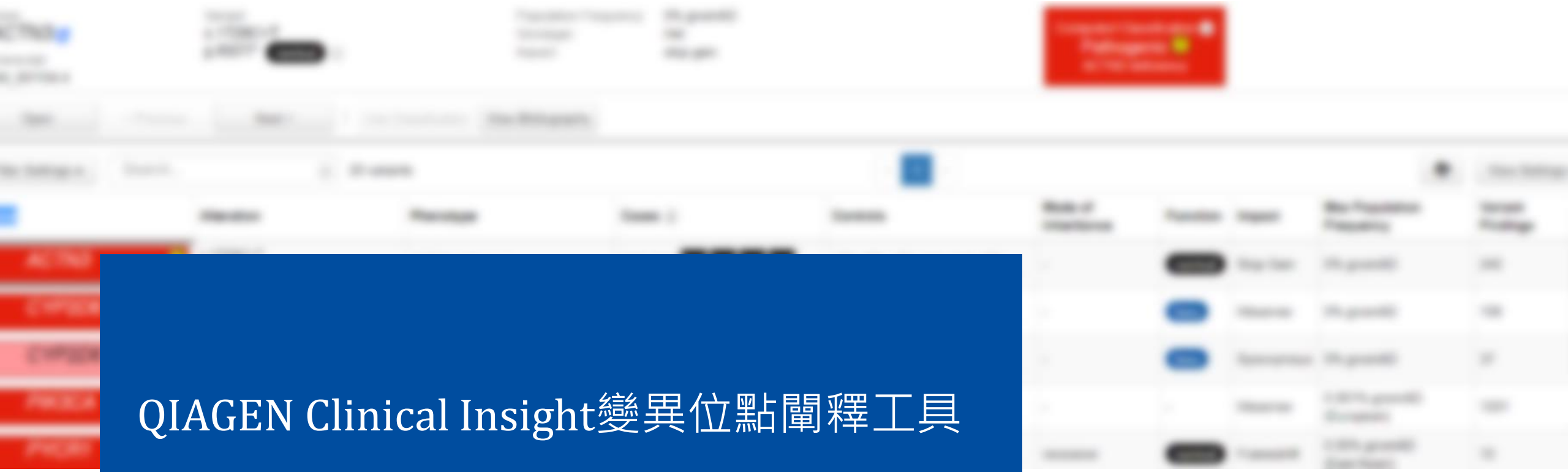
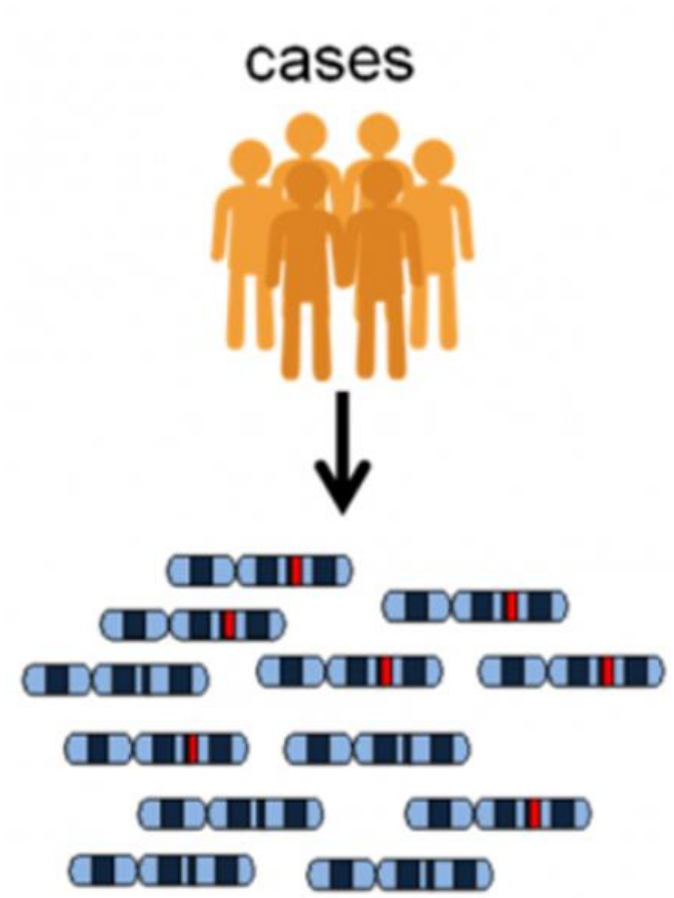


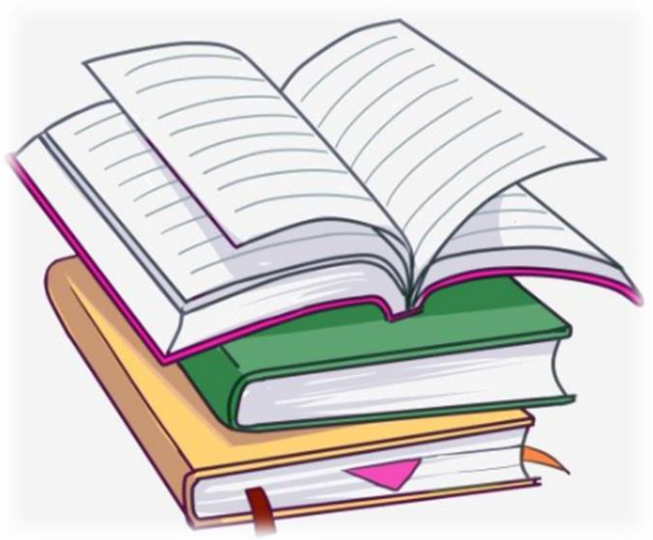
QIAGEN Clinical Insight變異位點闡釋工具

2021.09.09

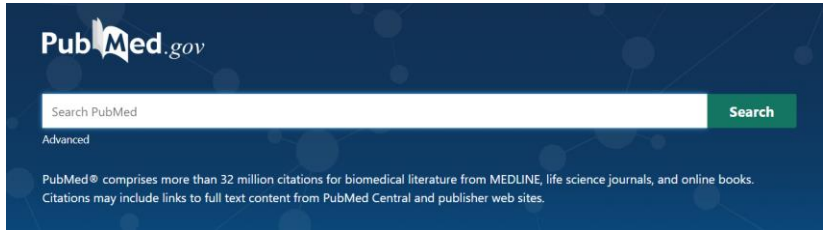
Clair Tsai 蔡宜庭 業務副理
Willis Cheng 鄭耀瑋 專案主任



?



Papers & References



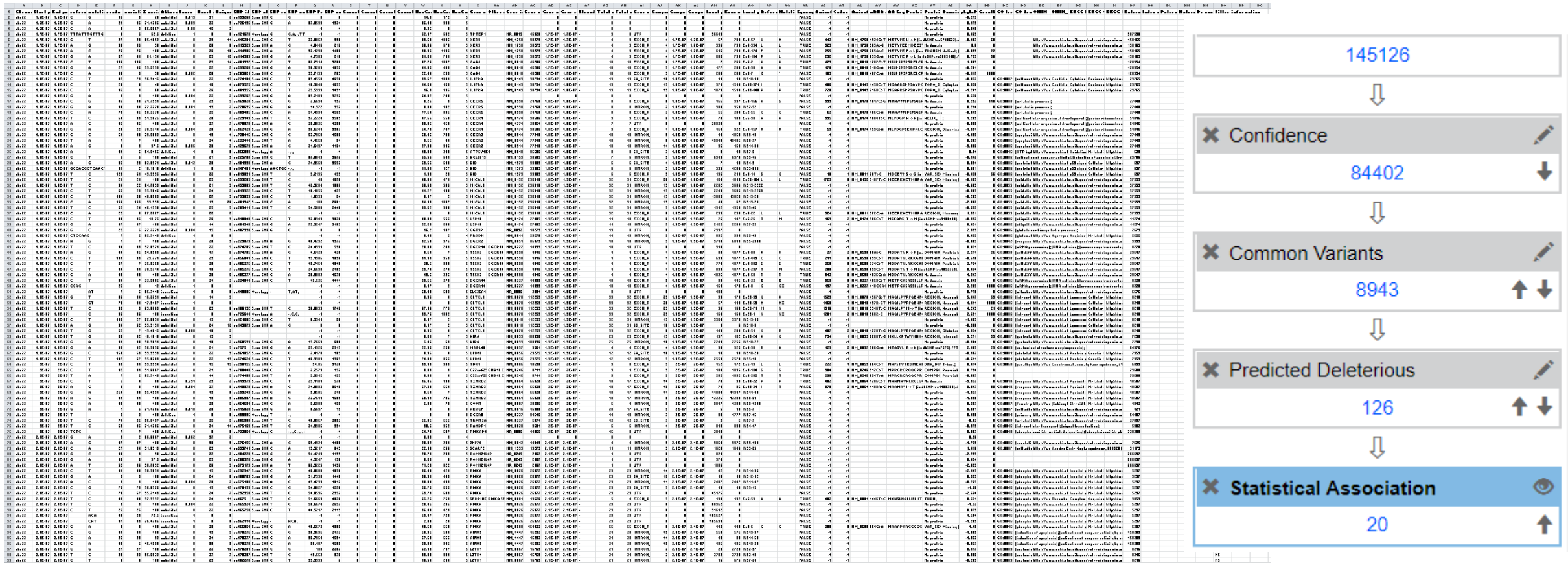
Pubmed or Google

https://reurl.cc/OXlYvv

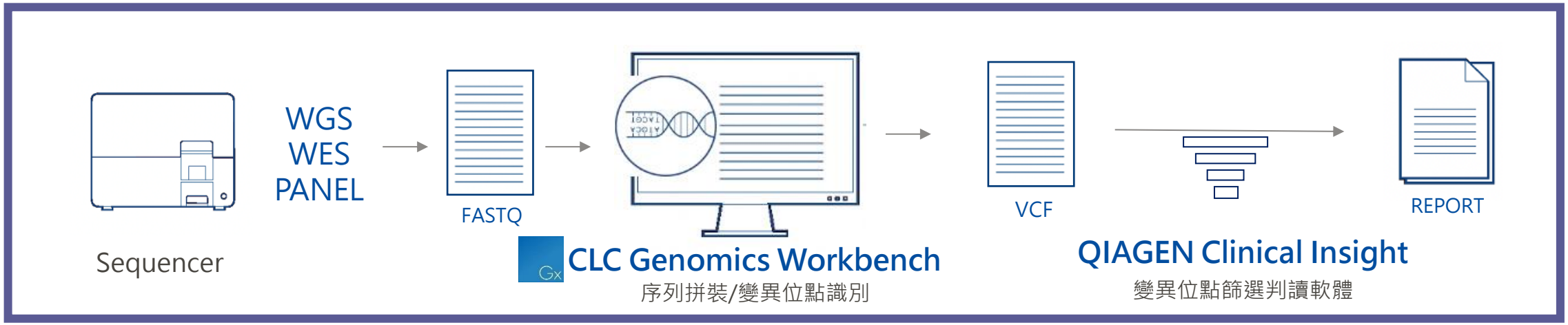
Identifying the Causal Variants the “Old Way”

“Curation of 90 to 127 variants in each participant required a median of 54 minutes (range, 5-223 minutes) per genetic variant”

FE Dewey et al, JAMA. 2014;311(10):1035-1044.



After sequencing, What's Next?



WES全外顯子約有
60,000,000bp

→

與人體參考基因組比對
約有30,000個變異位點

→

如何找到關鍵制病位點?

1. 常見位點篩選
2. 位點信心程度篩選
3. 資料庫評斷位點制病性
4. 位點與疾病的相關程度
5. 位點用藥資訊
6. 制病位點報告生成

*WGS有30億個鹼基對

NGS Variant Analysis Service

Variants number

1: sequencing & base call [FastQ]

```
@FCC078PACXX:2:1101:1184:3385#ACTACAAG/2
GGGGCATCAATGATAGTCACATAGTACTTGCTGGTCTCAAATTTCCACAAGGAGATATCAATGGTGATACCACGTTCACGCTCAGCTTTC
+
_bbeeeeegfgfgehhgfghhhfighiiiiiiiihbchfhiiihhiiiigdegfhdfgghihfdddfgdhihdgfgge6dcdb6dcd6
@FCC078PACXX:2:1101:1162:3439#ACTACAAG/2
CTGCTTTCCCTTTCCTTGCATATTCTGGAATCCCAGCAGTTTCTTTCTGTTTTTGCGAGGACACAGATTATCCTTATTTGGGTACCACCA
+
[__ceeeeacecg`beadf`gbg`chfdhhhhiihibd_^cefhhhe]aYaefdffhafff[`cdgddbbd]dccdcccc`]Z`bbbb8B
@FCC078PACXX:2:1101:1210:3447#ACTACAAG/2
GCGCCGCATCACTGTGCCTGCTTGAGGTATAAGCCGCTGAGTCCCCCGAGTGAAGACATGGCCCTGACTGCACTCAATCTGGATGCCCCG
+
a_bceeceeggeghaeefhff]]ebcf^bggffhie]fafh[beghifggG\ZZZ__`Z`dbdccc^]bbcbc]bX]bbb6bbY_^`ba_
⋮
```

2: Variants calling[VCF]

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT
chr1	978603	rs3588118	CCT	C	2883.6	PASS	AC=1;AF=	GT:AD:DP:GQ:PL
chr1	1163811	rs1476947	G	GGACA	2118.64	PASS	AC=1;AF=	GT:AD:DP:GQ:PL
chr1	1177918	rs3835300	CT	C	1614.6	PASS	AC=1;AF=	GT:AD:DP:GQ:PL
chr1	1223465	rs5808105	GC	G	375.6	PASS	AC=1;AF=	GT:AD:DP:GQ:PL
chr1	1247578	.	T	TGG	13	PASS	P=0.9462	GT:VR:RR:DP:GQ

3 : variants annotation [annotation file]

Gene	Type	Position	AAchange	dbsnp	AlleleFreq	gnomAD_e	gnomAD_g	primaryACMGEvid	primaryACMGLe
TSHR	nonsynony	chr14:816(	NM_000369.3:exon9:c.	rs1895064	0.0019230	0|22|-	0|3|-	pm1 pm2 pm5 pp3	Likely pathogen
LZTR1	nonsynony	chr22:213(	NM_006767.4:exon14:c	rs1331354	5.57165e-(	0|1|-	.	pm1 pm2 pm5	Likely pathogen
C7	frameshift	chr5:4095(	NM_000587.4:exon11:c	rs7458262	0.0006426	0|5|-	0|1|-	pvs1 pm2	Likely pathogen
CACNA1A	nonsynony	chr19:133(	NM_001127221.1:exon	rs5741943	0.0008409	0|15|-	.	pp2 pm1 pm2 pp3	Likely pathogen
CACNA1A	nonframes	chr19:133(	NM_001127222.2:exon	.	.	.	.	pm2 bp3	VUS
HSD17B4	nonsynony	chr5:1188(	NM_000414.4:exon4:c.	rs5444551	0.0006524	0|12|-	.	pm1 pm2 pp3	VUS
COL11A1	nonsynony	chr1:1035(	NM_001854.4:exon3:c.	rs1158320	.	.	.	pm1 pm2 bp4	VUS
KMT2C	stopgain	chr7:1519(	NM_170606.3:exon18:c	rs5852856	.	.	.	pvs1 pm2	Likely pathogen

4 : Clinical report

Gene	Position	Transcript	HGVS(.c) (Exon)	HGVS(.p)	Type	dbSNP	MAF	Heterozygosity	Clinical Level	Disease and Inheritance
MYH7	chr14:23902865-23902865	NM_000257.3	c.77C>T (exon3)	p.A26V	nonsynonymous SNV	rs186964570	0.0069	het	Likely pathogenic	Cardiomyopathy, hypertrophic, 1 AD

Format

FASTQ

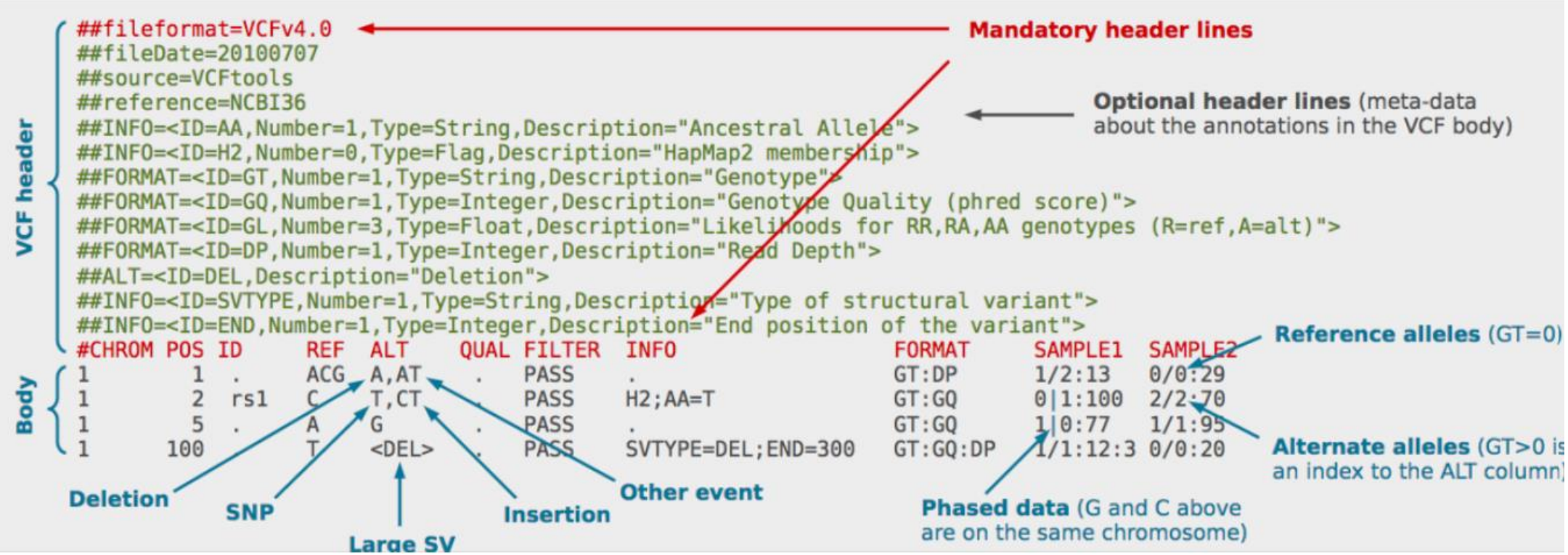
VCF

Excel / TXT

Excel / TXT

PDF

VCF(Variant Calling Format)

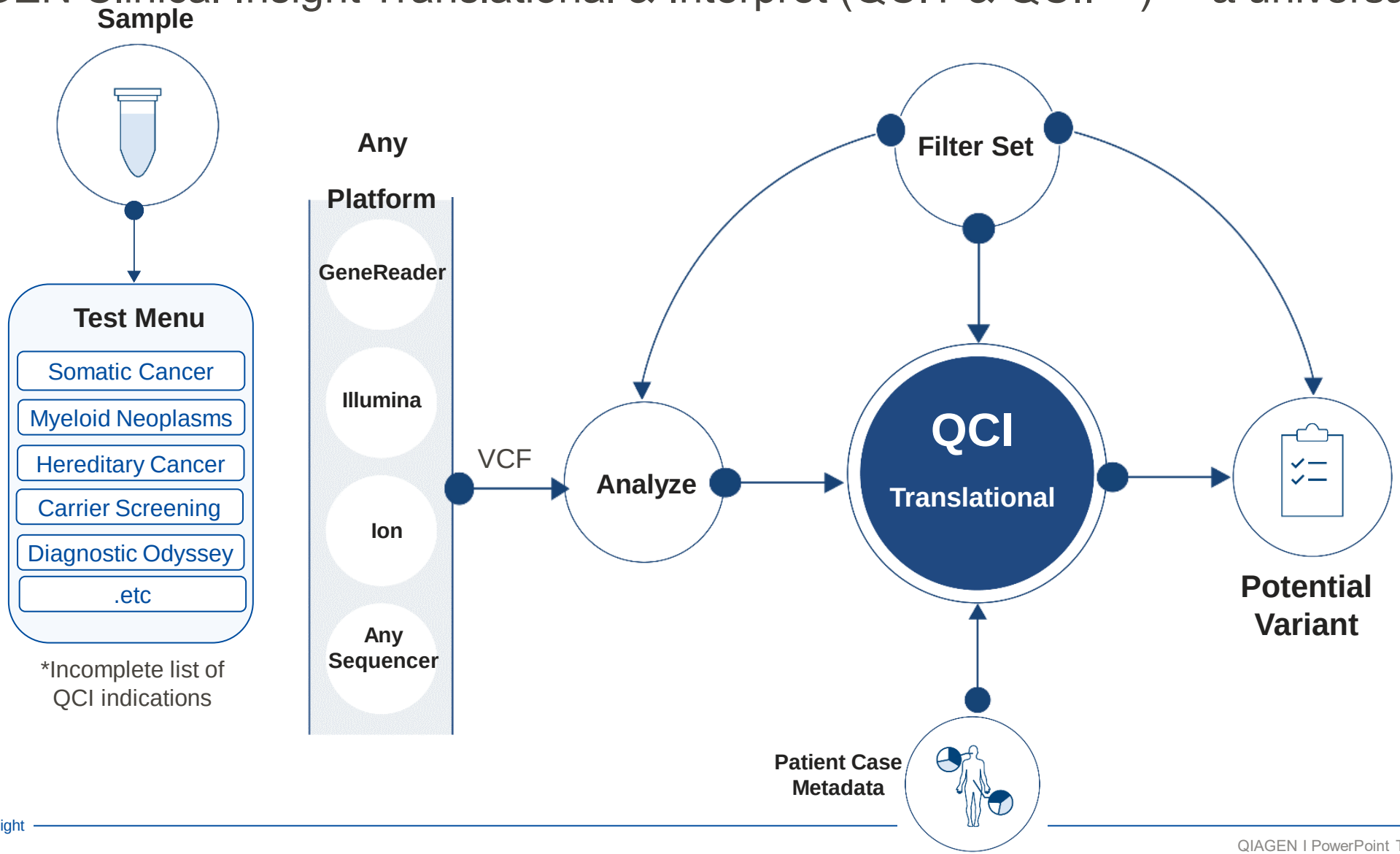


https://weitinglin.com/2017/05/29/vcfvariant-call-format-%E5%9F%BA%E5%9B%A0%E7%AA%81%E8%AE%8A%E8%B3%87%E6%96%99%E5%84%B2%E5%AD%98%E6%A0%BC%E5%BC%8F/comment-page-1/

Database in QCII/QCIT (Free access in QCII/QCIT software)

Public Databases	Licensed Databases	QIAGEN Databases	Clinical Guidelines	Others
TCGA	COSMIC	Clinical Cases	ACMG	CADD
Clinvar	ICGC	Clinical & Functional Studies	AMP	Polyphen
dbSNP	OMIM	HGMD	NCCN	SIFT
1000 Genome	BIC	PGMD	ASCO	PhyloP
ESP	Cento MD	Curated variants	CAP	Blosum
gnomAD		Pathway and causal network	ESMO	MaxEntScan
ExAC		Allele Frequency Community	FDA	Mutation Taster
Clinical Trials			EMA	

QIAGEN Clinical Insight Translational & Interpret (QCIT & QCII™) – a universal solution



QCII/QCIT difference

	QCIT(偏研究使用)	QCII(偏臨床使用)
功能	註解工具與篩選位點	註解工具與篩選位點 臨床與藥物資料提供臨床判讀使用
應用	• 可單一或多樣本分析，或是家族(trio)分析 • 可做群組分析(cohort study)	• 出具臨床報告
優勢	• Qiagen內建database(含ACMG guideline) • 有權限管理系統 • 可設定多種分析流程	• Qiagen內建database(含ACMG&AMP guideline) • 可客製報告模板 • 有權限管理系統 • 可設定多種分析流程(TPP) • 可設定報告簽核系統
輸出	Excel表格(註釋資料)	Excel表格與檢測報告

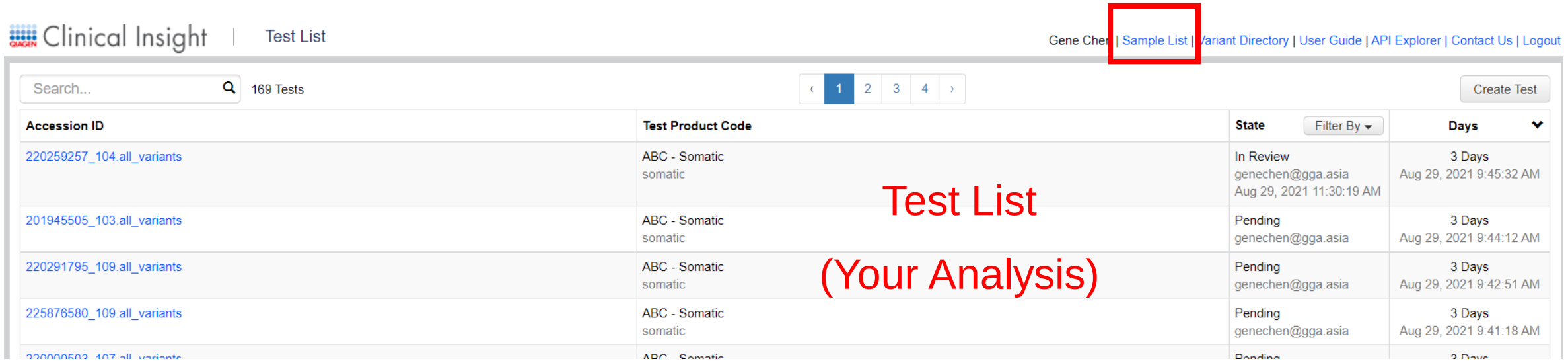
How to log in QCI System

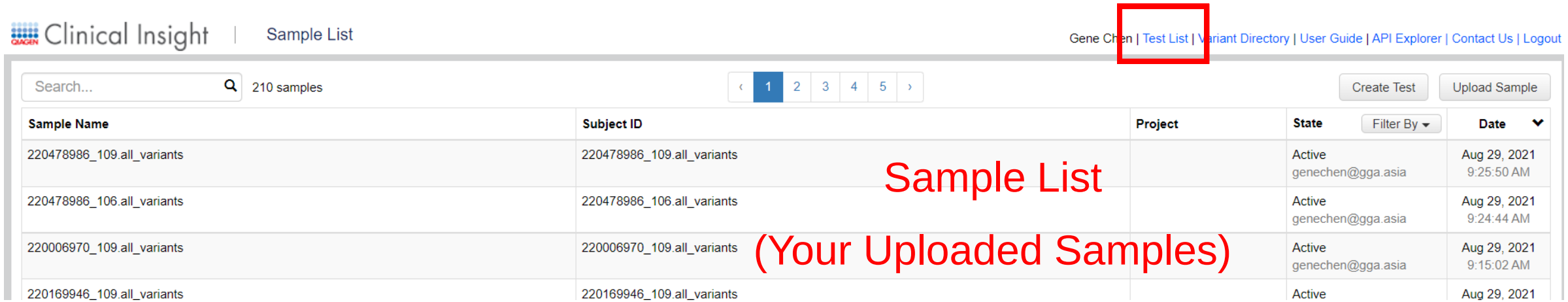
QCI Interpret Translational for USA QCIT

QCI Interpret Translational for China

QCI Interpret for USA QCII

Sample & Test List



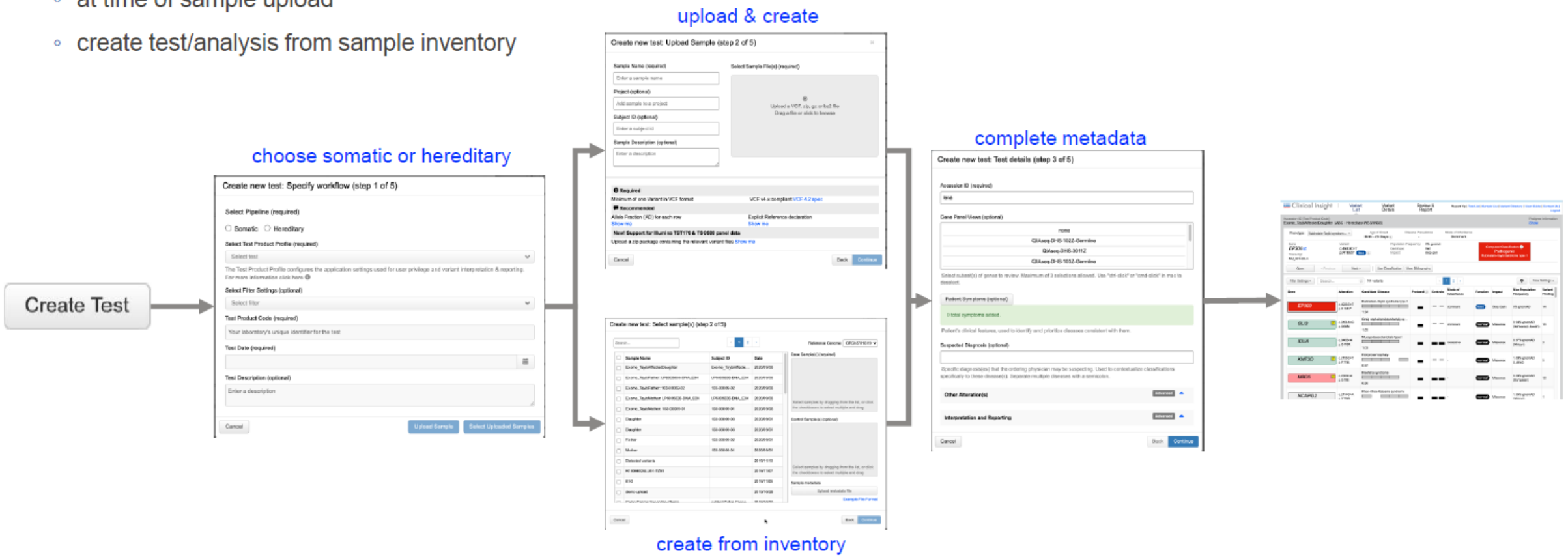


Create Your Variant Analysis Strategically

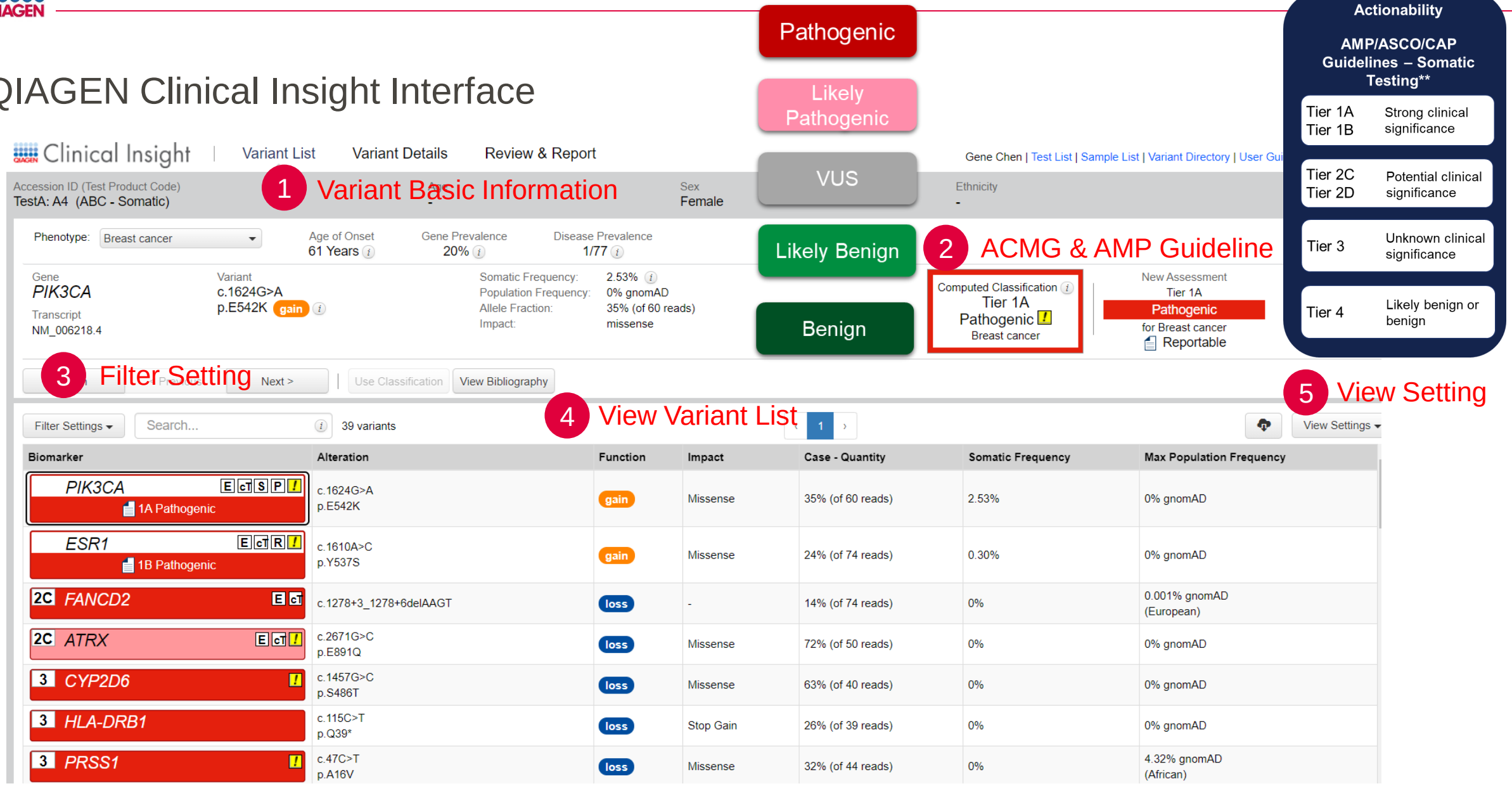
New Create Test button to start test creation workflow

Create new test/analysis

- at time of sample upload
- create test/analysis from sample inventory

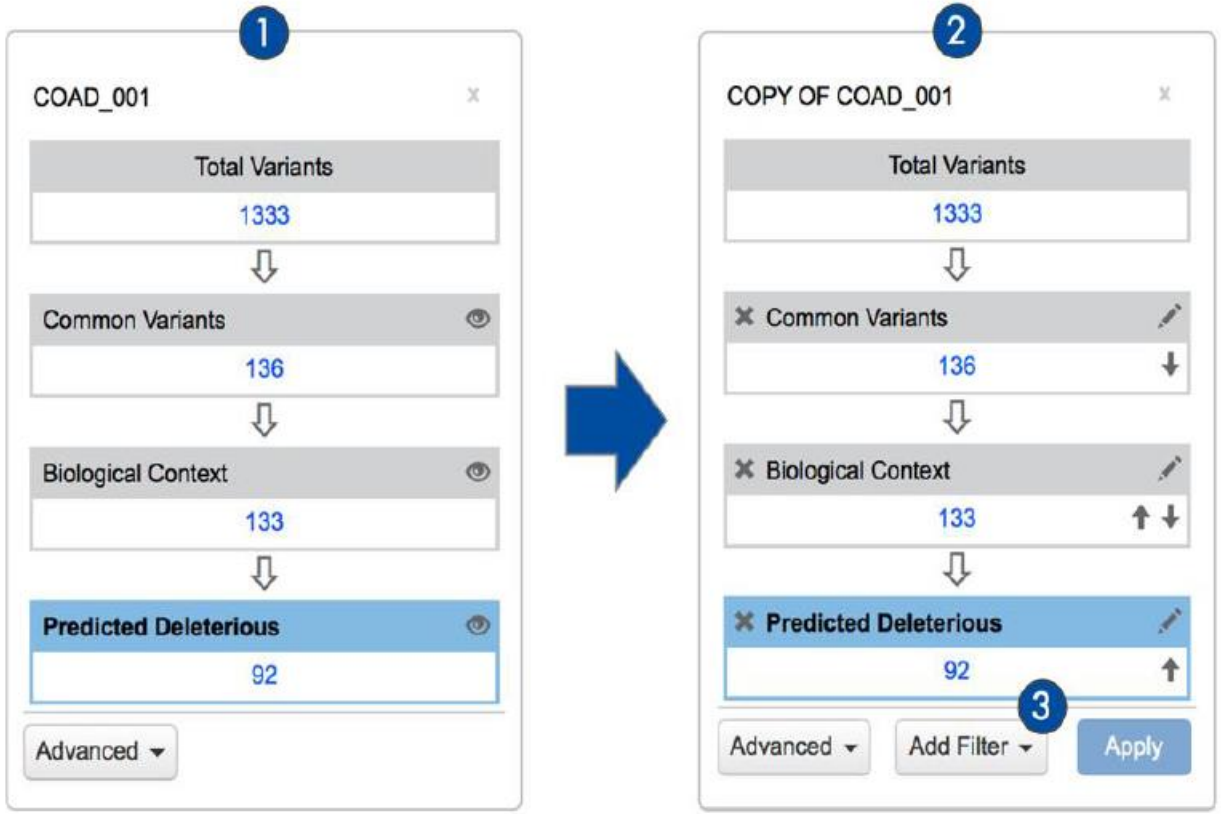


QIAGEN Clinical Insight Interface



Characterizing the Potential Variants by Databases and Filter Set

- QCI users can view the filtering strategy to see how and where variants are filtered out
- Clicking on blue numbers updates the variant table with list of filtered variants



Default user

Director user

Variant Filtering Sets

The following 4 filters are introduced to QCI variant filtering:

- Common Variants
  - remove/include variants based on population frequency cut off
- Confidence
  - remove/include variants based on variant calling metrics
- Predicted Deleterious
  - select or variants based on ACMG, HGMD, ClinVar , literature evidence, or in silico predictions
- Biological Context
  - based on relationships (direct/indirect) between gene and biological terms within QIAGEN ontology/QKB

Test Performed: **Somatic Panel**

Report Date Nov 8, 2020
Status -

Patient		Client		Specimen	
Patient Name	Michelle Doe	Client	General Hospital	Accession ID	TestA: A4
Date of Birth		Client ID	ABC123	Specimen	biopsy
Age		Physician	Dr. E Smith	Collection	Nov 9, 2020
Sex	Female	Pathologist	Dr. R Jones	Accession	Nov 9, 2020
Ethnicity				Primary Tumor Site	Breast
Diagnosis	Breast Cancer				

Result: Positive

2	5	8	22
Clinically Significant Variants	Therapies Associated with Resistance	Therapies with Potential Clinical Benefit	Clinical Trials

Report Summary

PIK3CA E542K was identified and is associated with an available treatment. One alteration is associated with resistance to aromatase inhibitor therapy.

######

Actionable Variants With Associated Therapies

		Approved Therapies			
Gene / Variant	**Allelic Fraction**	**Breast Cancer**	**Other Indications**	**Associated With Resistance**	**Clinical Trials**
PIK3CA c.1624G>A p.E542K g.179218294G>A **Tier 1A** Pathogenic	35.0% (of 60 reads)	alpelisib alpelisib /fulvestrant lapatinib /letrozole letrozole	-	-	19
ESR1 c.1610A>C p.Y537S g.152098788A>C **Tier 1B** Pathogenic	24.0% (of 74 reads)	fulvestrant neratinib tamoxifen toremifene	-	anastrozole aromatase inhibitor fulvestrant letrozole tamoxifen	3

Gene / Variant	Trial Title Trial ID	Treatments	Trial Phase	Location / Contact
ESR1 p.Y537S g.152098788A>C **Tier 1B** Pathogenic	A Phase 1 Study of SY 5609, an Oral, Selective CDK7 Inhibitor, in Adult Patients With Select Advanced Solid Tumors NCT04247126	SY-5609 fulvestrant	Phase 1	United States: MI, OK, PA, TN, TX Kimberley Caliri; kcaliri@syros.com; 617-674-9053;
ESR1 p.Y537S g.152098788A>C **Tier 1B** Pathogenic	INTERACT- Integrated Evaluation of Resistance and Actionability Using Circulating Tumor DNA in HR Positive Metastatic Breast Cancers NCT04256941	anastrozole letrozole ribociclib abemaciclib /letrozole letrozole /palbociclib palbociclib abemaciclib fulvestrant letrozole /ribociclib	Phase 2	United States: TX Senthilkumar Damodaran; sdamodaran@mdanderson.org; 713-792-2817;

Individual Variant Interpretations

Gene ***PIK3CA*** Exon 10 Nucleotide NM_006218.4: g.179218294G>A c.1624G>A Amino Acid p.E542K Function gain Allelic Fraction 35.0% (of 60 reads) Classification **Tier 1A** Assessment Pathogenic	**Interpretation** PIK3CA encodes the protein p110-alpha, which is the catalytic subunit of phosphatidylinositol 3-kinase (PI3K). The PI3K pathway is involved in cell signaling that regulates a number of critical cellular functions, including cell growth, proliferation, differentiation, motility, and survival [16, 6]. PIK3CA mutations are not mutually exclusive with EGFR or KRAS or BRAF mutations, and are associated with increased PI3K signaling and increased activation of Akt [22, 10]. Activating missense mutations in PIK3CA, including the E542K, E545K, and H1047R hotspot mutations, predominantly occur in the helical (exon 9) and kinase (exon 20) domains [17, 2].
Gene ***ESR1*** Exon 10 Nucleotide NM_001122742.1: g.152098788A>C c.1610A>C Amino Acid p.Y537S Function gain Allelic Fraction 24.0% (of 74 reads) Classification **Tier 1B** Assessment Pathogenic	**Interpretation** ESR1 encodes estrogen receptor alpha (ER-alpha), one of the major estrogen receptor isoforms in humans; binding of estrogen to ER-alpha promotes its translocation to the nucleus and the transcriptional activation of genes involved in cell cycle progression and survival [14]. Mutation or amplification of ESR1 and activation of ER-alpha may result in the upregulation of genes involved in cell cycle progression and survival, and ER-alpha signaling has been implicated in a number of cancer types [14, 12, 4, 21, 15]. However, ER-alpha may act as a tumor suppressor in some cancers [23, 3, 1, 5].

CASE STUDY

1. 針對單一病人表型之癌症病患篩選位點並提供用藥資訊
2. 針對同群體病人表型來判讀變異位點資訊

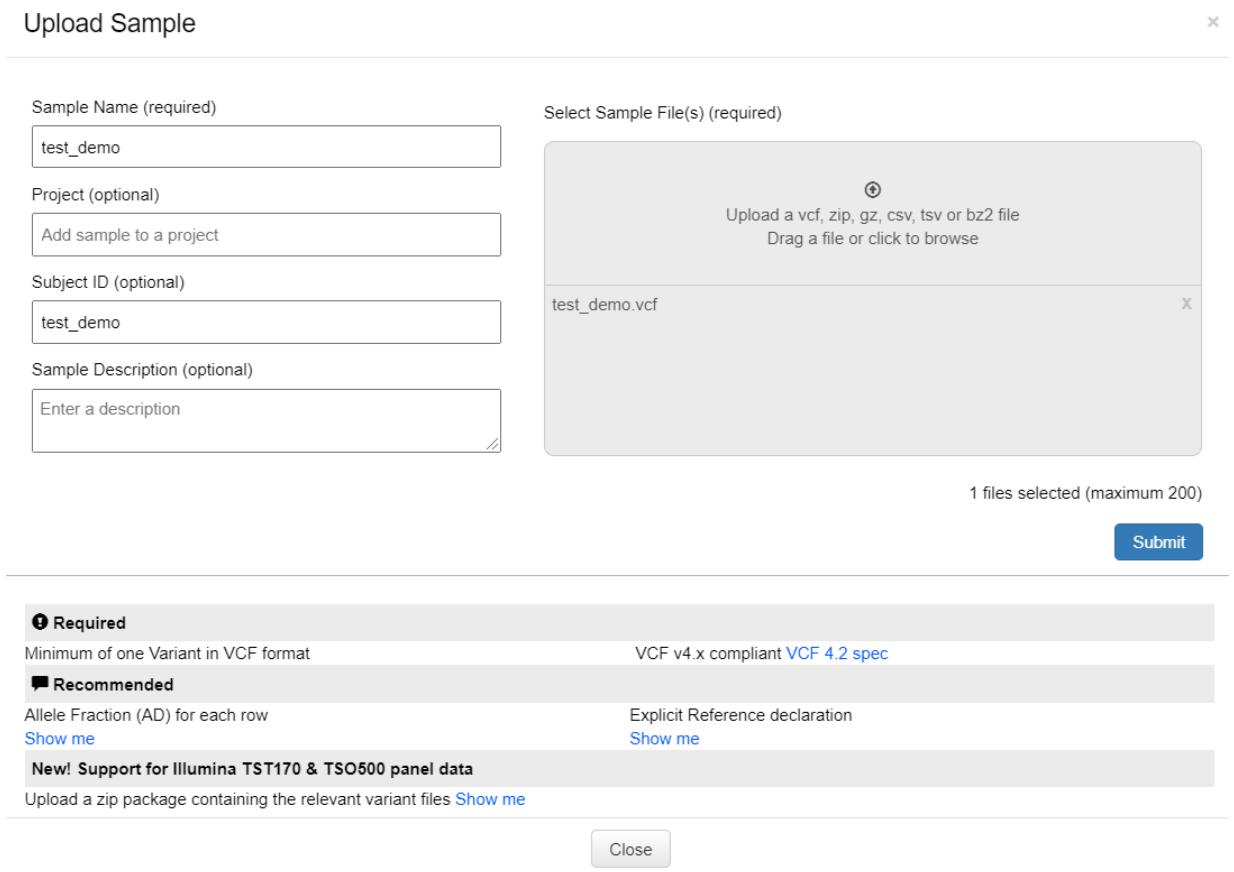
- Test-demo: 4 samples
  - Breast cancer

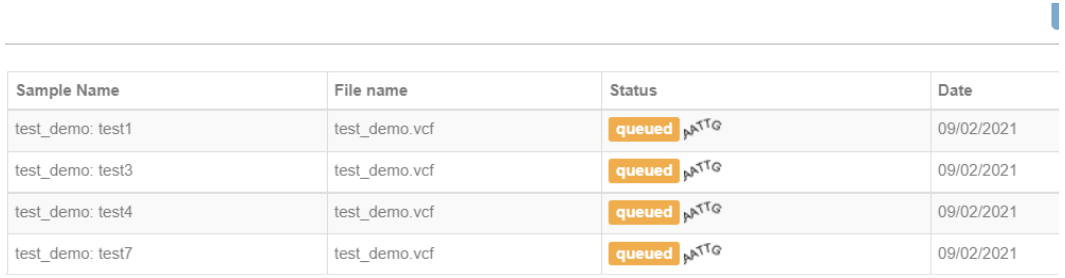
test_demo.vcf

test_demo: test7
test_demo: test4
test_demo: test3
test_demo: test1

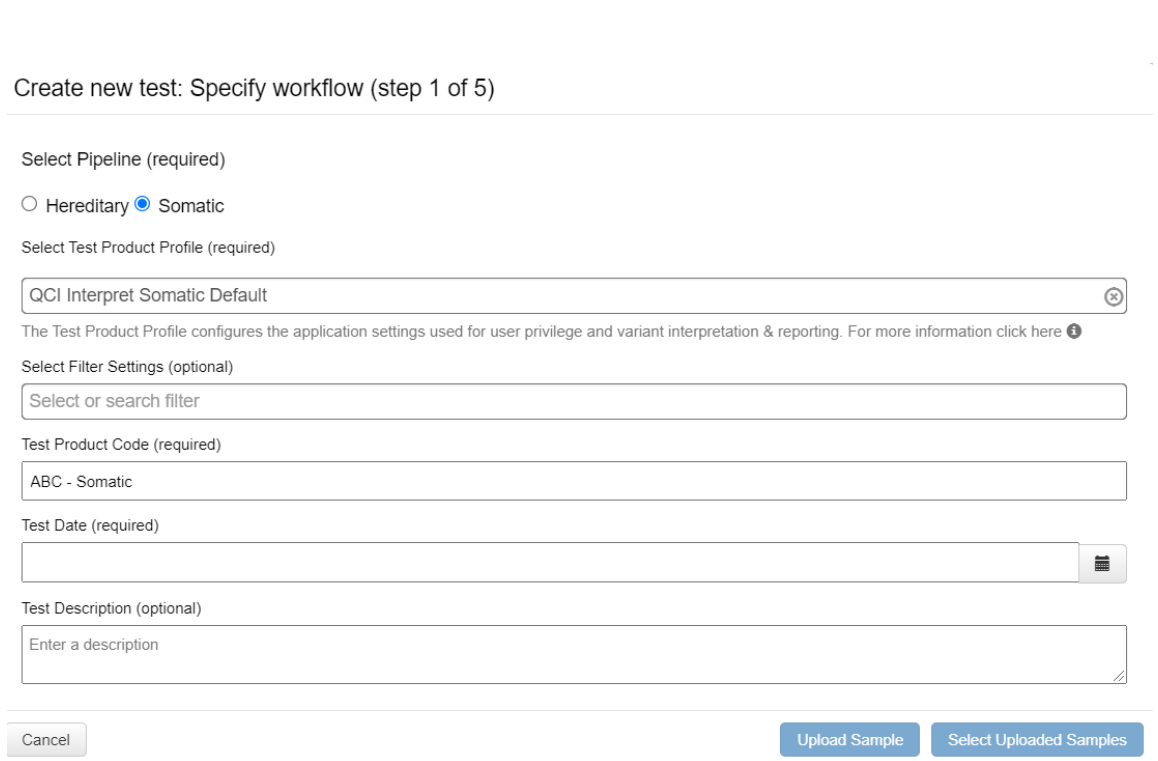
Sample Upload

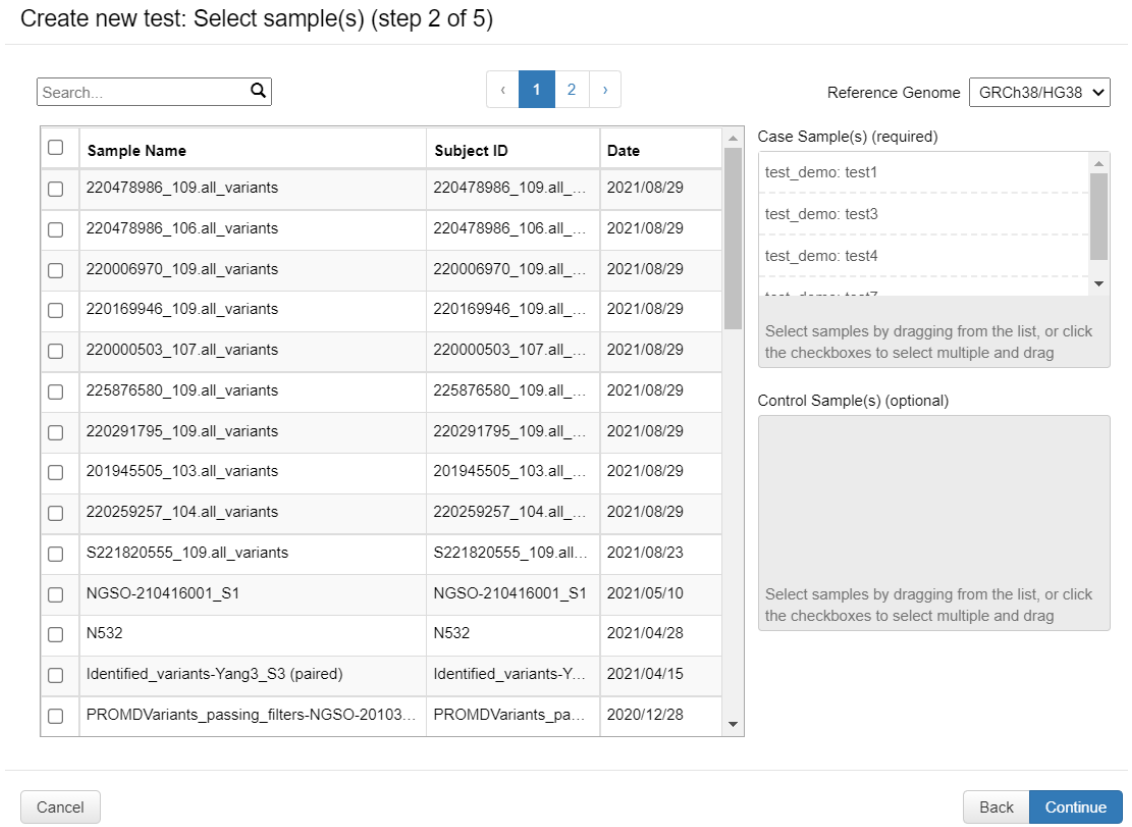
- Samples in multiple VCF files or in single VCF file will be separate into individual sample record.





Set a new analysis





Create new test: Test details (step 3 of 5)

Accession ID (required)

test_demo: KMU

Keep only confidently detected variants (optional)

Keep only variants that pass upstream pipeline quality filtering (VCF FILTER column is PASS or '.')

Gene Panel Views (optional)

- QIAact-BRCA-UMI-FFPE_QIAGEN
- Illumina® TruSight™ Tumor 170
- Illumina® TruSight™ Oncology 500
- QIAact-Lung-Plasma-Track

Select subset(s) of genes to review. Maximum of 3 selections allowed. Use "ctrl-click" or "cmd-click" in mac to deselect.

Diagnosis (required)

Breast Cancer

This diagnosis will be used to match treatments and trials and for display on the report.

Diagnosis Stage (optional)

-- Select diagnosis stage --

This is used to contextualize clinical trials to the selected stage. If left unselected, all clinical trials pertinent to the diagnosis will be displayed.

Cancel | Back | Continue

Create new test: Patient Information (step 4 of 5)

Information about the subject

Sex (optional)

Male Female

This is used to limit to gender-appropriate clinical trials.

Ethnicity (optional)

<select ethnicity>

Date of Birth (optional)

Age (recommended)

This is used to limit to age-appropriate clinical trials (e.g. pediatric for children).

Cancel | Back | Continue

Create new test: Reporting (step 5 of 5)

The information below is used only on the final report. For validation samples, you can use the data that has been pre-filled for you below, or hit Clear Data and enter your own information.

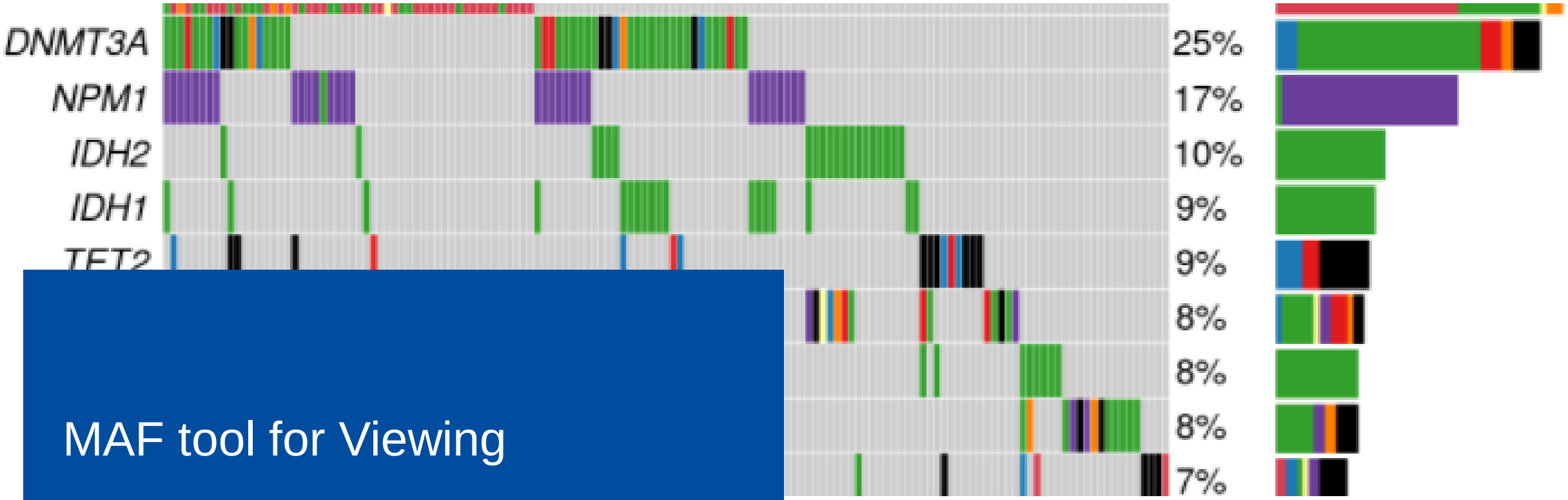
Demo Data | Clear Data | Reset Data

Subject Name (optional)

e.g. Michelle Doe

Client Name (optional)

e.g. General Hospital



MAF tool for Viewing

MAF Format

- MAF (Mutation Annotation Format)
  - A Mutation Annotation Format (MAF) file (.maf) is a **tab-delimited text file** that lists mutations. The format originates from The Cancer Genome Atlas (TCGA) project and is described in detail here. As such, the format pertains to human genomes.
  - In the context of human cancer, MAF files come in two types--protected and somatic. These two types extend conceptually to (1) mutation files that contain all sequenced mutations--however mutations are defined--e.g. against matched normal tissue, against a reference much like for the VCF format, or against another tumor stage, and (2) mutation files that filter the mutation types that are listed based on set criteria, e.g. only somatic mutations.
  - https://software.broadinstitute.org/software/igv/MutationAnnotationFormat

Requirement of MAF File

Field	Description	Allowed values
Hugo_Symbol	HUGO gene symbol	-
Chromosome	Chromosome no.	1-22, X, Y
Start_Position	Event start position	Numeric
End_Position	Event end position	Numeric
Reference_Allele	Positive strand reference allele	A, T, C, G
Tumor_Seq_Allele2	Primary data genotype	A, T, C, G
Variant_Classification	Translational effect of variant allele	Frame_Shift_Del, Frame_Shift_Ins, In_Frame_Del, In_Frame_Ins, Missense_Mutation, Nonsense_Mutation, Silent, Splice_Site, Translation_Start_Site, Nonstop_Mutation, 3'UTR, 3'Flank, 5'UTR, 5'Flank, IGR, Intron, RNA, Targeted_Region, De_novo_Start_InFrame, De_novo_Start_OutOfFrame, Splice_Region, Unknown
Variant_Type	Variant Type	SNP, DNP, INS, DEL, TNP and ONP
Tumor_Sample_Barcode	Sample ID	Either a TCGA barcode, or for non-TCGA data, a literal SAMPLE_ID as listed in the clinical data file

https://github.com/umccr/MAF-summary#maf-field-requirements

Example of MAF

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	Hugo_Syn	Entrez_Ge	Center	NCBI_Bu	Chromoso	Start_Posit	End_Posit	Strand	Variant_C	Variant_T	Reference_	Tumor_Se	Tumor_Se	Tumor_Sa	Protein_Cl	i_TumorV	i_transcript_name	
2	AHDC1			38	1	27552023	27552024		Frame_Sh	INS			CC	A1	p.P32fs*8	37.84		
3	DPYD			38	1	97883329	97883329		Missense_	SNP	A	A	G	A1	p.C29R	51.16		
4	FANCD2			38	3	10046723	10046726			DEL	AGTA	AGTA		A1		58.9		
5	TREX1; ATRIP-TREX1; ATR			38	3	48467186	48467186		synonymo	SNP	T	T	C	A1	p.Y38Y; p	100		
6	FRG2C			38	3	75664413	75664413		Missense_	SNP	T	T	C	A1	p.C12R	84.21		
7	FRG2C			38	3	75665147	75665147		Missense_	SNP	A	A	G	A1	p.Q93R; p	18.75		
8	FRG2C			38	3	75665186	75665186		Missense_	SNP	T	T	G	A1	p.I105S; p	20		
9	ZNF717; MIR4273			38	3	75737105	75737105		Nonstop_	SNP	T	T	A	A1	p.K840*;	60.76		
10	ZNF717; MIR4273			38	3	75737127	75737127		Missense_	SNP	A	A	C	A1	p.H832Q;	58.57		
11	ZNF717; MIR4273			38	3	75737676	75737676		Nonstop_	SNP	A	A	T	A1	p.C599*;	53.85		
12	ZNF717; MIR4273			38	3	75737948	75737949		Frame_Sh	DEL	CT	CT		A1	p.E560fs*	28.81		
13	HLA-DRB1			38	6	32584174	32584174		Missense_	SNP	G	G	C	A1	p.A102G	100		
14	HLA-DRB1			38	6	32584364	32584364		Nonstop_	SNP	G	G	A	A1	p.Q39*	74.07		
15	CYP3A7-CYP3A51P; CYP3A			38	7	99709062	99709062		Missense_	SNP	G	G	C	A1	p.T409R	41.43		
16	PRSS1			38	7	1.43E+08	1.43E+08		Missense_	SNP	C	C	T	A1	p.A16V	29.87		
17	LRRC6			38	8	1.33E+08	1.33E+08		Nonstop_	SNP	G	G	A	A1	p.R60*; p.	37.23		

How to Make MAF File

- Straight Forward:
  - Coding
    - No matter you use Python, R, Perl, or other languages.
    - The goal is to make a tab-delimited text file for the data structure of MAF format
    - The only limitation is that almost the profiles are from different styles of data by users
  - By Program from other' programmers
    - Format specific!
  - Using Excel (Maybe another nice choice)

Install R

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CRAN

R Project

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The R Project for Statistical Computing

Getting Started

R is a free software environment for statistical computing and graphics. It compiles and runs on a wide variety of UNIX platforms, Windows and MacOS. To **download R**, please choose your preferred CRAN mirror.

If you have questions about R like how to download and install the software, or what the license terms are, please read our answers to frequently asked questions before you send an email.

News

- **R version 4.1.1 (Kick Things)** has been released on 2021-08-10.
- **R version 4.0.5 (Shake and Throw)** was released on 2021-03-31.
- Thanks to the organisers of useR! 2020 for a successful online conference. Recorded tutorials and talks from the conference are available on the R Consortium YouTube channel.
- You can support the R Foundation with a renewable subscription as a supporting member

News via Twitter

The R Foundation Retweeted

Dirk Eddelbuettel
@eddelbuettel
#ThankYouCRAN, and congratulations on another round number of #RStats packages -- now at 18,000. Just wow.

Available Packages

Currently, the CRAN package repository features 18000 available packages.

https://cran.csie.ntu.edu.tw/

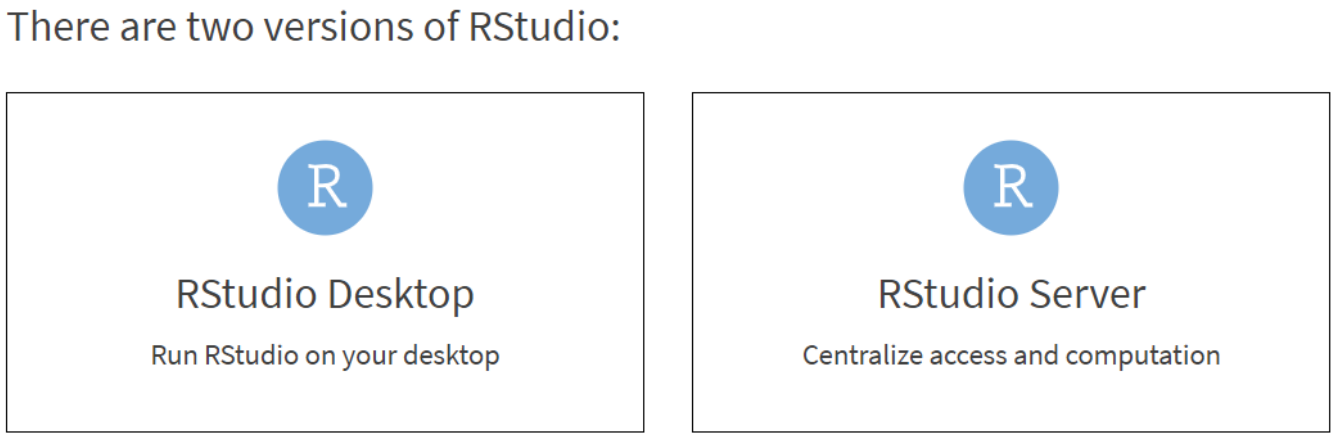
Download and Install R

Precompiled binary distributions of the base system and contribut R:

- Download R for Linux (Debian, Fedora/Redhat, Ubuntu)
- Download R for macOS
- Download R for Windows

Install Rstudio (Option)

https://www.rstudio.com/products/rstudio/



https://joe11051105.gitbooks.io/r_basic/content/environment_settings/install_RStudio.html

https://joe11051105.gitbooks.io/r_basic/content/environment_settings/RStudio_introduction.html

Installation of maftools

- On R console:

- Code:

```
if (!require("BiocManager"))
    install.packages("BiocManager")
BiocManager::install("maftools")
```

Coding Approach

- Key point:
  - Using data frame (in Python) or data table (in R) to sort out all the required information from VCF files or other text files.
  - Using String processing method to retrieve the best information what you want
    - General key word on languages (R, python)
      - split
      - re (regular expression)
  - **Make sure the information is fitted to the data structure and value.**
    - E.g.:
      - Reference allele ≠ Tumor_Sample_allele1
      - The value of Allele frequency is split from original attribute in VCF (GT)
  - Export tab-delimited format and the extension of file is “.maf”

Note on R

- rbind, cbind, or ‘$’ operator to generate column
  - DataTable
  - Combine columns to make your maf file
  - Save as txt files

Make your MAF Format by yourself

```
library("maftools")

### Make MAF from your csv
# Read csv files from QCII

df <- read.csv([redacted]/test_demo_QCII.csv",sep=",",header=TRUE)

### Retrieve the key columns from dataframe

maf <- df['Gene.Symbol']
column?(maf) <- "HUGO"
maf$Entrez_Gene_Id <- NA
maf$Center <- NA
maf$NCBI_Build <- 38
maf$Chromosome <- df['Chromosome']
maf$Start_Position <- df['Position']
maf$End_Position <- df['End_Position']
maf$Strand <- NA
maf$Variant_Classification <- df['Translation.Imp?ct']
maf$Variant_Type <- df['Variation.Type']
```

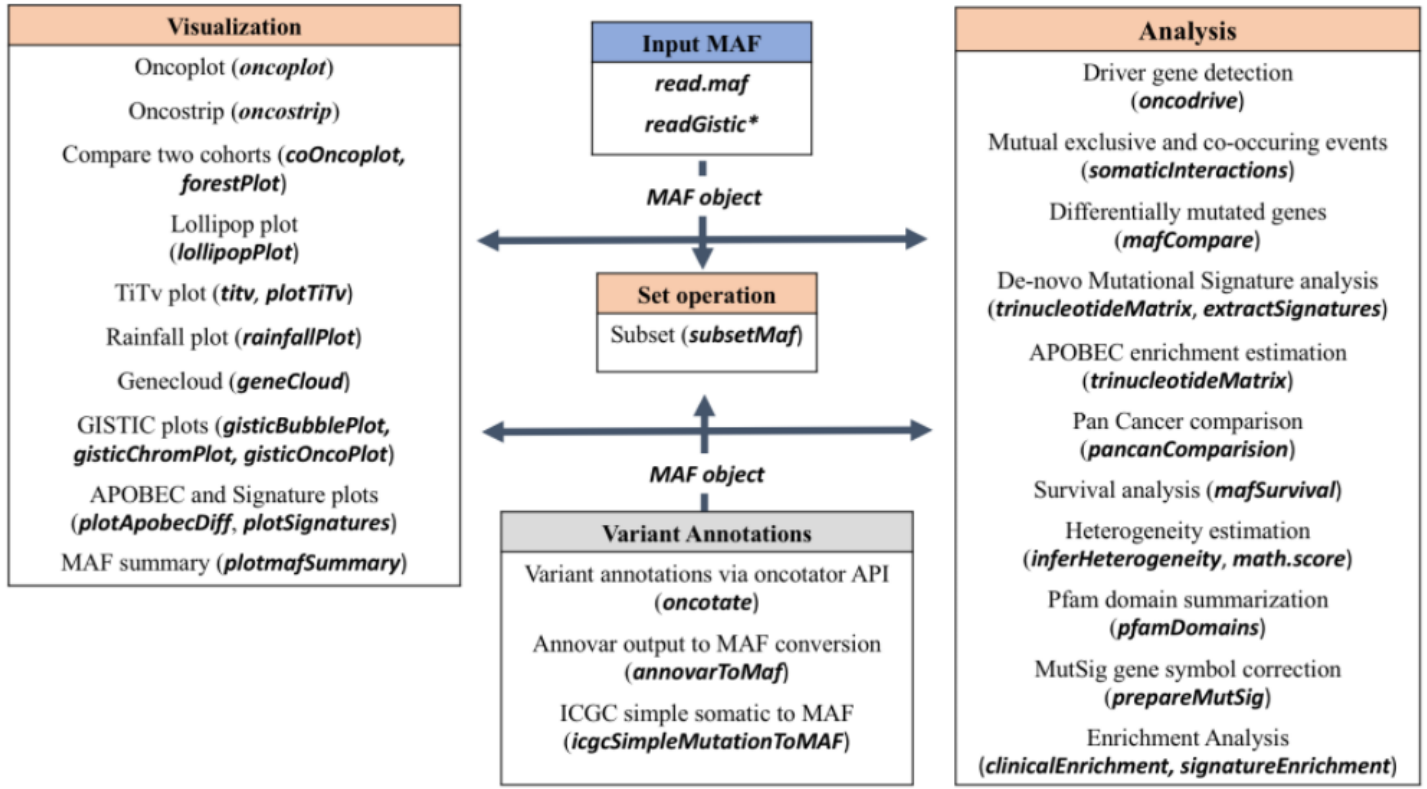
Let’s see the script of R!

Regular Expression

- A sequence of **characters** that define a search **pattern**
  - Be useful to search the pattern of word in your data
- Example:
  - re.findall('(\d+)[:]',str(df['Location']))
    - (\d+)[:] means:
      - I want the **number of INTEGER or FLOAT (or more) before the pattern ':'** and the data is from the attribute of **Location** in the DataFrame called **df**
- Disadvantages:
  - Not easy to learn… very complicated!

maftools on Bioconductor of R

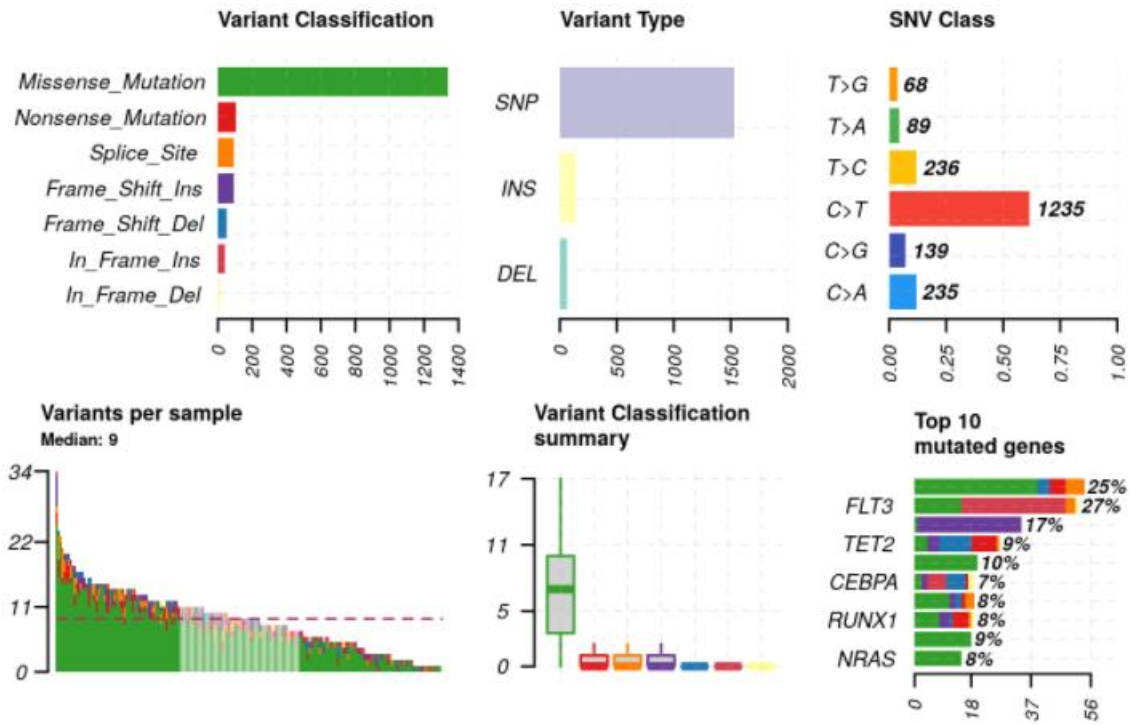
- The common visualization way to visualize the variants in your data
- The example of visualization
  - Oncoplot
    - Rainfall plot
  - Statistics plot (MAF summary)
  - Bar plot



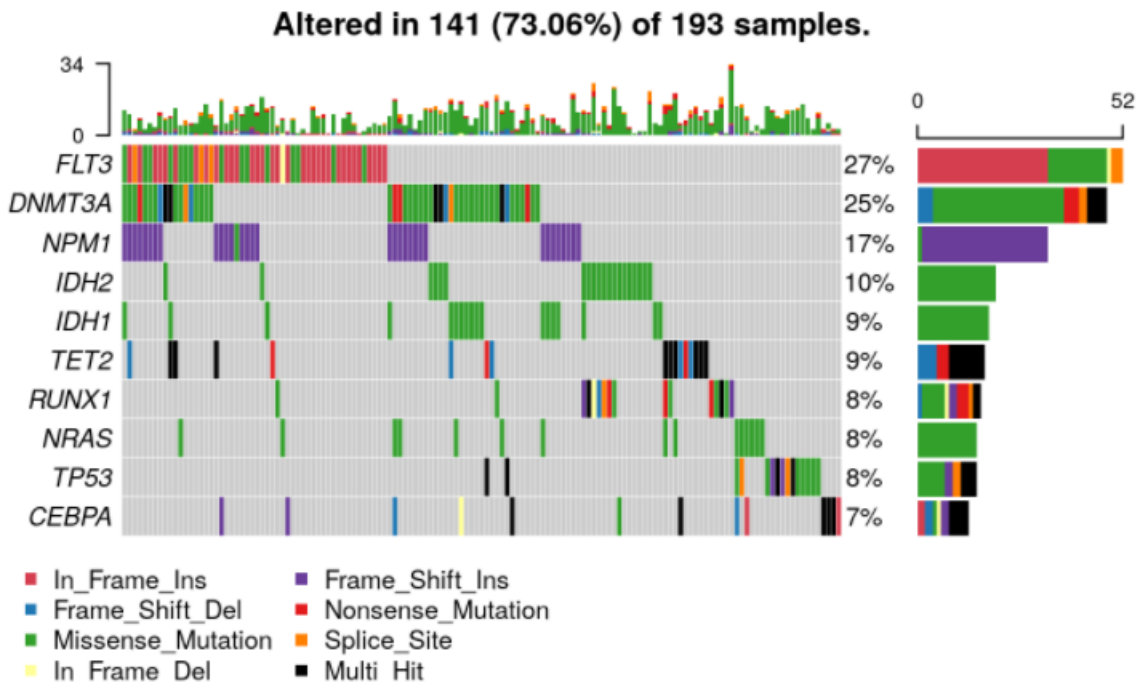
- https://www.bioconductor.org/packages/release/bioc/vignettes/maftools/inst/doc/maftools.html

The output is generated by maftools

MAF Summary



Oncoplot



Key Points on maftools

- No other defined words in Variant Classification section
- No **synonymous** variants in your OncoPlot
- If you have multiple-hit on the specific genes, it will be classified as “Multi-Hit”
- Samples are defined in the **Tumor Sample Barcode** attribute

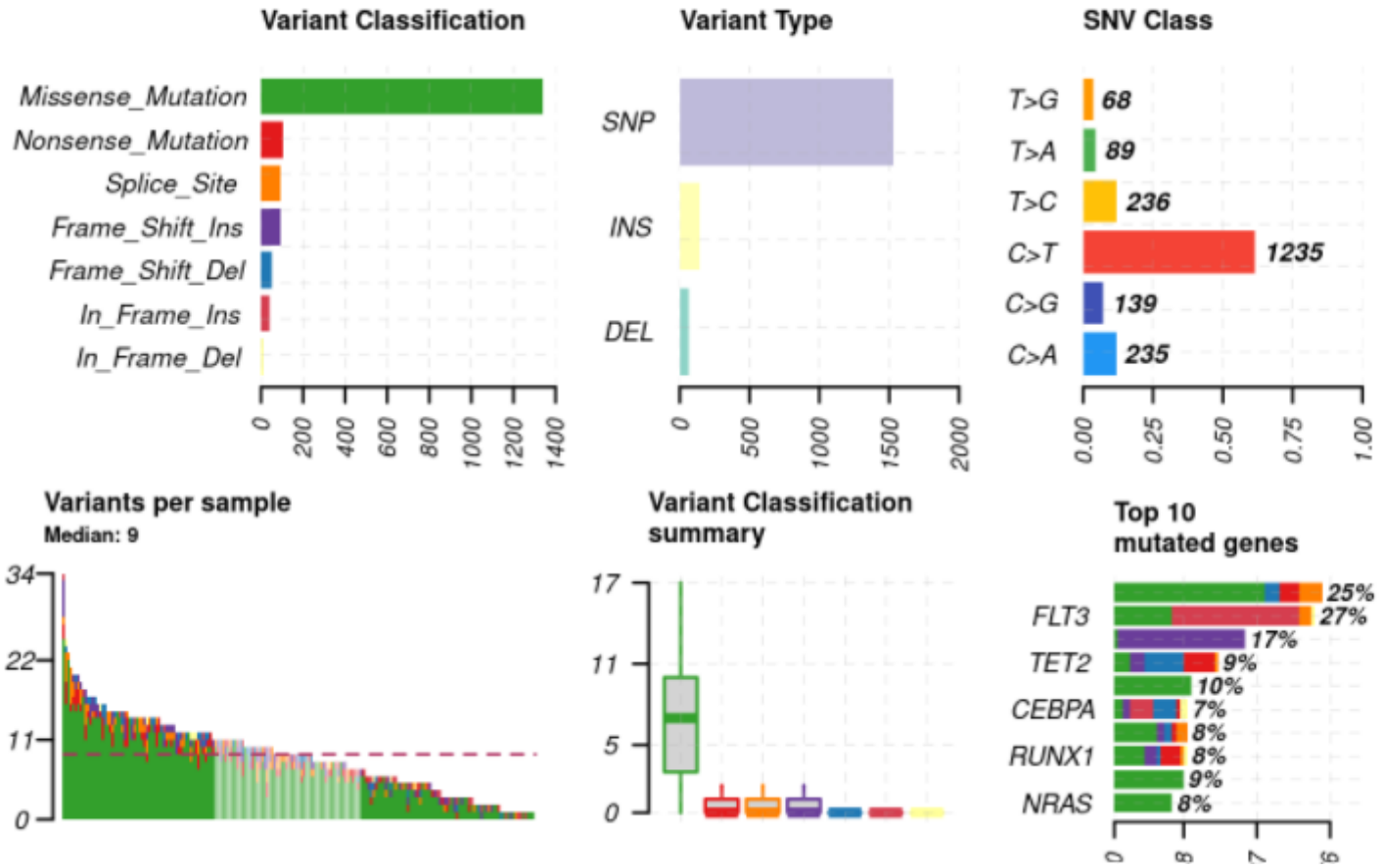
- References:
  - http://tardis.cgu.edu.tw/maftools.html
  - https://github.com/umccr/MAF-summary#maf-field-requirements

Example small script on R

```
library(maftools)


df <- data.table::fread("D:/WillisCheng/Client/share/all_ABC_specific.maf")


syn <- c("synonymous",NA,"","-")
vc <- names(table(df$Variant_Classification))
nonSyn <- setdiff(vc,syn)
colors <- rainbow(length(nonSyn))
names(colors) <- nonSyn
ALL.maf = read.maf(maf = "D:/WillisCheng/Client/share/all_ABC_specific.maf",vc_nonSyn = nonSyn, verbose=TRU

plotmafSummary(ALL.maf, rmOutlier = TRUE, addStat = 'median', dashboard = TRUE, titvRaw = FALSE, color = co
#
vc_cols = RColorBrewer::brewer.pal(n = 8, name = 'Paired')
nonSyn[8]="Multi_Hit"
names(vc_cols) = nonSyn

oncoplot(maf = ALL.maf, colors=vc_cols, clinicalFeatures = "Tumor_Sample_Barcode",top=20,removeNonMutated =
ALL.titv = titv(maf = ALL.maf,plot = FALSE, useSyn = TRUE, )
plotTiTv(res = ALL.titv)
```

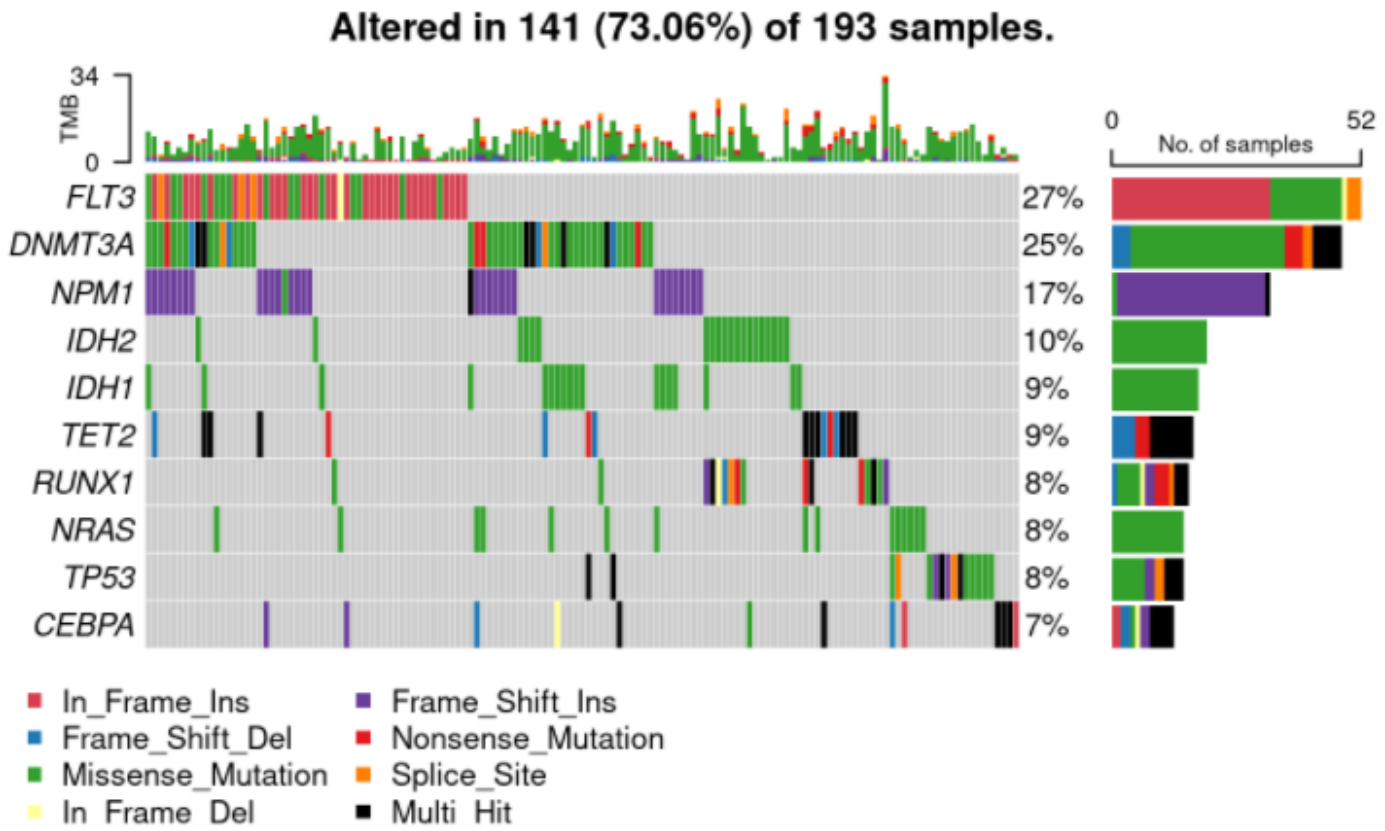
plotmafSummary

```
plotmafSummary(maf = laml, rmOutlier = TRUE, addStat = 'median', dashboard = TRUE, titvRaw = FALSE)
```



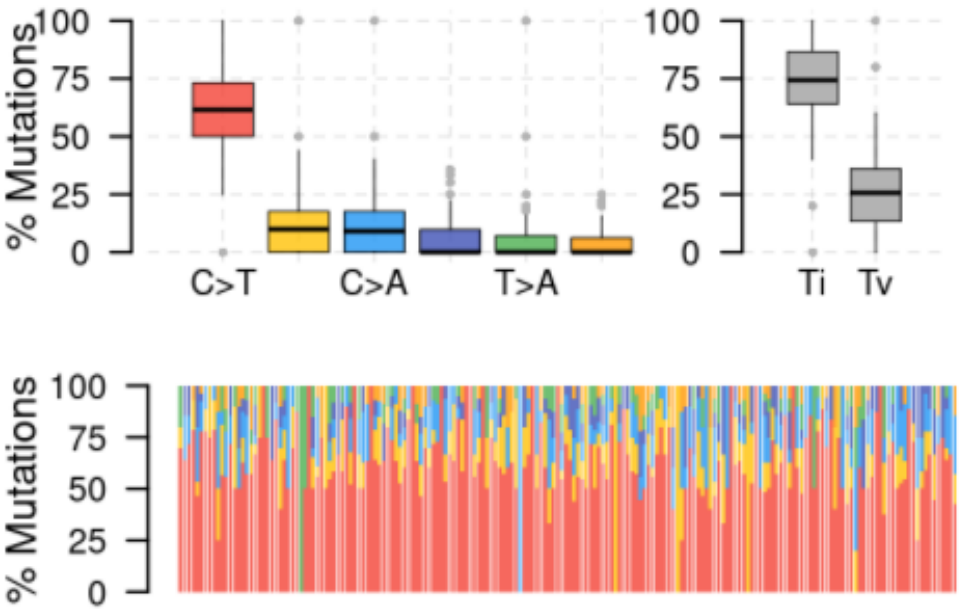
Oncoplot

```
#oncoplot for top ten mutated genes.
oncoplot(maf = laml, top = 10)
```



TiTv plot

```
laml.titv = titv(maf = laml, plot = FALSE, useSyn = TRUE)
#plot titv summary
plotTiTv(res = laml.titv)
```



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