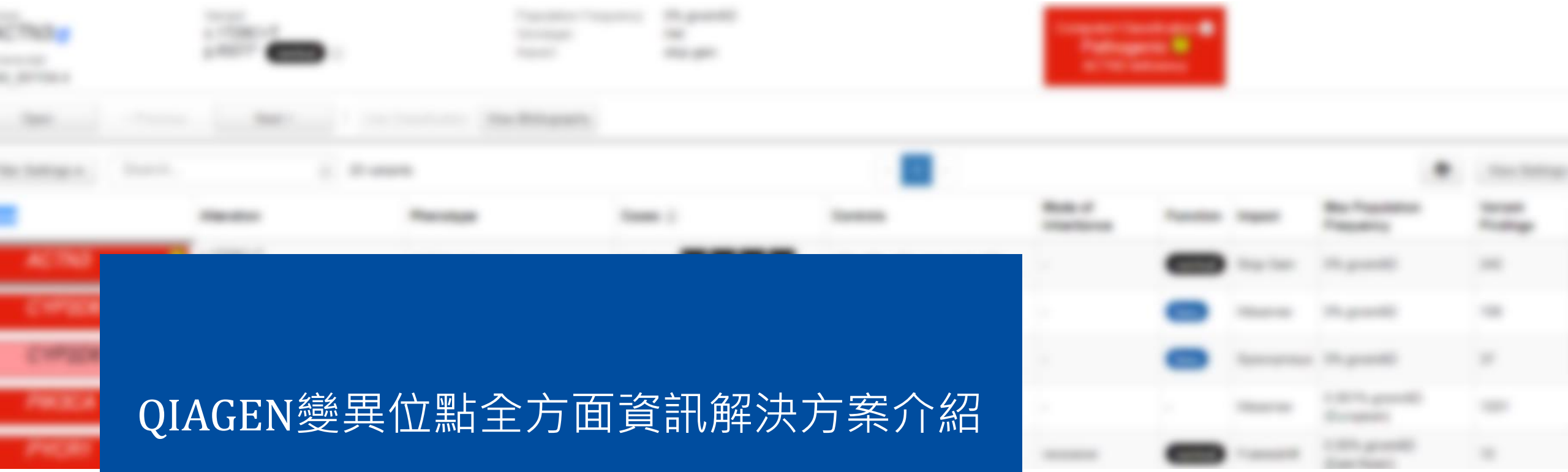
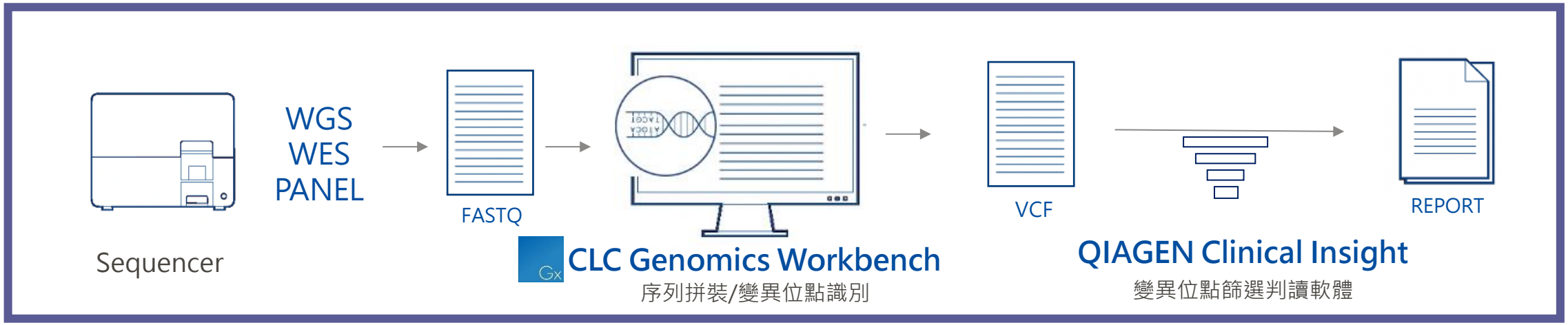


QIAGEN變異位點全方面資訊解決方案介紹

2021.05.06

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

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

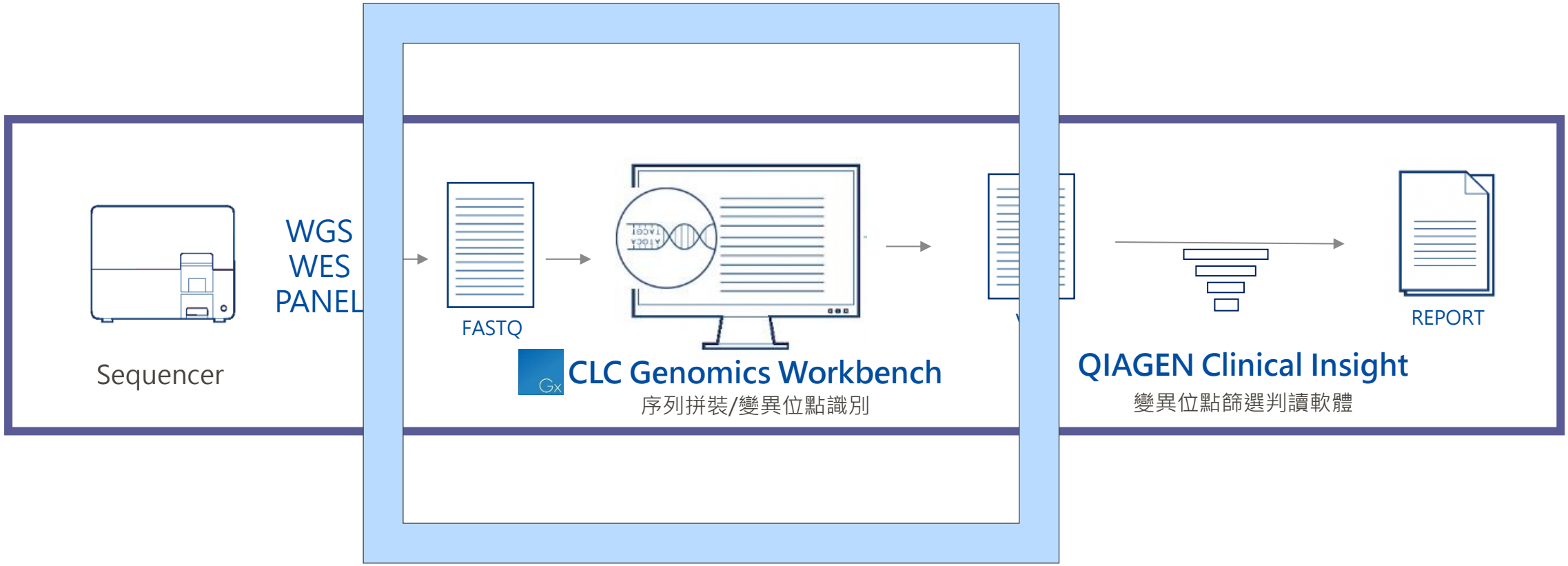
→

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

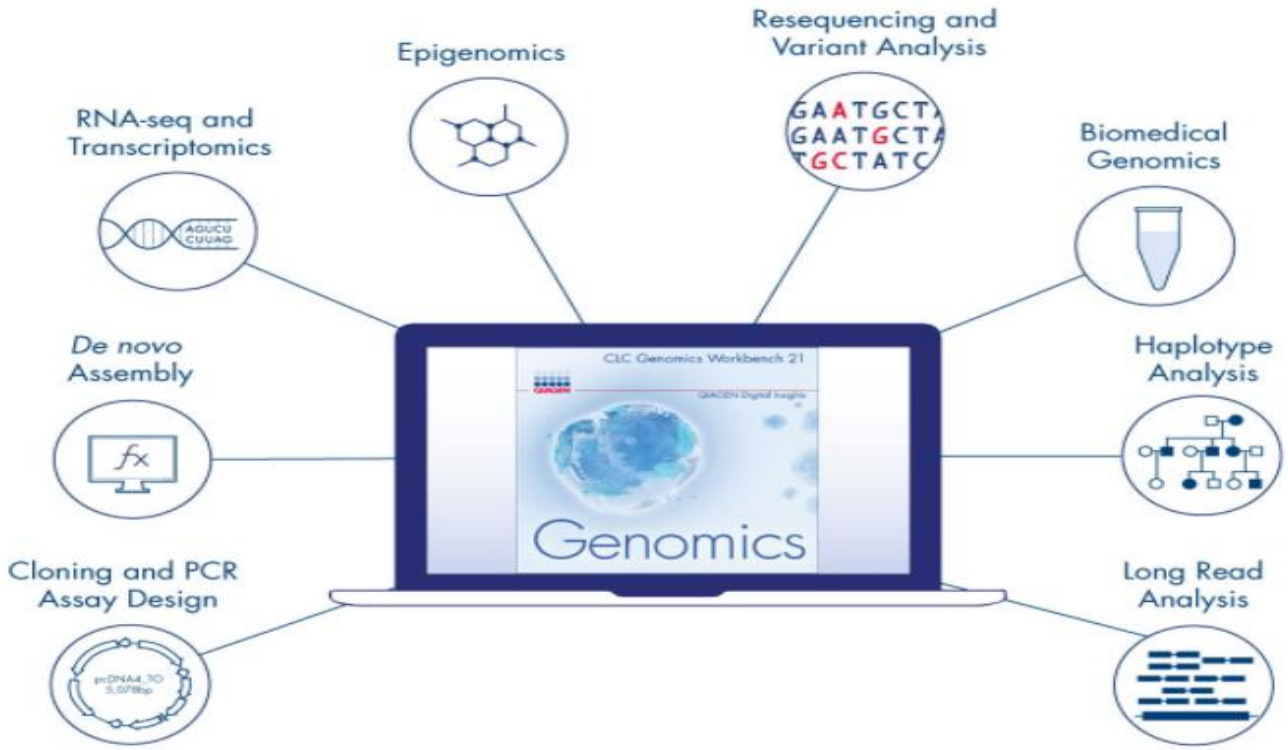
*WGS有30億個鹼基對

CLC Genomics Workbench

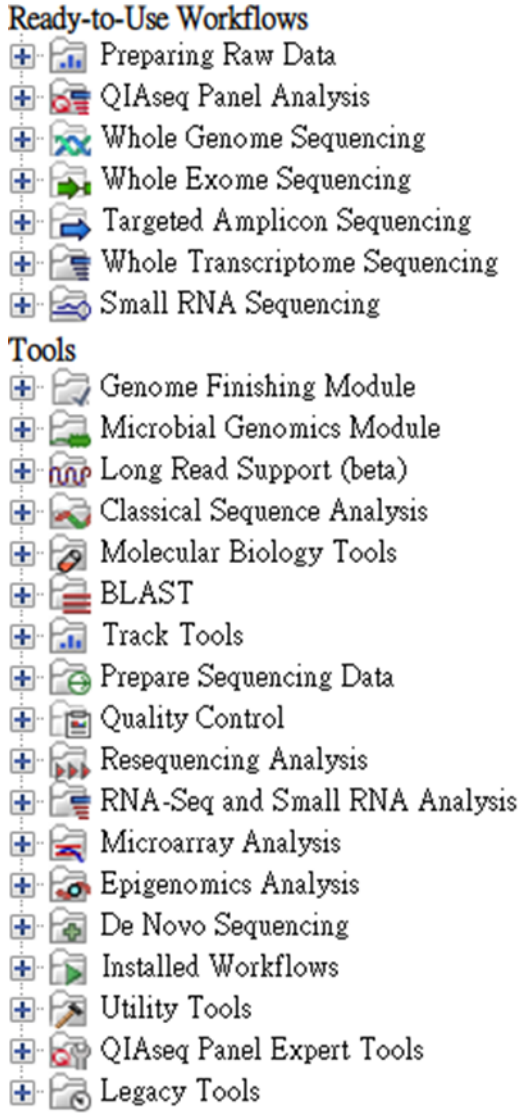


QIAGEN CLC Genomics Workbench

- Cross-platform desktop genomics application
  - **User-friendly GUI interface**
    - Works on Windows, Mac and Linux
  - **Data Localization**
  - **Interactive visualization**
  - **Workflows**
    - For automated processing
    - For sharing with colleagues
  - **Modular design to add plugins**
  - **Compatible with most platforms**
    - Illumina, Ion Torrent, Oxford Nanopore, PacBio, BGI/MGI
  - **Fully documented** and supported
  - Developed under quality guidelines set forth by ISO 9001:2015
    - TUV Rheinland-certified

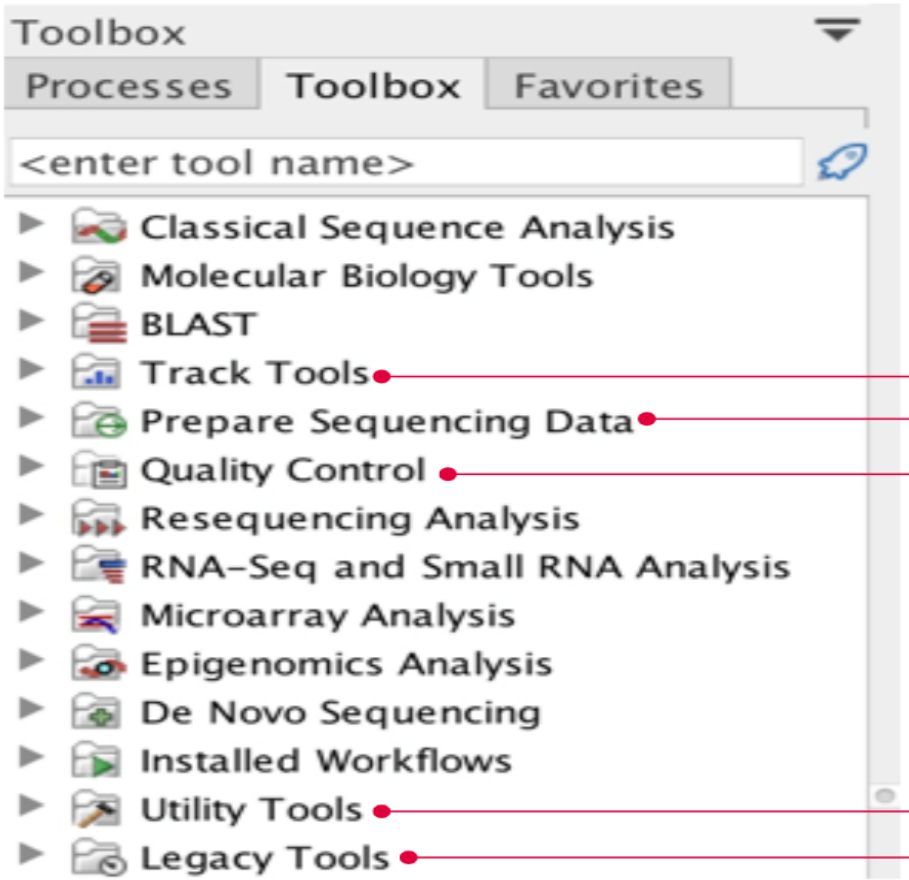


In CLC Workbenches you can..



CLC Genomics Workbench	QC & Reads Processing	●
	Resequencing (whole genome, exome, targeted)	●
	Transcriptomics (RNA-Seq)	●
	Single Cell RNA-Seq Analysis	●
	De novo assembly	●
	Epigenomics	●
	Long Reads Supports (Oxford Nanopore & Pacbio)	●
	QIAseq Panel Analysis – TMB & MSI & TSO500	●
	Workflow (Pipeline)	●
	Microarray Analysis	●
	Phylogeny Tools	●
	Blast, Sanger Sequencing, Cloning, Primer Design, …	●
Extended Modules	Microbiome Analysis (Microbial Genomics Module)	●
	Contigs Assembly (Genome Finishing Module)	●

Organization of Toolbox



For non-NGS data (e.g., multiple-sequence alignment, phylogenetics, cloning, Sanger etc.)

Tracks, Genome viewer

NGS QC, trim and demultiplex

Mapping QC, combine reports

Application-specific tools

Renaming, sampling and extraction

Tools to-be-retired in the next version

Plugins

Premium modules

Microbial Genomics Module

- Strain typing, epidemiology and antimicrobial resistance analysis
- Metagenomics community profiling, assembly and functional analysis
- Functional annotation tools
- Pre-built or user-customized databases
- Integrated support for QIAseq 16S/ITS panels

Genome Finishing Module

- Automated and manual tools for genome finishing and polishing
- Integrated support for PacBio + Illumina hybrid assembly and finishing

Single Cell Analysis Module

- RNA-seq, t-SNE, UMAP, clustering, cell type annotation (automatic and manual)

Free plugins

Free plugins

- Biomedical Genomics Analysis
- Long Read Support
- Whole Genome Alignment
- Ingenuity Pathway Analysis
- Ingenuity Variant Analysis

} Requires subscription

Publication Roundup: QIAGEN CLC Genomics Workbench

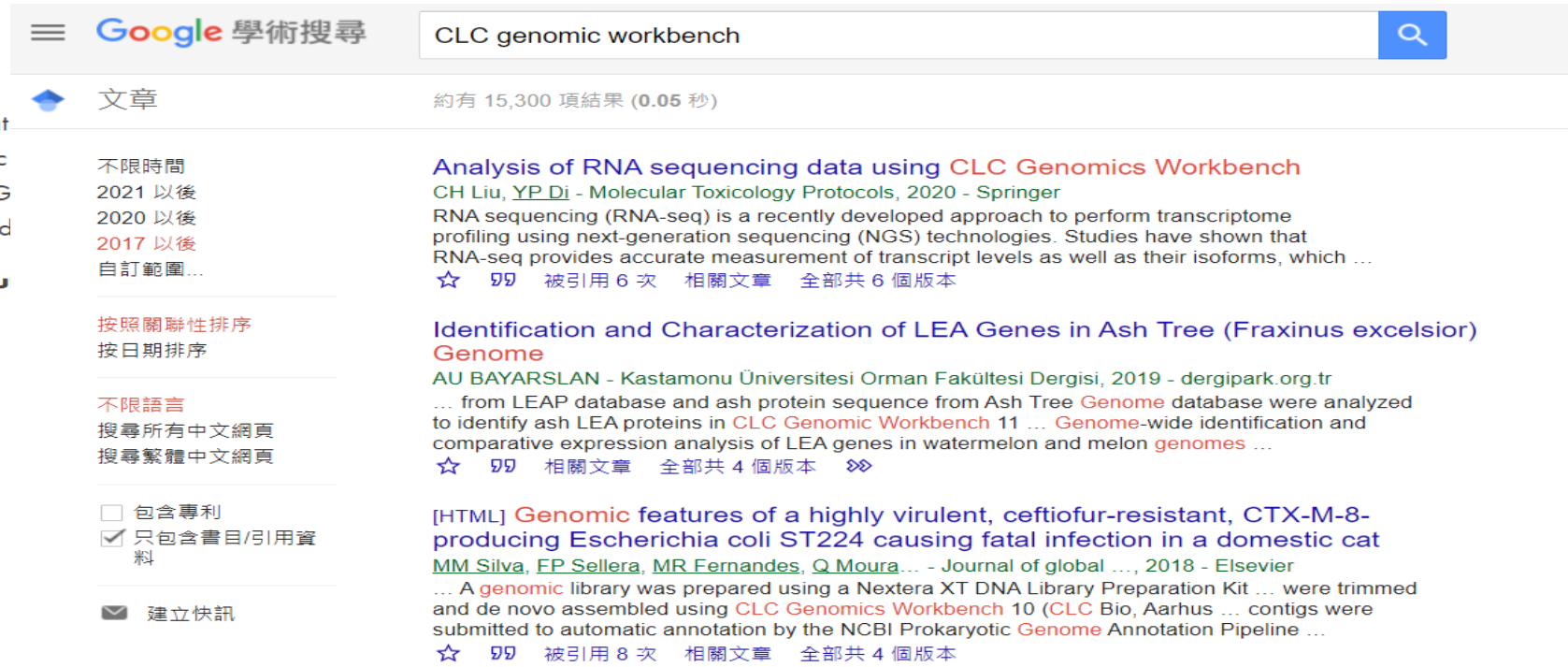
Recently, there have been many noteworthy papers citing QIAGEN CLC Genomics Workbench, a comprehensive, easy-to-use toolbox that ensures continuity in your NGS workflow. Here, we round up just a few of them to offer a sense of the diversity of the research for which QIAGEN CLC Genomics Workbench makes a difference. Below are some examples of how researchers from all over the world use this solution as a tool for metagenomic analysis to characterize dengue viruses and pathogens, create *de novo* assemblies or investigate ocular diseases.

Genomic characterization of SARS-CoV-2 identified in a reemerging COVID-19 outbreak in Beijing's Xinfadi market in 2020

First author: Yong Zhang

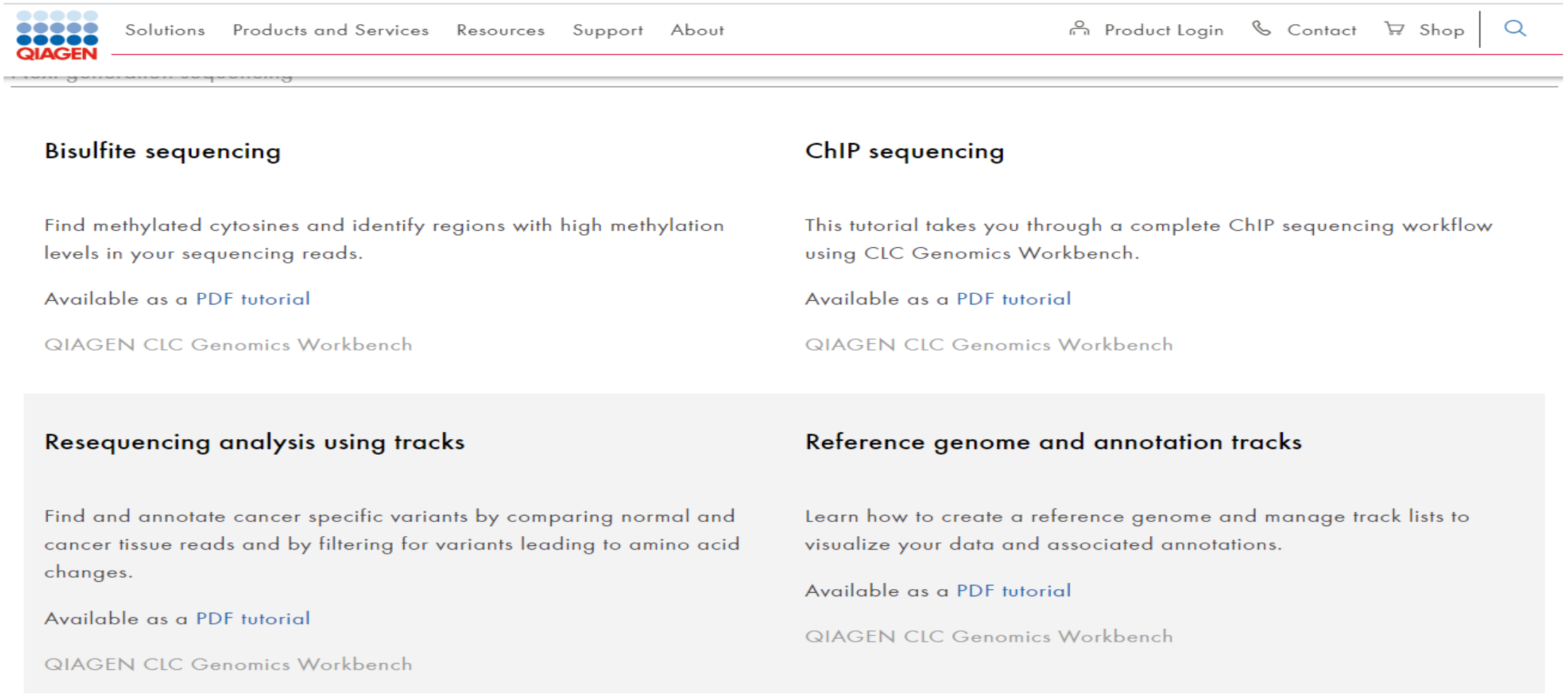
Should we be looking for new mutations in SARS-CoV-2 that
Center for Disease Control and Prevention perform genomic
reemerging outbreak in China. Discover how they use QIAG
source of the virus in this second outbreak in Beijing's Xinfad

Genetic tracing of HCoV-19 for the re-emerging ou



https://qiagen.pathfactory.com/gwb-trial/publication-roundup-/?cmpid=CM_QDI_DISC_CLC-Webpage_0221_PF_website_GWB

Document & Tutorial on Website

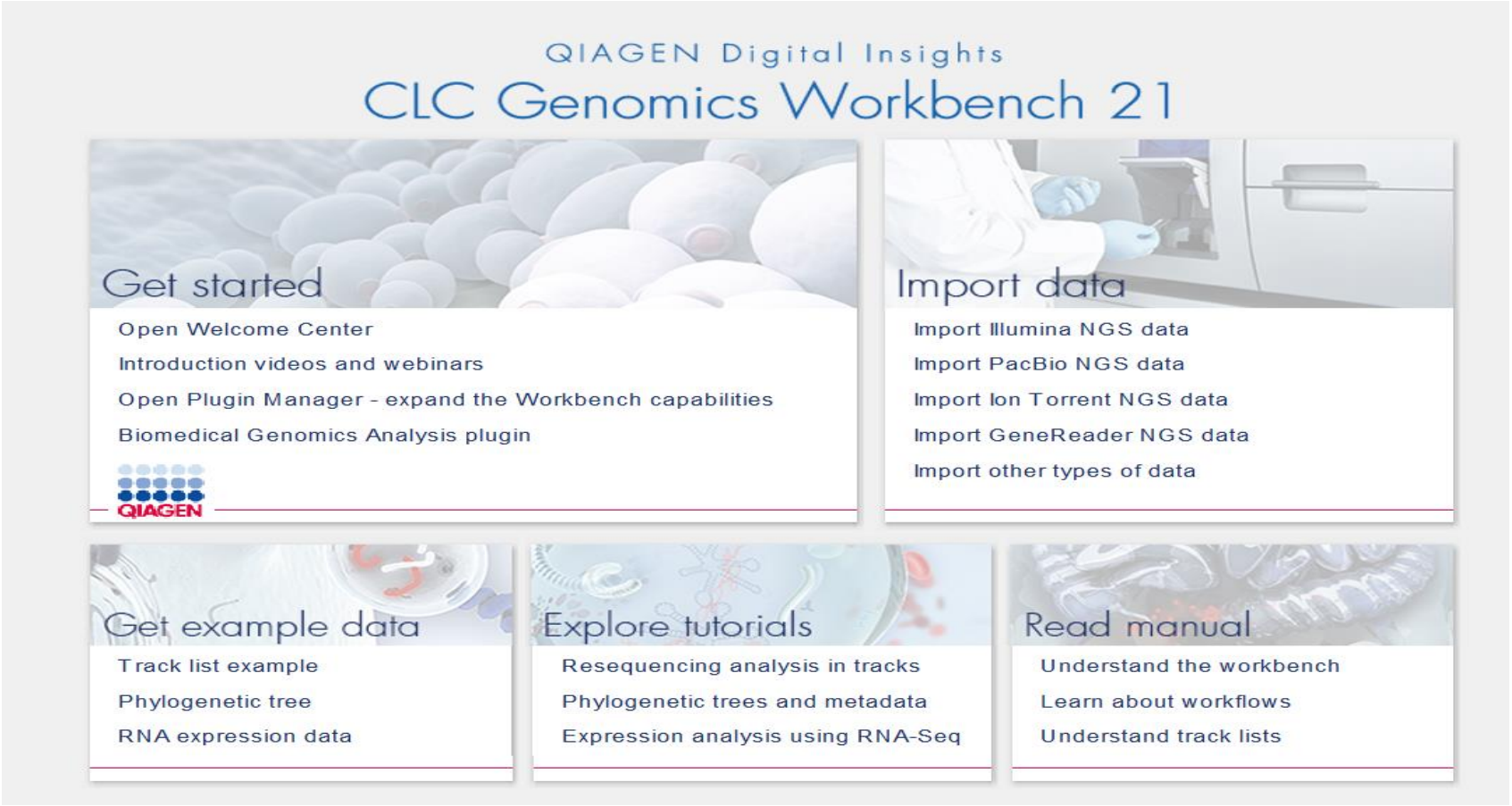


Q&A: https://qiagen.secure.force.com/KnowledgeBase/KnowledgeGemomicWorkbench

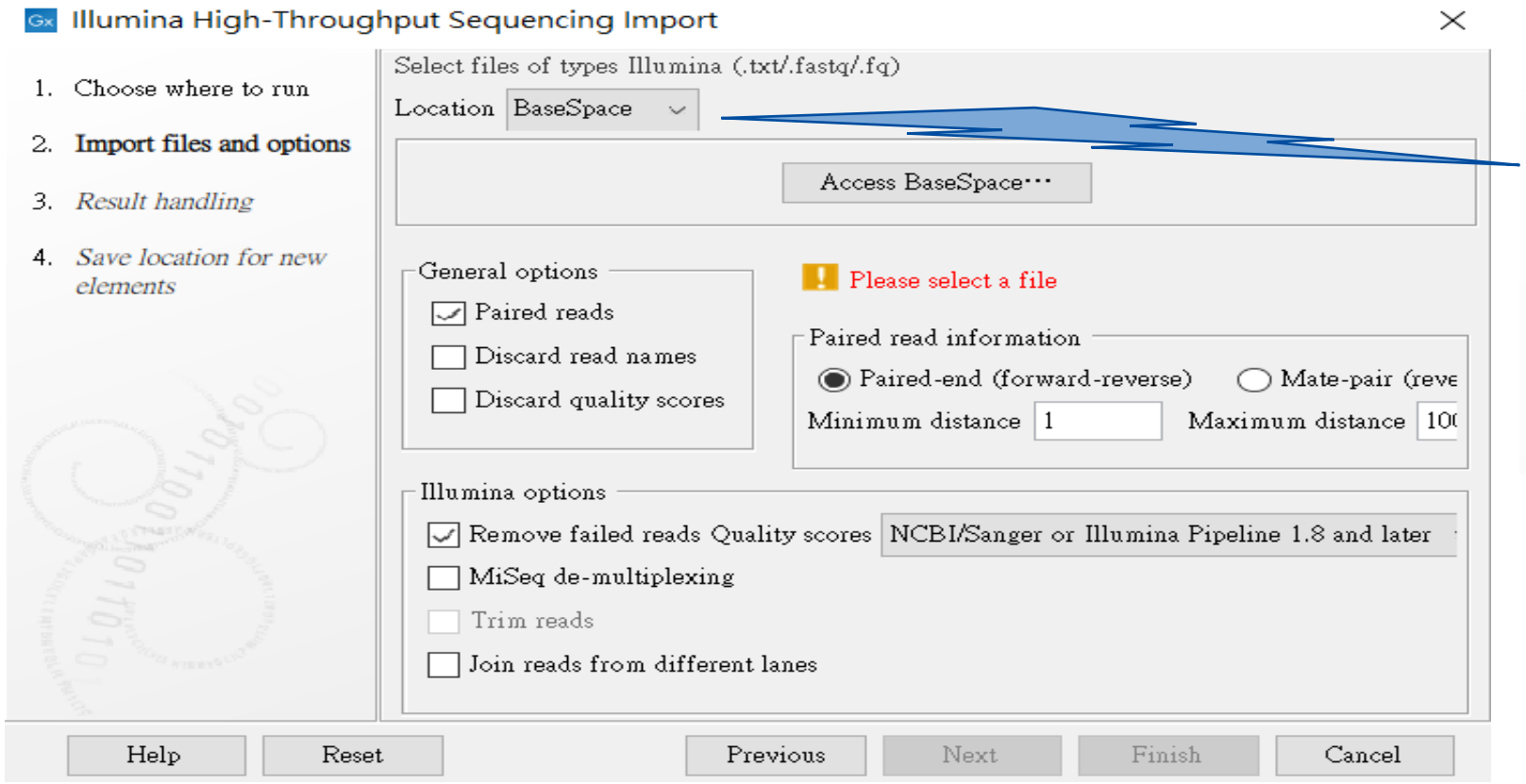
Tutorial: https://digitalinsights.qiagen.com/support/tutorials

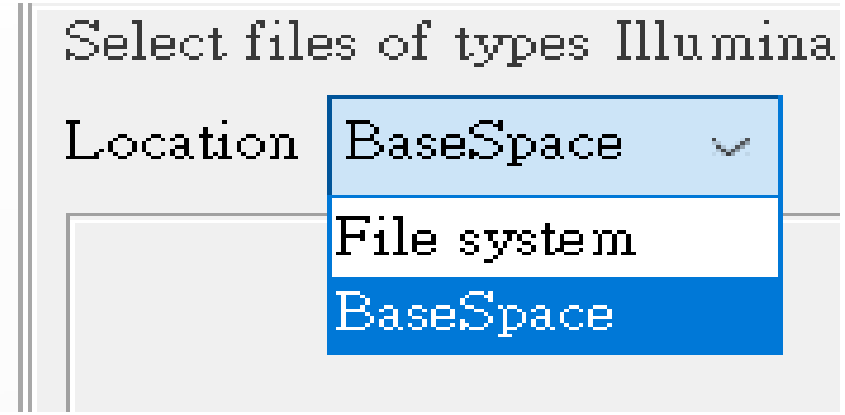
The new CLC Genomics Workbench 21

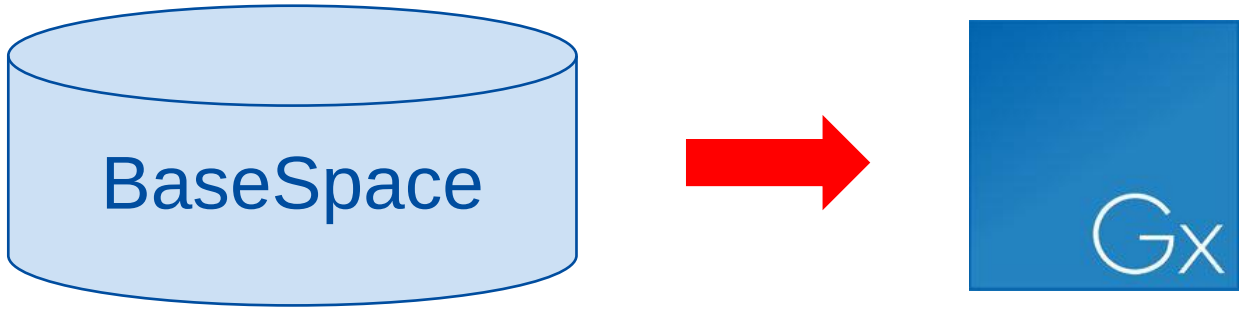
- Cloud Plugins
- Single Cell Analysis Plugins
- Biomedical Workflow
  - SARS-CoV-2 Workflow
  - TSO500 Panel Workflow
- RNA-Seq Analysis
  - Long Reads
- MGM
  - Functional Database



CLC Cloud Engine on BaseSpace







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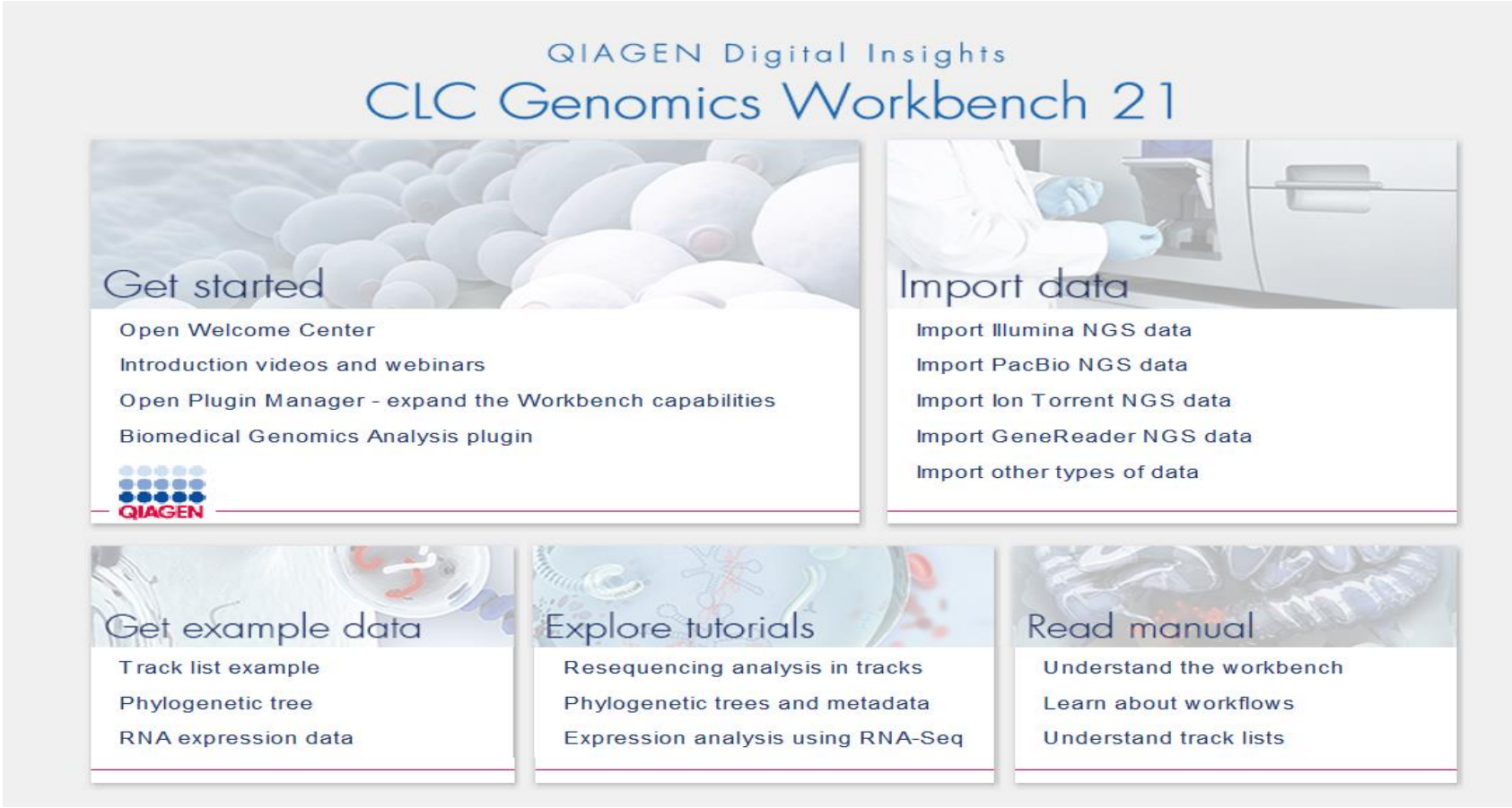
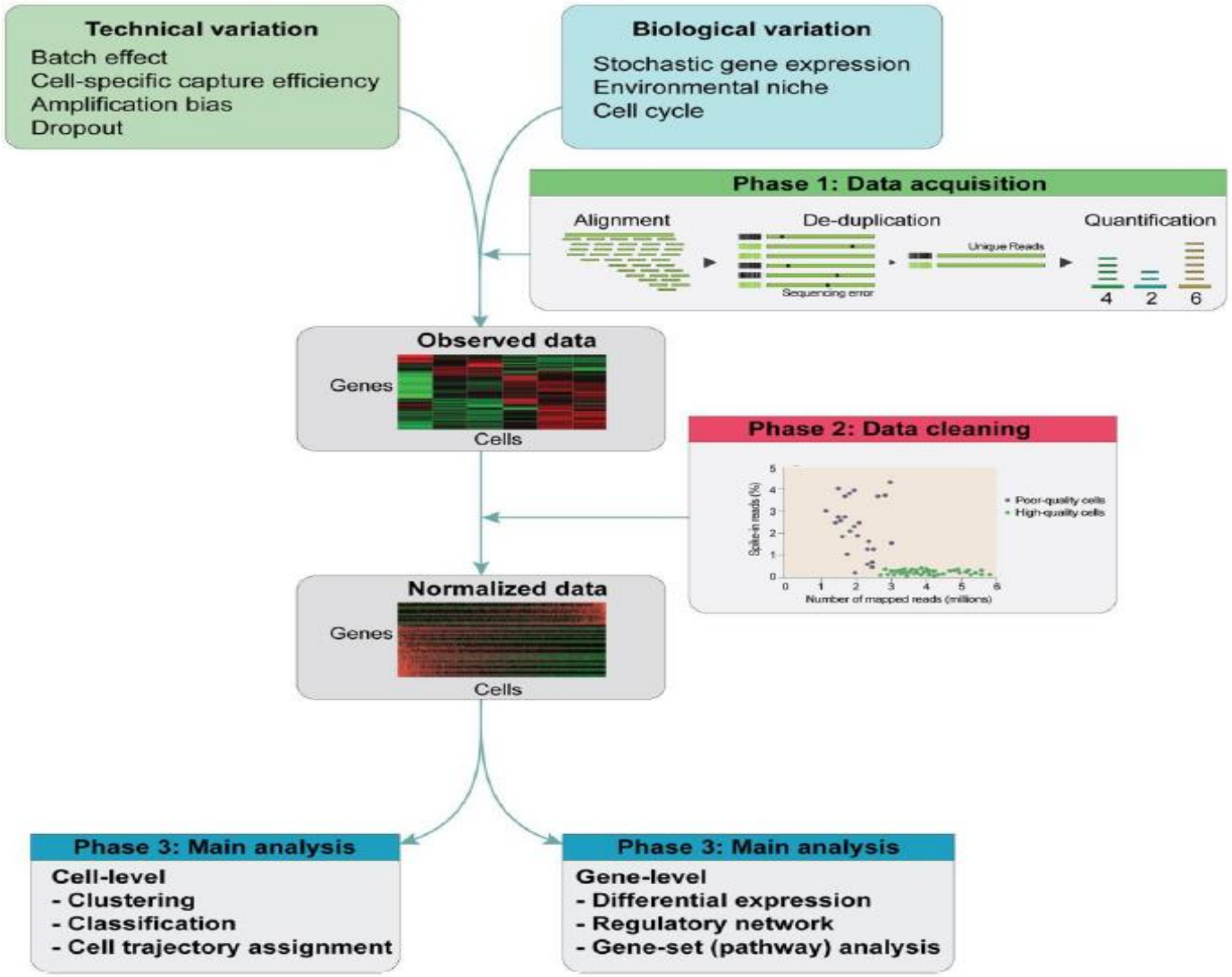


Single Cell Analysis

1. Raw sequencing data QC
2. Alignment to genome
3. Cellular barcode and UMI process
4. Generate gene expression matrix (zero-inflated matrix)
5. Cell QC and clean
6. Normalization
7. Estimate confounding factors
8. Cell-level and gene-level analysis



Single Cell Analysis

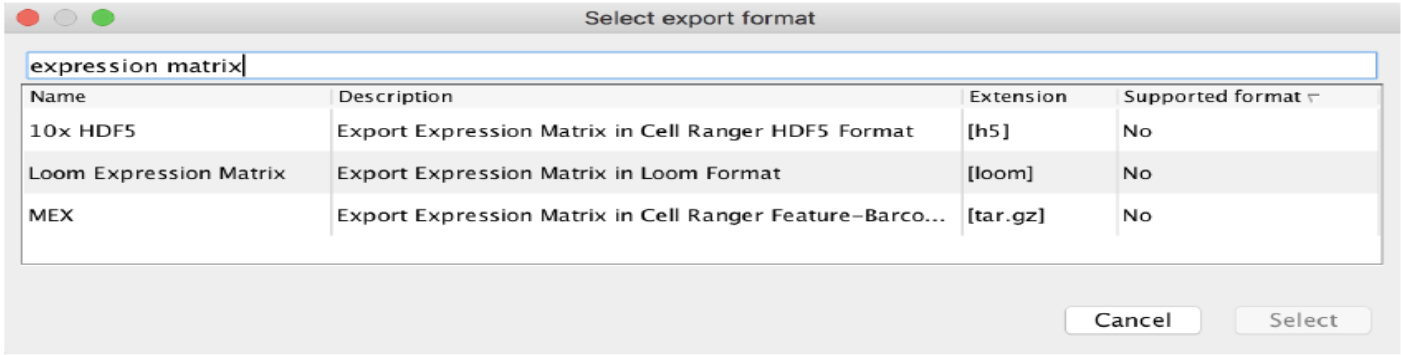
Tools

- Single Cell Analysis
  - Cell Preparation
    - Annotate Reads with Cell and UMI
    - Single Cell RNA-Seq Analysis
    - QC for Single Cell
    - Normalize Single Cell Data
    - Combine Cell Annotations
    - Combine Cell Clusters
    - Convert Metadata to Cell Annotations
  - Cell Annotation
    - Predict Cell Types
    - Train Cell Type Classifier
    - Cluster Single Cell Data
  - Dimensionality Reduction
    - UMAP for Single Cell
    - tSNE for Single Cell
    - Add Information to Plot
  - Expression Analysis
    - Differential Expression for Single Cell
    - Create Expression Plot
  - Workflows
    - Perform Single Cell Analysis from Expression Matrix
    - Perform Single Cell Analysis from Reads

Importers

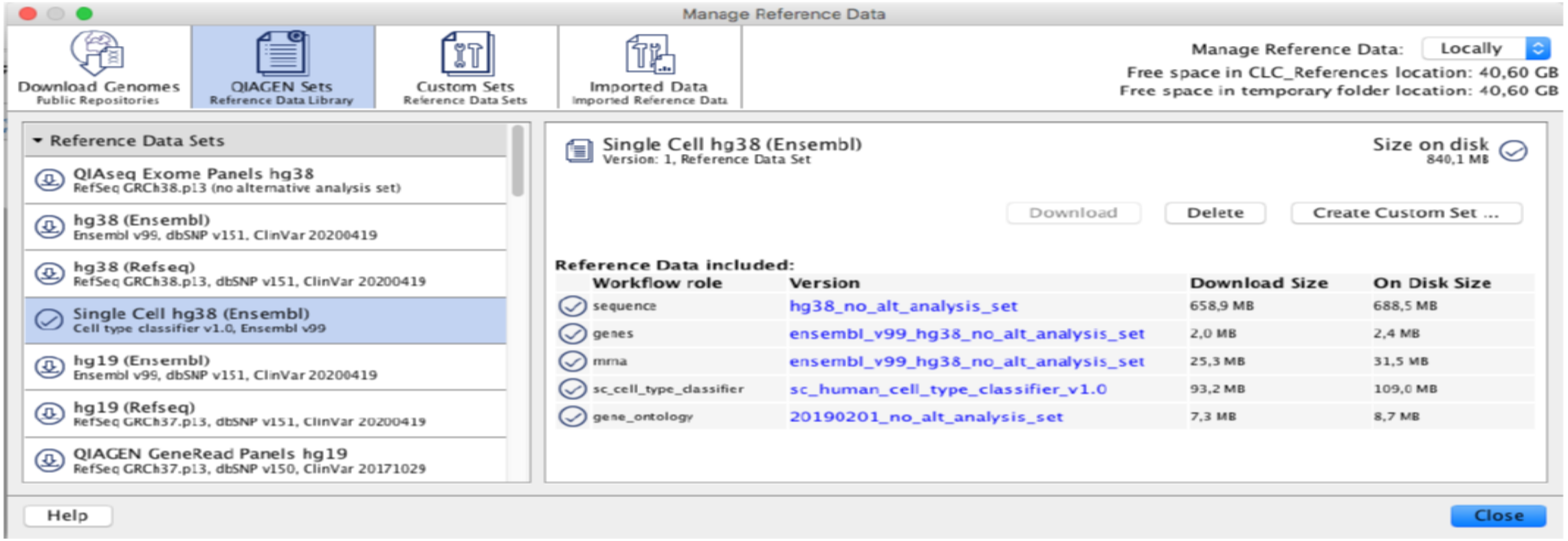
- Import Cell Annotations...
- Import Cell Clusters...
- Import Expression Matrix
  - Import Expression Matrix in Cell Ranger HDF5 Format...
  - Import Expression Matrix in Loom format...
  - Import Expression Matrix in MEX format...
  - Import Expression Matrix in MEX format (archive)...
  - Import Expression Matrix in CSV/TXT Format...

Exporters

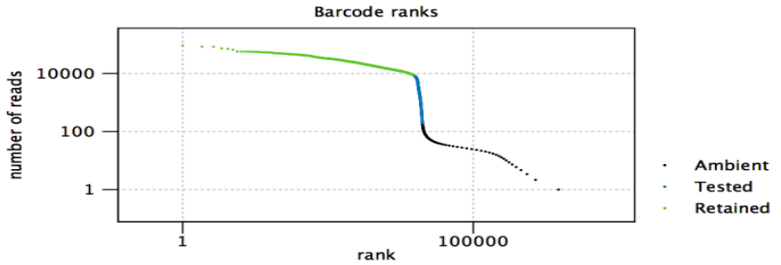


Single Cell Analysis

- Limitation on installed version: Human, Mouse



QC for Single Cell Report

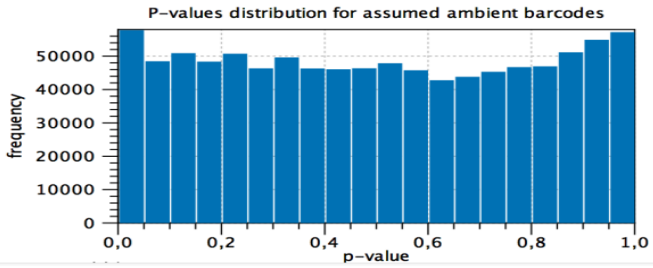


2 Cell calling for droplet data for 5k_pbmc_v3_S1_L001_R1

2.1 Summary

Minimum number of reads for retaining barcodes	3.225
Maximum number of reads for ambient barcodes	100
Estimated number of cells	4.989
Sufficient simulations	Yes
Number of barcodes with significant FDR-corrected p-value	1.008
Fraction of reads in cells	89,27
Median number of reads per cell	4.605,00
Median genes per cell	1.545,00

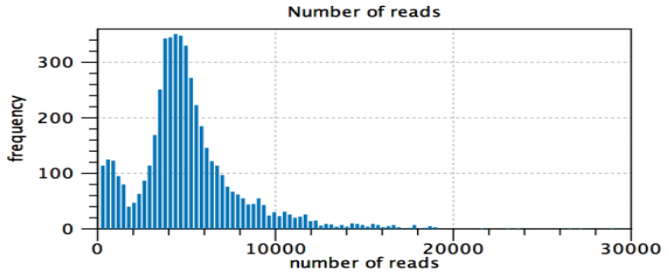
2.2 P-values distribution for assumed ambient barcodes



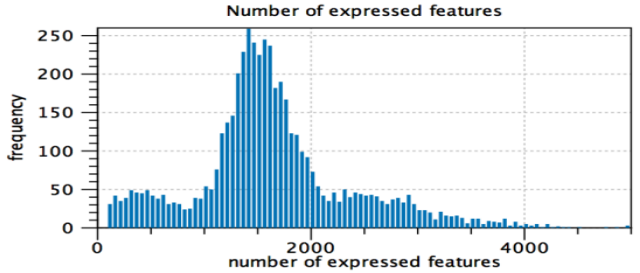
3.1 Summary

Input cells	4
Retained cells	4
Known retained cells	
Maximum mitochondrial reads (%)	
Cells with too many mitochondrial reads (%)	

3.2 Number of reads

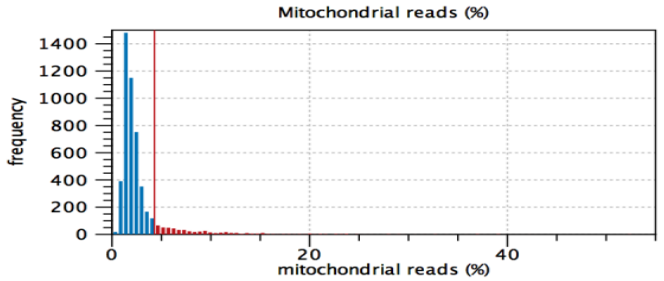


3.3 Number of expressed features



3.4 spike-in reads (%)

3.5 Mitochondrial reads (%)

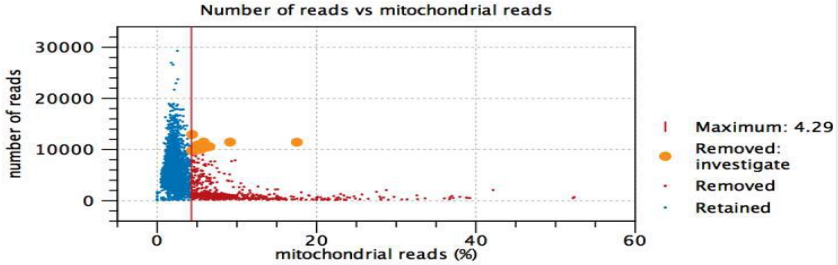


3.6 QC metrics relations

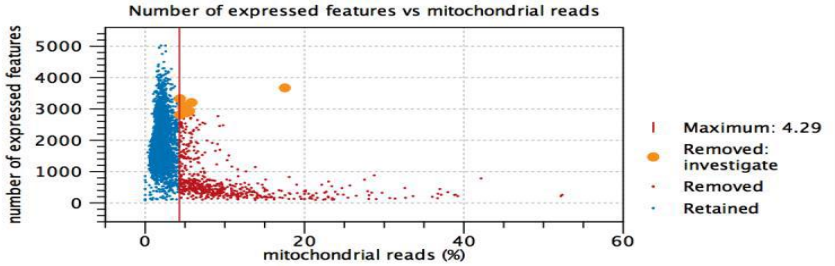
The relation between the mitochondrial reads (%) and the other QC metrics highlight if there are cells with both:
- many number of reads / expressed features and large mitochondrial reads, indicative of high-quality cells that are highly metabolically active;
- few spike-in and many mitochondrial reads (%), indicative of undamaged cells that are metabolically active.

These cells are highlighted in orange and should not necessarily be removed.

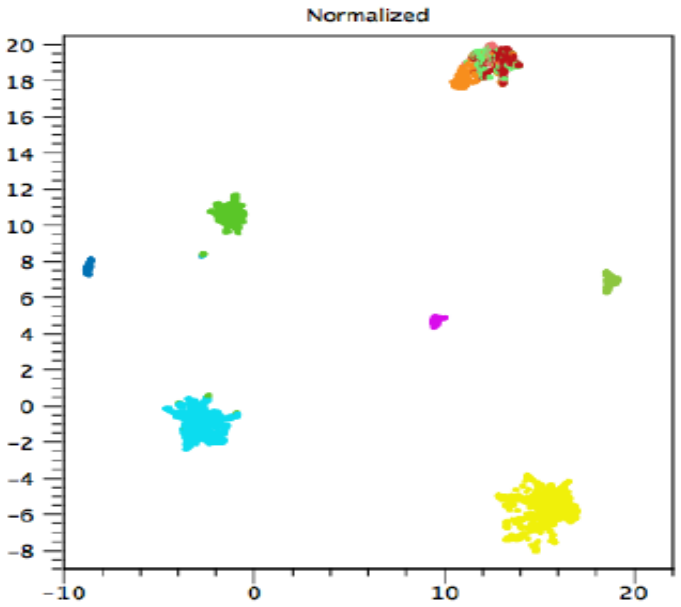
3.6.1 Number of reads vs mitochondrial reads

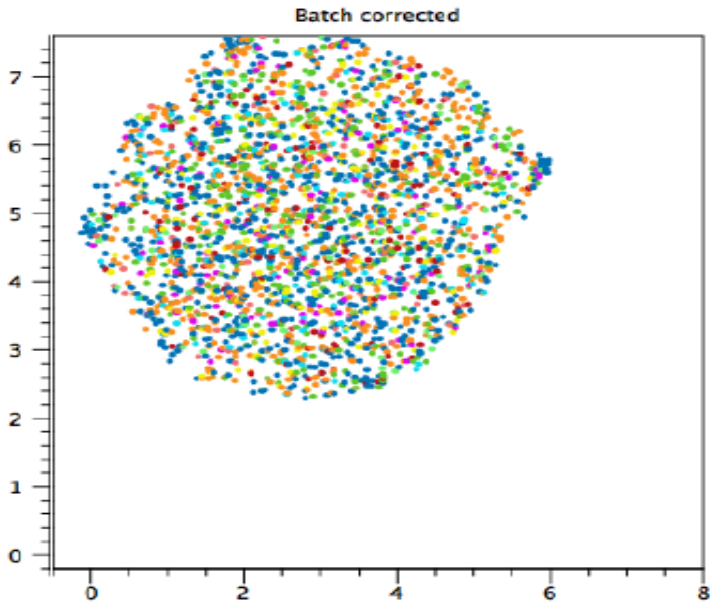


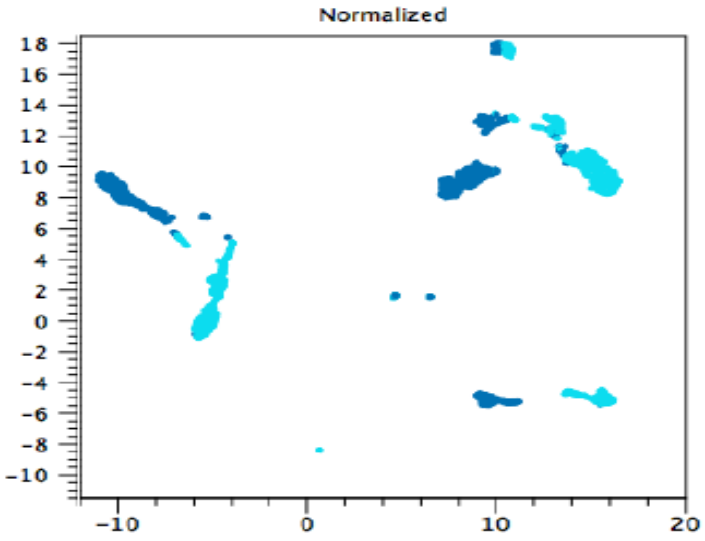
3.6.2 Number of expressed features vs mitochondrial reads

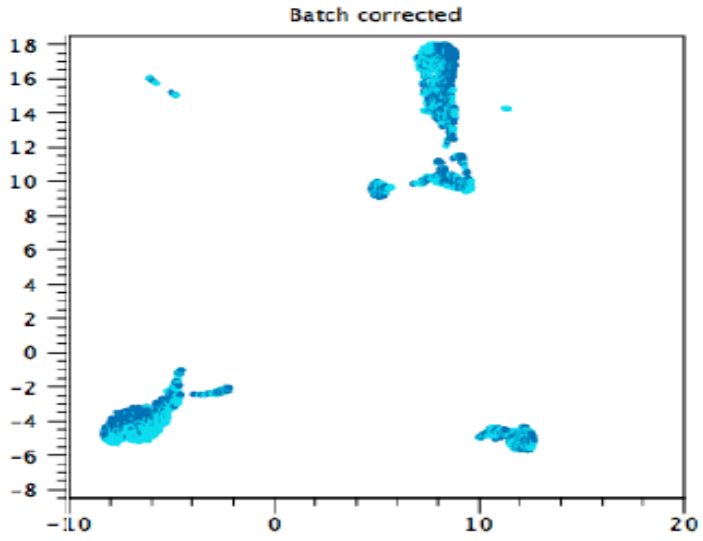


Normaliz

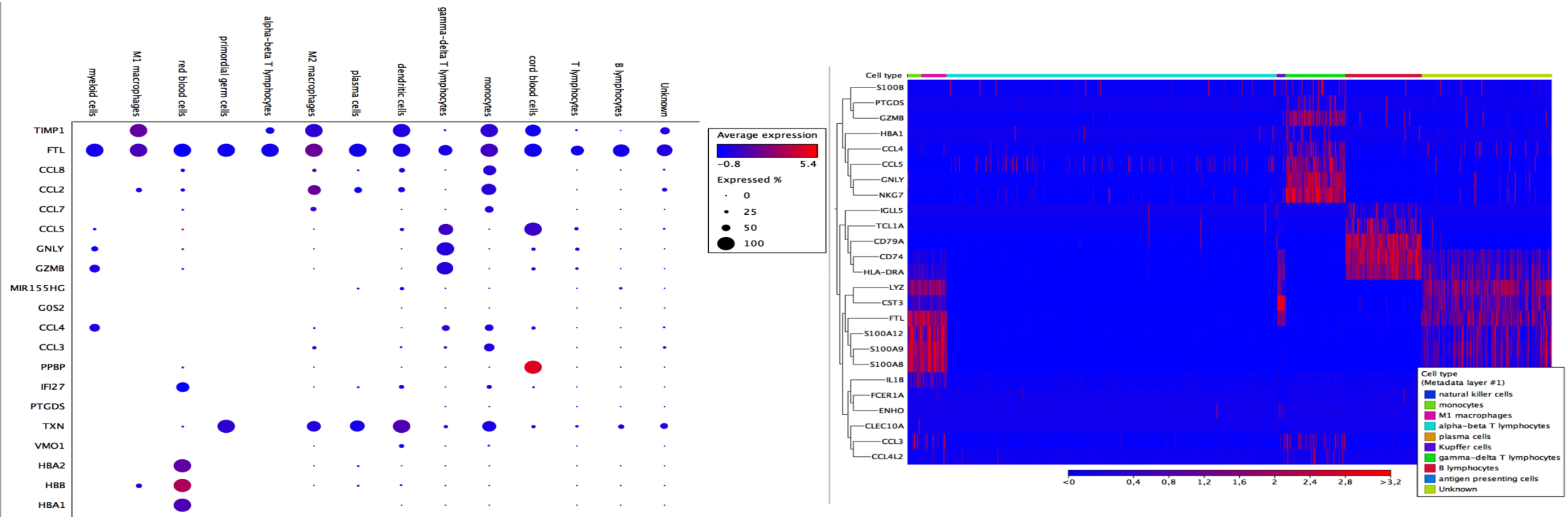








Expression Analysis on Single Cell



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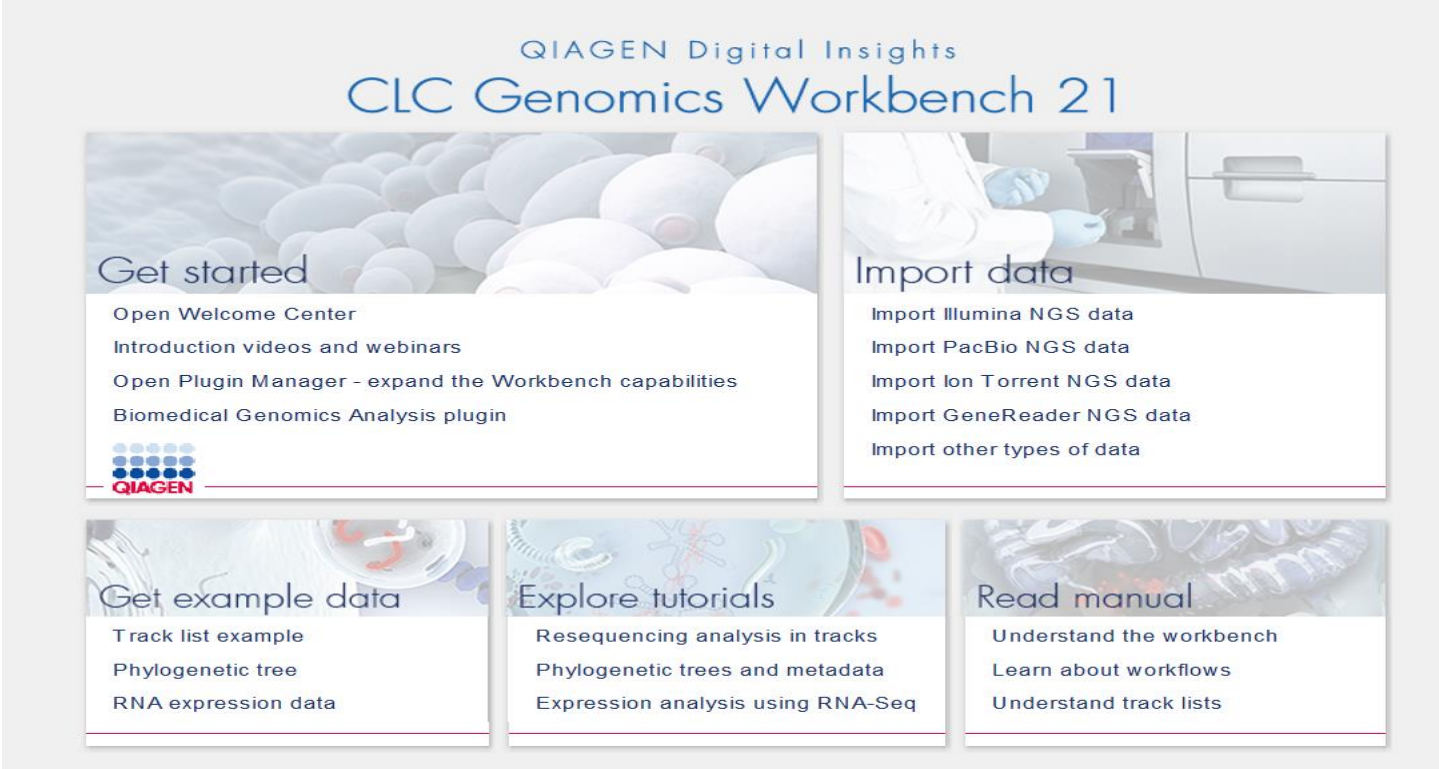
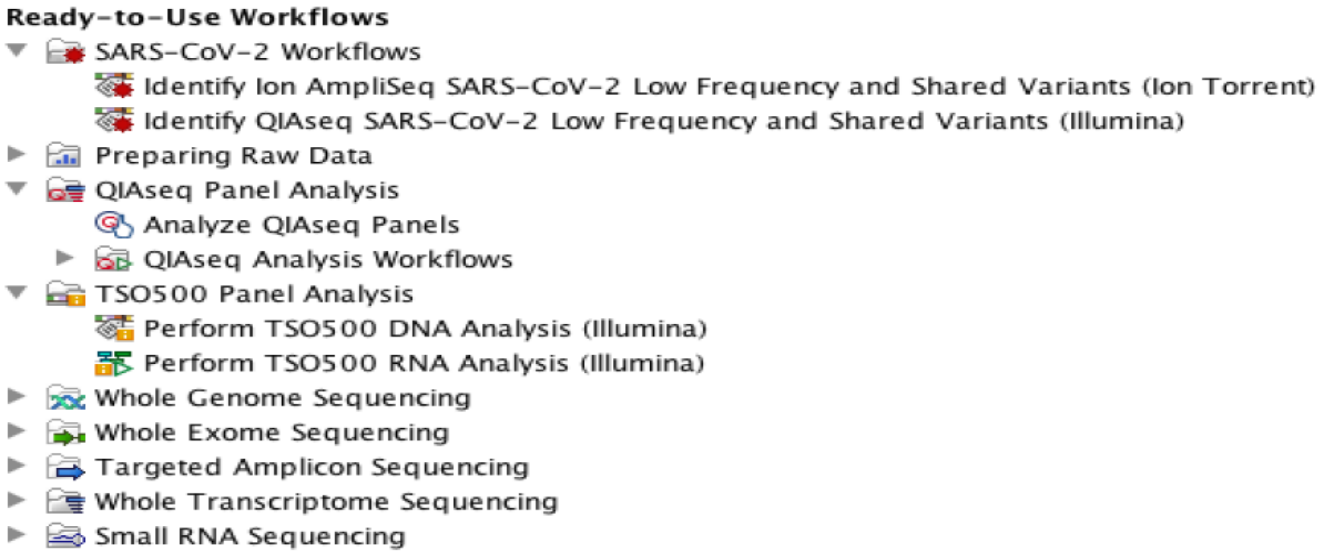


Biomedical Tools and Workflows

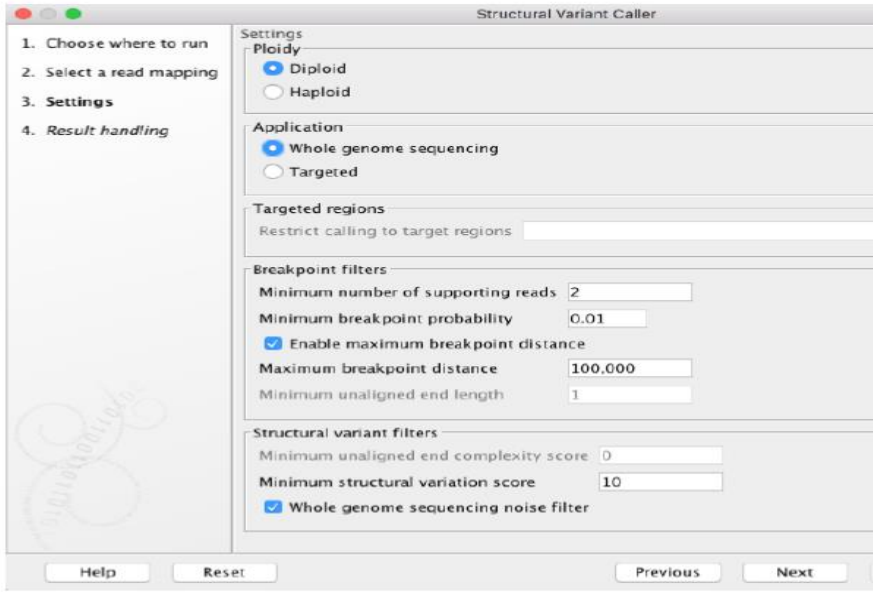
- New tools
  - Structural Variant Caller
  - Compare Immune Repertoires
  - Extract Reads with Primer
  - Remove Marginal Reads
  - Target Region Coverage Analysis
  - CNV and LOH Detection

- New workflows
  - SARS-CoV-2
  - TruSight Oncology 500 bundle
  - QIAseq
  - Reference data



Structural Variant Caller

- Can be used for whole genome or targeted analysis
- Better performance compared to old tools in CLC
- Detects germline as well as somatic variants



1 Variants

Chromosome	Length	Reads	Left breakpoints	Right breakpoints	Variants
1	248,956,422	1,272,783	202	186	3
2	242,193,529	1,255,428	229	211	2
3	198,295,559	1,075,417	193	170	3
4	190,214,555	563,830	125	125	1
5	181,538,259	795,898	141	124	2
6	170,805,979	1,050,319	219	190	8
7	159,345,973	753,297	131	141	0
8	145,138,636	688,069	126	149	1

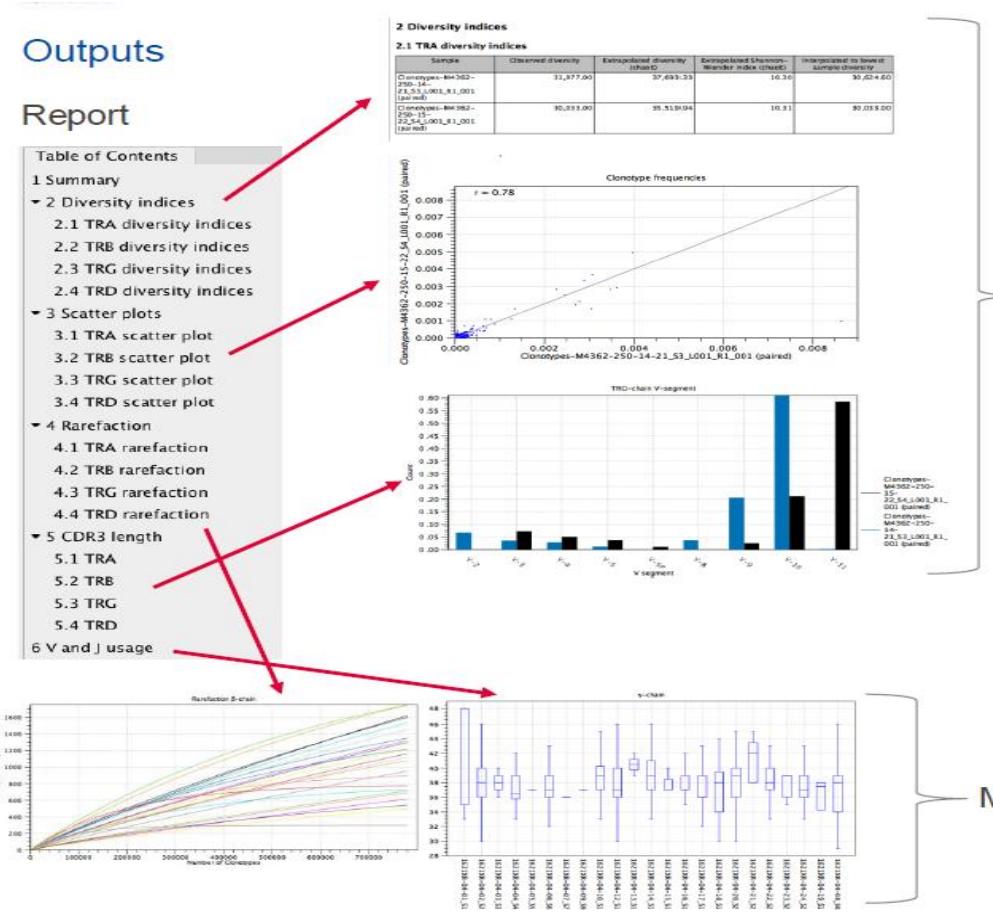
2 Summary

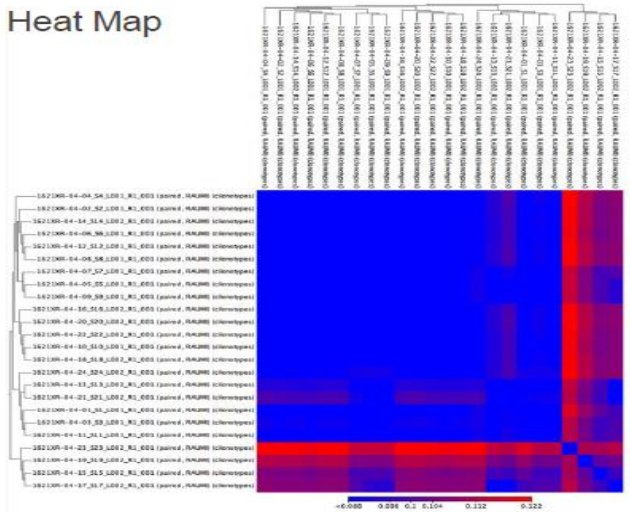
Chromosome	Total # variants	Insertion	Deletion	Tandem Duplication	Inversion	CNV Loss	CNV Gain
1	3	0	0	2	1	0	0
2	2	0	0	1	1	0	0
3	3	0	2	1	0	0	0
4	1	0	1	0	0	0	0
5	2	0	2	0	0	0	0

Indels_WGS

Chromosome	Region	Type	Score	Subtype	Complexity	Evidence	Left breakpoint	Right breakpoint
19	57018308..57018310	Deletion	28	Deletion	13	Single Breakpoint	57018307^57018308	
19	57174102..57174145	Deletion	44	Deletion	18	Single Breakpoint	57174101^57174102	
19	57499556..57499621	Deletion	48	Deletion	20	Single Breakpoint		57499621^57499622
19	245876..245978	Deletion	103	Deletion	30	Paired Breakpoints	245900^245901	245971^245972
19	245971^245972	Insertion	30	Insertion	23	Paired Breakpoints	245999^246000	245971^245972
19	269761..269826	Deletion	66	Deletion	20	Paired Breakpoints	269825^269826	269819^269820
19	302692^302693	Insertion	87	Tandem Dupli...	26	Paired Breakpoints	302778^302779	302692^302693
19	365492..365545	Deletion	79	Deletion	41	Paired Breakpoints	365541^365542	365545^365546

Compare Immune Repertoires





Sample	1621XR-04-01_S1...	1621XR-04-02_S2...	1621XR-04-03_S3...	1621XR-04-04_...	1621XR-04-05_S5...	1621XR-04-06_S6...
1621XR-04-01_S1_L...	1.00	0.92	0.91	0.92	0.92	0.92
1621XR-04-02_S2_L...	0.92	1.00	0.91	0.93	0.93	0.93
1621XR-04-03_S3_L...	0.91	0.91	1.00	0.91	0.92	0.91
1621XR-04-04_S4_L...	0.92	0.93	0.91	1.00	0.92	0.93
1621XR-04-05_S5_L...	0.92	0.93	0.92	0.92	1.00	0.94
1621XR-04-06_S6_L...	0.92	0.93	0.91	0.93	0.94	1.00

Similarity table

CNV and LoH Analysis

STATE
DIPLOID
DIPLOID
HOMOLOGUOUS_RECOMBINATION
HOMOLOGUOUS_RECOMBINATION
HOMOLOGUOUS_RECOMBINATION
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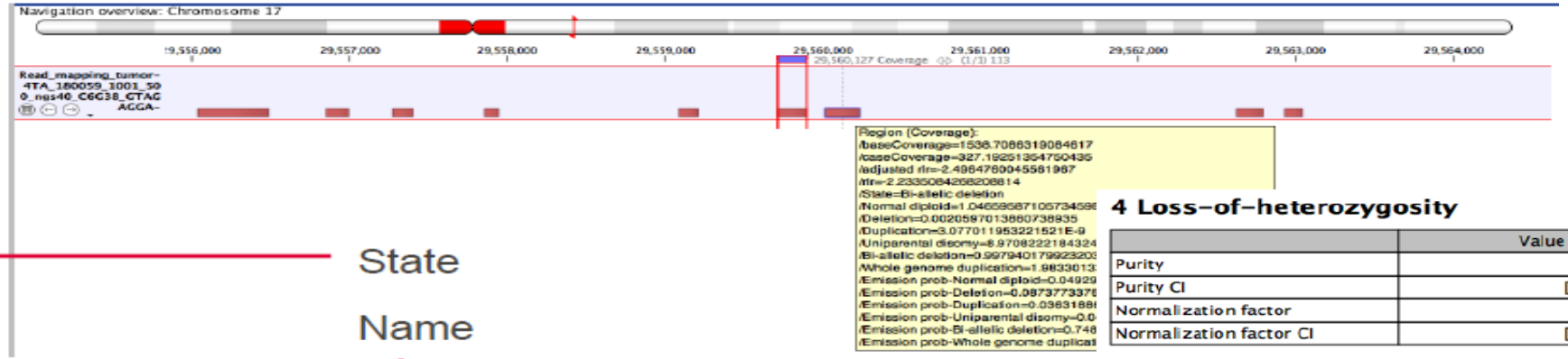


State

Name

Chrom...	Region	Name	baseCov...	caseCov...	adjuste...	rlr	State
17	41219620..41219717	Coverage	681.27	857.00	0.05	0.33	Uniparental disomy
17	41222940..41223260	Coverage	1,089.82	1,360.23	0.05	0.32	Uniparental disomy
17	41223093^41223094	VAF					Uniparental disomy
17	41226343..41226543	Coverage	1,305.49	1,484.75	-0.08	0.19	Uniparental disomy
17	41228500..41228636	Coverage	912.10	1,060.63	-0.06	0.22	Uniparental disomy
17	41231346..41231421	Coverage	1,531.68	1,911.34	0.06	0.32	Uniparental disomy
17	41234416..41234597	Coverage	1,034.78	1,455.48	0.22	0.49	Uniparental disomy
17	41234469^41234470	VAF					Uniparental disomy
17	41242956..41243054	Coverage	731.86	1,138.13	0.36	0.64	Uniparental disomy
17	41243447..41246882	Coverage	1,066.76	1,235.68	-0.06	0.21	Uniparental disomy
17	41243999^41244000	VAF					Uniparental disomy
17	41244434^41244435	VAF					Uniparental disomy
17	41244935^41244936	VAF					Uniparental disomy
17	41245236^41245237	VAF					Uniparental disomy
17	41245465^41245466	VAF					Uniparental disomy
17	41245470^41245471	VAF					Uniparental disomy
17	41247858..41247944	Coverage	624.47	765.00	0.01	0.29	Uniparental disomy

4 Loss-of-heterozygosity

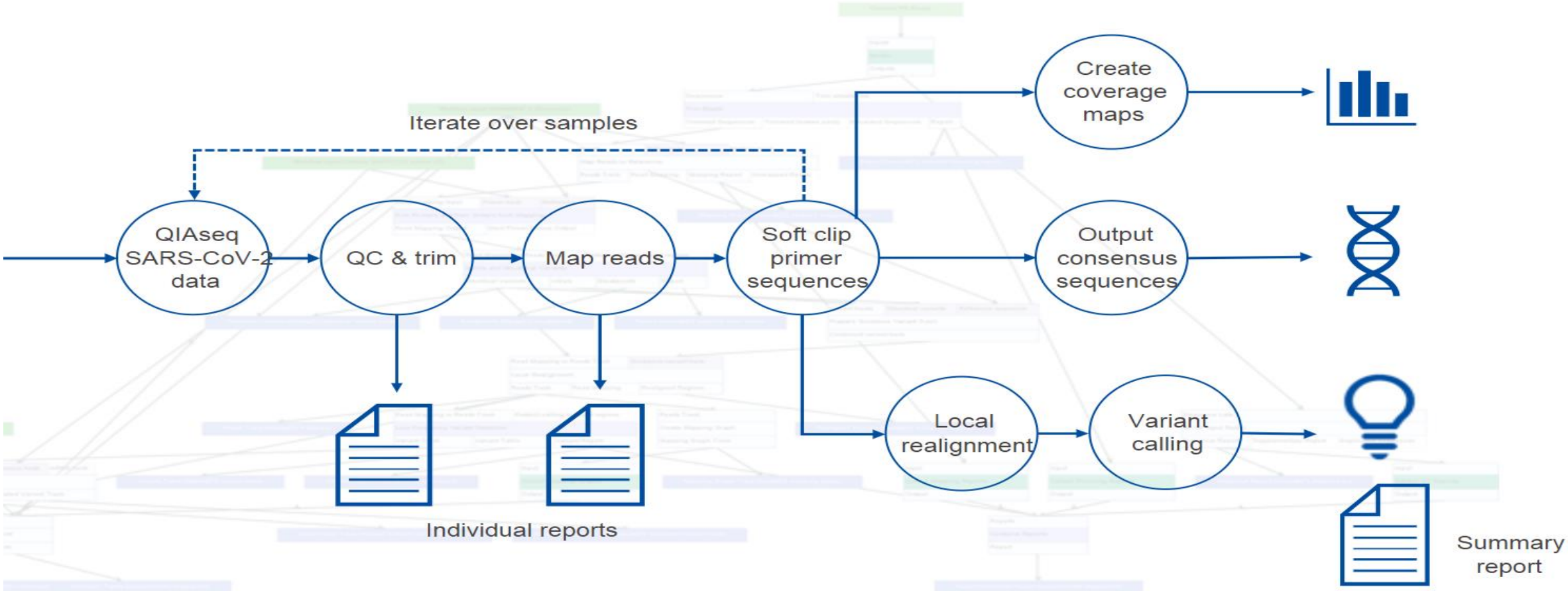
	Value
Purity	0.65
Purity CI	[0.623;0.672]
Normalization factor	0.74
Normalization factor CI	[0.720;0.753]

State	Count
Bi-allelic deletion	0
Deletion	292
Normal diploid	82
Uniparental disomy	0
Duplication	70
Whole genome duplication	0

State	Expected RLR	Avg RLR
Bi-allelic deletion	-1.51	-
Deletion	-0.56	-0.60
Normal diploid	0.00	-0.00
Uniparental disomy	0.00	-
Duplication	0.40	0.37
Whole genome duplication	0.72	-

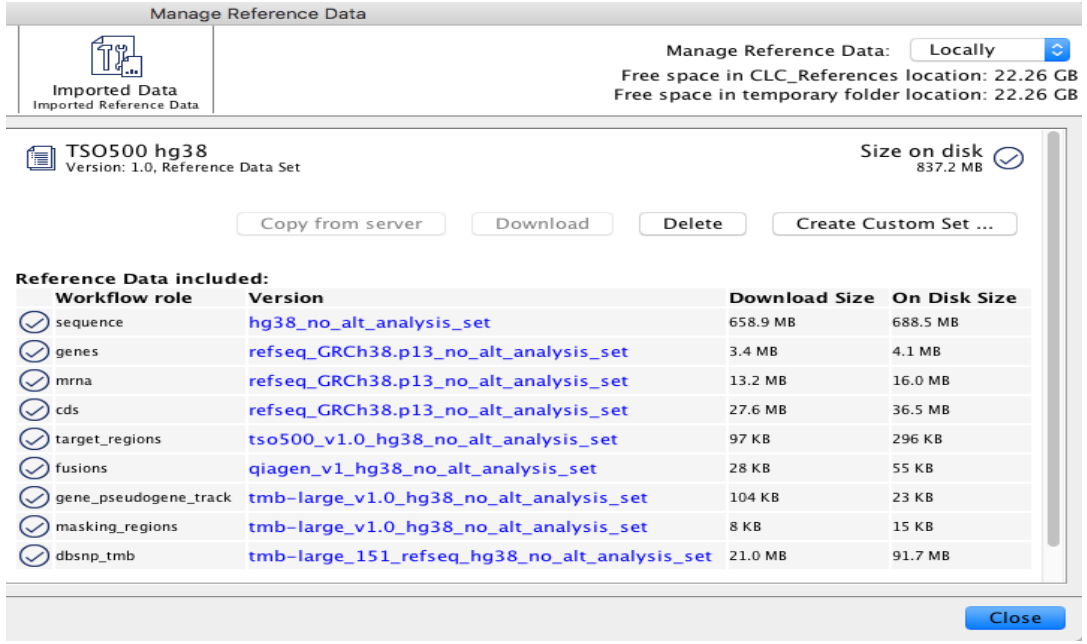
State	Expected BAF	Avg BAF
Bi-allelic deletion	0.50	-
Deletion	0.74	0.74
Normal diploid	0.50	0.50
Uniparental disomy	0.82	-
Duplication	0.62	0.65
Whole genome duplication	0.50	-

SARS-CoV-2 Workflow



TSO500 Panel Analysis

- TSO500 Panel Analysis
  - Perform TSO500 DNA Analysis (Illumina)
  - Perform TSO500 RNA Analysis (Illumina)



Outputs

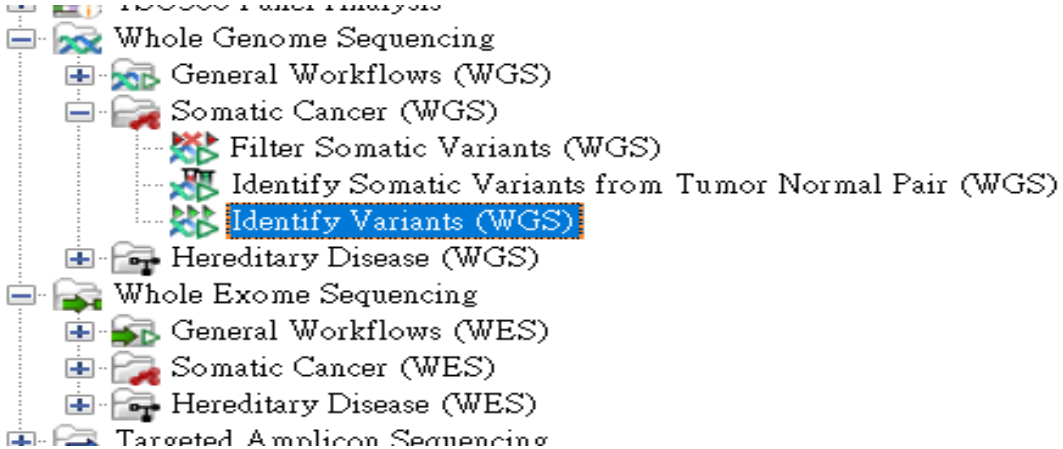
- TSO500_DNA
  - Workflow Result Metadata
  - QC & Reports
  - Tracks
    - Mapped_UMI_reads-DL017-UP01-19MAY20-ABU_S1_L001_R1_001 (paired)
    - Per-region_statistics_track-DL017-UP01-19MAY20-ABU_S1_L001_R1_001 (paired)
    - Unfiltered_variants-DL017-UP01-19MAY20-ABU_S1_L001_R1_001 (paired)
    - Amino acid track
    - TMB_somatic_variants-DL017-UP01-19MAY20-ABU_S1_L001_R1_001 (paired)
  - VCF Exportable Tracks
    - Variants_passing_filters-DL017-UP01-19MAY20-ABU_S1_L001_R1_001 (paired)
  - DNA_combined_report-DL017-UP01-19MAY20-ABU_S1_L001_R1_001 (paired)
  - Track List
  - Perform TSO500 DNA Analysis (Illumina) log
- TSO500_RNA
  - Workflow Result Metadata
  - QC & Reports
  - Gene_expression-RL008-UP16-19MAY20-SA_S16_L001_R1_001 (paired)
  - Tracks (WT)
    - RNA_read_mapping (WT)-RL008-UP16-19MAY20-SA_S16_L001_R1_001 (paired)
    - Fusion_genes_unaligned_ends (WT)-RL008-UP16-19MAY20-SA_S16_L001_R1_001 (paired)
    - Fusion_genes (WT)-RL008-UP16-19MAY20-SA_S16_L001_R1_001 (paired)
    - Read_mapping_refined (WT)-RL008-UP16-19MAY20-SA_S16_L001_R1_001 (paired)
  - RNA_combined_QC_report-RL008-UP16-19MAY20-SA_S16_L001_R1_001 (paired)
  - Tracks (fusion)
  - VCF Exportable Tracks
    - Final_fusion_genes (WT)-RL008-UP16-19MAY20-SA_S16_L001_R1_001 (paired)
  - Genome Browser View (Fusions)
  - Genome Browser view (WT)
  - Perform TSO500 RNA Analysis (Illumina) log

Summary

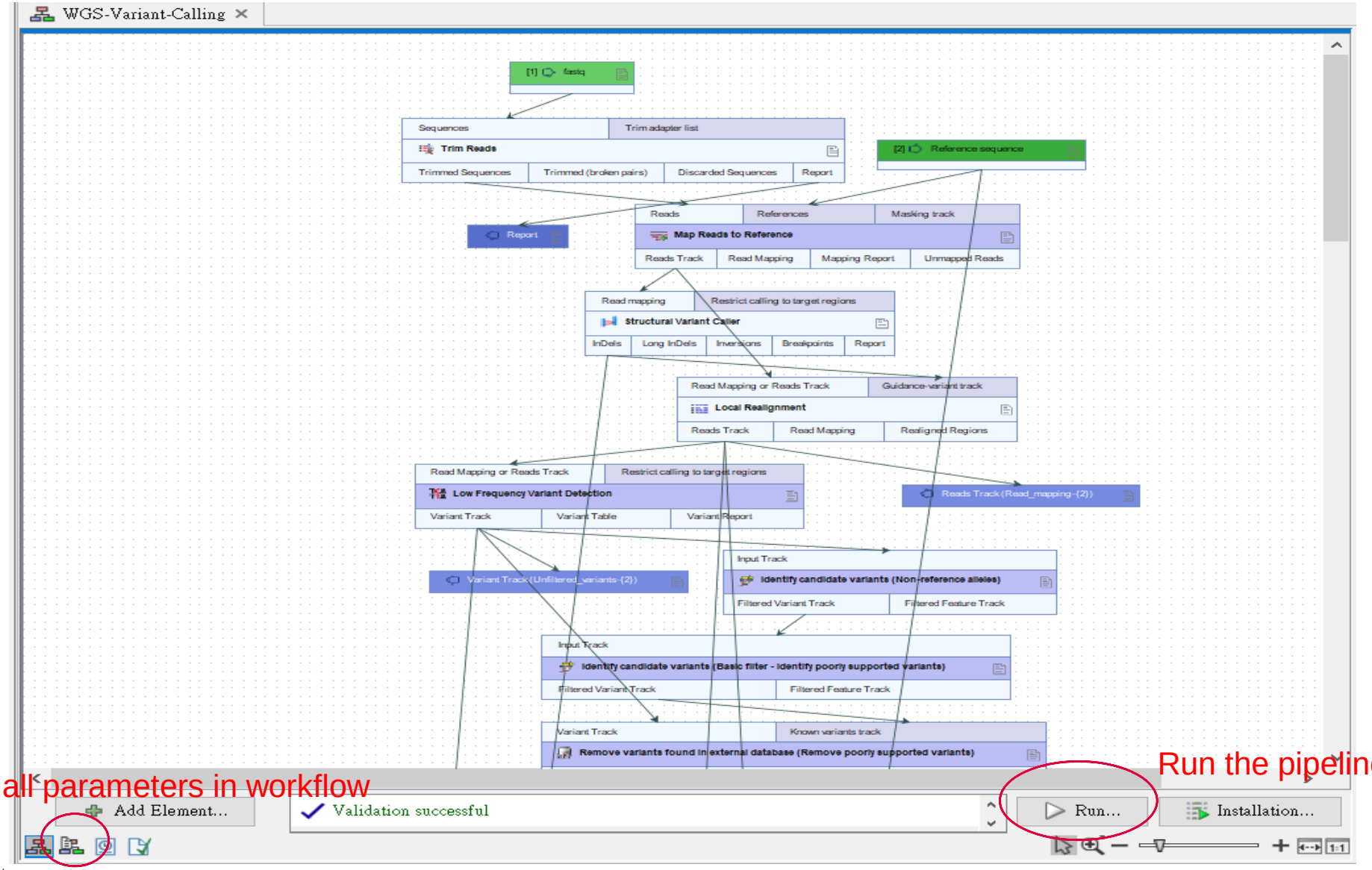
- One solution for genomics application with GUI
  - User-friendly interface
  - Interactive visualization to facilitate analysis
  - Ready-to-use and customizable workflows
    - For automated processing
    - For sharing with colleagues
  - Modular design to add plugins
  - Works with reads from most platform
    - Illumina, Ion Torrent, Oxford Nanopore, PacBio, BGI/MGI
  - Fully documented and supported

Workflow Methods

- The whole workflow is design when you have FASTQ files
  - Previous work: DNA-Seq Analysis for all your sample
  - Input:
    - FASTQ data
    - Metadata
  - Output:
    - VCF files with filtering variants



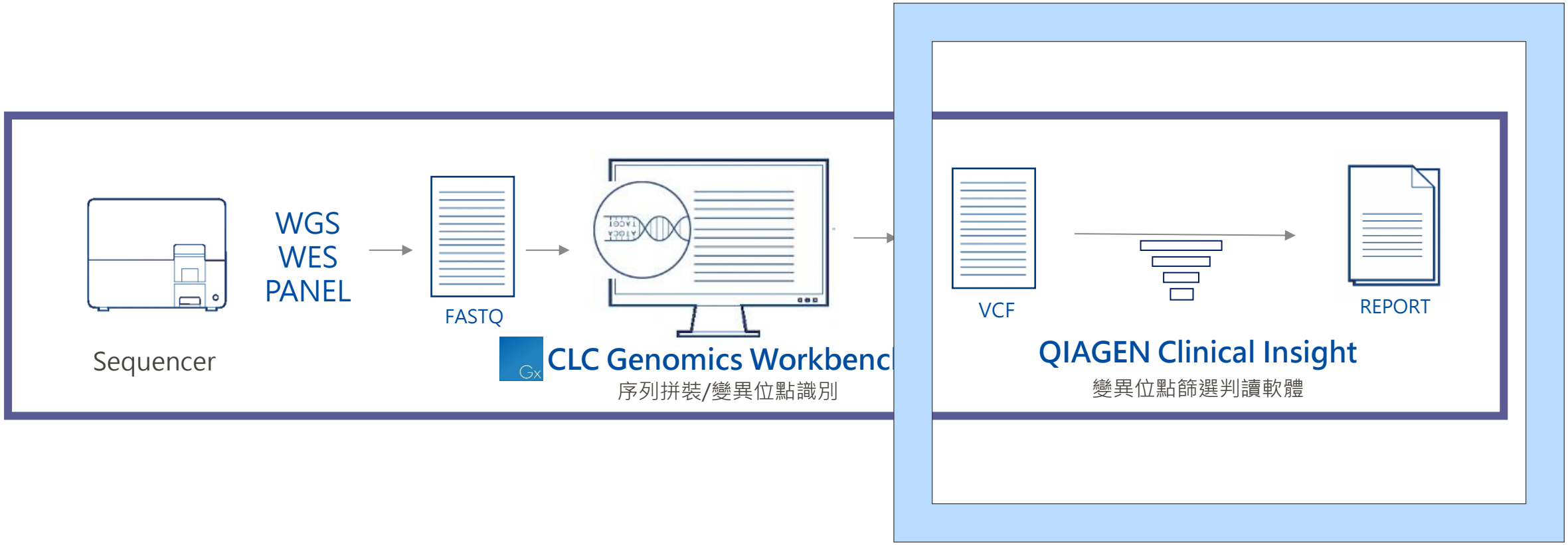
Customized full workflow



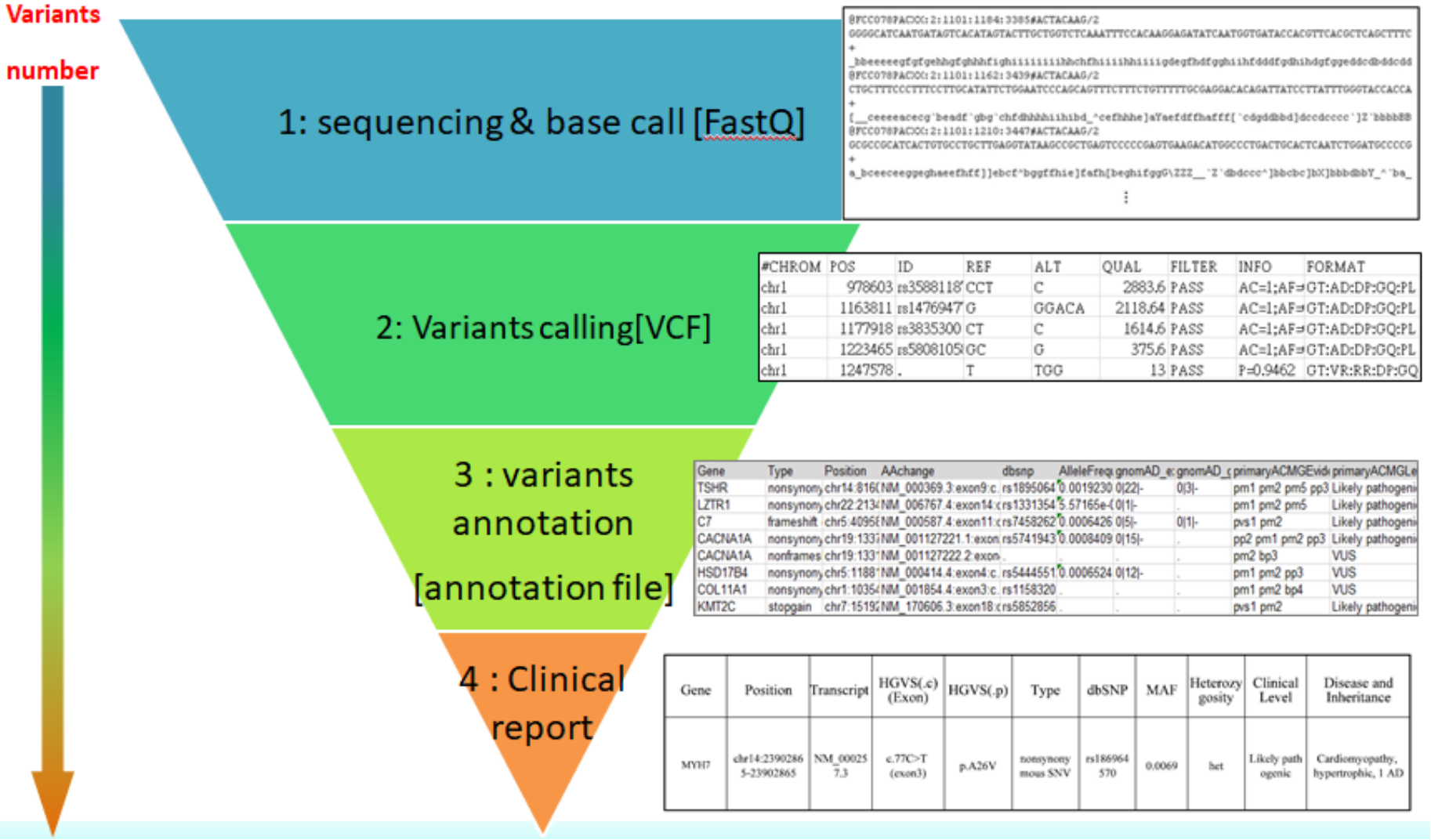
Change all parameters in workflow

Run the pipeline

QIAGEN Clinical Insight System



NGS Variant Analysis Service



@FCC078PACXX:2:1101:1184:3385#ACTACAAG/2
GGGGCATCAATGATAGTCACATAGTACTTGCTGGTCTCAAATTTCCACAAGGAGATATCAATGGTGATACCACGTTCACGCTCAGCTTTC
+
_bbeeeeegfgfgehhgfghhhfighiiiiiiiihbchfhiiihhiiiigdegfhdfgghihfdddfgdhihdgfgged6dcdbddcdd
@FCC078PACXX:2:1101:1162:3439#ACTACAAG/2
CTGCTTTCCCTTTCCTTGCATATTCTGGAATCCCAGCAGTTTCTTTCTGTTTTTGCGAGGACACAGATTATCCTTATTTGGGTACCACCA
+
[__ceeeeacecg`beadf`gbg`chfdhhhhiihibd_^cefhhhe]aYaefdffhafff[`cdgddbbd]dccdcccc`]Z`bbbb88
@FCC078PACXX:2:1101:1210:3447#ACTACAAG/2
GCGCCGCATCACTGTGCCTGCTTGAGGTATAAGCCGCTGAGTCCCCCGAGTGAAGACATGGCCCTGACTGCACTCAATCTGGATGCCCCG
+
a_bceeceeggeghaeefhff]]ebcf^bggffhie]fafh[beghifggG\ZZZ__`Z`dbdccc^]bbcbc]bX]bbbdbbY_^`ba_
⋮

#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

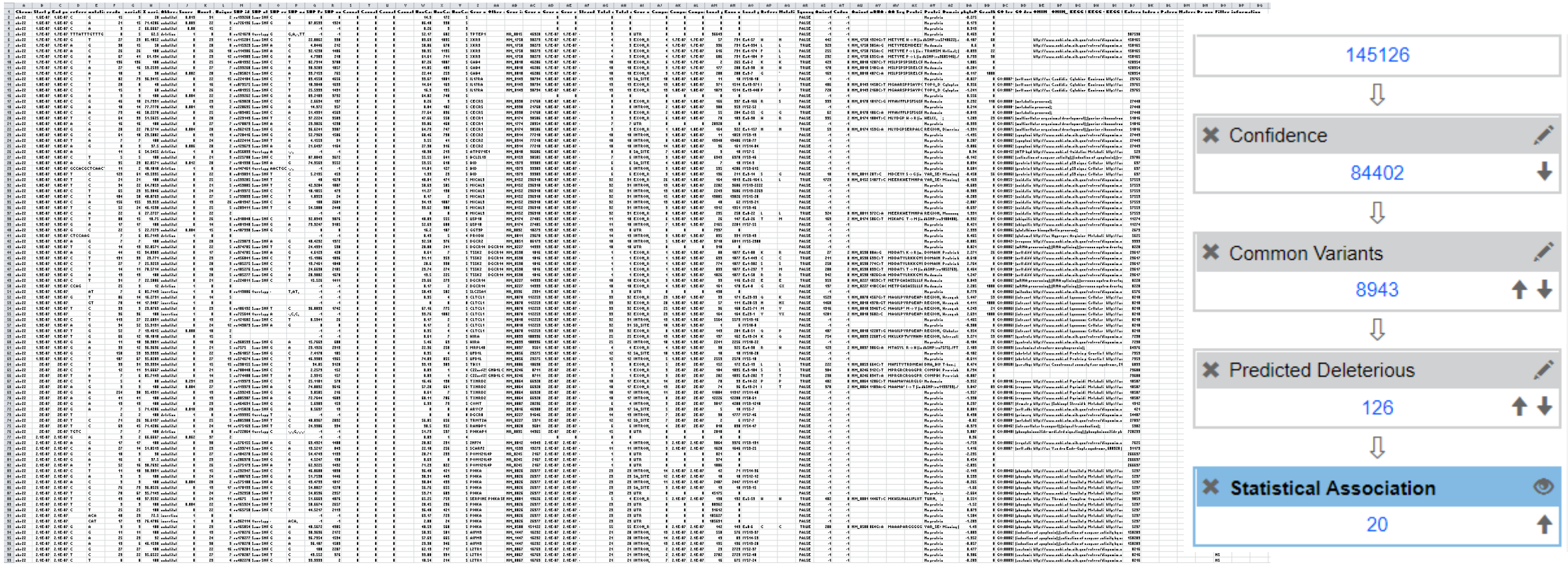
Gene	Type	Position	AAchange	dbsnp	AlleleFreq	gnomAD_e	gnomAD_g	primaryACMGEvid	primaryACMGLe
TSHR	nonsynony	chr14:816(	NM_000369.3:exon9:c.	rs1895064	0.0019230	0|22|-	0|3|-	pm1 pm2 pm5 pp3	Likely pathogen
LZTR1	nonsynony	chr22:213(	NM_006767.4:exon14:c	rs1331354	5.57165e-(	0|1|-	.	pm1 pm2 pm5	Likely 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

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

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”

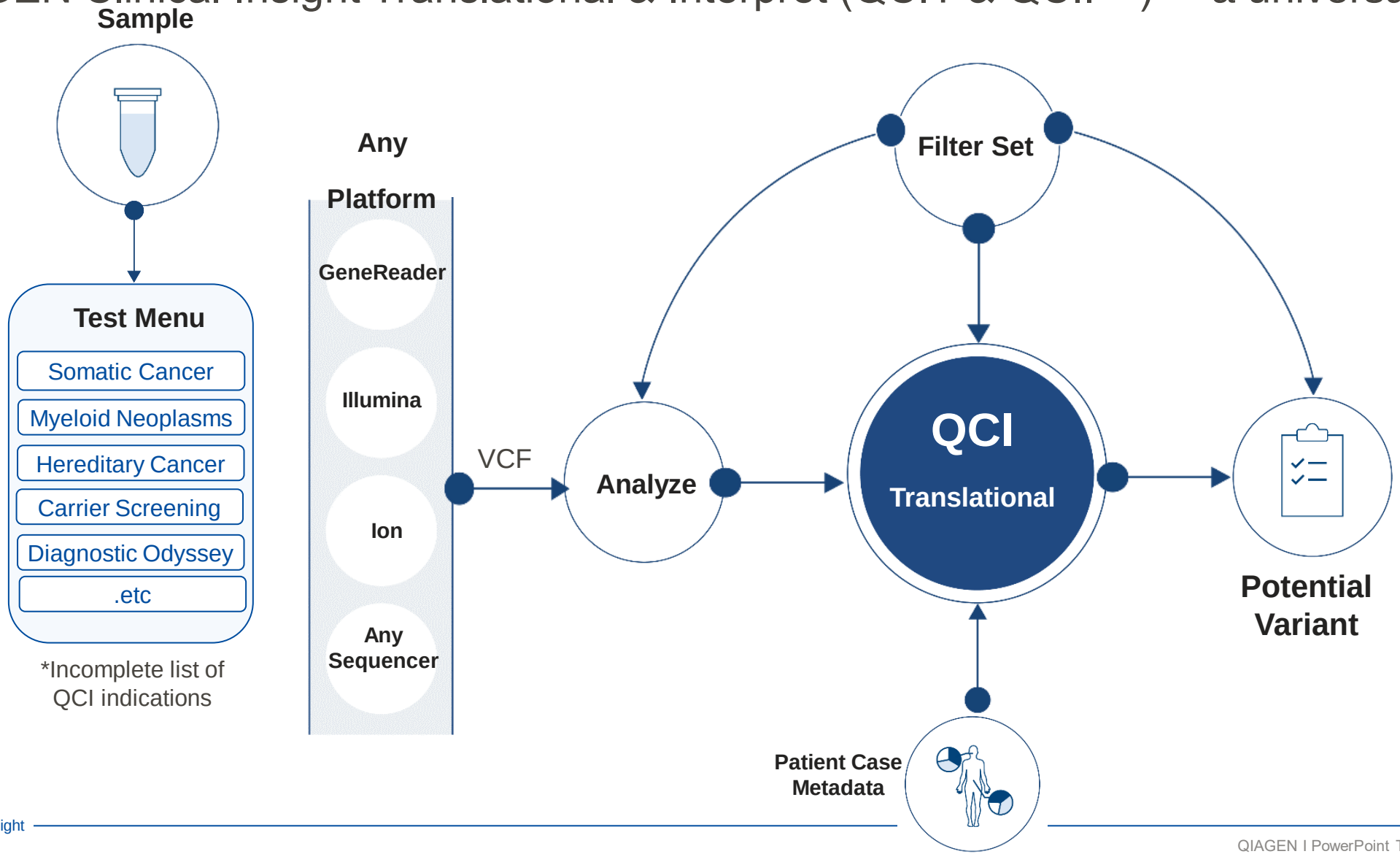
FE Dewey et al, JAMA. 2014;311(10):1035-1044.



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

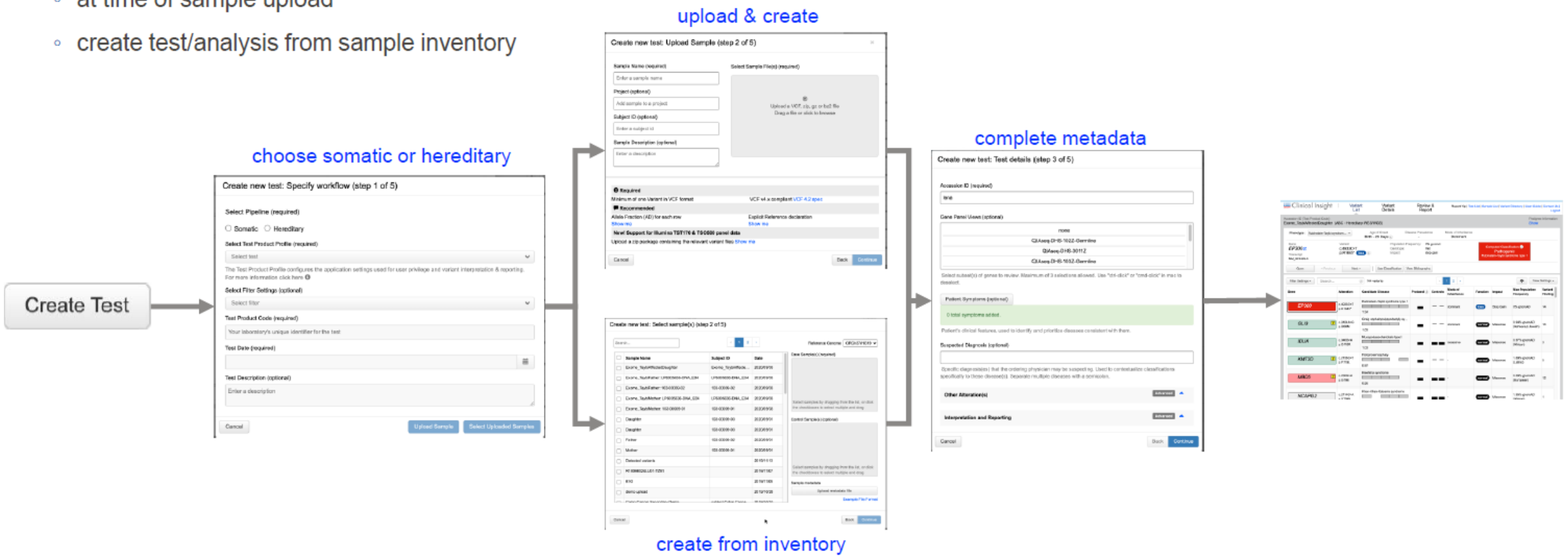
	QCIT(偏研究使用)	QCII(偏臨床使用)
功能	註解工具與篩選位點	註解工具與篩選位點 臨床與藥物資料提供臨床判讀使用
應用	• 可單一或多樣本分析，或是家族(trio)分析 • 可做群組分析(cohort study)	• 出具臨床報告
優勢	• Qiagen內建database(含ACMG guideline) • 有權限管理系統 • 可設定多種分析流程	• Qiagen內建database(含ACMG&AMP guideline) • 可客製報告模板 • 有權限管理系統 • 可設定多種分析流程(TPP) • 可設定報告簽核系統
輸出	Excel表格(註釋資料)	Excel表格與檢測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

Create Your Variant Analysis Strategically

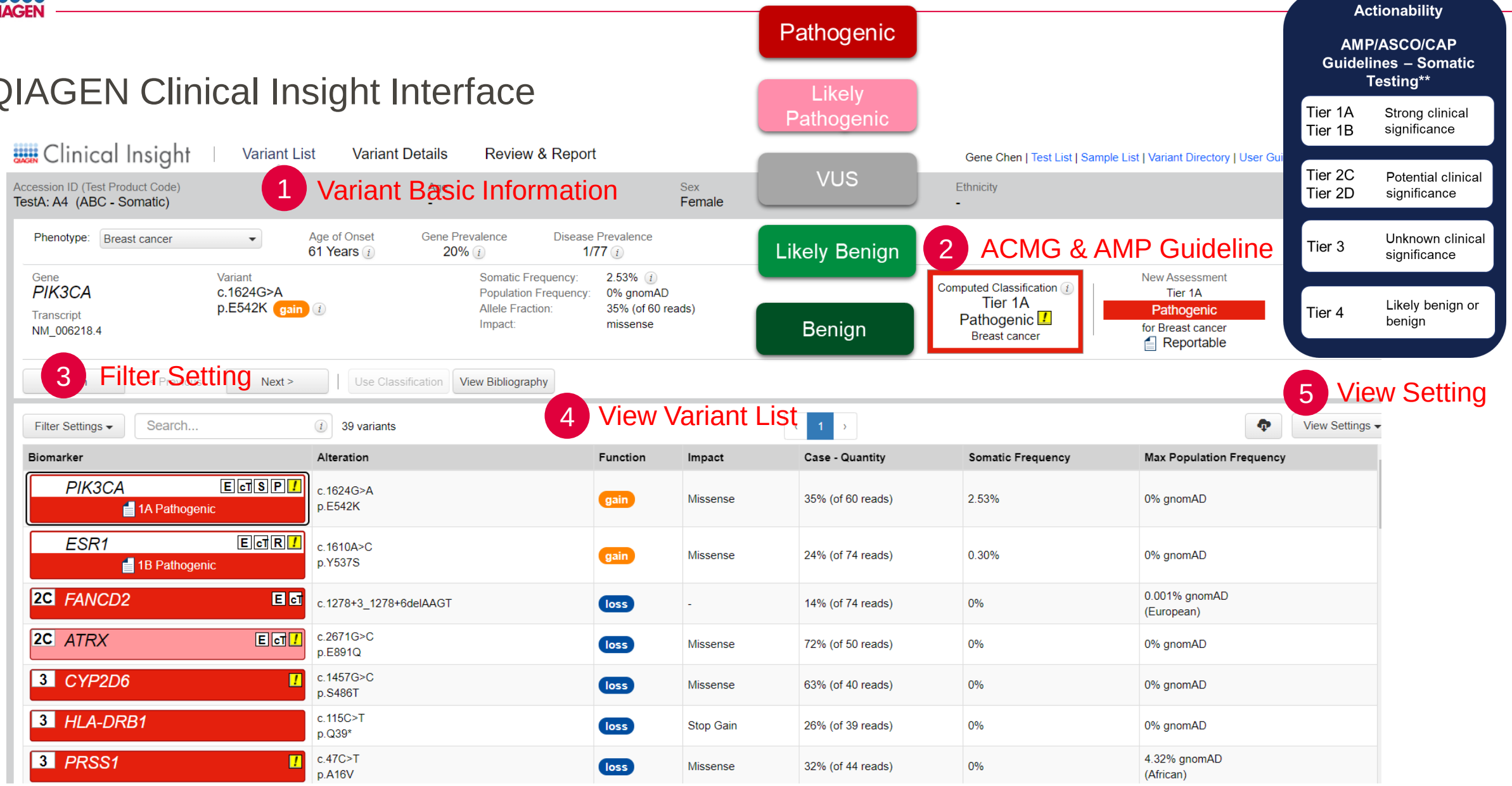
New Create Test button to start test creation workflow

Create new test/analysis

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



QIAGEN Clinical Insight Interface



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

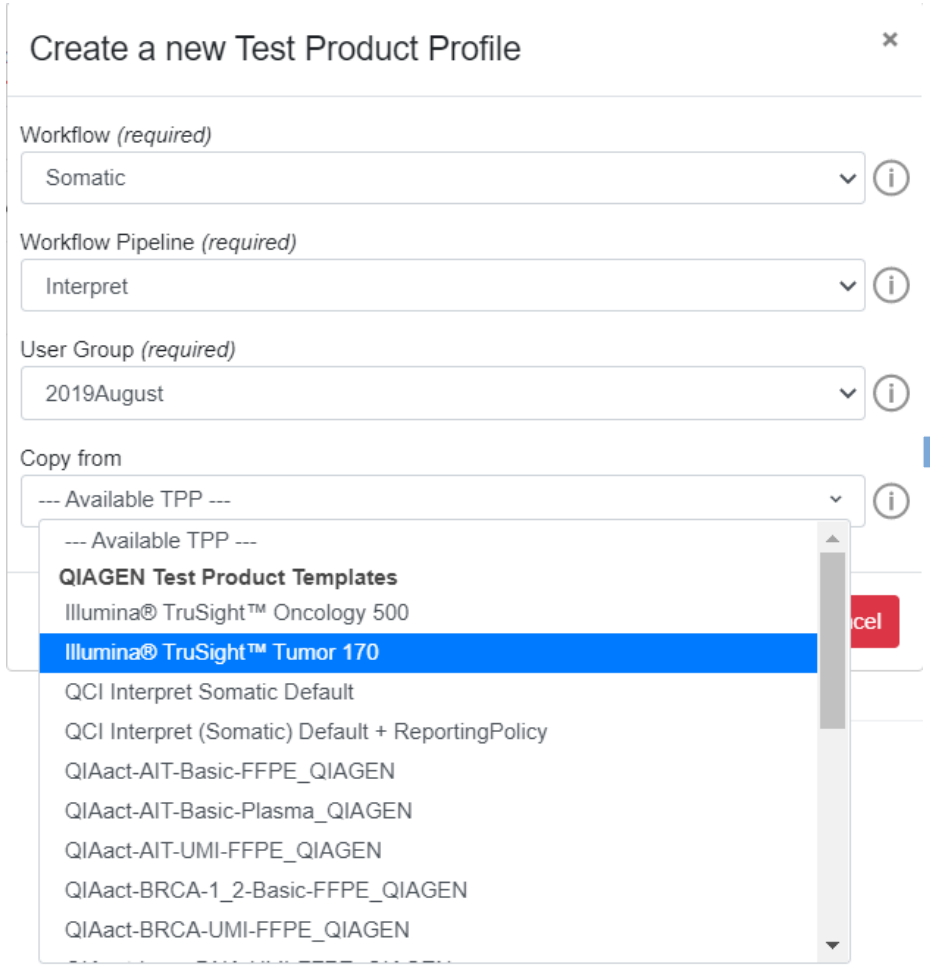
		Approved Therapies			
Gene / Variant	**Allelic Fraction**	**Breast Cancer**	**Other Indications**	**Associated With Resistance**	**Clinical Trials**
PIK3CA c.1624G>A p.E542K g.179218294G>A **Tier 1A** Pathogenic	35.0% (of 60 reads)	alpelisib alpelisib /fulvestrant lapatinib /letrozole letrozole	-	-	19
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

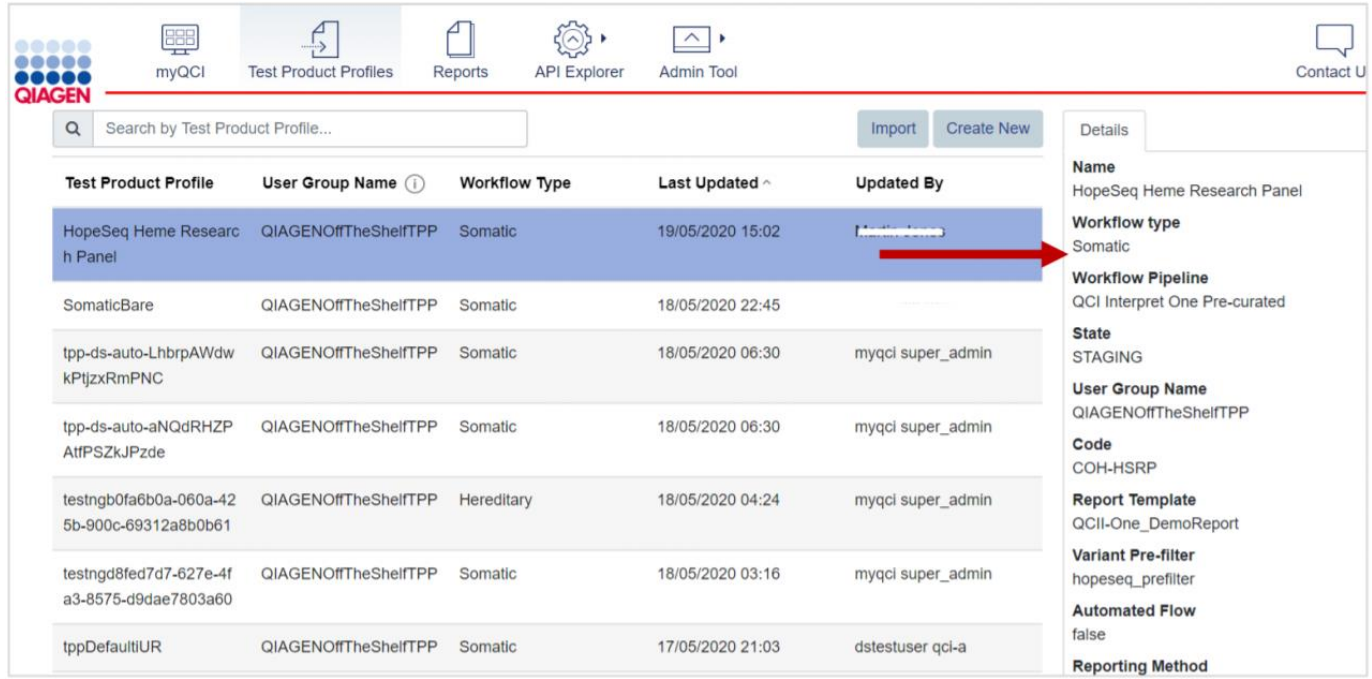
Gene / Variant	Trial Title Trial ID	Treatments	Trial Phase	Location / Contact
ESR1 p.Y537S g.152098788A>C **Tier 1B** Pathogenic	A Phase 1 Study of SY 5609, an Oral, Selective CDK7 Inhibitor, in Adult Patients With Select Advanced Solid Tumors NCT04247126	SY-5609 fulvestrant	Phase 1	United States: MI, 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].

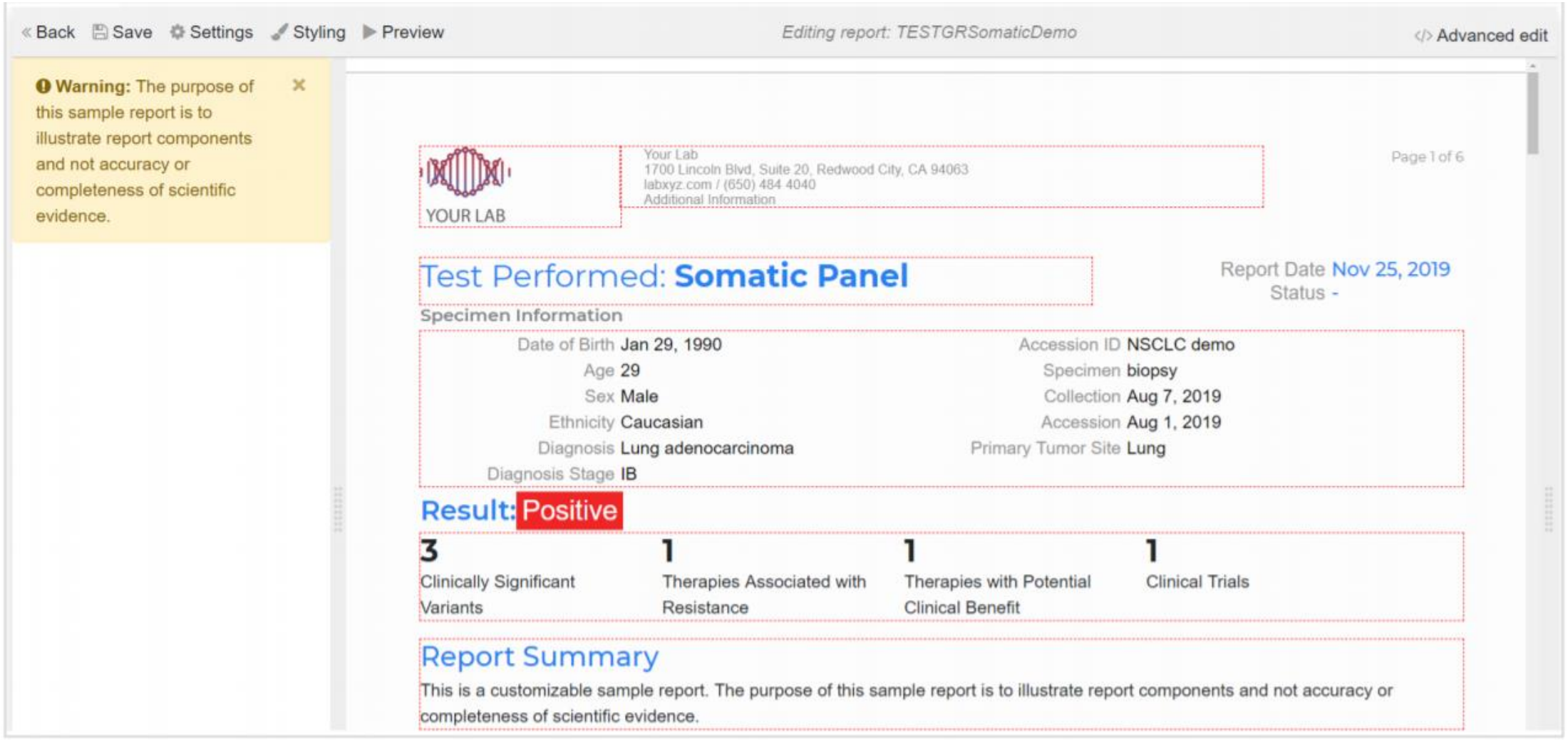
MyQCI – Test Product Profile (TPP) Configuration



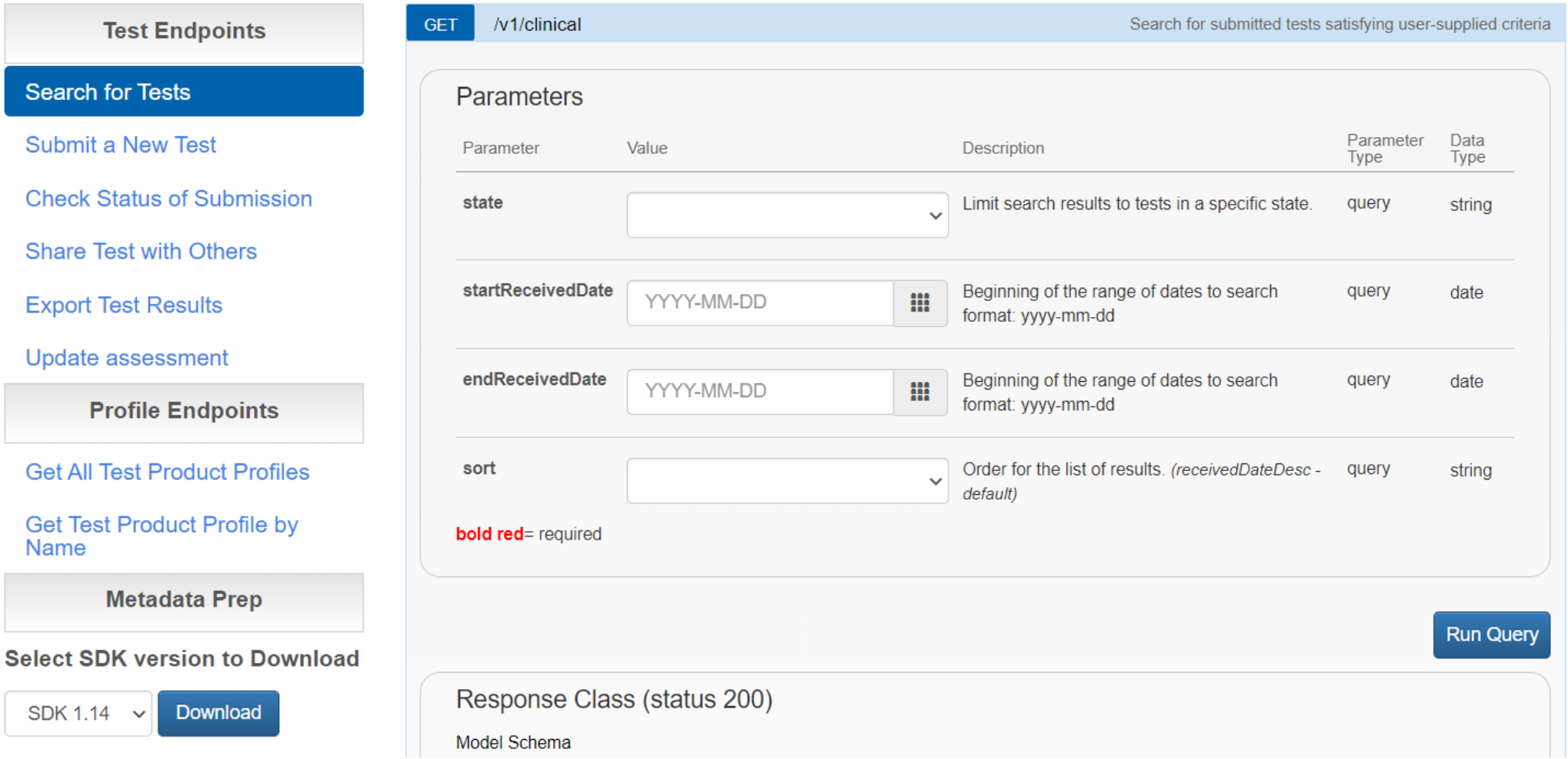


MyQCI - Report

MyQCI – Customize Your Report Style



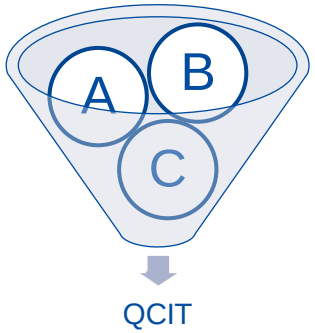
MyQCI - API Explorer (**Another License Required**)



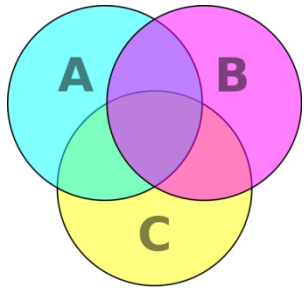
CASE STUDY

1.針對不同表型之癌症病患篩選位點並提供用藥資訊

- Characterizing the variants specific to the different subtypes
  - Samples
    - Subtype A: 9 samples
    - Subtype B: 9 samples
    - Subtype C: 9 samples

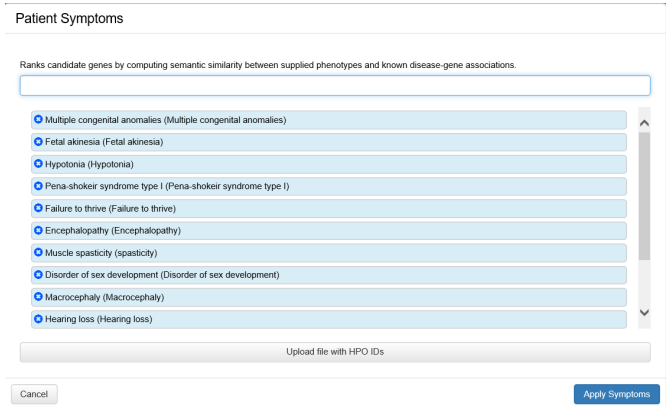


QCIT

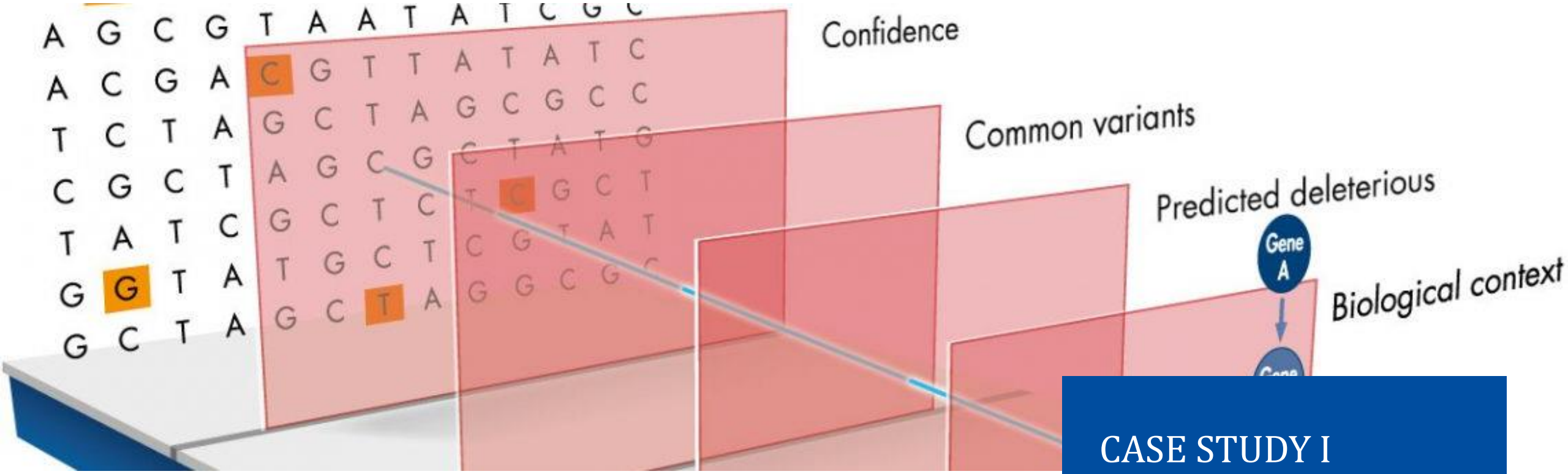


2. 以Trio家族性遺傳性分析評斷多表型遺傳症狀之變異位點

- Heredity: Trio Analysis on multiple-phenotypes genetic disease
  - Samples
    - Proband
      - Father
      - Mother
  - Goal:
    - To find the genetic-linkage variants on specific phenotypes on case children



LIVE DEMO

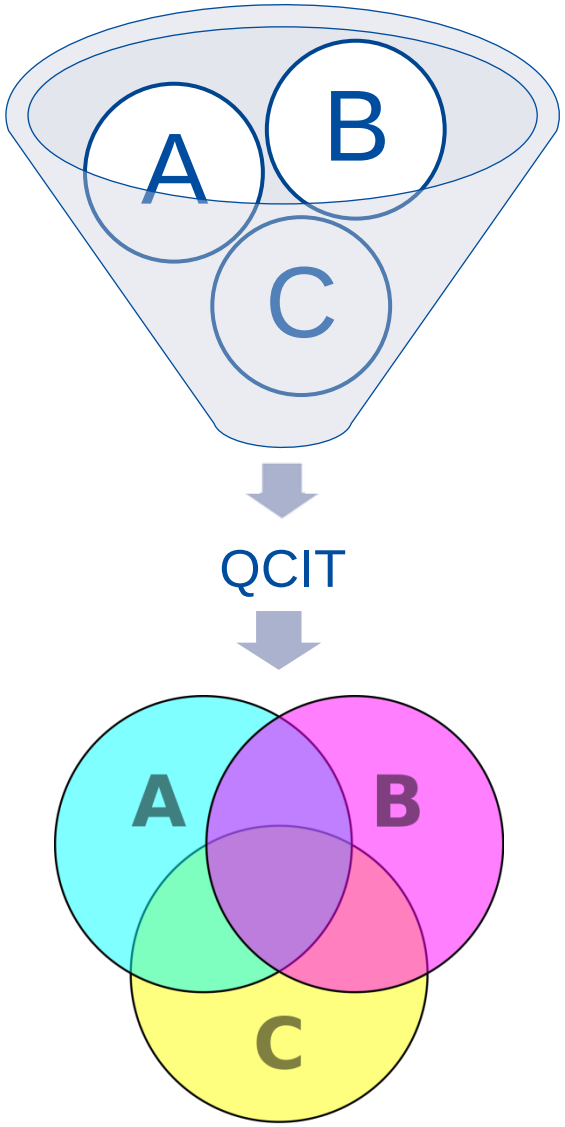


CASE STUDY I

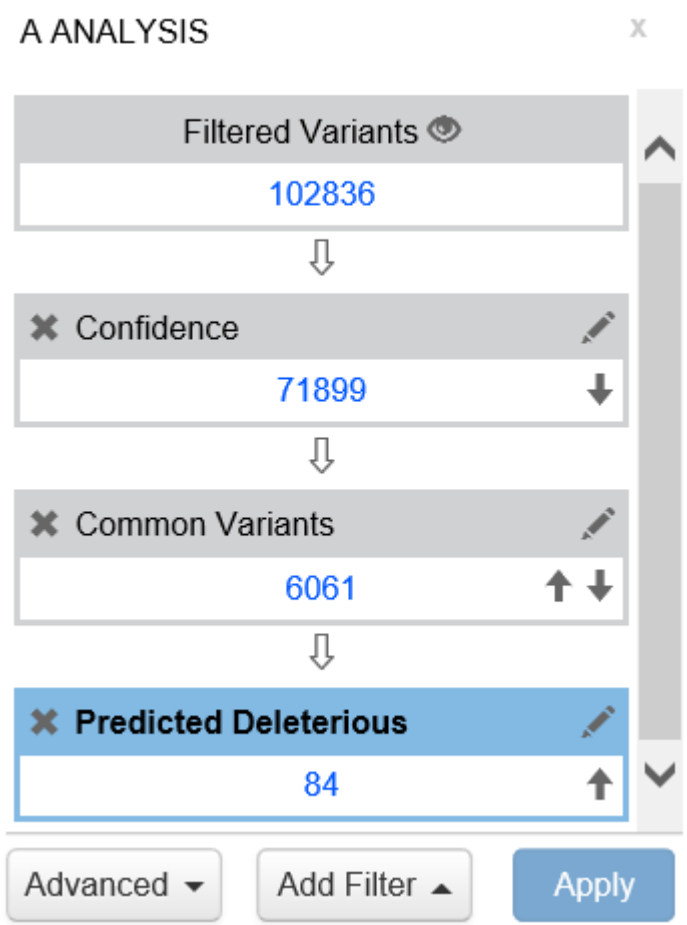
針對不同表型之癌症病患篩選位點並提供用藥資訊

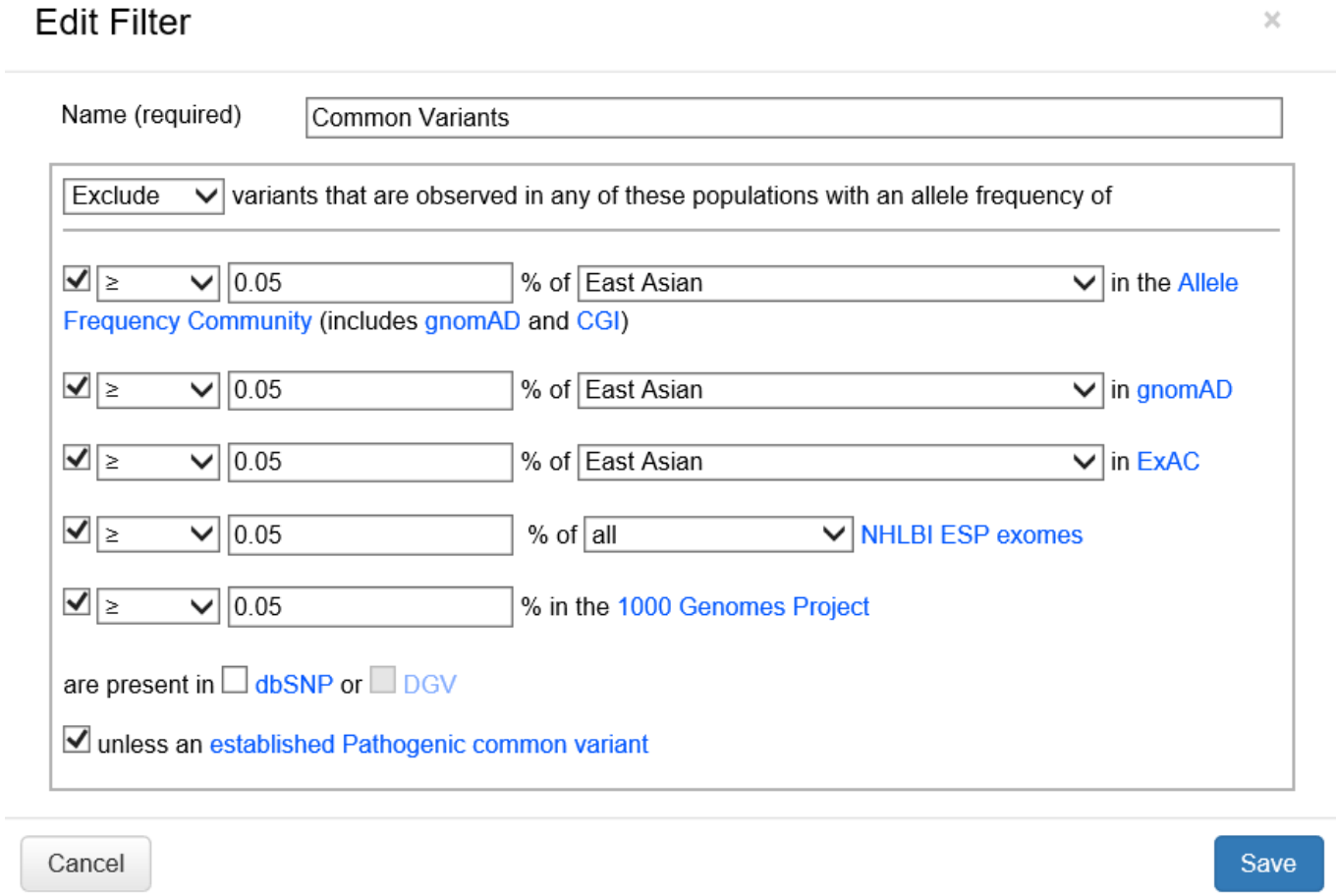
Case Studies I:針對不同表型之乳癌病患篩選位點並提供用藥資訊

- Characterizing the variants specific to the different subtypes
  - Samples
    - Subtype A: 9 samples
    - Subtype B: 9 samples
    - Subtype C: 9 samples
  - Goal:
    - To find the specific variants between different subtypes
  - Ways:
    - Filter out the false positive variants – Confident Filter
    - Filter out common variants – Common Variant Filter
    - Search for reportable pathogenic variants by ACMG guideline – Predict Deleterious
    - Pool all potential variant and find the intersection and specific variants

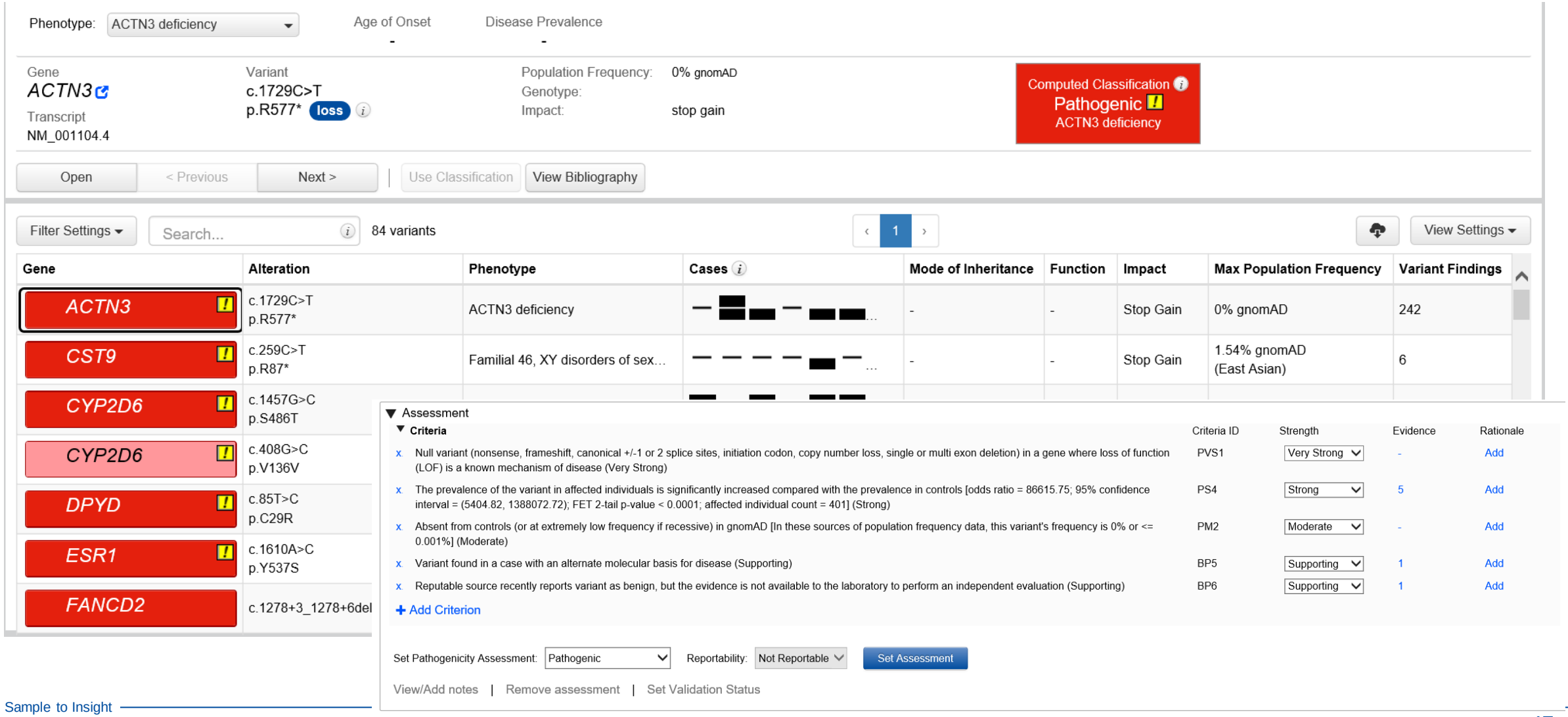


Step III: Change Filter Settings





Step IV: View the Variant Results AND Report



Step V: Further Analysis on Your Own Pipeline

A subtype specific variant lists (Pathogenic Variant)

Chromosome	Position	End Position	Reference Allele	Sample Allele	Variation Type	Gene Region	Gene Symbol	Protein Variant	Variant Findings	Translation Impact	ACMG
1	45332088	45332088	T	C	SNV	Splice Site; Intronic	MUTYH	-	195	-	Pathogenic
3	75737893	75737894	-	CTT	Insertion	Promoter; Exonic; Intronic	ZNF717; MIR4273	p.F577delins*V; p.F527delins*V	1	in-frame	Pathogenic
3	1.79E+08	1.79E+08	G	A	SNV	Exonic	PIK3CA	p.E542K	3679	missense	Pathogenic
3	1.79E+08	1.79E+08	C	A	SNV	Exonic	PIK3CA	p.Q546K	762	missense	Pathogenic
3	1.79E+08	1.79E+08	G	A	SNV	Exonic	PIK3CA	p.E726K	430	missense	Pathogenic
6	1.52E+08	1.52E+08	A	C	SNV	Exonic; Intronic; 3'UTR	ESR1	p.Y536S; p.Y537S; p.Y276S; p.Y539S	364	missense	Pathogenic
8	1.33E+08	1.33E+08	G	A	SNV	Exonic; ncRNA; Intronic	LRRC6	p.R60*; p.R180*; p.R98*	2	stop gain	Pathogenic
10	8073787	8073787	C	T	SNV	Exonic	GATA3	p.R367*; p.R366*	39	stop gain	Pathogenic
12	1.03E+08	1.03E+08	T	C	SNV	Exonic	PAH	p.Y204C	537	missense	Pathogenic
17	7674179	7674179	A	C	SNV	Splice Site	**TP53**	-	19	-	Pathogenic
17	31352348	31352348	C	T	SNV	Exonic	NF1	p.R2517*; p.R2496*	66	stop gain	Pathogenic
M	12338	12338	T	C	SNV	Exonic	MT-ND5	p.M1T	10	start loss	Pathogenic

ESR1 Treatment Info from QCII

Phenotype: Breast cancer

Age of Onset	Gene Prevalence	Disease Prevalence
61 Years	8.42%	1/77

Gene: *ESR1*
Transcript: NM_001122742.1

Variant: c.1610A>C
p.Y537S gain

Somatic Frequency: 0.30%
Population Frequency: 0% gnomAD
Allele Fraction: 24% (of 74 reads)
Impact: missense

Computed Classification
Tier 1B
Pathogenic
Breast cancer

Variant List | < Previous | Next > | Use Classification | View Bibliography

▼ Treatment Information

Treatments Report All Showing | Unreport All Showing

Change Phenotype To: All Cancers 1 treatment(s) ineligible

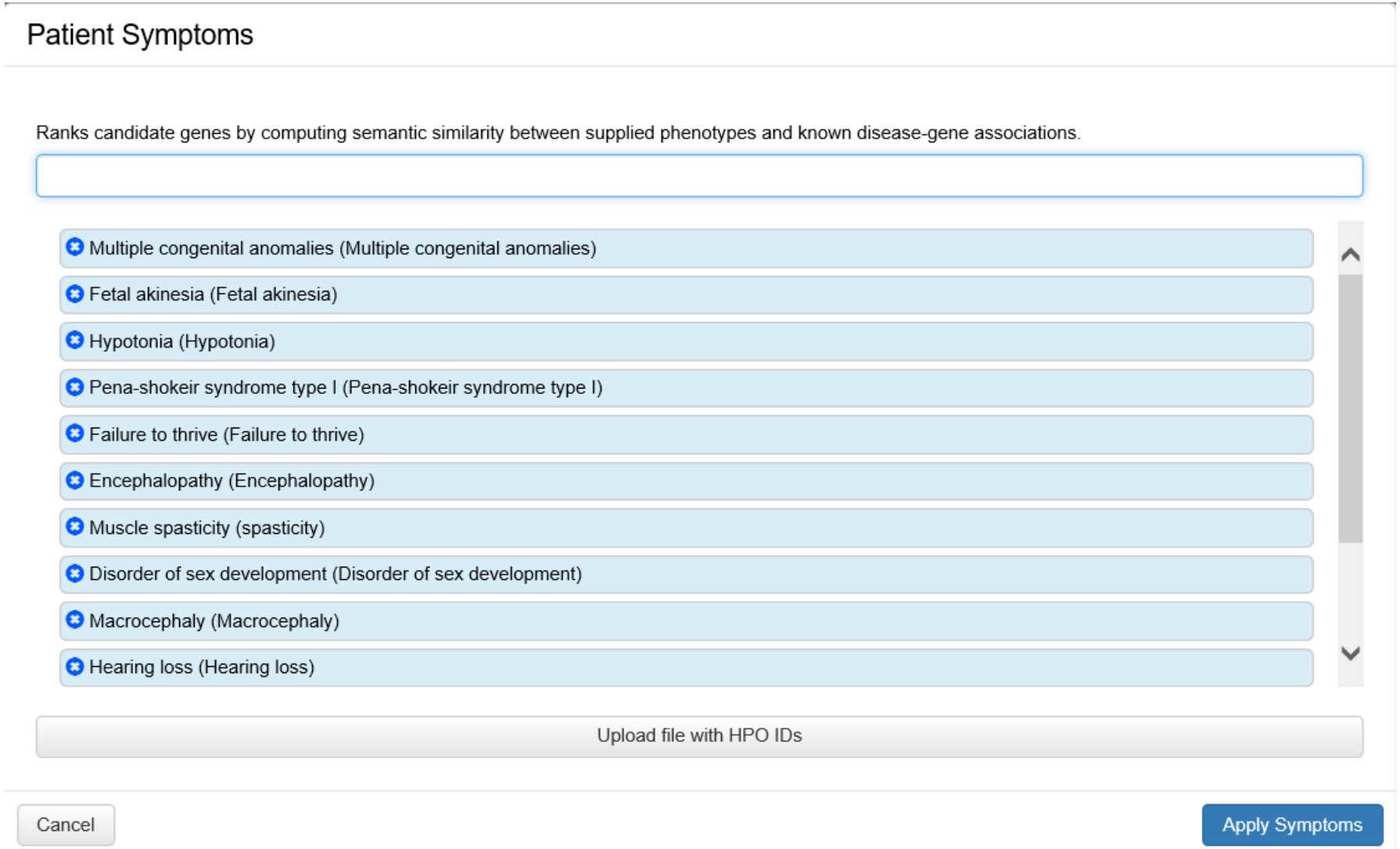
Treatment	Response	Evidence	Specificity	Indication	References
aromatase inhibitor	Resistant	1B	exact variant	Breast cancer	Clinical Studies
anastrozole	Resistant	2D	exact variant	Ductal breast carcinoma	Clinical Studies
fulvestrant	Resistant	2D	exact variant	Breast cancer	Clinical Studies
letrozole	Resistant	2D	exact variant	Ductal breast carcinoma	Clinical Studies
tamoxifen	Resistant	3	same position p.Y537N	Breast cancer	Clinical Studies

以Trio家族性遺傳性分析評斷多表型遺傳症狀之變異位點

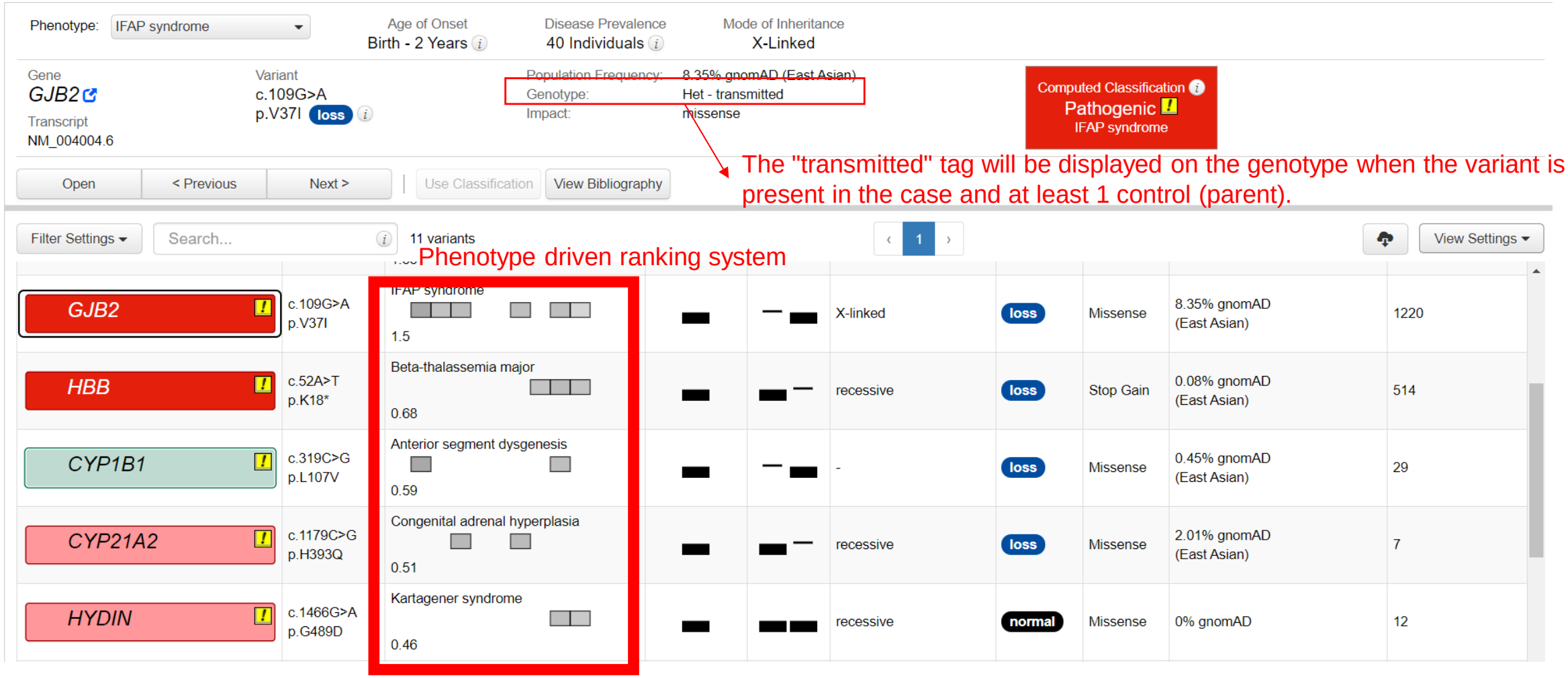
Case Studies II:以Trio家族性遺傳性分析評斷多表型遺傳症狀之變異位點

- Heredity: Trio Analysis on multiple-phenotypes genetic disease
  - Samples
    - Proband
      - Father
      - Mother
  - Goal:
    - To find the genetic-linkage variants on specific phenotypes on case children
  - Ways:
    - Heredity Analysis
    - Filter out the false positive variants – Confident Filter
    - Filter out common variants – Common Variant Filter
    - Search for reportable pathogenic variants by ACMG guideline – Predict Deleterious

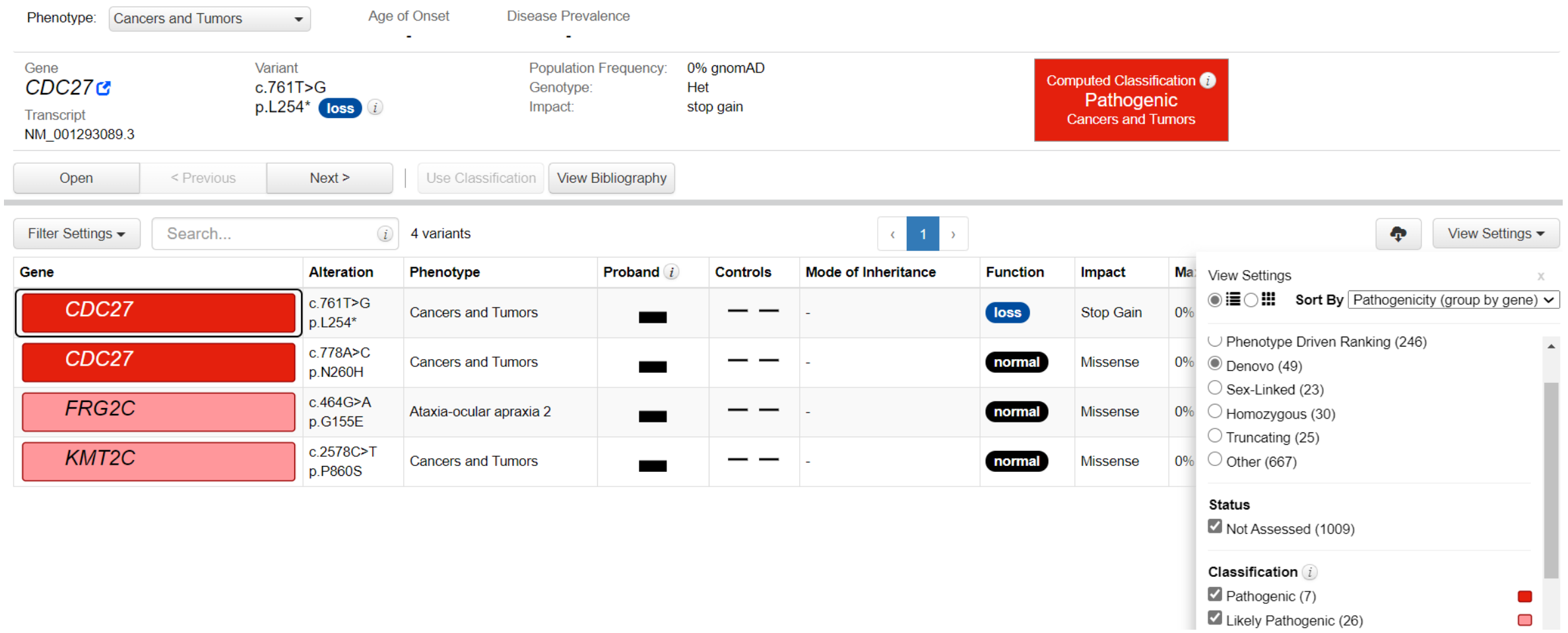
Step II: Patient Phenotypes (Optional)



Step III: View and Interpret



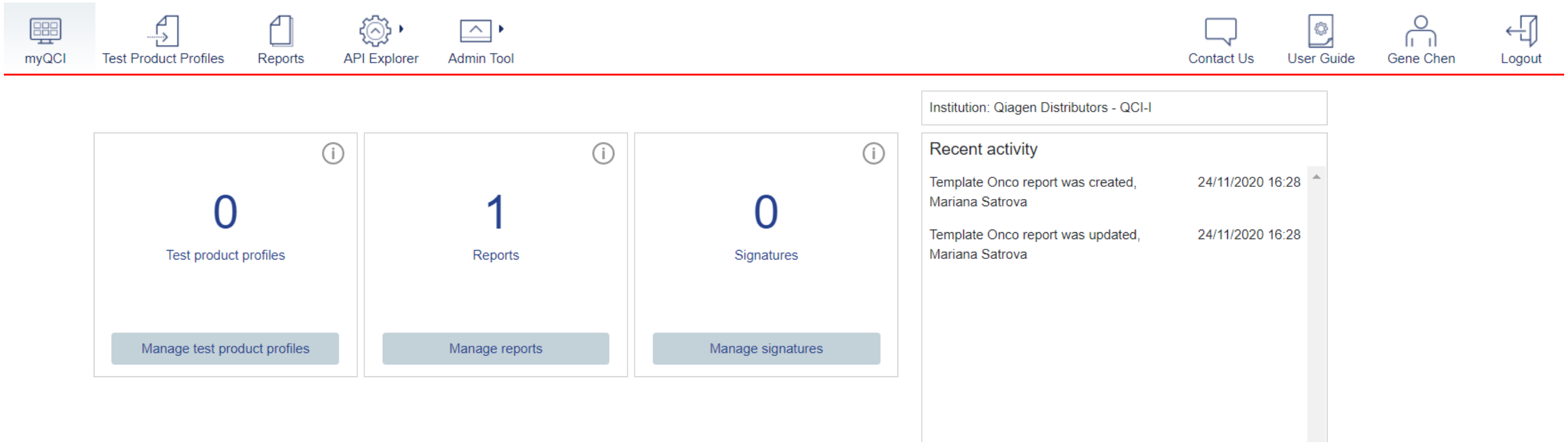
Step IV: Change View by Viewing Settings



LIVE DEMO

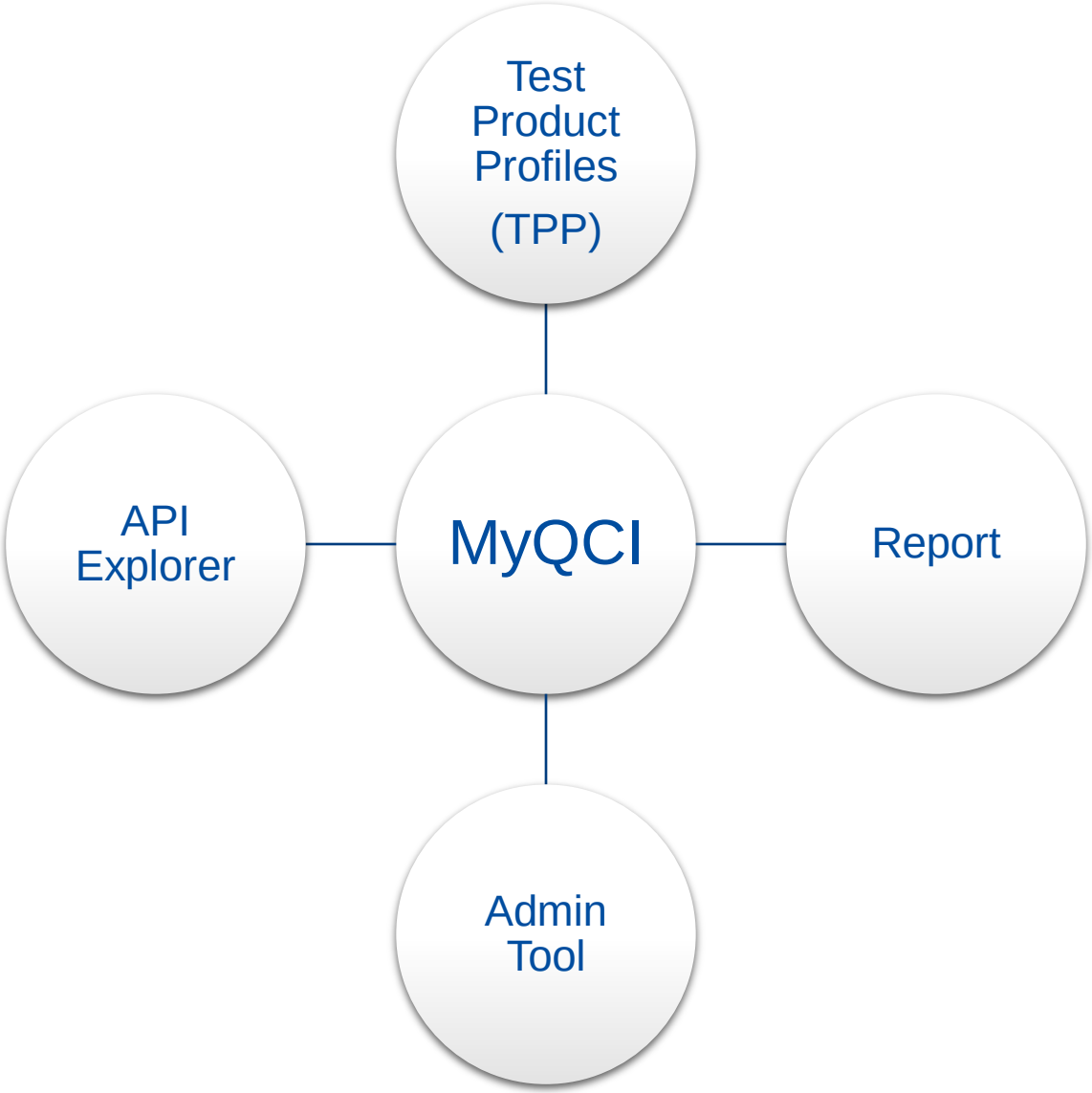
MyQCI – An Administrative Application for QCI Product

- MyQCI is an administrative application for QCI products providing a flexible and easy-to-use platform for managing, configuring, and customizing key components of your test menu including test configuration, PDF report template, and electronic signature.

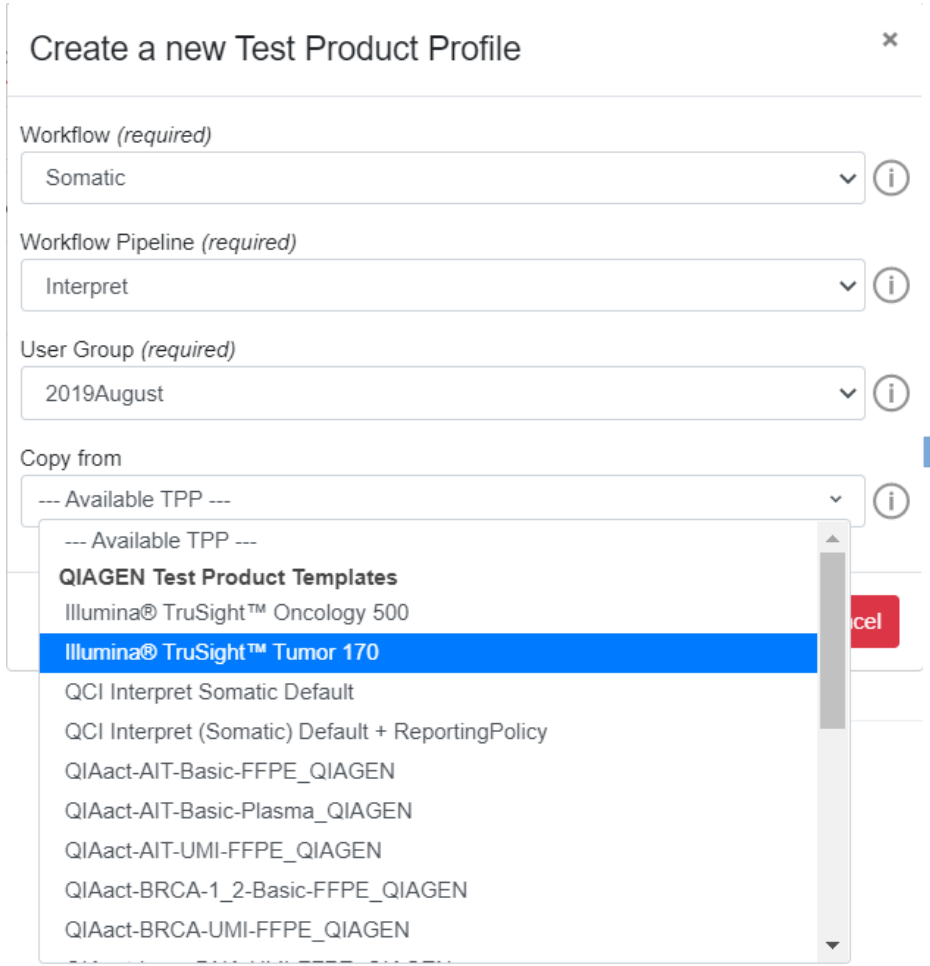


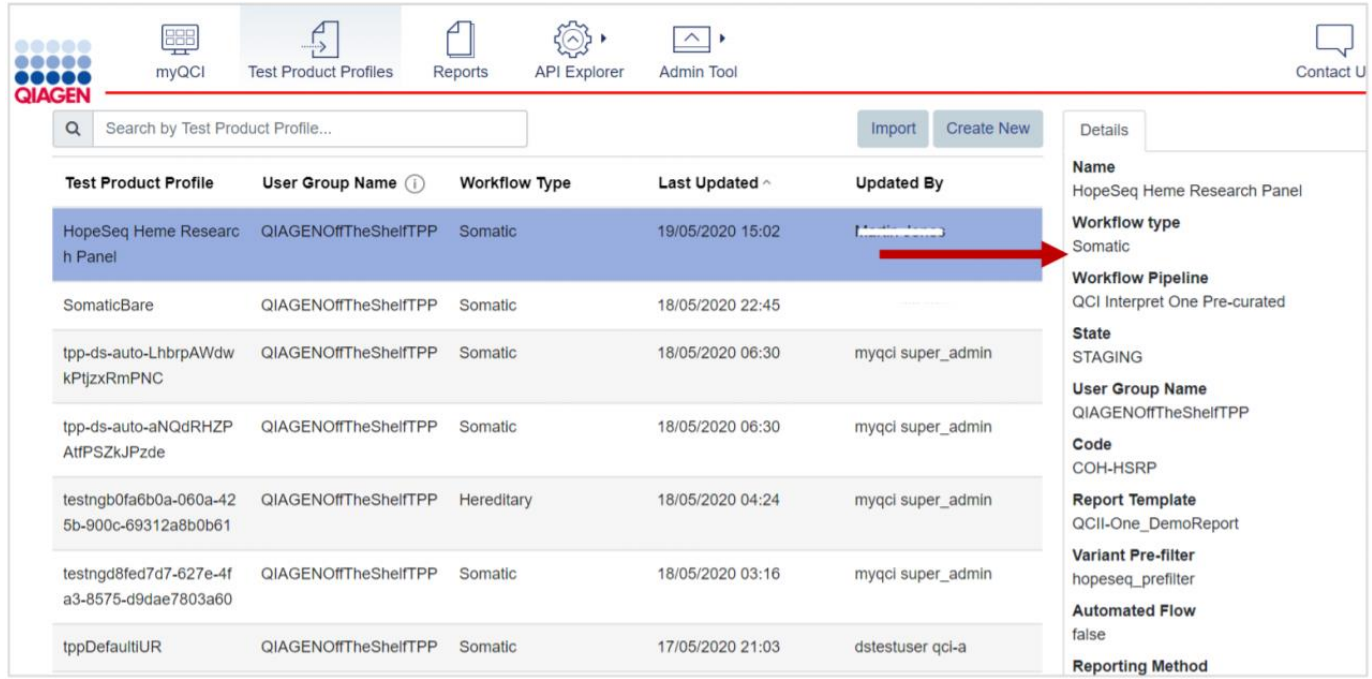
Need to activate the function for any account

Applications on MyQCI



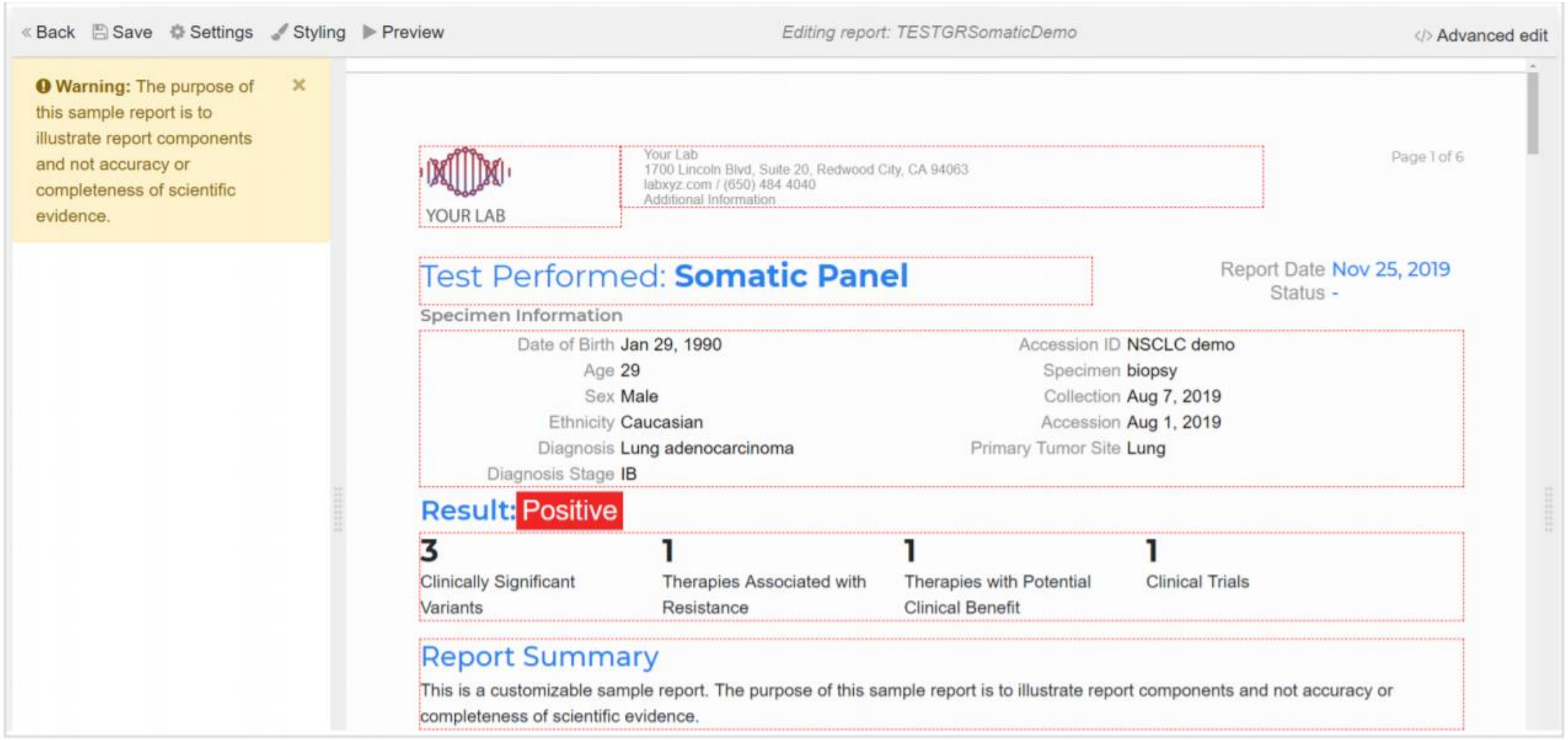
MyQCI – Test Product Profile (TPP) Configuration



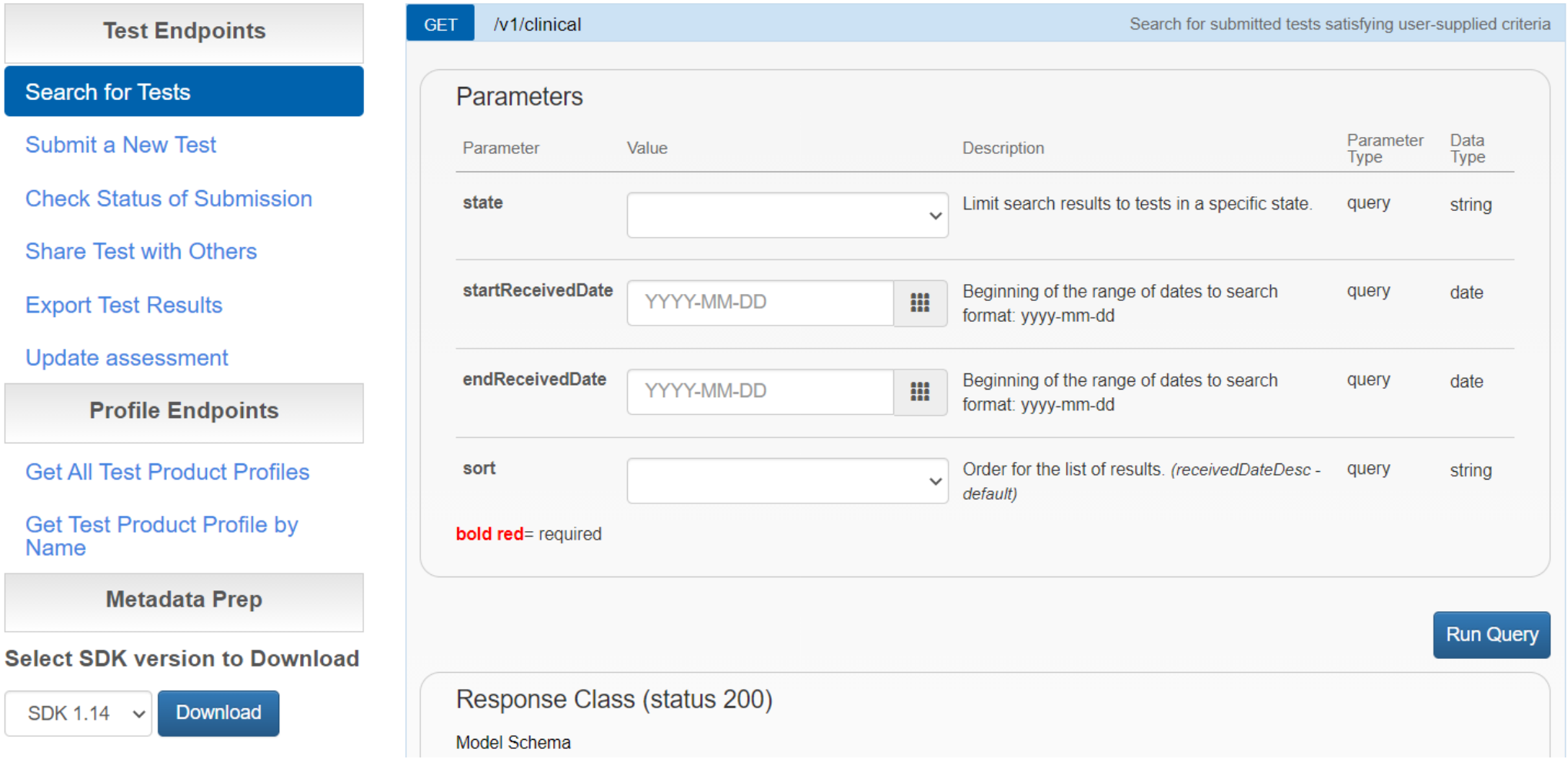


MyQCI - Report

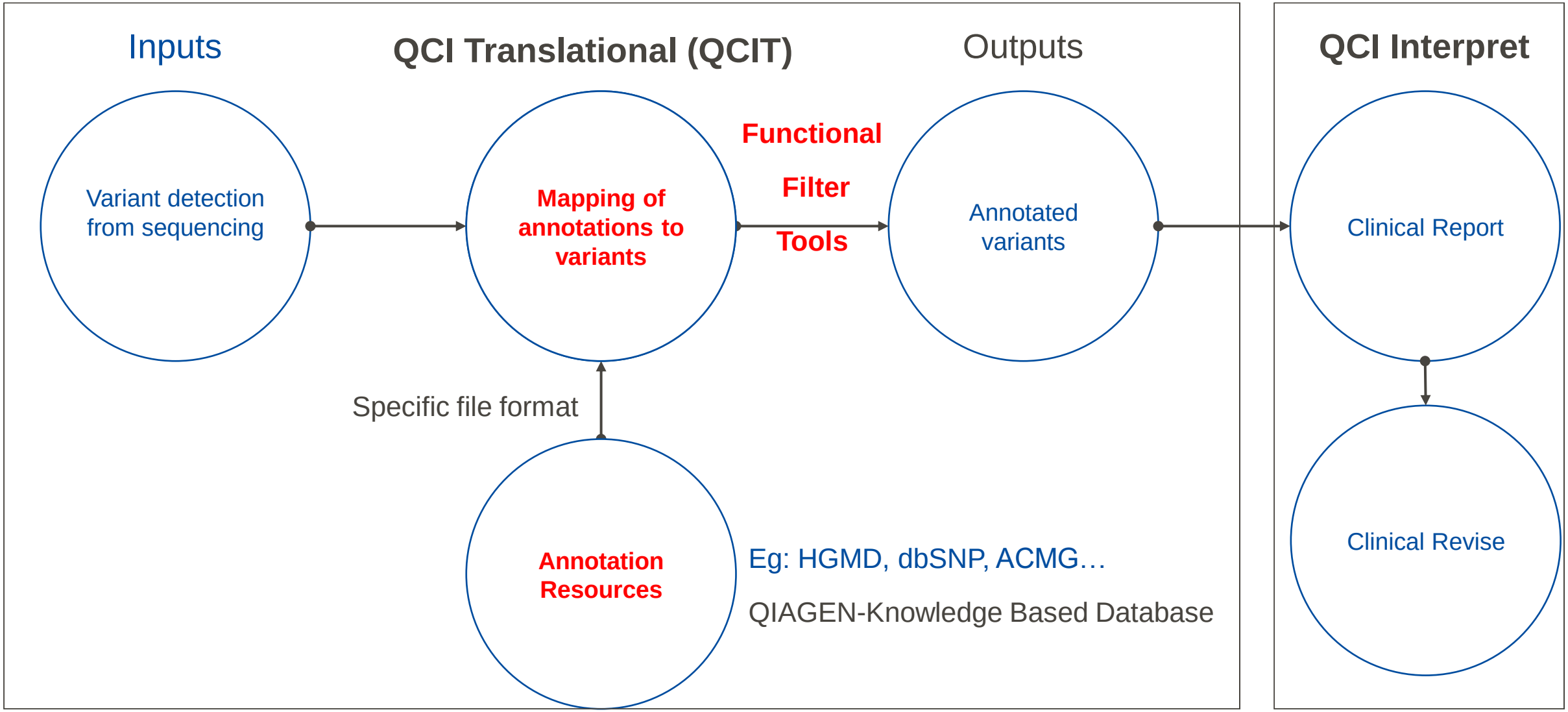
MyQCI – Customize Your Report Style



MyQCI - API Explorer (**Another License Required**)



The QIAGEN Knowledge Base System Support Full Variant Analysis



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