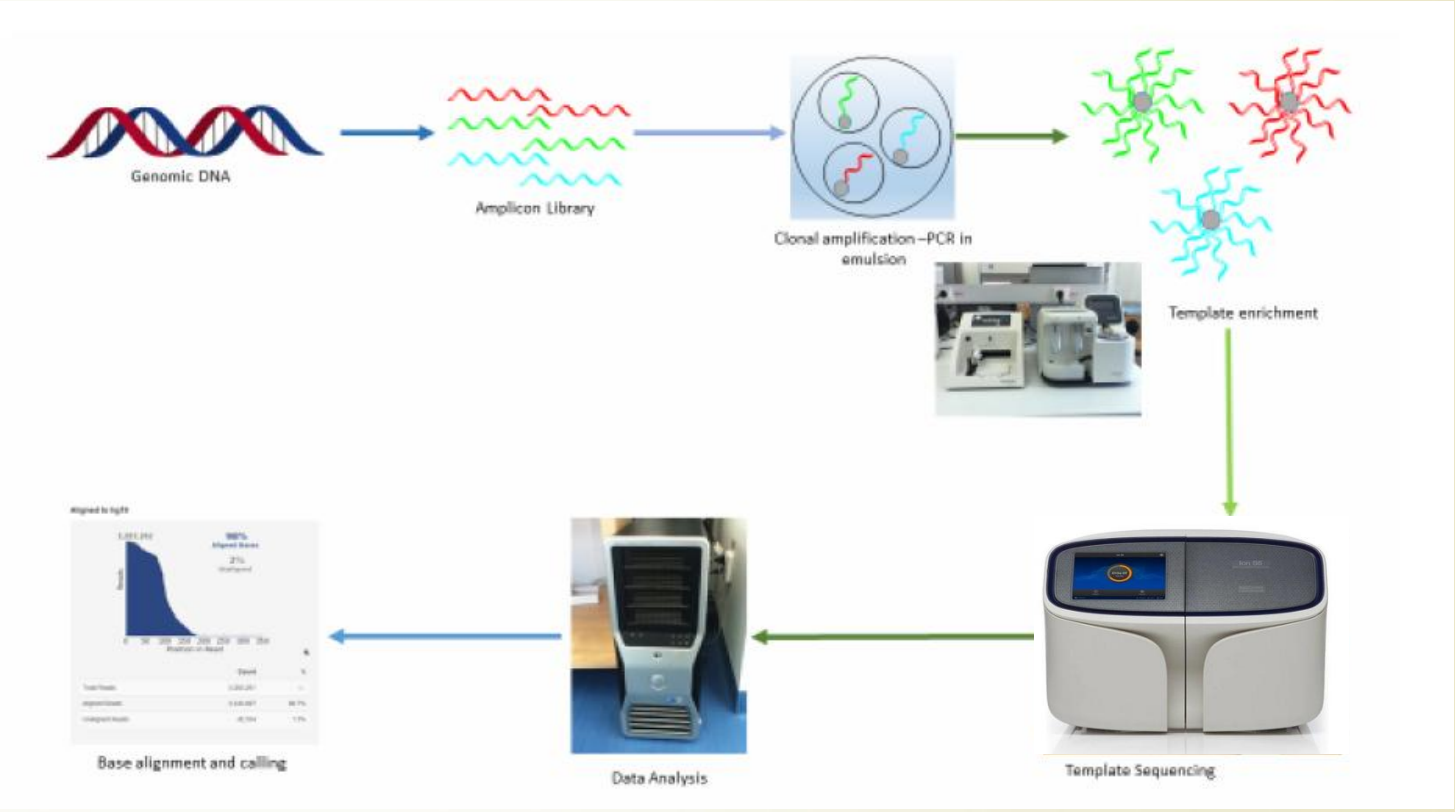


Ενίσχυση βιβλιοθηκών με Emulsion PCR

Αλληλούχιση



Βιοπληροφορική ανάλυση



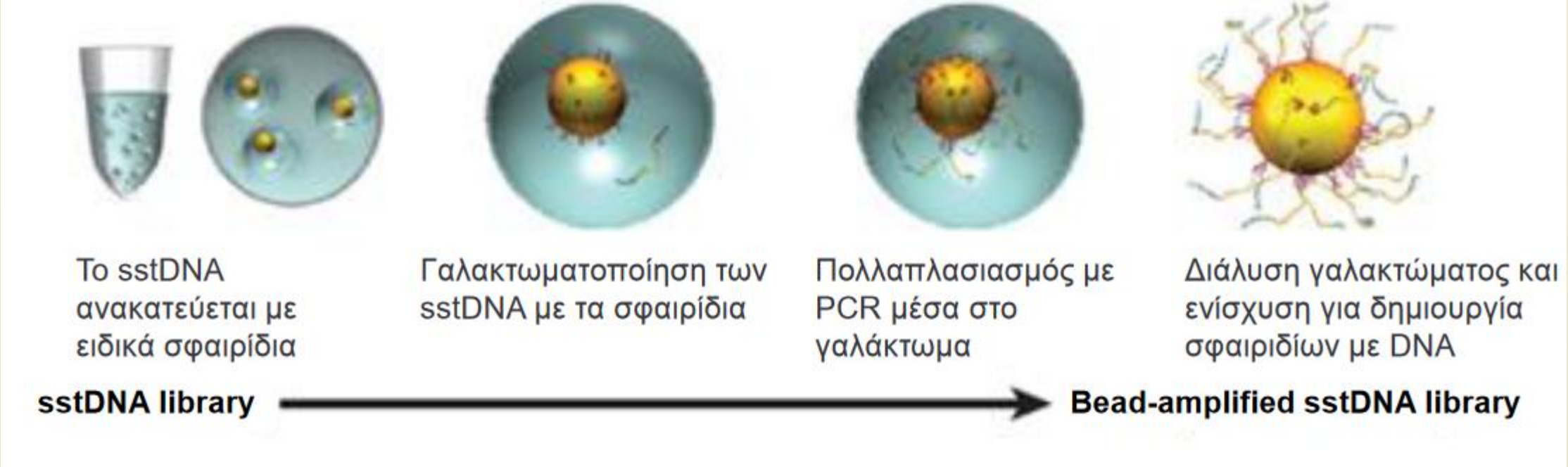
Lucan et al., 2016

Emulsion PCR

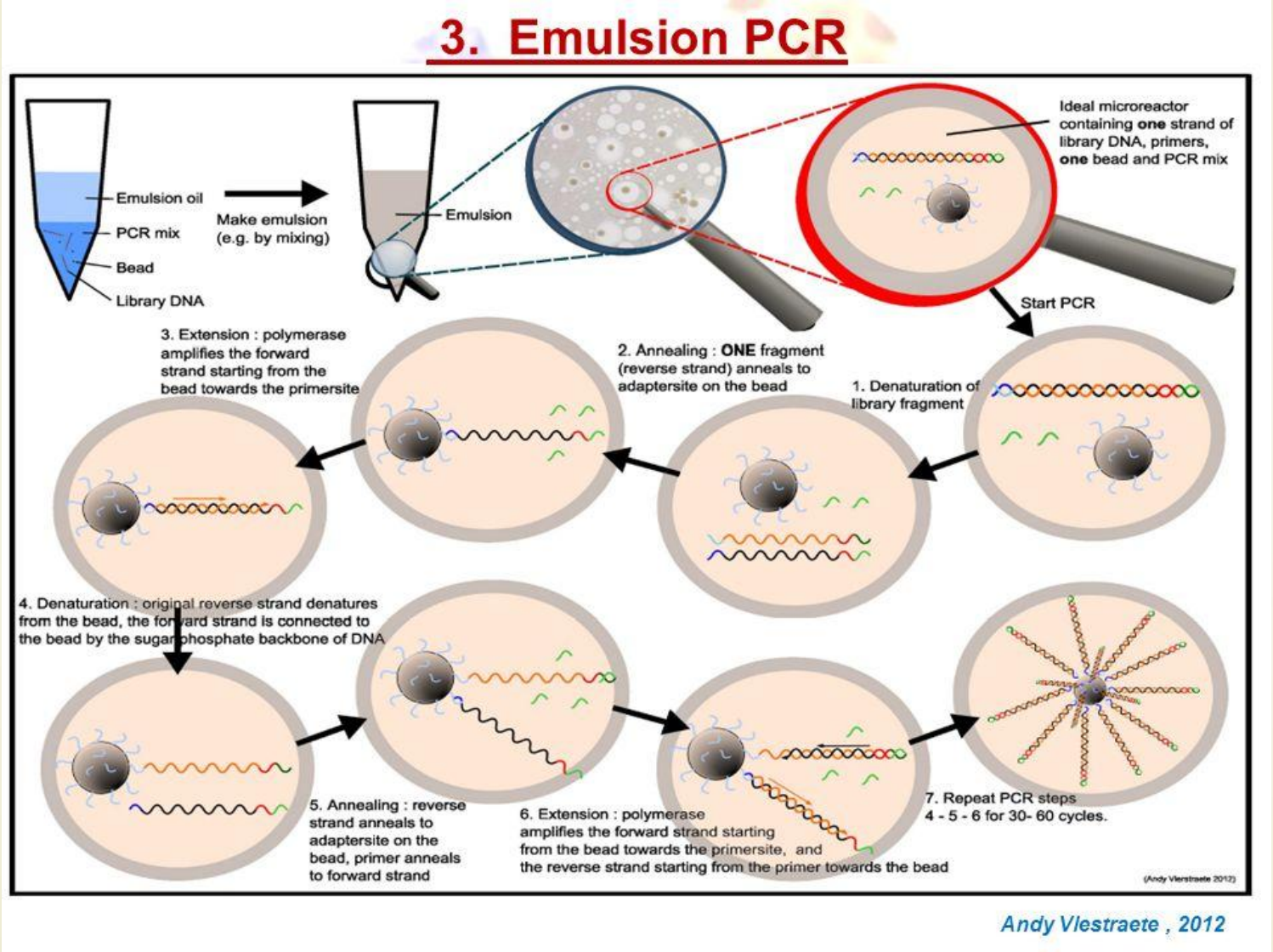
Ποσοτικοποίηση βιβλιοθηκών με Qubit

Ισομοριακό δείγμα – (όλα τα δείγματα, ίση συγκέντρωση και όγκο, σε 1 σωληνάριο)

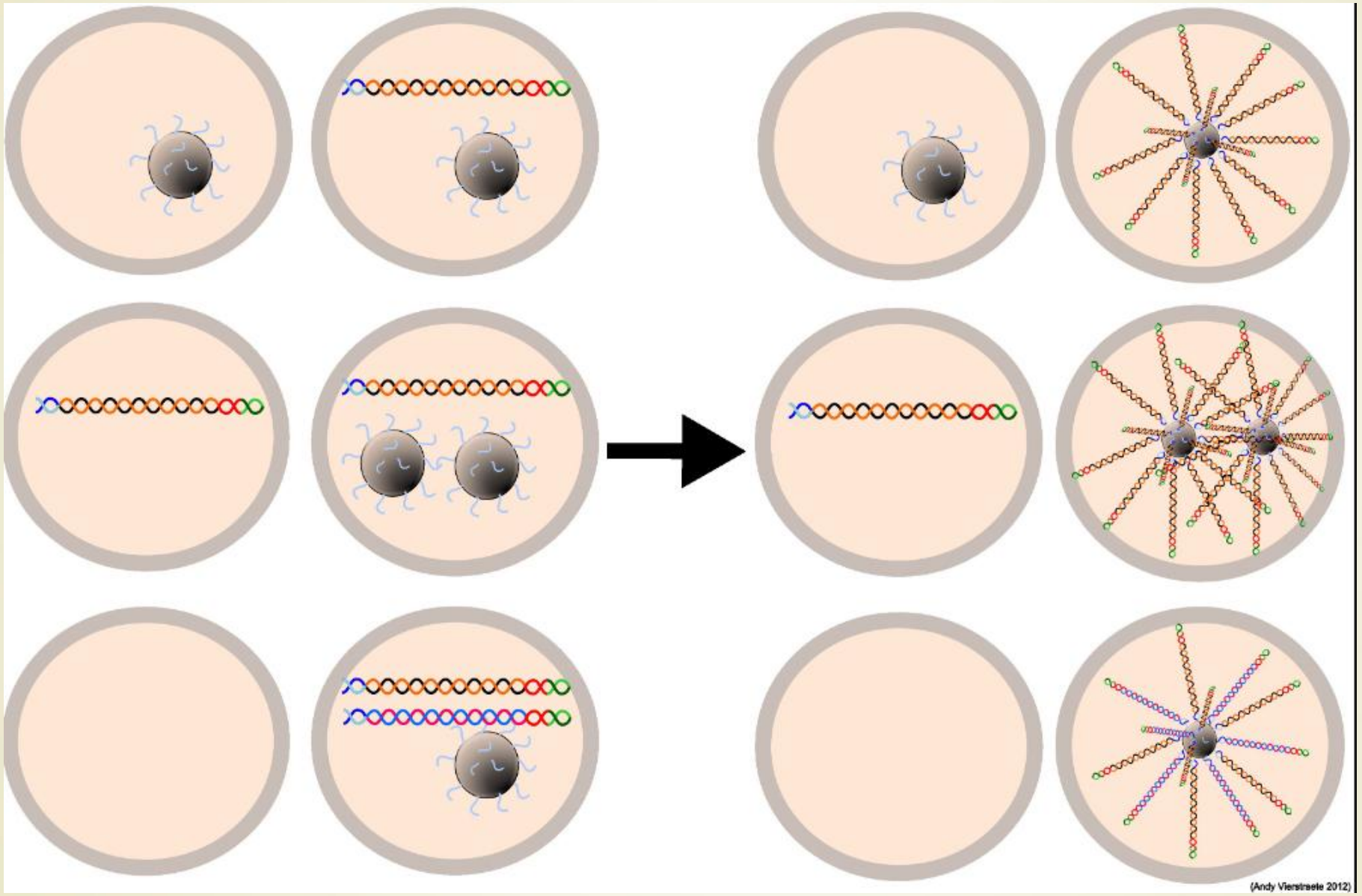
Ακολουθεί One Touch με Emulsion PCR: 1 κλώνος βιβλιοθήκης σε ένα κυστίδιο το οποίο περιέχει ISPs, πολυμεράση, εκκινητές, dNTPS



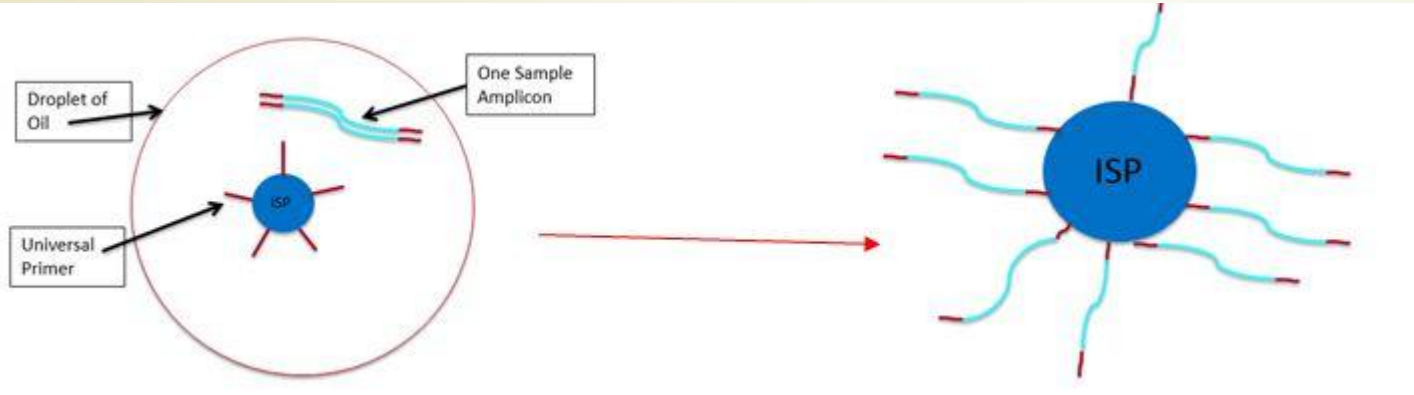
Emulsion PCR

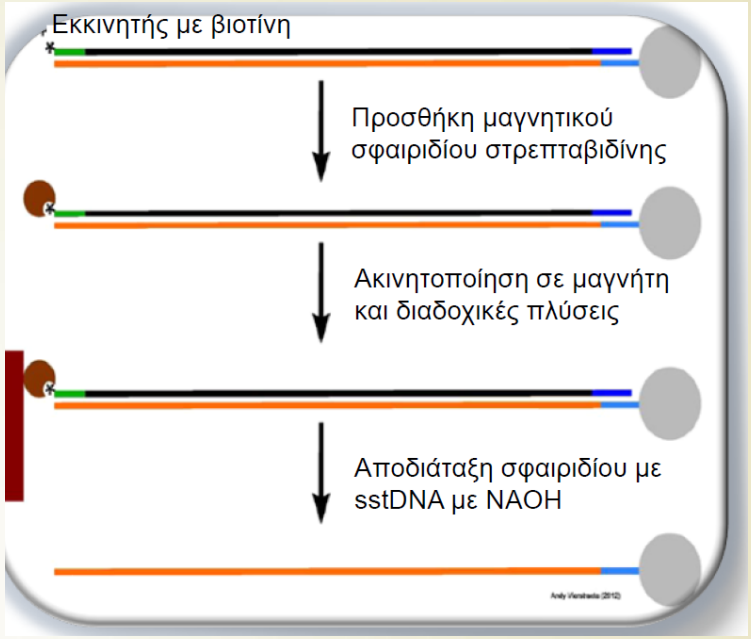


Emulsion PCR

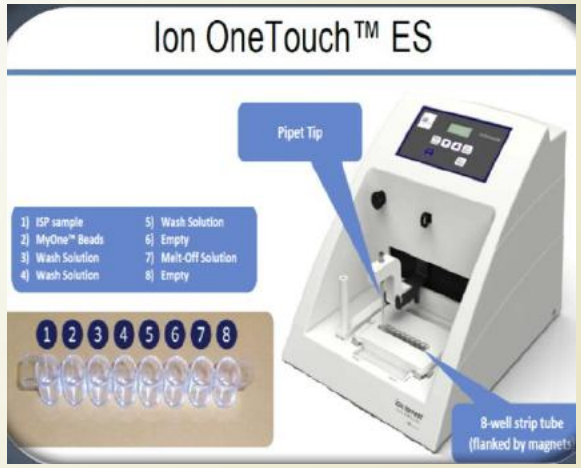


Enrichment: Ενίσχυση βιβλιοθηκών



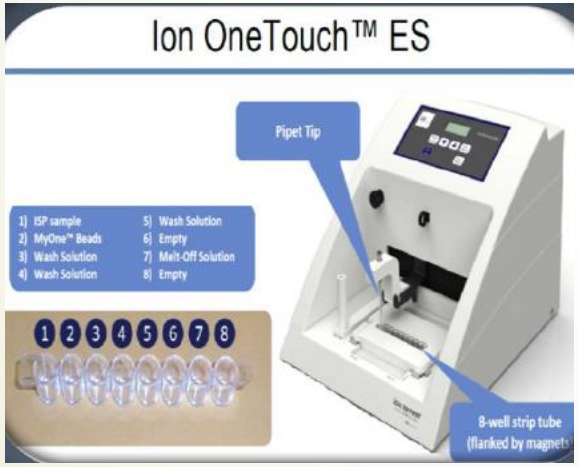


Η διαδικασία εμπλουτισμού του εκμαγείου αλληλούχησης όπου απομακρύνονται τα κενά ISPs, εκείνα δηλαδή στα οποία δεν έχει γίνει ενίσχυση της βιβλιοθήκης μας

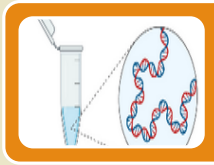


Ion Chef Instrument

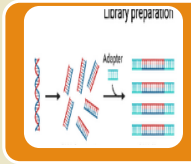
Ion One Touch 2 Instrument and Ion One Touch ES instrument



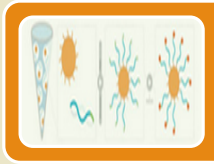
Ροή εργασίας NGS σε δείγματα DNA με τη χρήση πλατφόρμας Ion torrent



Απομόνωση DNA από δείγμα ιστού FFPE

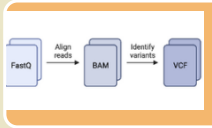


Προετοιμασία βιβλιοθηκών DNA, PCR με τους εκκινητές του panel

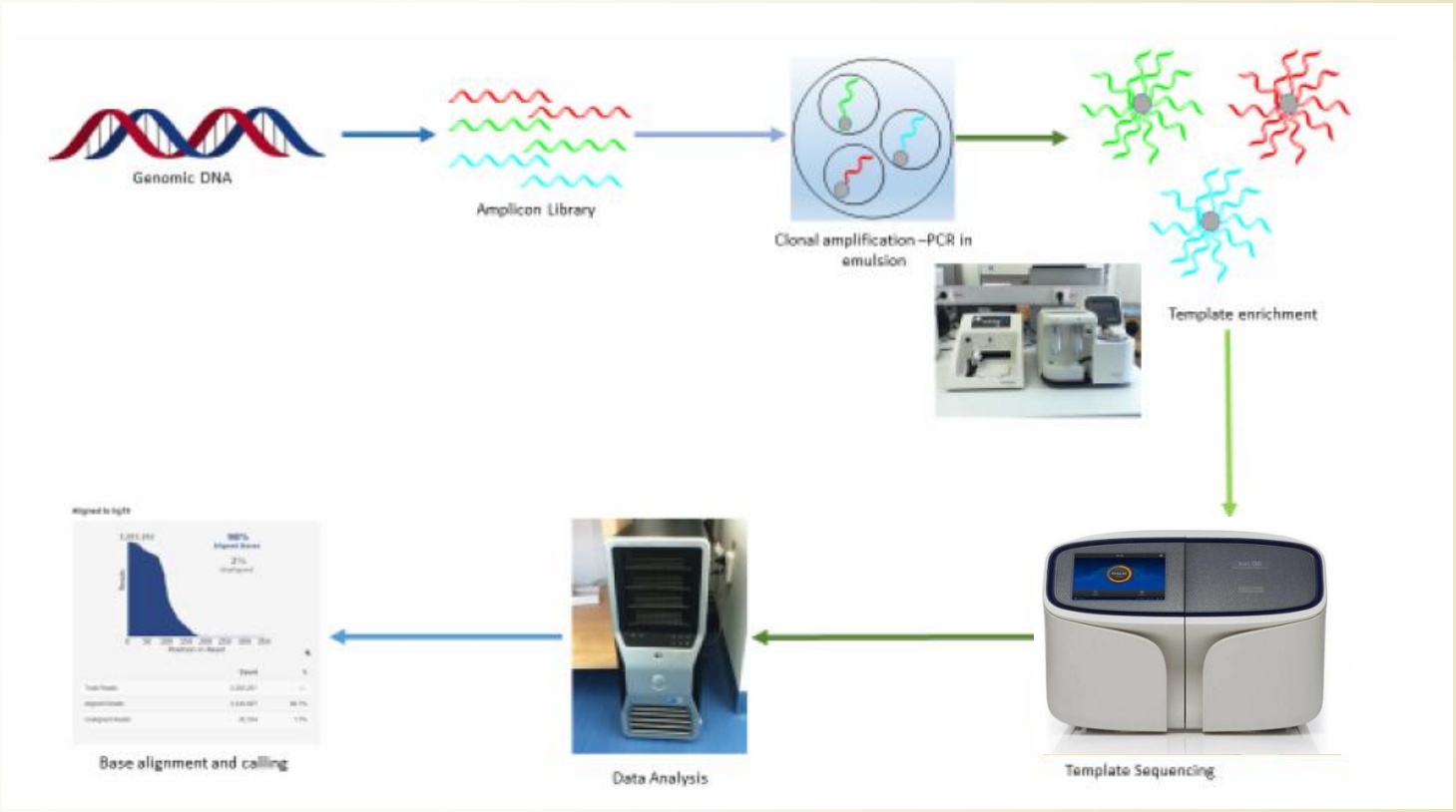


Ενίσχυση βιβλιοθηκών με Emulsion PCR

Αλληλούχιση



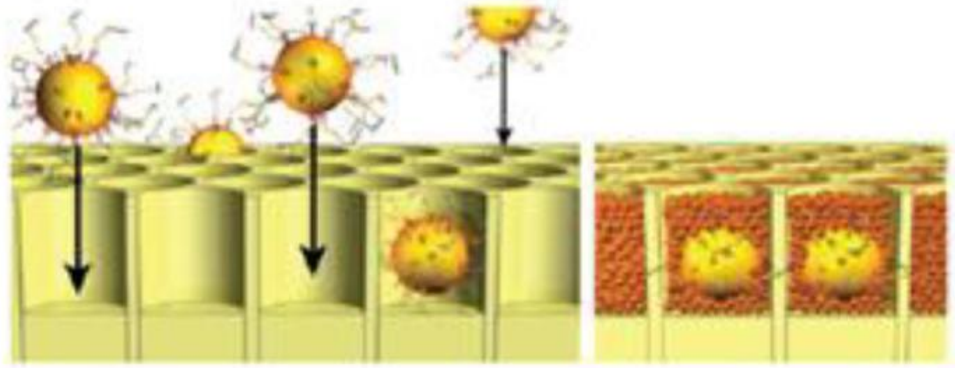
Βιοπληροφορική ανάλυση

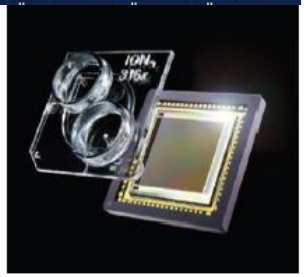


Lucan et al., 2016

Αλληλούχιση

- Αυτοματοποιημένη φόρτωση chip από τον Ion Chef
- Τοποθέτηση του chip στο μηχάνημα αλληλούχισης

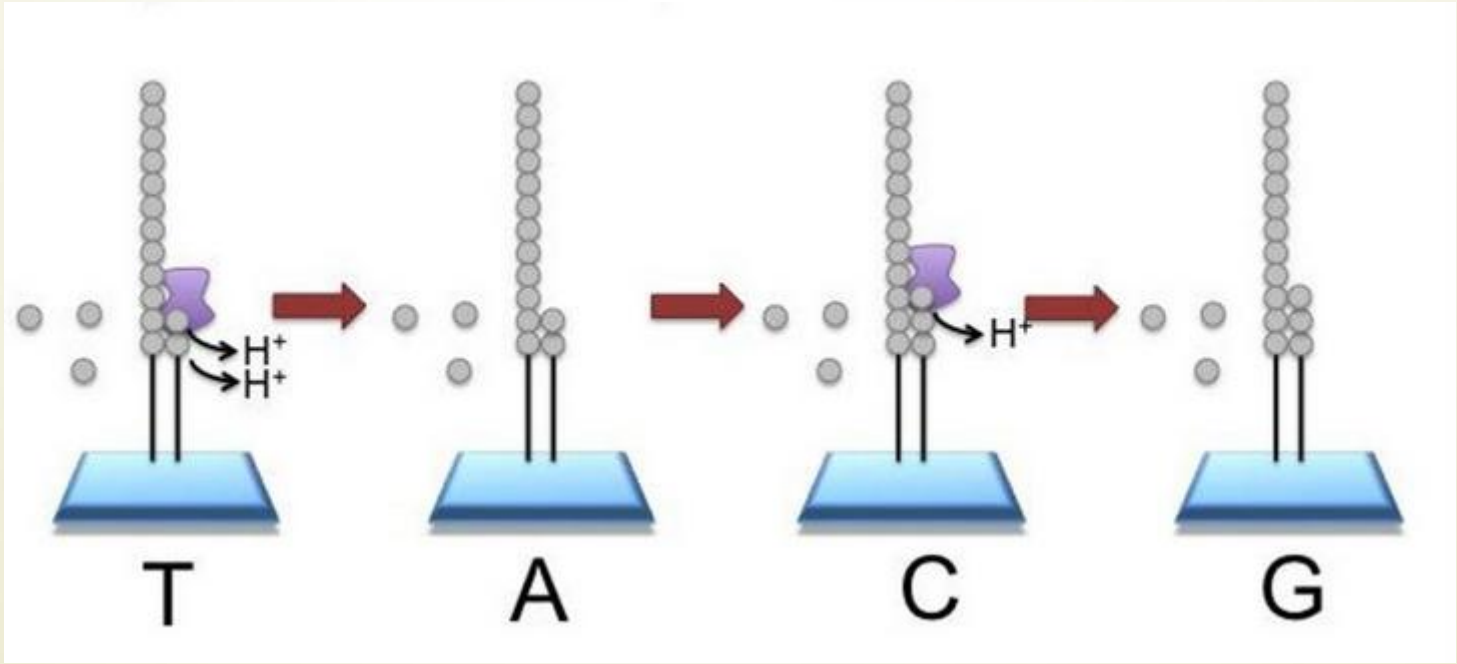




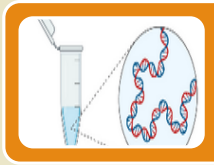
Amplified sstDNA library beads → Quality filtered bases

Ion Torrent sequencing

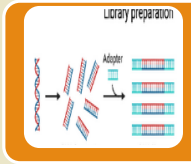
- Ion torrent and Ion proton sequencing δεν χρησιμοποιούν οπτικά σήματα
- Βασίζονται στο γεγονός ότι όταν προστίθεται ένα dNTP στη νεοσυντιθέμενη αλυσίδα DNA απελευθερώνεται ένα ιόν υδρογόνου (H^+ ion)



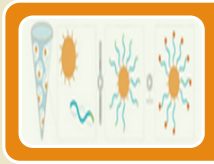
Ροή εργασίας NGS σε δείγματα DNA με τη χρήση πλατφόρμας Ion torrent



Απομόνωση DNA από δείγμα ιστού FFPE

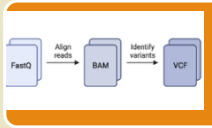


Προετοιμασία βιβλιοθηκών DNA, PCR με τους εκκινητές του panel

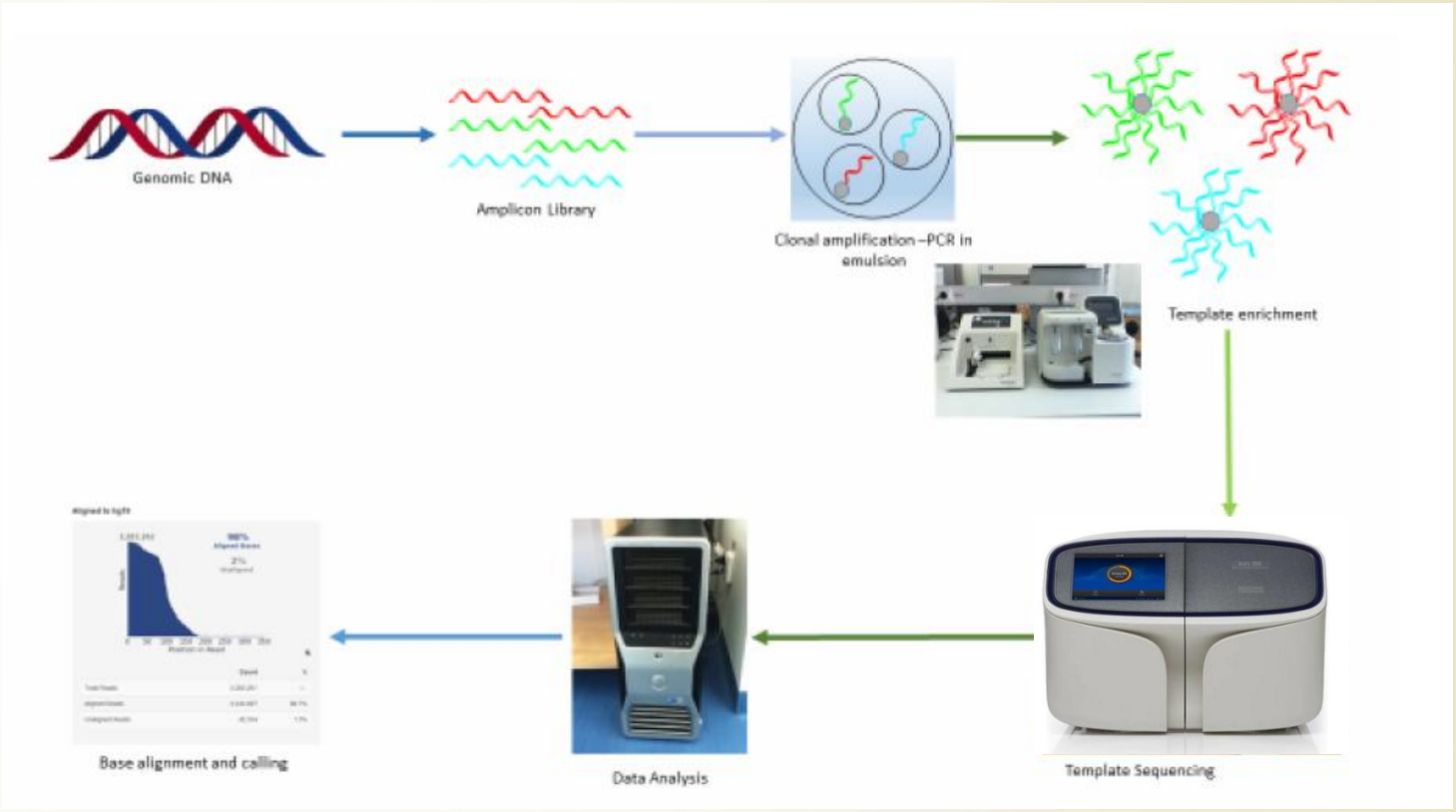


Ενίσχυση βιβλιοθηκών με Emulsion PCR

Αλληλούχιση

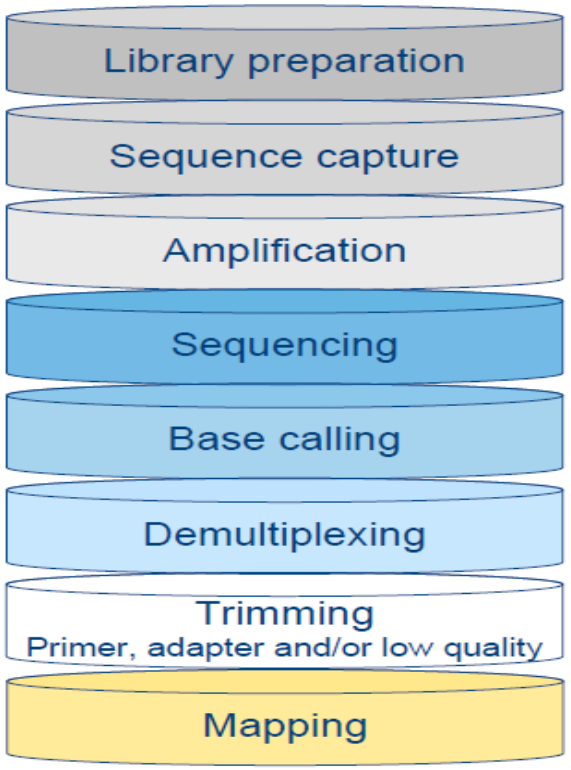


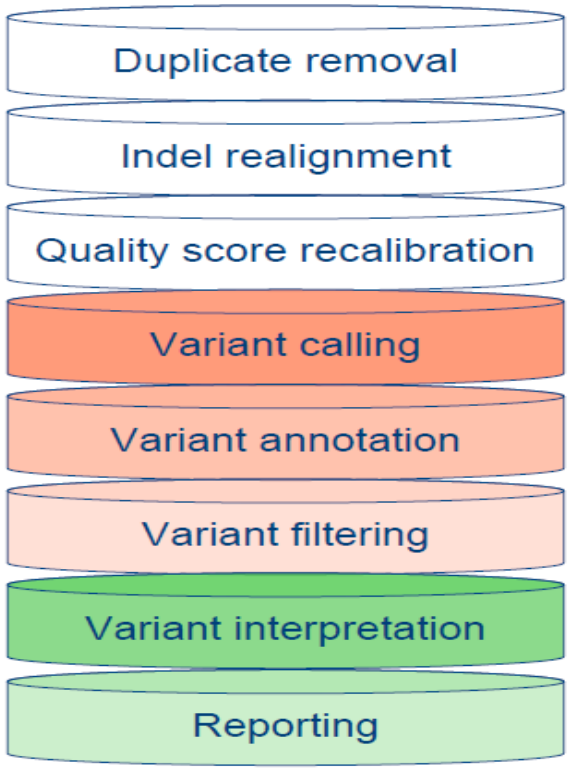
Βιοπληροφορική ανάλυση



Lucan et al., 2016

NGS workflow





Souche E., NGS quality control

NGS: ορισμοί

Table 2. Definitions

Term	Definition
Alignment	To compare a sequence read to another sequence and determine where it belongs. There are 2 types of alignment: de novo assembly or resequencing.
De novo assembly	A sequence read is compared to all the other sequence reads of that sample to determine a consensus sequence.
Resequencing	A sequence read is compared to a reference sequence (eg, the reference human genome). Also referred to as *mapping*.
Bait	An artificial construct that is able to target the sequence of interest (eg, a complementary DNA or RNA sequence) and can be used to isolate that target sequence. Used for sequence capture target enrichment.
Demultiplex	Separate an individual sample's reads from the pooled reads of multiple samples by unique identifier codes that were attached before pooling.
Map/mapping	To compare a sequence read to a reference and determine where it belongs. See also Alignment, Resequencing.
Read	May refer to either the sequence result of a single base pair position or to the sequence result of a sequential length of base pair reads from a single clonally amplified DNA cluster.

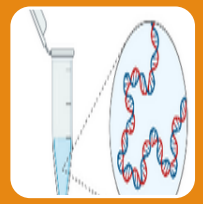
Mapping: ευθυγράμμιση των περιοχών που έχουν αλληλουχιθεί με το ανθρώπινο γονιδίωμα

Reads / Διαβάσματα: Πόσες φορές έχει διαβαστεί μία βάση/ μια αλληλουχία που έχει παραχθεί

Προαναλυτική διαδικασία- Βασικά προαναλυτικά κριτήρια

1ο σημείο ελέγχου

- Επάρκεια ιστού
- Ποσοστό Καρκινικών κυττάρων
- **Ποιότητα του ιστού**

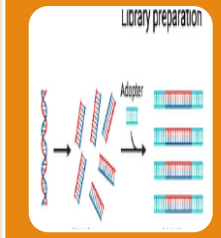


Απομόνωση DNA από δείγμα ιστού FFPE

2ο σημείο ελέγχου

Ποσότητα και ποιότητα γενετικού υλικού

- Συγκέντρωση ng/μl
- Ratio 260/280nm



Προετοιμασία βιβλιοθηκών DNA, PCR με τους εκκινητές του panel

3ο σημείο ελέγχου

Προετοιμασία βιβλιοθηκών

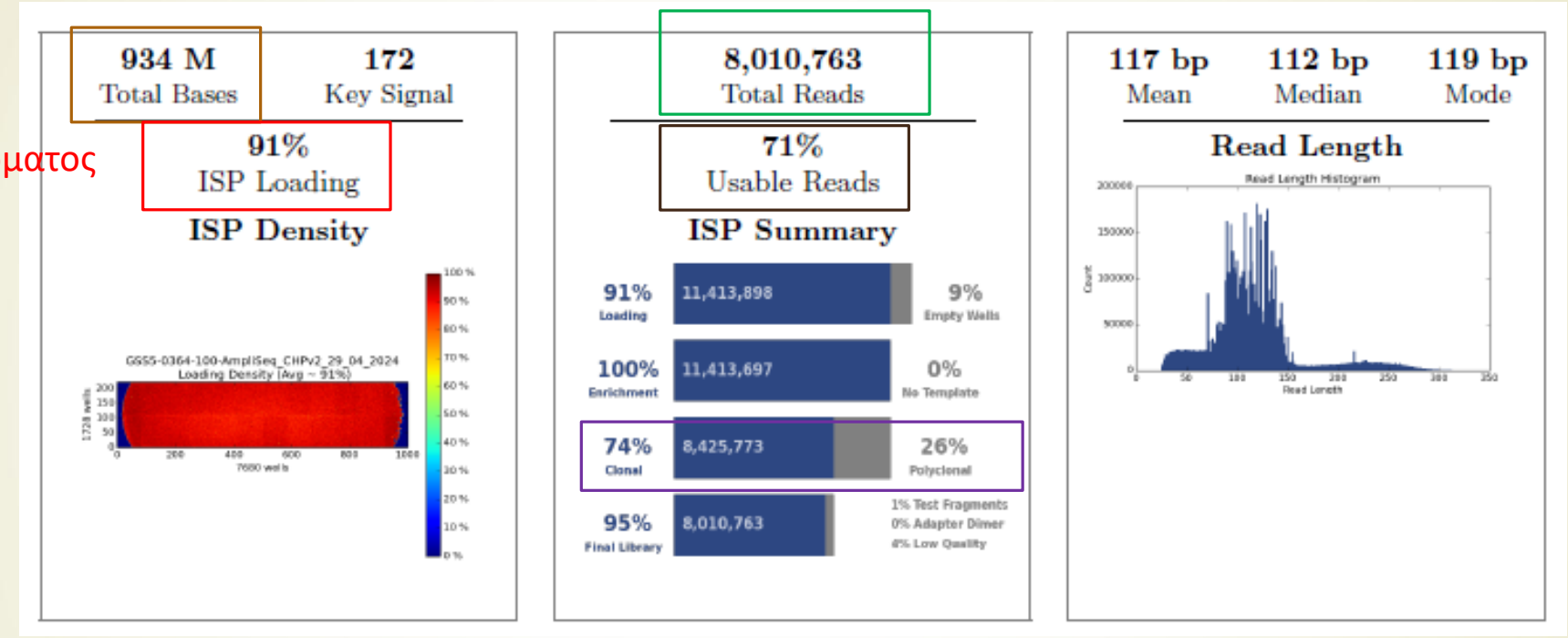
Ποσοτικοποίηση βιβλιοθηκών ng/mL

Ποιοτικά κριτήρια πειράματος

1) Συνολικό αριθμό των βάσεων

3) Συνολικό αριθμό διαβασμάτων

2) ποσοστό ISPs φορτώματος του chip



5) Κλωνικότητα των ISPs

4) Αξιοποιήσιμα διαβάσματα

Ποιοτικά χαρακτηριστικά του πειράματος:

1)Συνολικό αριθμό των βάσεων

2)ποσοστό ISPs φορτώματος του chip

3)Συνολικό αριθμό διαβασμάτων

4)Αξιοποιήσιμα διαβάσματα

5)Κλωνικότητα των ISPs

Αναλυτικά ποιοτικά κριτήρια δειγμάτων

Ποιοτικά χαρακτηριστικά του κάθε δείγματος:

Στοιχισμένα διαβάσματα (mapped reads) >100.000

Μέσο Βάθος διαβασμάτων (Mean depth) >500x

On target: Στοιχισμένα διαβάσματα/ συνολικά διαβάσματα

Uniformity: Η ομοιομορφία κατανομής των συνολικών διαβασμάτων

Barcode Name	Sample	Mapped Reads	On Target	Mean Depth	Uniformity
IonXpress_016	19676	384,610	98.71%	1,755	91.59%
IonXpress_017	19695	935,282	98.43%	9,813	89.92%
IonXpress_018	19720	475,874	98.60%	5,035	88.77%
IonXpress_019	19728	323,547	97.95%	3,311	92.12%
IonXpress_020	19733	227,987	19.53%	395.3	79.53%
IonXpress_021	19736	109,689	0.07%	0.421	93.44%
IonXpress_022	19635	503,421	99.52%	2,289	86.49%
IonXpress_023	19643	600,291	96.95%	2,624	86.46%
IonXpress_024	19616 rna	86,628	4.92%	11.38	4.26%

Barcode Name	Sample	Bases	>=Q20 Bases	Reads	Mean Read Length	Read Length Histogram	Files
No barcode	None	8,991,288	8,505,627	69,372	129 bp	0 50 100 150 200 250 300	UBAM BAM BAI
IonXpress_016	19676	48,842,080	46,706,885	384,855	126 bp	0 50 100 150 200 250 300	UBAM BAM BAI
IonXpress_017	19695	140,015,662	132,889,326	935,628	149 bp	0 50 100 150 200 250 300	UBAM BAM BAI
IonXpress_018	19720	60,715,525	57,136,318	476,047	127 bp	0 50 100 150 200 250 300	UBAM BAM BAI
IonXpress_019	19728	39,067,395	37,541,083	323,729	120 bp	0 50 100 150 200 250 300	UBAM BAM BAI
IonXpress_020	19733	29,207,295	27,511,648	228,871	127 bp	0 50 100 150 200 250 300	UBAM BAM BAI
IonXpress_021	19736	9,185,029	8,727,309	110,267	83 bp	0 50 100 150 200 250 300	UBAM BAM BAI
IonXpress_022	19635	64,591,927	61,745,949	503,713	128 bp	0 50 100 150 200 250 300	UBAM BAM BAI
IonXpress_023	19643	66,765,275	64,072,042	600,714	111 bp	0 50 100 150 200 250 300	UBAM BAM BAI
IonXpress_024	19616 rna	8,839,351	8,522,992	87,332	101 bp	0 50 100 150 200 250 300	UBAM BAM BAI

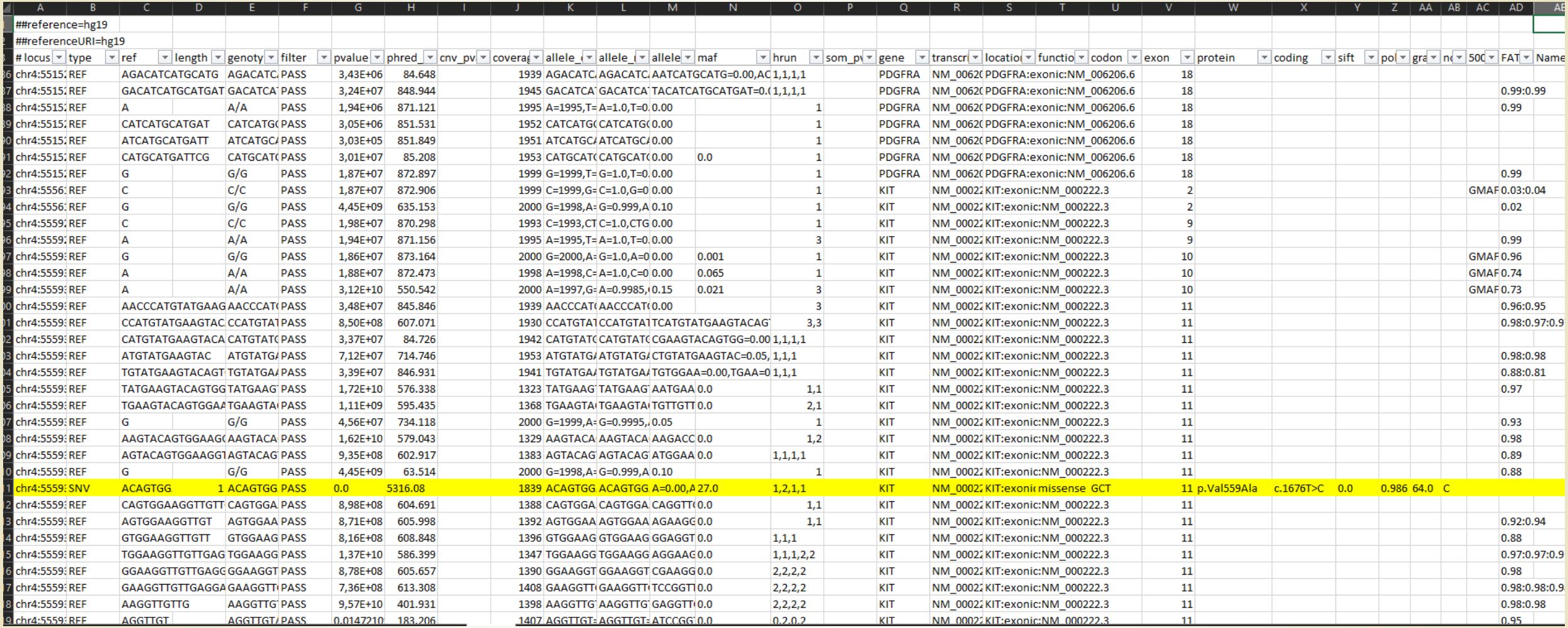
1 2 3 4 10 items per page 1 - 10 of 33 items

Αξιολόγηση αποτελεσμάτων

##reference=hg19
##referenceURI=hg19

# locus	type	ref	length	genoty	filter	pvalue	phred_	cnv_pv	coverag	allele_coverage	allele_ratio	allele_frequency	maf	hrun	som_pv	gene	transcr	locatio	functio	codon	exon	protein	coding	s
chr5:149433646	REF	A		A/A	PASS	2,64E+12	357.846		820	A=820,G=0,T=0	A=1.0,G=0.0,T=0.0	G=0.00,T=0.00		1,1		HMGXB3	NM_0149	HMGXB3:c			22			
chr5:149453044	REF	A		A/A	PASS	8,78E+09	505.641		1879	A=1876,G=3,T=0	A=0.9984,G=0.0016,	G=0.16,T=0.00		2,2		CSF1R	NM_0052	CSF1R:exonic:NM_005211.3			7			
chr5:170837543	REF	C		C/C	PASS	3,30E+10	548.213		1256	C=1256,CTCTG=0	C=1.0,CTCTG=0.0	0.00		1		NPM1	NM_0025	NPM1:exonic:NM_002520.7			11			
chr5:170837544	REF	T		T/T	PASS	3,28E+09	548.406		1256	T=1256,TCTGC=0	T=1.0,TCTGC=0.0	0.00		1		NPM1	NM_0025	NPM1:exonic:NM_002520.7			11			
chr5:170837545	REF	C		C/C	PASS	3,25E+10	548.863		1257	C=1257,CTGCA=0,CTGCG=	C=1.0,CTGCA=0.0,CT	CTGCA=0.00,CTGCG=0.00,CTGCT=		1,1,1,1,1,1		NPM1	NM_0025	NPM1:exonic:NM_002520.7			11			
chr5:170837546	REF	T		T/T	PASS	3,49E+10	545.701		1250	T=1250,TGCCA=0,TGCCG=	T=1.0,TGCCA=0.0,TG	TGCCA=0.00,TGCCG=0.00,TGTAA=		2,2,2,2,2,2,2		NPM1	NM_0025	NPM1:exonic:NM_002520.7			11			
chr5:170837547	REF	G		G/G	PASS	3,49E+09	545.721		1250	G=1250,GCAGA=0,GCCGA	G=1.0,GCAGA=0.0,G	GCAGA=0.00,GCCGA=0.00,GGCCA		1,1,2		NPM1	NM_0025	NPM1:exonic:NM_002520.7			11			
chr5:170837551	REF	G		G/G	PASS	3,64E+09	543.875		1246	G=1246,GAGAC=0,GAGGA	G=1.0,GAGAC=0.0,G	GAGAC=0.00,GAGGA=0.00,GAGG		0,0,0,0		NPM1	NM_0025	NPM1:exonic:NM_002520.7			11			
chr5:170837552	REF	T		T/T	PASS	3,61E+10	544.295		1247	T=1247,G=0	T=1.0,G=0.0	0.00		1		NPM1	NM_0025	NPM1:exonic:NM_002520.7			11			
chr5:170837577	REF	GT		GT/GT	PASS	4,81E+09	53.183		1233	GT=1233,G=0	GT=1.0,G=0.0	0.00		3		NPM1	NM_0025	NPM1:utr_3:NM_002520.7			11			
chr7:55211080	REF	G		G/G	PASS	1,85E+07	873.348		2000	G=2000,A=0	G=1.0,A=0.0	0.00		1		EGFR	NM_0052	EGFR:exonic:NM_005228.5			3			
chr7:55221821	REF	G		G/G	PASS	4,17E+12	337.961		1416	G=1413,A=3	G=0.9979,A=0.0021	0.21		1		EGFR	NM_0052	EGFR:exonic:NM_005228.5			7			
chr7:55221822	REF	C		C/C	PASS	9,29E+10	403.198		1416	C=1414,A=0,T=2	C=0.9986,A=0.0,T=0.	A=0.00,T=0.14		2,2		EGFR	NM_0052	EGFR:exonic:NM_005228.5			7			
chr7:55233037	REF	C		C/C	PASS	9,32E+08	603.037		1683	C=1682,T=1	C=0.9994,T=6.0E-4	0.06		3		EGFR	NM_0052	EGFR:exonic:NM_005228.5			15			
chr7:55233043	REF	G		G/G	PASS	4,59E+08	733.839		1680	G=1680,T=0	G=1.0,T=0.0	0.00		2		EGFR	NM_0052	EGFR:exonic:NM_005228.5			15			
chr7:55241644	REF	G		G/G	PASS	4,35E+09	536.108		1748	G=1746,A=2	G=0.9989,A=0.0011	0.11		1		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55241656	REF	G		G/G	PASS	2,27E+08	764.334		1751	G=1751,T=0	G=1.0,T=0.0	0.00		1		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55241660	REF	T		T/T	PASS	2,57E+11	458.949		1753	T=1750,C=3	T=0.9983,C=0.0017	0.17		1		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55241677	REF	GAA		GAA/GAA	PASS	4,94E+08	630.651		1751	GAA=1750,AAA=1,CAT=0	GAA=0.9994,AAA=6.	AAA=0.06,CAT=0.00		2,2		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55241678	REF	A		A/A	PASS	4,05E+09	539.251		1756	A=1754,C=0,G=2,T=0	A=0.9989,C=0.0,G=0.	C=0.00,G=0.11,T=0.00		3,3,3		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55241686	REF	T		T/T	PASS	4,75E+07	632.362		1755	T=1754,C=1	T=0.9994,C=6.0E-4	0.06		2		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55241687	REF	T		T/T	PASS	2,20E+07	765.689		1754	T=1754,C=0	T=1.0,C=0.0	0.00		2		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55241706	REF	GG		GG/GG	PASS	2,49E+07	760.309		1742	GG=1742,TT=0	GG=1.0,TT=0.0	0.00		3		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55241707	REF	G		G/G	PASS	2,44E+08	761.179		1744	G=1744,A=0,T=0	G=1.0,A=0.0,T=0.0	A=0.00,T=0.00		3,3		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55241708	REF	G		G/G	PASS	4,64E+09	533.312		1741	G=1739,A=2,C=0	G=0.9989,A=0.0011,(	A=0.11,C=0.00		3,3		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55241711	REF	C		C/C	PASS	7,26E+08	613.899		1716	C=1715,T=1	C=0.9994,T=6.0E-4	0.06		2		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55241713	REF	G		G/G	PASS	3,31E+07	748.048		1714	G=1714,A=0	G=1.0,A=0.0	0.00		2		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55241714	REF	G		G/G	PASS	3,30E+07	748.132		1714	G=1714,C=0	G=1.0,C=0.0	0.00		2		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55241722	REF	G		G/G	PASS	6,06E+08	521.729		1711	G=1709,A=2	G=0.9988,A=0.0012	0.12		2		EGFR	NM_0052	EGFR:exonic:NM_005228.5			18			
chr7:55242418	REF	C		C/C	PASS	3,03E+10	551.886		2000	C=1997,T=3	C=0.9985,T=0.0015	0.15		1		EGFR	NM_0052	EGFR:exonic:NM_005228.5			19			
chr7:55242423	REF	G		G/G	PASS	3,12E+10	550.537		2000	G=1997,A=3	G=0.9985,A=0.0015	0.15		2		EGFR	NM_0052	EGFR:exonic:NM_005228.5			19			
chr7:55242427	REF	C		C/C	PASS	1,85E+07	873.359		2000	C=2000,T=0	C=1.0,T=0.0	0.00		3		EGFR	NM_0052	EGFR:exonic:NM_005228.5			19			
chr7:55242428	REF	C		C/C	PASS	4,56E+07	734.115		2000	C=1999,T=1	C=0.9995,T=5.0E-4	0.05		3		EGFR	NM_0052	EGFR:exonic:NM_005228.5			19			
chr7:55242430	REF	G		G/G	PASS	4,45E+07	635.139		2000	G=1998,A=2	G=0.999,A=0.001	0.10		1		EGFR	NM_0052	EGFR:exonic:NM_005228.5			19			

Αξιολόγηση αποτελεσμάτων



# locus	type	ref	length	genoty	filter	pvalue	phred_	cnv_pv	coverag	allele_	allele_	allele	maf	hrun	som_pv	gene	transcr	locatio	functio	codon	exon	protein	coding	sift	po	gra	n	500	FAT	Name
##reference=hg19																														
##referenceURI=hg19																														
chr4:5515	REF	AGACATCATGCATG		AGACATC	PASS	3,43E+06	84.648		1939	AGACATC	AGACATC	AATCATGCATG=0.00,AC		1,1,1,1		PDGFRA	NM_0062	PDGFRA:exonic:NM_006206.6			18									
chr4:5515	REF	GACATCATGCATGAT		GACATCA	PASS	3,24E+07	848.944		1945	GACATCA	GACATCA	TACATCATGCATGAT=0.(		1,1,1,1		PDGFRA	NM_0062	PDGFRA:exonic:NM_006206.6			18									0.99:0.99
chr4:5515	REF	A		A/A	PASS	1,94E+06	871.121		1995	A=1995,T=	A=1.0,T=0.	0.00		1		PDGFRA	NM_0062	PDGFRA:exonic:NM_006206.6			18									0.99
chr4:5515	REF	CATCATGCATGAT		CATCATG	PASS	3,05E+06	851.531		1952	CATCATG	CATCATG	0.00		1		PDGFRA	NM_0062	PDGFRA:exonic:NM_006206.6			18									
chr4:5515	REF	ATCATGCATGATT		ATCATGC	PASS	3,03E+05	851.849		1951	ATCATGC	ATCATGC	0.00		1		PDGFRA	NM_0062	PDGFRA:exonic:NM_006206.6			18									
chr4:5515	REF	CATGCATGATTCG		CATGCAT	PASS	3,01E+07	85.208		1953	CATGCAT	CATGCAT	0.00	0.0	1		PDGFRA	NM_0062	PDGFRA:exonic:NM_006206.6			18									
chr4:5515	REF	G		G/G	PASS	1,87E+07	872.897		1999	G=1999,T=	G=1.0,T=0.	0.00		1		PDGFRA	NM_0062	PDGFRA:exonic:NM_006206.6			18									0.99
chr4:5556	REF	C		C/C	PASS	1,87E+07	872.906		1999	C=1999,G=	C=1.0,G=0	0.00		1		KIT	NM_0002	KIT:exonic:NM_000222.3			2								GMAF	0.03:0.04
chr4:5556	REF	G		G/G	PASS	4,45E+09	635.153		2000	G=1998,A=	G=0.999,A	0.10		1		KIT	NM_0002	KIT:exonic:NM_000222.3			2									0.02
chr4:5559	REF	C		C/C	PASS	1,98E+07	870.298		1993	C=1993,CT	C=1.0,CTG	0.00		1		KIT	NM_0002	KIT:exonic:NM_000222.3			9									
chr4:5559	REF	A		A/A	PASS	1,94E+07	871.156		1995	A=1995,T=	A=1.0,T=0.	0.00		3		KIT	NM_0002	KIT:exonic:NM_000222.3			9									0.99
chr4:5559	REF	G		G/G	PASS	1,86E+07	873.164		2000	G=2000,A=	G=1.0,A=0	0.00	0.001	1		KIT	NM_0002	KIT:exonic:NM_000222.3			10								GMAF	0.96
chr4:5559	REF	A		A/A	PASS	1,88E+07	872.473		1998	A=1998,C=	A=1.0,C=0	0.00	0.065	1		KIT	NM_0002	KIT:exonic:NM_000222.3			10								GMAF	0.74
chr4:5559	REF	A		A/A	PASS	3,12E+10	550.542		2000	A=1997,G=	A=0.9985,(	0.15	0.021	3		KIT	NM_0002	KIT:exonic:NM_000222.3			10								GMAF	0.73
chr4:5559	REF	AACCCATGTATGAAG		AACCCAT	PASS	3,48E+07	845.846		1939	AACCCAT	AACCCAT	0.00		3		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.96:0.95
chr4:5559	REF	CCATGTATGAAGTAC		CCATGTA	PASS	8,50E+08	607.071		1930	CCATGTA	CCATGTA	TCATGTATGAAGTACAG		3,3		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.98:0.97:0.9
chr4:5559	REF	CATGTATGAAGTACA		CATGTAT	PASS	3,37E+07	84.726		1942	CATGTAT	CATGTAT	CGAAGTACAGTGG=0.00		1,1,1,1		KIT	NM_0002	KIT:exonic:NM_000222.3			11									
chr4:5559	REF	ATGTATGAAGTAC		ATGTATG	PASS	7,12E+07	714.746		1953	ATGTATG	ATGTATG	CTGTATGAAGTAC=0.05,		1,1,1		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.98:0.98
chr4:5559	REF	TGTATGAAGTACAGT		TGTATGA	PASS	3,39E+07	846.931		1941	TGTATGA	TGTATGA	TGTGGAA=0.00,TGAA=0		1,1,1		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.88:0.81
chr4:5559	REF	TATGAAGTACAGTGG		TATGAAG	PASS	1,72E+10	576.338		1323	TATGAAG	TATGAAG	AATGAA	0.0	1,1		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.97
chr4:5559	REF	TGAAGTACAGTGGAA		TGAAGTA	PASS	1,11E+09	595.435		1368	TGAAGTA	TGAAGTA	TGTTGTT	0.0	2,1		KIT	NM_0002	KIT:exonic:NM_000222.3			11									
chr4:5559	REF	G		G/G	PASS	4,56E+07	734.118		2000	G=1999,A=	G=0.9995,(	0.05		1		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.93
chr4:5559	REF	AAGTACAGTGGAAG		AAGTACA	PASS	1,62E+10	579.043		1329	AAGTACA	AAGTACA	AAGACC	0.0	1,2		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.98
chr4:5559	REF	AGTACAGTGGAAGGT		AGTACAG	PASS	9,35E+08	602.917		1383	AGTACAG	AGTACAG	ATGGAA	0.0	1,1,1,1		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.89
chr4:5559	REF	G		G/G	PASS	4,45E+09	63.514		2000	G=1998,A=	G=0.999,A	0.10		1		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.88
chr4:5559	SNV	ACAGTGG	1	ACAGTGG	PASS	0.0	5316.08		1839	ACAGTGG	ACAGTGG	A=0.00,A	27.0	1,2,1,1		KIT	NM_0002	KIT:exonic	missense	GCT	11	p.Val559Ala	c.1676T>C	0.0	0.986	64.0	C			
chr4:5559	REF	CAGTGGAAGGTTGTT		CAGTGGA	PASS	8,98E+08	604.691		1388	CAGTGGA	CAGTGGA	CAGGTT	0.0	1,1		KIT	NM_0002	KIT:exonic:NM_000222.3			11									
chr4:5559	REF	AGTGGAAGGTTGT		AGTGGAA	PASS	8,71E+08	605.998		1392	AGTGGAA	AGTGGAA	AGAAGG	0.0	1,1		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.92:0.94
chr4:5559	REF	GTGGAAGGTTGTT		GTGGAAG	PASS	8,16E+08	608.848		1396	GTGGAAG	GTGGAAG	GGAGGT	0.0	1,1,1		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.88
chr4:5559	REF	TGGAAGGTTGTTGAG		TGGAAGG	PASS	1,37E+10	586.399		1347	TGGAAGG	TGGAAGG	AGGAAG	0.0	1,1,1,2,2		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.97:0.97:0.9
chr4:5559	REF	GGAAGGTTGTTGAGG		GGAAGGT	PASS	8,78E+08	605.657		1390	GGAAGGT	GGAAGGT	CGAAGG	0.0	2,2,2,2		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.98
chr4:5559	REF	GAAGGTTGTTGAGGA		GAAGGTT	PASS	7,36E+08	613.308		1408	GAAGGTT	GAAGGTT	TCCGGT	0.0	2,2,2,2		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.98:0.98:0.9
chr4:5559	REF	AAGGTTGTTG		AAGGTTG	PASS	9,57E+10	401.931		1398	AAGGTTG	AAGGTTG	GAGGTT	0.0	2,2,2,2		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.98:0.98
chr4:5559	REF	AGGTTGT		AGGTTGT	PASS	0.0147210	183.206		1407	AGGTTGT=	AGGTTGT=	ATCCGG	0.0	0,2,0,2		KIT	NM_0002	KIT:exonic:NM_000222.3			11									0.95

1. Διαβάσματα θέσης
2. Ποσοστό μεταλλαγής VAF >5%
3. Ταυτοποίηση μεταλλαγής πχ p.Val559Ala, c.1676 T>C
4. Στοιχεία- παθογόνος ή μη

→

Βάσεις δεδομένων: ClinVar, COSMIC,IARC TP53, Varsome, UniProt, etc

Mutation

COSV55388782

Overview
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Pathways affected
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Overview

This section shows a general overview of the selected mutation. It describes the source of the mutation i.e gene name/sample name/tissue name with unique ID, and also shows the mutation syntax at the amino acid and nucleotide sequence level. You can see more information on our help pages.

Genomic Mutation ID	COSV55388782
Legacy Identifier	COSM1255
Gene name	KIT
AA mutation	p.V559A (Substitution - Missense, position 559, V→A)
CDS mutation	c.1676T>C (Substitution, position 1676, T→C)
Nucleotides inserted	n/a
Genomic coordinates	GRCh37, 4:55593610..55593610, view Ensembl contig
CDD	NP_001087241.1
HomoloGene	n/a
Ever confirmed somatic?	Yes
Remark	n/a
Recurrent	n/a
Drug resistance	n/a
Alternative Ids	n/a

COSV55388782
Overview
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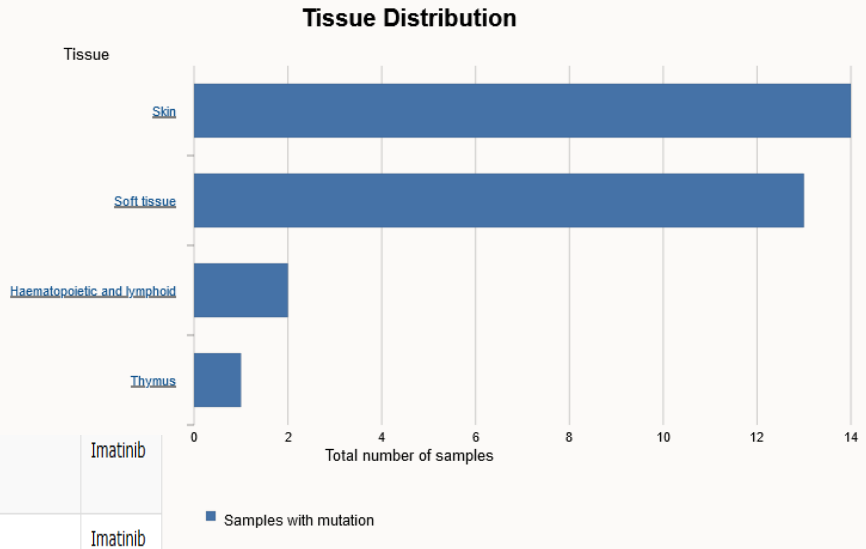
Tissue distribution

This section displays the distribution of mutated samples and tissue types (top 5). You can see more information on our help pages.



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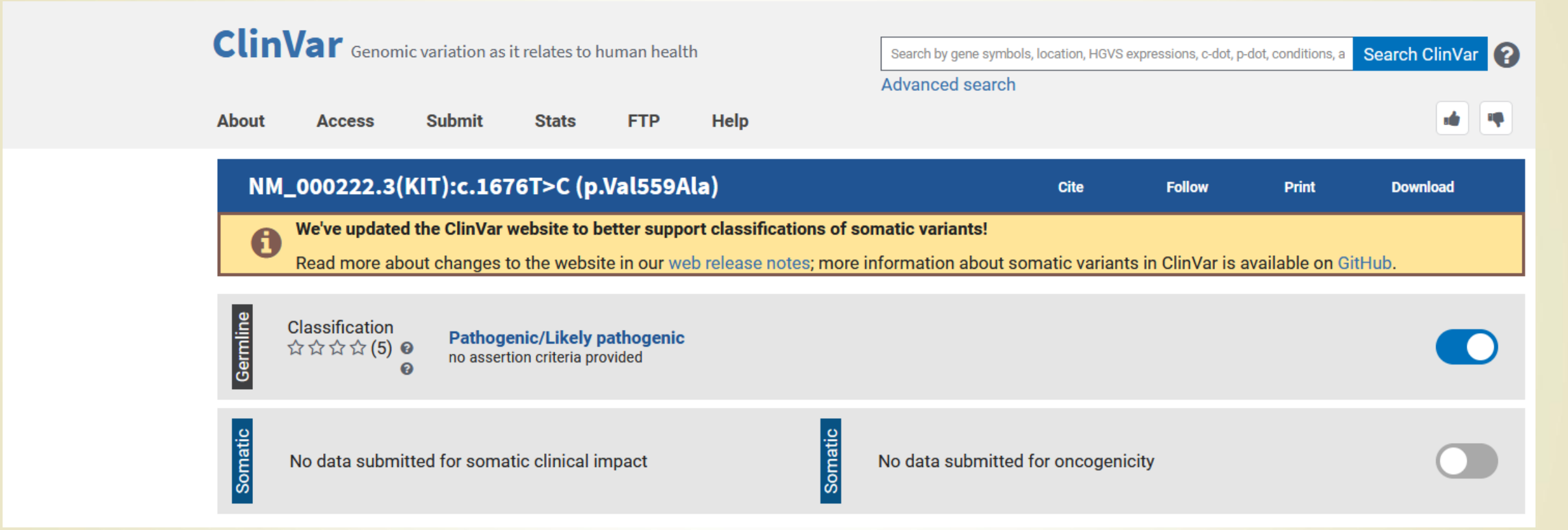
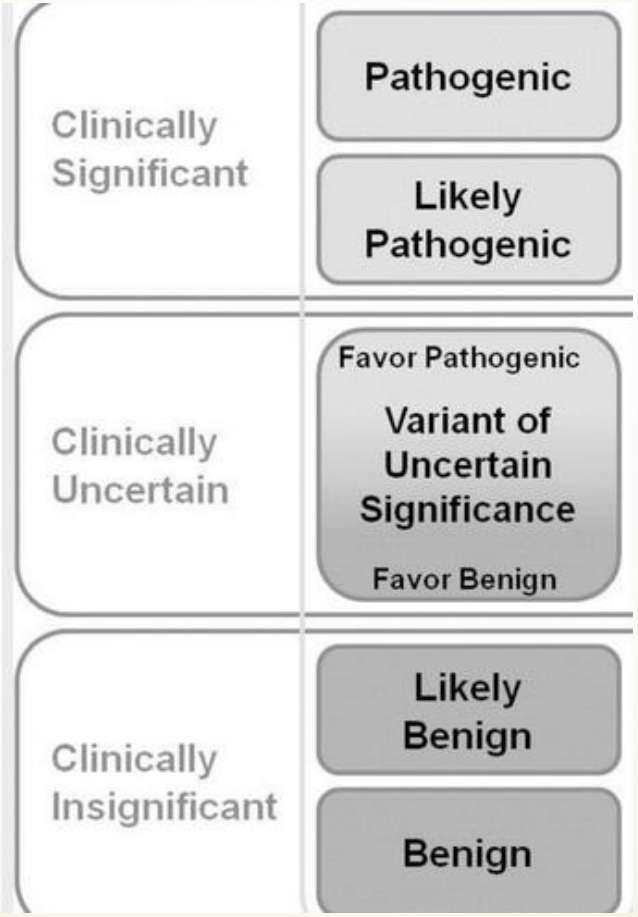
1217444	KIT	ENST00000288135.5	Soft tissue	Fibrous tissue and uncertain origin	Gastrointestinal stromal tumour	NS	19096980	Heterozygous	Previously Reported	Tumour Sample	Unknown	-	Imatinib
1245664	KIT	ENST00000288135.5	Soft tissue	Fibrous tissue and uncertain origin	Gastrointestinal stromal tumour	Spindle and epithelioid	19384074	Heterozygous	Previously Reported	Tumour Sample	Unknown	-	
1516538	KIT	ENST00000288135.5	Soft tissue	Fibrous tissue and uncertain origin	Gastrointestinal stromal tumour	NS	20861712	Heterozygous	Previously Reported	Tumour Sample	Unknown	-	


ClinVar
Genomic variation as it relates to human health
Search by gene symbols, location, HGVS expressions, c-dot, p-dot, conditions, a
Search ClinVar
Advanced search
About
Access
Submit
Stats
FTP
Help
NM_000222.3(KIT):c.1676T>C (p.Val559Ala)
Cite
Follow
Print
Download
We've updated the ClinVar website to better support classifications of somatic variants!
Read more about changes to the website in our web release notes; more information about somatic variants in ClinVar is available on GitHub.
Germline
Classification
(5)
Pathogenic/Likely pathogenic
no assertion criteria provided
Somatic
No data submitted for somatic clinical impact
Somatic
No data submitted for oncogenicity

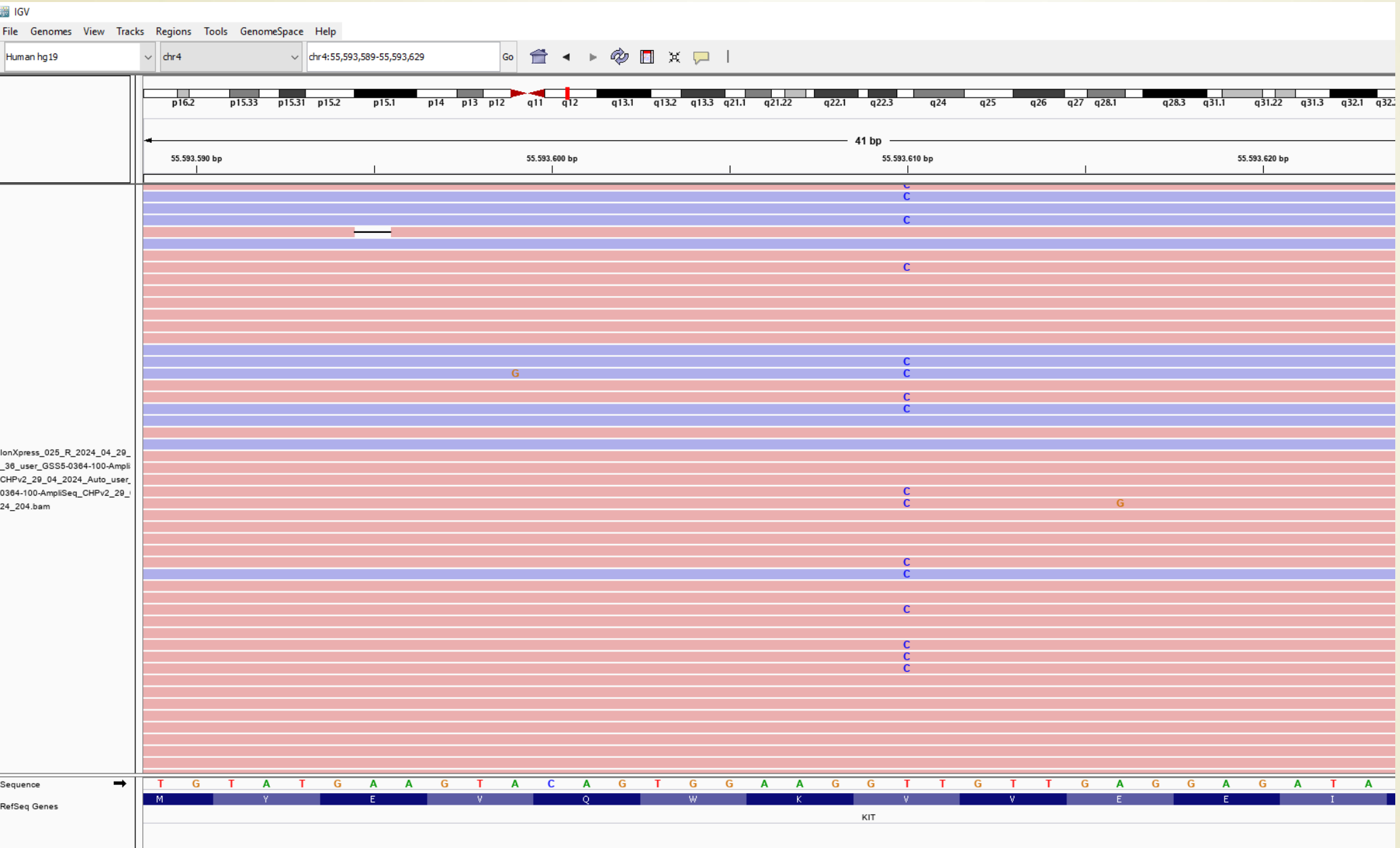
Κατηγοριοποίηση μεταλλαγής



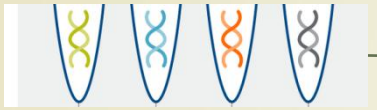
Hoskinson DC, et al 2017

Αξιολόγηση αποτελεσμάτων (IGV)

p.Val559Ala, c.1676 T>C



RNA

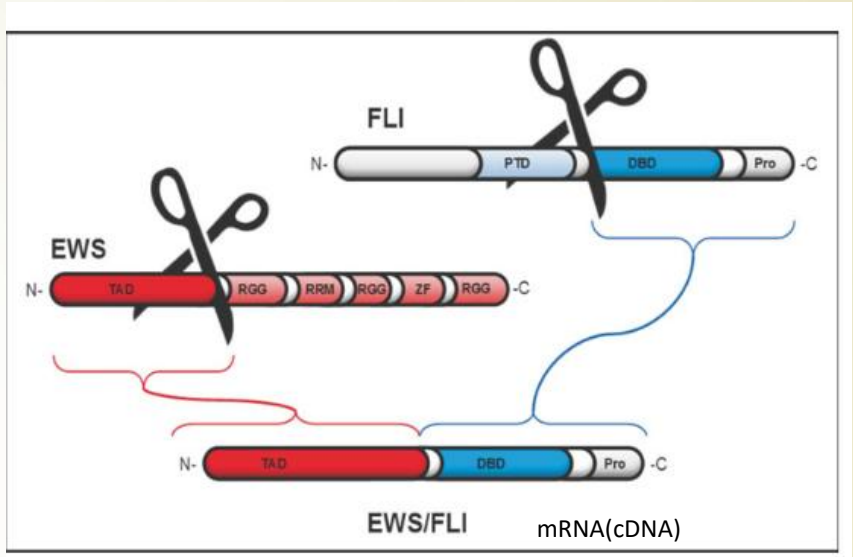


Αντίστροφη μεταγραφή-cDNA

Προετοιμασία Βιβλιοθηκών PCR με τους εκκινητές του panel

Ενίσχυση Βιβλιοθηκών με emulsion PCR

Αλληλούχιση με NGS



Γονίδια σύντηξης στη διάγνωση

National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2025
Soft Tissue Sarcoma

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PRINCIPLES OF ANCILLARY TECHNIQUES USEFUL IN THE DIAGNOSIS OF SARCOMAS

TUMOR	ABERRATION	GENE(S) INVOLVED
Malignant Round Cell Tumors		
Alveolar RMS	t(2;13)(q35;q14) t(1;13)(p36;q14) t(X;2)(q13;q35)	*PAX3::FOXO1* *PAX7::FOXO1* *PAX3::AFX*
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	*EWSR1::WT1*
Embryonal RMS	Complex alterations	Multiple, *MYOD1, KRAS, HRAS, TP53, NF1, NRAS, PIK3CA, FBXW7, FGFR4, BCOR*
Ewing sarcoma/peripheral neuroectodermal tumor	t(11;22)(q24;q12) t(21;22)(q22;q12) t(2;22)(q33;q12) t(7;22)(p22;q12) t(17;22)(q12;q12) inv(22)(q12q;12) t(16;21)(p11;q22)	*EWSR1::FLI1* *EWSR1::ERG* *EWSR1::FEV* *EWSR1::ETV1* *EWSR1::E1AF* *EWSR1::ZSG* *FUS::ERG*
Undifferentiated round cell sarcoma	t(4;19)(q35;q13) or t(10;19)(q26;q13) inv(X)(p11.4p11.22)	*CIC::DUX4*[4] *BCOR::CCNB3*[5]
Lipomatous Tumors		
ALT/WDLPS	Supernumerary ring chromosomes; giant marker chromosomes	Amplification of region 12q13-15, including *MDM2, CDK4, HMGA2, SAS, GLI*
Dedifferentiated liposarcoma	Same as for ALT/WDLPS	Same as for ALT/WDLPS
Myxoid/round cell liposarcoma	t(12;16)(q13;p11) t(12;22)(q13;q12)	*FUS::DDIT3* *EWSR1::DDIT3*
Pleomorphic liposarcoma	Complex alterations	Unknown

[4] Yoshimoto T, Tanaka M, Homme M, et al. CIC-DUX4 induces small round cell sarcomas distinct from Ewing sarcoma. Cancer Res 2017;77:2927-2937.
[5] Kao YC, Owosho AA, Sung YS, et al. BCOR-CCNB3-fusion positive sarcomas: A clinicopathologic and molecular analysis of 36 cases with comparison to morphologic spectrum and clinical behavior of other round cell sarcomas. Am J Surg Path 2018;42:604-615.

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TUMOR	ABERRATION	GENE(S) INVOLVED
Other Sarcomas		
Alveolar soft part sarcoma	der(17)t(X;17)(p11;q25)	*ASPL::TFE3*
Angiomatoid fibrous histiocytoma	t(12;22)(q13;q12) t(2;22)(q33;q12) t(12;16)(q13;p11)	*EWSR1::ATF1* *EWSR1::CREB1* *FUS::ATF1*
Clear cell sarcoma	t(12;22)(q13;q12) t(2;22)(q33;q12)	*EWSR1::ATF1* *EWSR1::CREB1*
Congenital/infantile fibrosarcoma	t(12;15)(p13;q25)	*ETV6::NTRK3*[6]
Dermatofibrosarcoma protuberans	t(17;22)(q21;q13) and derivative ring chromosomes	*COL1A1::PDGFB*
Desmoid fibromatosis	Trisomy 8 or 20; loss of 5q21	*CTNNB1* or *APC* mutations
High-grade endometrial stromal sarcoma	t(10;17)(q22;p13) t(x;22)(p11;q13)	*YWHAE::NUTM2* *ZC3H7B::BCOR*[7]
Epithelioid hemangioendothelioma	t(1;3)(p36;q25) t(X;11)(p11.23; q22.1)	*WWTR1::CAMTA1* *YAP1::TFE3*
Epithelioid sarcoma	Inactivation, deletion, or mutation of *INI1 (SMARCB-1)*	*INI1 (SMARCB-1)*
Extrarenal rhabdoid tumor	Inactivation of *INI1 (SMARCB-1)*	*INI1 (SMARCB-1)*
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12) t(9;17)(q22;q11) t(9;15)(q22;q21) t(3;9)(q11;q22)	*EWSR1::NR4A3* *TAF2N::NR4A3* *TCF12::NR4A3* *TFG::NR4A3*
Sporadic and familial GIST Carney-Stratakis syndrome (gastric GIST and paraganglioma)	Activating kinase mutations Krebs cycle mutation	*KIT* or *PDGFRA* Germline *SDH* subunit mutations

[6] Yamamoto H, Yoshida A, Taguchi K, et al. ALK, ROS1 and NTRK3 gene rearrangements in inflammatory myofibroblastic tumours. Histopathology 2016;69:72-83.
[7] Lewis N, Soslow RA, Delair DF, et al. ZC3H7B-BCOR high-grade endometrial stromal sarcomas: a report of 17 cases of a newly defined entity. Mod Pathol 2018;31:674-684.

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PRINCIPLES OF ANCILLARY TECHNIQUES USEFUL IN THE DIAGNOSIS OF SARCOMAS

TUMOR	ABERRATION	GENE(S) INVOLVED
Inflammatory myofibroblastic tumor (IMT)	t(1;2)(q22;p23) t(2;19)(p23;p13) t(2;17)(p23;q23) t(2;2)(p23;q13) t(2;11)(p23;p15) inv(2)(p23;q35)	*TPM3::ALK*[6] *TPM4::ALK*[6] *CLTC::ALK*[6] *RANBP2::ALK*[6] *CARS::ALK*[6] *ATIC::ALK*[6] *ETV6::NTRK3*[6,8] *TFG::ROS1*[8-10]
Leiomyosarcoma (LMS)	Complex alterations	Unknown
Low-grade fibromyxoid sarcoma/sclerosing epithelioid fibrosarcoma	t(7;16)(q33;p11) t(11;16)(p11;p11)	*FUS::CREB3L2* *FUS::CREB3L1*
Malignant peripheral nerve sheath tumor (MPNST)		*NF1*, *CDKN2A* and *EED* or *SUZ12*
Mesenchymal chondrosarcoma	t(8;8)(q13;q21)	*HEY1::NCOA2*
NTRK-rearranged spindle cell neoplasm[a,11]	Multiple	*NTRK 1, 2, 3*
Solitary fibrous tumor	inv(12)(q13q13)	*NAB2::STAT6*
Synovial sarcoma	t(X;18)(p11;q11); t(X;18)(p11;q11); t(X;18)(p11;q11)	*SS18::SSX1; SS18::SSX2; SS18::SSX4*
Tenosynovial giant cell tumor/pigmented villonodular synovitis (TGCT/PVNS)	*CSF1* rearrangements	Multiple[12-14]

[a] Emerging entity; NGS testing is preferred.

[6] Yamamoto H, Yoshida A, Taguchi K, et al. ALK, ROS1 and NTRK3 gene rearrangements in inflammatory myofibroblastic tumours. Histopathology 2016;69:72-83.
[8] Taylor MS, Chougule A, MacLeay AR, et al. Morphologic overlap between inflammatory myofibroblastic tumor and IgG4-related disease: Lessons from next-generation sequencing. Am J Surg Pathol 2019;43:314-324.
[9] Lopez-Nunez O, John I, Panasiti RN, et al. Infantile inflammatory myofibroblastic tumors: clinicopathological and molecular characterization of 12 cases. Mod Pathol 2020;33:576-590.
[10] Lovly CM, Gupta A, Lipson D, et al. Inflammatory myofibroblastic tumors harbor multiple potentially actionable kinase fusions. Cancer Discov 2014;4:889-895.
[11] Davis JL, Al-Ibraheemi A, Rudzinski ER, Surrey LF. Mesenchymal neoplasms with NTRK and other kinase gene alterations. Histopathology 2022;80:4-18.
[12] Ho J, Peters T, Dickson BC, et al. Detection of CSF1 rearrangements deleting the 3' UTR in tenosynovial giant cell tumors. Genes Chromosomes Cancer 2020;59:96-105.
[13] West RB, Rubin BP, Miller MA, et al. A landscape effect in tenosynovial giant-cell tumor from activation of CSF1 expression by a translocation in a minority of tumor cells. Proc Natl Acad Sci 2006;103:690-695.
[14] Cupp JS, Miller MA, Montgomery KD, et al. Translocation and expression of CSF1 in pigmented villonodular synovitis, tenosynovial giant cell tumor, rheumatoid arthritis and other reactive synovitides. Am J Surg Pathol 2007;31:970-976.

Note: All recommendations are category 2A unless otherwise indicated.

Πάνελ γονιδίων για σαρκώματα

PAX7(7) - FOXO1(2) COSF287	FUS(6) - CREB3L2(5) COSF330	FUS(8) - ERG(9) COSF318	COL1A1(23) - PDGFB(2) COSF543	EWSR1(7) - ERG(11) COSF145	EWSR1(12) - NR4A3(3) COSF352
THRAP3(1) - USP6(1) COSF1410	FUS(6) - CREB3L2(5) COSF858	FUS(9) - CREB3L1(5) COSF912	COL1A1(20) - PDGFB(2) COSF819	EWSR1(7) - ERG(8) COSF146	EWSR1(13) - DDIT3(2) COSF271
THRAP3(1) - USP6(1) COSF1411	FUS(6) - CREB3L2(5) COSF335	FUS(9) - CREB3L1(5) COSF913	COL1A1(19) - PDGFB(2) COSF539	EWSR1(7) - ERG(9) COSF152	EWSR1(13) - DDIT3(3) COSF281
MEAF6(5) - PHF1(2) COSF1450	FUS(6) - CREB3L2(5) COSF325	FUS(9) - DDIT3(3) COSF867	COL1A1(18) - PDGFB(2) COSF531	EWSR1(7) - ETV1(12) COSF163	EWSR1(13) - NR4A3(3) COSF367
TPM3(7) - ALK(20) COSF439	FUS(6) - CREB3L1(5) COSF942	FUS(8) - DDIT3(2) COSF301	COL1A1(17) - PDGFB(2) COSF597	EWSR1(7) - ETV4(8) COSF208	TFE3(5) - ASPSCR1(8) COSF797
PAX3(7) - FOXO1(2) COSF247	FUS(6) - CREB3L2(5) COSF331	FUS(8) - ERG(11)	COL1A1(16) - PDGFB(2) COSF596	EWSR1(7) - FEV(2) COSF275	TFE3(4) - ASPSCR1(8) COSF782
PAX3(7) - NCOA1(14) COSF1042	FUS(6) - CREB3L2(5) COSF925	FUS(8) - ERG(9) COSF318	COL1A1(16) - PDGFB(2) COSF595	EWSR1(7) - FLI1(5) COSF165	TFE3(4) - ASPSCR1(8) COSF798
PAX3(7) - NCOA2(12) COSF1040	FUS(6) - DDIT3(2) COSF303	FUS(9) - CREB3L1(5) COSF912	COL1A1(15) - PDGFB(2) COSF1293	EWSR1(7) - FLI1(5) COSF167	
PAX3(6) - NCOA1(15) COSF289	FUS(6) - CREB3L2(5) COSF332	FUS(9) - CREB3L1(5) COSF913	COL1A1(13) - PDGFB(2) COSF593	EWSR1(7) - FLI1(6) COSF180	
TFG(7) - NR4A3(3) COSF1187	FUS(6) - CREB3L2(5) COSF336	FUS(9) - DDIT3(3) COSF867	COL1A1(11) - PDGFB(2) COSF605	EWSR1(7) - FLI1(7) COSF173	
CNBP(1) - USP6(1) COSF1417	FUS(6) - CREB3L2(5) COSF334	FUS(10) - FEV(2) COSF295	COL1A1(9) - PDGFB(2) COSF603	EWSR1(7) - FLI1(8) COSF227	
CNBP(1) - USP6(2) COSF1416	FUS(6) - CREB3L2(5) COSF922	FUS(11) - DDIT3(2) COSF1018	COL1A1(8) - PDGFB(2) COSF654	EWSR1(7) - NR4A3(2) COSF353	
ACTB(3) - GLI1(5) COSF1384	FUS(6) - CREB3L2(5) COSF389	FUS(13) - DDIT3(2) COSF293	COL1A1(7) - PDGFB(2)COSF564	EWSR1(7) - PBX1(5) COSF350	
ACTB(3) - GLI1(6) COSF1380	FUS(6) - CREB3L2(5) COSF948	CDH11(2) - USP6(1) COSF1403	COL1A1(6) - PDGFB(2) COSF591	EWSR1(7) - SMARCA5(5) COSF1208	
ACTB(3) - GLI1(7) COSF1378	FUS(6) - ERG(11) COSF309	CDH11(2) - USP6(2) COSF1405	COL1A1(5) - PDGFB(2) COSF1289	EWSR1(7) - SP3(6) COSF278	
ACTB(2) - GLI1(6) COSF1379	FUS(6) - CREB3L2(5) COSF952	CDH11(1) - USP6(1) COSF1399	COL1A1(1) - USP6(1) COSF1414	EWSR1(8) - ATF1(4) COSF217	
JAZF1(3) - SUZ12(2) COSF568	FUS(6) - CREB3L2(5) COSF337	CDH11(1) - USP6(2) COSF1401	COL1A1(1) - USP6(1) COSF1415	EWSR1(8) - FLI1(5) COSF204	
COL1A2(1) - PLAG1(2) COSF1098	FUS(6) - CREB3L1(5) COSF943	COL1A1(49) - PDGFB(2) COSF650	COL1A1(1) - USP6(2) COSF1397	EWSR1(8) - FLI1(6) COSF183	
COL1A2(1) - PLAG1(3) COSF1097	FUS(6) - CREB3L2(5) COSF338	COL1A1(48) - PDGFB(2) COSF817	CLTC(30) - ALK(20) COSF470	EWSR1(8) - NFATC2(3) COSF1180	
CREB3L2(5) - FUS(6) COSF861	FUS(6) - CREB3L2(5) COSF340	COL1A1(47) - PDGFB(2) COSF646	ASPSCR1(7) - TFE3(5) COSF395	EWSR1(8) - PATZ1(1) COSF283	
CREB3L2(5) - FUS(7) COSF848	FUS(6) - CREB3L2(5) COSF341	COL1A1(46) - PDGFB(2) COSF644	ASPSCR1(7) - TFE3(6) COSF393	EWSR1(8) - PBX1(5) COSF349	
CREB3L2(5) - FUS(8) COSF847	FUS(6) - CREB3L2(5) COSF390	COL1A1(45) - PDGFB(2) COSF642	SS18(10) - SSX1(5) COSF513	EWSR1(8) - SP3(6) COSF279	
CREB3L2(5) - FUS(6) COSF860	FUS(6) - CREB3L2(5) COSF852	COL1A1(44) - PDGFB(2) COSF1291	SS18(10) - SSX1(5) COSF514	EWSR1(8) - ZNF444(5) COSF1342	
CREB3L2(1) - FUS(8) COSF842	FUS(6) - CREB3L2(5) COSF918	COL1A1(43) - PDGFB(2) COSF547	SS18(10) - SSX1(6) COSF499	EWSR1(8) - ZNF444(5) COSF1343	
HEY1(4) - NCOA2(13) COSF1211	FUS(6) - DDIT3(2) COSF304	COL1A1(42) - PDGFB(2) COSF638	SS18(10) - SSX4(3) COSF507	EWSR1(9) - FLI1(4) COSF175	
OMD(1) - USP6(1) COSF1412	FUS(6) - ERG(11) COSF316	COL1A1(41) - PDGFB(2) COSF599	SS18(10) - SSX4(6) COSF502	EWSR1(9) - FLI1(6) COSF174	
ETV6(4) - NTRK3(15) COSF823	FUS(6) - ERG(9)	COL1A1(40) - PDGFB(2) COSF648	SS18(10) - SSX4(7) COSF525	EWSR1(9) - ATF1(4) COSF299	
ETV6(5) - NTRK3(15) COSF571	FUS(7) - CREB3L2(5) COSF339	COL1A1(39) - PDGFB(2) COSF634	SS18(9) - SSX1(4) COSF527	EWSR1(9) - ATF1(5) COSF223	
ATF1(3) - EWSR1(10) COSF259	FUS(7) - CREB3L2(5) COSF863	COL1A1(38) - PDGFB(2) COSF562	SS18(9) - SSX1(5) COSF505	EWSR1(9) - DDIT3(2) COSF273	
GLI1(6) - ACTB(4) COSF1387	FUS(7) - CREB3L2(5) COSF950	COL1A1(37) - PDGFB(2) COSF545	TPM4(7) - ALK(20) COSF441	EWSR1(9) - ERG(8) COSF150	
HMGA2(3) - LPP(7) COSF969	FUS(7) - CREB3L2(5) COSF845	COL1A1(36) - PDGFB(2) COSF541	TPM4(7) - ALK(20)	EWSR1(9) - FEV(2) COSF195	
HMGA2(3) - LPP(9) COSF962	FUS(7) - CREB3L2(5) COSF927	COL1A1(34) - PDGFB(2) COSF601	CIC(20) - DUX4(1) COSF1374	EWSR1(9) - FLI1(5) COSF169	
NTRK3(14) - ETV6(6) COSF825	FUS(7) - CREB3L2(5) COSF846	COL1A1(33) - PDGFB(2) COSF626	NFATC2(2) - EWSR1(9) COSF1182	EWSR1(9) - FLI1(5) COSF171	
FUS(3) - DDIT3(2) COSF294	FUS(7) - CREB3L2(5) COSF856	COL1A1(32) - PDGFB(2) COSF535	SS18L1(10) - SSX1(6) COSF1123	EWSR1(9) - FLI1(6) COSF1302	
FUS(5) - CREB3L2(6) COSF944	FUS(7) - DDIT3(2) COSF291	COL1A1(31) - PDGFB(2) COSF552	EWSR1(7) - ERG(10) COSF149	EWSR1(9) - FLI1(7) COSF176	
		COL1A1(30) - PDGFB(2)		EWSR1(11) - NR4A3(1)	

RNA- ποιοτικά κριτήρια

Γονίδια ελέγχου- εσωτερικός έλεγχος

Συνολικά διαβάσματα >20.000

Διαβάσματα διαμετάθεσης

RNA αποτελέσματα (ION REPORTER)

Δείγμα 1

Analysis Results

MyVariants | Download | Visualize | Selected Variants | Send to Report Role | Switch To | Generate Report

Analysis Name: 18953 sarc_v1_c1517_2023-12-14-09-26-19-901
Total Unmapped Reads: 66066

Fusion Sample QC: PASS,[TotalMappedFusionPanelReads>20000;M... **Fusion Overall Call**: POSITIVE ,[DriverGene=FLI1,IsoformsDetected=... **Total Mapped Fusion Panel Reads**: 51716

To learn more about reviewing your results, visit the help guide.

Fusions

FLI1 | Go | Preferences

Genes (Exons)	Read Counts	Detection	3'/5' Imbala...	Ratio To Wild Type	Norm Count Within Gene	COSMIC/NCBI	Variant ID	Rea
EWSR1(9) - FLI1(4)	0	Absent, READ_COUNT<= and NORM_COUNT<=				COSF175	EWSR1-FLI1.E9F4.COSF175	
EWSR1(9) - FLI1(6)	0	Absent, READ_COUNT<= and NORM_COUNT<=				COSF1302	EWSR1-FLI1.E10F6.COSF1302	
EWSR1(8) - FLI1(6)	0	Absent, READ_COUNT<= and NORM_COUNT<=				COSF183	EWSR1-FLI1.E8F6.COSF183	
EWSR1(7) - FLI1(8)	0	Absent, READ_COUNT<= and NORM_COUNT<=				COSF227	EWSR1-FLI1.E7F8.COSF227	
EWSR1(9) - FLI1(7)	0	Absent, READ_COUNT<= and NORM_COUNT<=				COSF176	EWSR1-FLI1.E10F7.COSF176	
EWSR1(7) - FLI1(6)	5442	Present, .				COSF180	EWSR1-FLI1.E7F6.COSF180	

Filter Options

Variants
- **Filtered In Variants (13)**
- Hidden Variants (0)
- Filtered Out Variants (0)

Samples
- **Fusions Sample:** 18953 sarc_v1
  - **Gender :** Unknown
  - **Sample Type :** DNA

Chromosome: All

Filter Chains: No Filter

No filters selected

Save Filter Chain

Ewing sarcoma/peripheral neuroectodermal tumor	**t(11;22)(q24;q12)**	***EWSR1::FLI1***
	t(21;22)(q22;q12)	***EWSR1::ERG***
	t(2;22)(q33;q12)	***EWSR1::FEV***
	t(7;22)(p22;q12)	***EWSR1::ETV1***
	t(17;22)(q12;q12)	***EWSR1::E1AF***
	inv(22)(q12q;12)	***EWSR1::ZSG***
	t(16;21)(p11;q22)	***FUS::ERG***

Αξιολόγηση αποτελεσμάτων

Fusion

GRCh38 · COSMIC v99

Summary
Genomic Details
Related Breakpoints
Samples
Reset page

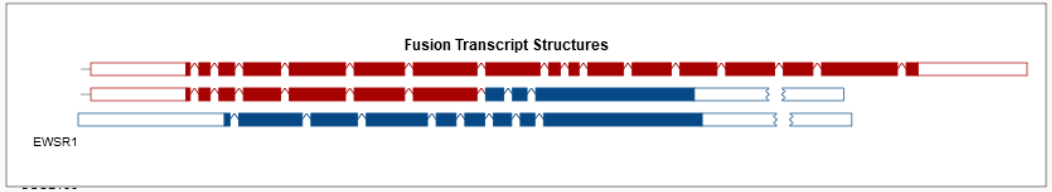
Summary

This section shows a summary of the selected fusion. You can see more information on the help pages.

Mutation Id COSF180

Type This fusion structure is derived from the range of fusion mRNAs reported.

Translocation Name ENST00000397938.6(EWSR1):r.1_1112::ENST00000527786.6(FLI1):r.1211_4127



Genomic Details

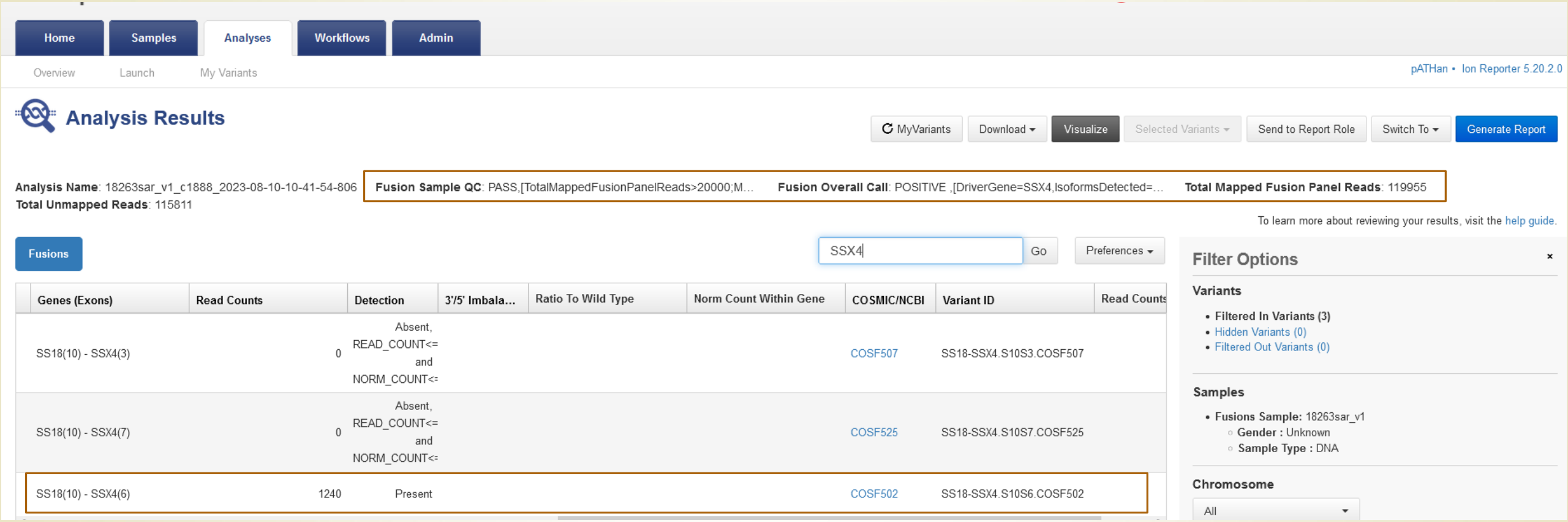
This section shows the details of the genomic breakpoints. You can see more information on the help pages.

Genomic Details

Gene Name	Chr	Genome start from	Genome start to	Genome stop from	Genome stop to	Strand
EWSR1_ENST00000397938	22	29268018	29268018	29287134	29287134	+
FLI1	11	128807180	128807180	128813267	128813267	+

RNA αποτελέσματα (ION REPORTER)

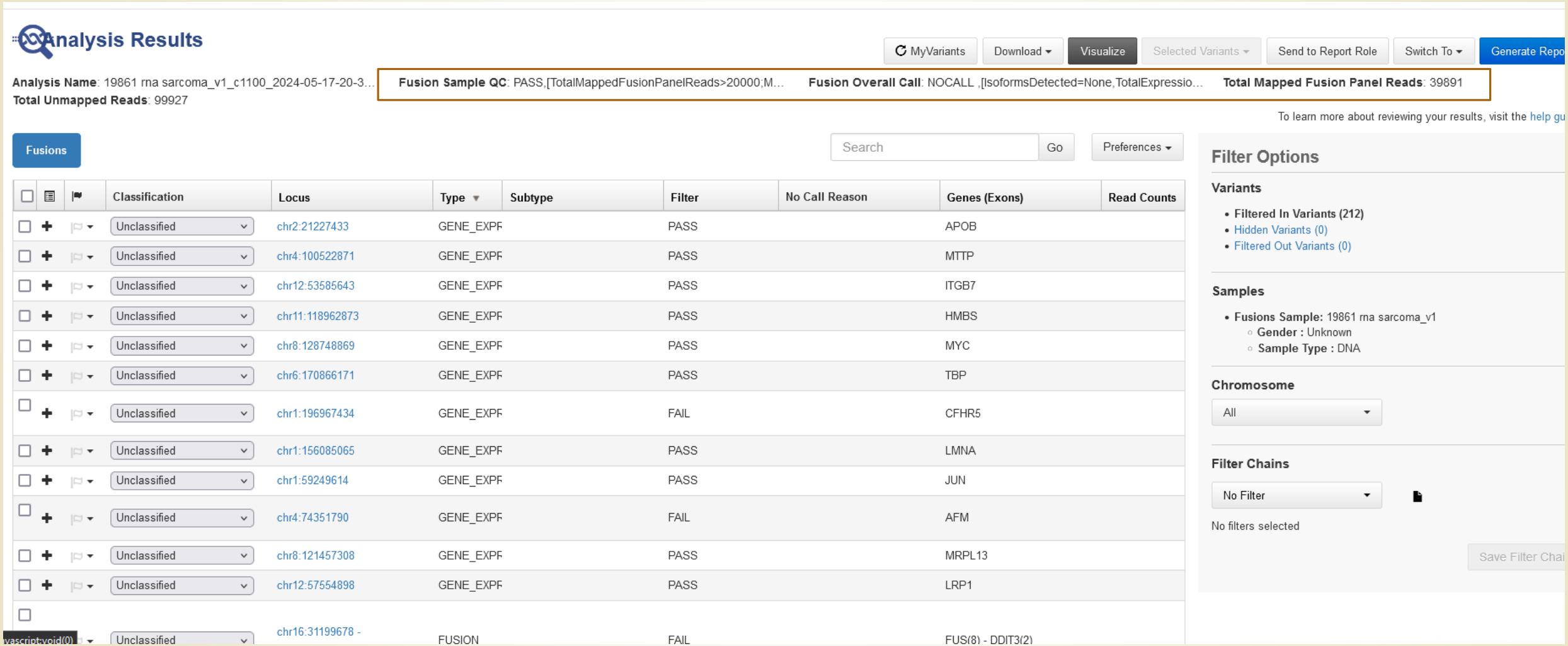
Δείγμα 2



Synovial sarcoma	**t(X;18)(p11;q11); t(X;18)(p11;q11); t(X;18)(p11;q11)**	***SS18::SSX1; SS18::SSX2; SS18::SSX4***

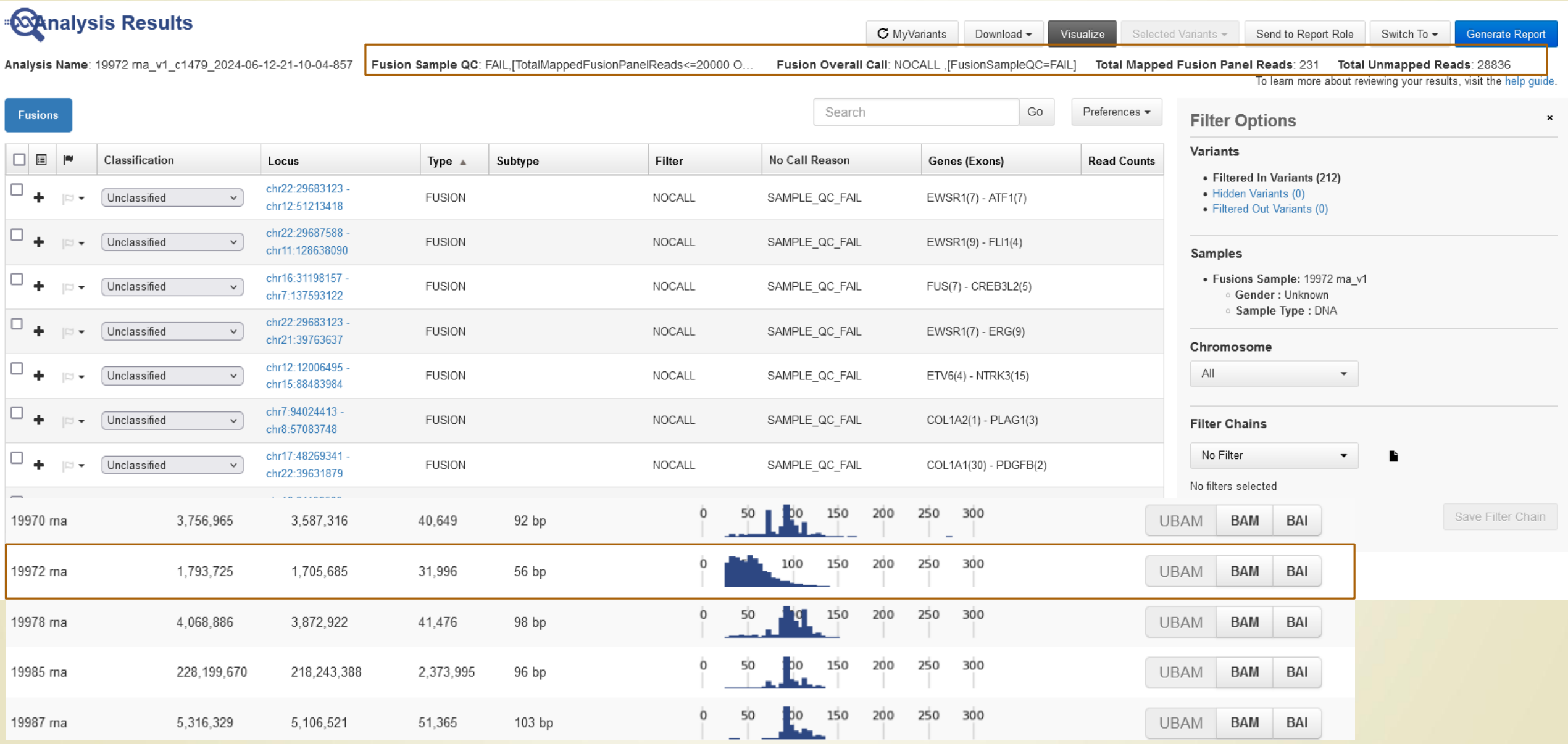
RNA αποτελέσματα (ION REPORTER)

Δείγμα 3



RNA αποτελέσματα (ION REPORTER)

Δείγμα 4



Αποτελέσματα DNA/RNA

Μεταλλαγές

Τα αποτελέσματα αφορούν μόνο τις περιοχές που έχουν αναλυθεί

Δεν αποκλείεται η ύπαρξη μεταλλαγών εκτός των αναλυμένων περιοχών

Διαμεταθέσεις

Τα αποτελέσματα αφορούν μόνο τις διαμεταθέσεις για τις οποίες είναι σχεδιασμένο το πανελ

Δεν αποκλείεται η ύπαρξη διαμεταθέσεων μεταξύ άλλων γονιδίων

Συμπεράσματα

- NGS χρήσιμη τεχνολογία για την παράλληλη ανάγνωση αλληλουχιών για την ανίχνευση μεταλλαγών σε επίπεδο DNA / ανίχνευση σύντηξης γονιδίων → στοχευμένη και εξατομικευμένη ιατρική, διαφορική διάγνωση και στην ανακάλυψη νέων βιοδεκτών

- Βασική προϋπόθεση τα δείγματα προς ανάλυση να ακολουθούν τα προ-αναλυτικά κριτήρια και εφόσον τα πληρούν να ακολουθούν και τα ποιοτικά κριτήρια κατά τη βιοπληροφορική ανάλυση

Ευχαριστώ πολύ για την προσοχή σας