MOHCCN QC GATES
Sequencing (WGTS) Guideline

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1. Data Standards for WGTS

- Whole genome and RNA-seq as a baseline, with tissue and nucleic acids compatible for broader profiling within each study (e.g. ctDNA, single cell)
    - Tumour WGS: 80X minimum median coverage
    - Normal WGS: 30X minimum median coverage
    - Tumour total RNA-seq with ribodepletion: 80M read pairs minimum
    - Does not have to be CAP-accredited but should operate on a set of standards that ensure interoperability (i.e. clinical-grade)
- Pathology-review of all tissues, digitize and share H&E slides, cancer cell enrichment when possible (macro/microdissection)

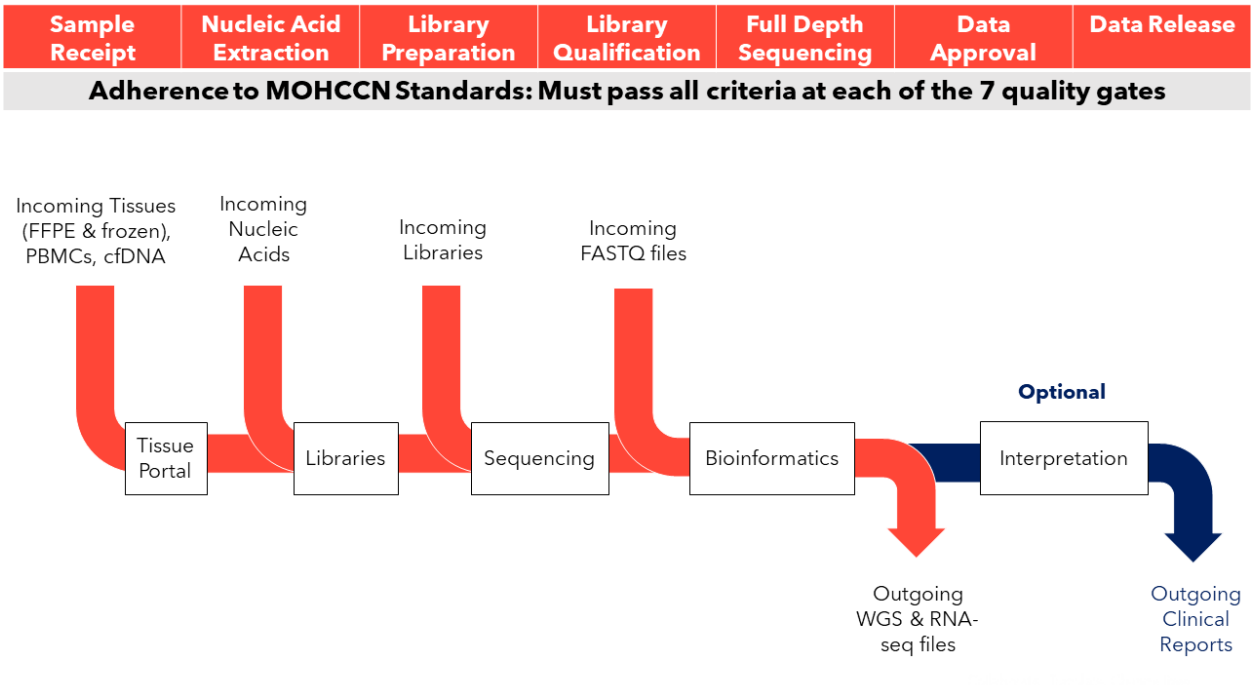

Figure 1. From tissues to report: On-ramps and off-ramps to accredited pipeline

2. Sample Receipt

Verify samples received, appropriately labelled and sufficient quantity.

QC GATES	MOHCCN CONSORTIA					
	BC2C	**PM2C**	**MOH-Q**	**PR2C**	**ACC**	**Consensus**
Sample receipt						
Initial sample QC	Visual inspection of container integrity & sample volume	Visual inspection of container integrity & sample volume	Visual inspection of container integrity & sample volume	Visual inspection of container integrity & sample volume	Visual inspection of container integrity & sample volume	Visual inspection of container integrity & sample volume
Receipt temperature appropriate	Receipt temperature appropriate	Receipt temperature appropriate	Receipt temperature appropriate	Receipt temperature appropriate.	Receipt temperature appropriate	Receipt temperature appropriate
The appropriate amount of material for tissues and blood samples	The appropriate amount of material for tissues and blood samples	The appropriate amount of material for tissues and blood samples		The appropriate amount of material for tissues and blood samples	The appropriate amount of material for tissues and blood samples	The appropriate amount of material for tissues and blood samples
Container IDs match SSF	Container IDs match sample submission form	Container IDs match sample submission form	Container IDs match MGC sample manifest	Container ID match sample submission worksheet	Container ID match sample submission worksheet	Container ID match sample submission worksheet/manifest
DNA volume	25-40uL	>12uL (RUO)	25-100uL	20-60uL	25-100uL	>12uL
DNA quantity	0.1-120 ng (Qubit 1X dsDNA HS)	0.1-120 ng (Qubit 1X dsDNA HS)	100-1000 ng (Qubit 1X dsDNA HS)	Fresh: 30-125 ng (Qubit 1X dsDNA HS) FFPE: 10ng	0.1-120 ng (Qubit 1X dsDNA HS)	0.1-120 ng (Qubit 1X dsDNA HS)

QC GATES	MOHCCN CONSORTIA					
	BC2C	PM2C	MOH-Q	PR2C	ACC	Consensus
Sample receipt						
RNA volume	10uL	>12uL (RUO)	10-20uL	>=11uL	10-20uL	10uL
RNA quantity	5-250 ng (Agilent RNA 6000 Nano Kit or PerkinElmer RNA Assay LabChip GX)	4-200 ng (Qubit 1X RNA HS)	100-1000 ng	1-125 ng (Qubit 1X RNA HS)	4-200 (Qubit 1X RNA HS)	5-200 ng (Qubit 1X RNA HS, Agilent RNA 6000 Nano Kit or PerkinElmer RNA Assay LabChip GX)

3. Nucleic Acid Extraction

Ensure sufficient DNA and RNA quantity to proceed to library construction.

QC GATES	MOHCCN CONSORTIA					
	BC2C	PM2C	MOH-Q	PR2C	ACC	Consensus
Extraction						
DNA positive controls	Mouse lung FFPE tissue	Sigma Aldrich (Cat# ERMAD442K)	None	None	None	None
RNA positive controls	HeLaS3	Fisher Scientific (Cat# AM7155M)	None	None	None	None
Appropriate volume	>=25 uL	>=11 uL		>=25uL	>=25uL	>=11 uL
Tumour DNA quantity	DNA (Flash Frozen tissue) >=50 ng	DNA (Flash Frozen tissue) >=50 ng	DNA (FFPE) > 400ng	DNA (PCR-free preps) >=100ng	DNA (Flash Frozen tissue) >=50 ng	DNA (Flash Frozen tissue) >=50 ng

QC GATES	MOHCCN CONSORTIA					
	BC2C	**PM2C**	**MOH-Q**	**PR2C**	**ACC**	**Consensus**
Extraction						
	DNA (FFPE) >=100 ng	DNA (FFPE) >=100 ng		[abs min 25ng] DNA FFPE >= 100ng [abs min 15ng]	DNA (FFPE) >=100 ng	DNA (FFPE) >=100 ng
Tumour RNA quantity	RNA (Flash Frozen tissue/cells) >=50 ng RNA (FFPE) >400 ng	RNA (Flash Frozen tissue/cells) >=60 ng RNA (FFPE) >220 ng	RNA (FFPE) > 250ng and DV200 >20%	RNA fresh frozen >= >=50ng [abs min 10ng] RNA FFPE >= 150ng [abs min 20ng] RNA (FFPE) >85-100ng	RNA (Flash Frozen tissue/cells) >=50 ng RNA (FFPE) >200 ng	50 ng frozen tissues 200 ng FFPE
Normal DNA quantity	DNA (Buffy coat or whole blood) >=250ng	DNA (Buffy coat or whole blood) >=200ng		DNA (PCR-free preps) >=100ng [abs min 25ng] DNA FFPE >= 100ng [abs min 15ng]	DNA (Buffy coat or whole blood) >=200ng	200 ng

4. Library Preparation

Confirm that DNA/RNA sequencing libraries were successfully generated.

QC GATES	MOHCCN CONSORTIA					
	BC2C	PM2C	MOH-Q	PR2C	ACC	Consensus
Library preparation						
Total RNA integrity	Caliper or Agilent Bioanalyzer	Fragment Analyzer or Tapestation	Tapestation or Bioanalyzer (FFPE)	TapeStation RIN > 2 or DV200 >55	TapeStation	Tapestation, Fragment Analyzer, or Caliper
Batch controls (DNA)	HL60	NA12878 (Coriell Institute)	NA12878 (Coriell Institute)	None	None	None
Batch controls (RNA)	UHR (500 ng & matching sample amount)	AM7852 (Thermo Fisher)	We can add NA12878 RNA control if requested/ UHR (FFPE)	UHR (matching sample amount, per run)	None	None
Negative controls	DEPC water & PCR brew control	Water NTC	We don't add any usually	None	None	None
Average Size Distribution	225 bp (FFPE) to 600 bp by Caliper/Agilent Bioanalyzer	300 bp (FFPE) to 700 bp by Fragment Analyzer or Tapestation	250 bp to 700 bp by Fragment Analyzer	Insert size 185-200 bp (Fragment size minus adapters)	200-600 bp	200-600 bp
Adapter contamination	<10% of area under the curve between lower and upper markers: adapter peak between 130-	<10% of area under the curve between lower size marker and 170bp; adapter peak between 130-	<10% Adapter peak between 100-140bp	<1% After secondary bead cleanup	<1%	<10% of area under the curve between lower size marker and 170bp; adapter peak between 130-

QC GATES	MOHCCN CONSORTIA					
	BC2C	**PM2C**	**MOH-Q**	**PR2C**	**ACC**	**Consensus**
Library preparation						
	140bp	140bp				140bp
RNA-seq final library yield (Qubit)	>3.0 nM in 10 uL (200-500 bp)	>4 nM (0.8 ng/uL)	>2 nM in 50 ul >1nM (FFPE)	>0.5 nM (for main peak on TapeStation)	>1 nM	>1 nM
PCR-free WGS (qPCR)	>2.5 nM in 15 uL	Not applicable	>2 nM in 25 ul >1nM (FFPE)	>0.75 nM (using XP loading)	>1 nM	>0.75 nM

5. Library Qualification

Confirm the WGS/WTS libraries can be sequenced.

QC GATES	MOHCCN CONSORTIA					
	BC2C	**PM2C**	**MOH-Q**	**PR2C**	**ACC**	**Consensus**
QPCR (QC)						
Library quantification	qPCR for TruSeq genome libraries	qPCR for TruSeq genome libraries	qPCR and LabChip	Individual libraries are run twice on Kapa qPCR plates	Qubit dsDNA Broad Range and Aglient D1000 Tapestation	qPCR for TruSeq genome libraries
Instrument	MiSeq Nano QC before NovaSeq (RUO)	MiSeq QC before NovaSeq (RUO). NextSeq 550		StepOne/QuantStudio	NovaSeq 6000	None
% Bases Over Q30	>75 at 2x150	>80 at 2x150 bp			>80 at 2x100 bp	>75 at 2x150
Min Clusters (PF)	>50% of target	>500K/lane			>50% of target	>50% of target cluster count for flow cell type
Spike-in controls	pCR-TOPO4 tracking plasmid (~0.1%)	PhiX (~0.1-2%)			PhiX (~1%)	PhiX or other tracking plasmid
Low-pass sequencing, optional						
		qPCR for TruSeq genome libraries	MiSeq QC for FFPE DNA_T samples			
		MiSeq QC before NovaSeq (RUO). NextSeq 550				

QC GATES	MOHCCN CONSORTIA					
	BC2C	PM2C	MOH-Q	PR2C	ACC	Consensus
		>80 at 2x150 bp				
		100,000 - 4,000,000				
		0.1X - 0.4X				
		≥25%				
ichorCNA solution is accurate/logical		Yes (Trained Personnel)				

Library Qualification of Challenging Clinical Samples

Low cancer cell content has been a common failure mode in PM2C.

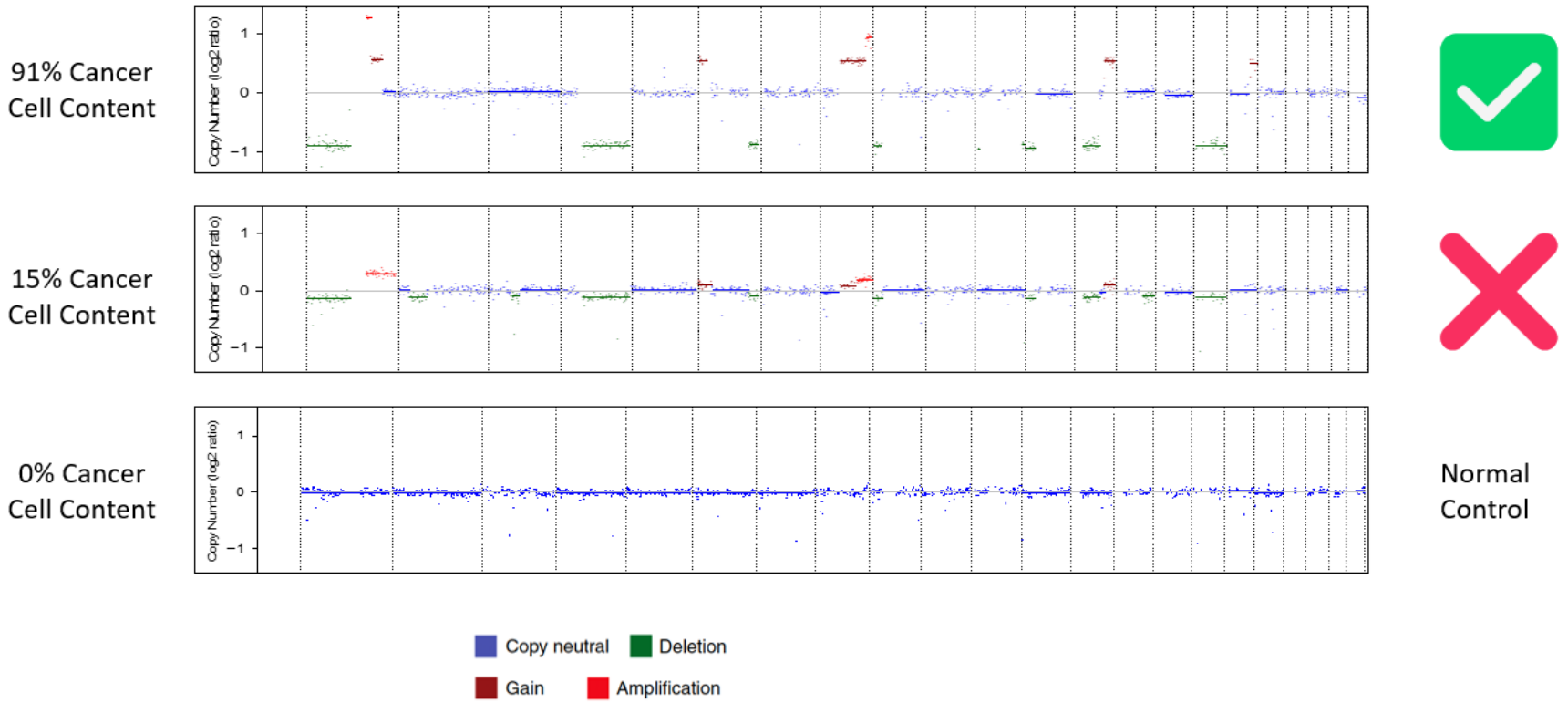

Figure 2. Cancer cell content example

6. Full-Depth Sequencing

QC GATES	MOHCCN CONSORTIA					
	BC2C	**PM2C**	**MOH-Q**	**PR2C**	**ACC**	**Consensus**
Full-depth sequencing						
Instrument	NovaSeq 6000 or NovaSeq X Plus	NovaSeq 6000 or NovaSeq X Plus	NovaSeq 6000	NovaSeq 6000	NovaSeq 6000	NovaSeq 6000 or NovaSeq X Plus
% Bases Over Q30	>75 at 2x150 bp	>75 at 2x150 bp	>75 at 2x150 bp	>75 at 2x150 bp	>75 at 2x150 bp	>75 at 2x150 bp
Min Reads Delivered (PF)	18B reads per NovaSeq S4 flowcell	S4 Flow Cell = 1.6B/lane	2.4B per lane	10B reads per S4 flowcell passing filters (Illumina spec)	10B reads per S4 flow cell	10B reads per S4 flow cell
Sequencing control	PhiX Control (1%)	PhiX Control (0.1%)	PhiX Control (1%)	PhiX Control (1%)	PhiX Control (1%)	PhiX Control (1%)
WGS Minimum Coverage (Deduplicated)	80x T, 30x N We calculate the insert size from the sequenced data by getting the mean of the insert sizes of non-chastity-failed, primary-aligned, properly paired reads. We use some simple custom code for this.	80x T, 30x N Calculated by Picard CollectWgsMetrics.	80x T, 30x N As reported by *qualimap bamqc* with the --skip-duplicated option enabled	80x T, 30x N Coverage would be the total length as reported by "samtools stat" with the remove-overlaps options enabled, divided by 2.9e9.	80x T, 30x N Calculated by Picard CollectWgsMetrics.	80x T, 30x N Calculated by Picard CollectWgsMetrics.
WGS Mean Insert Size	>150bp	>150bp	>150bp >125bp (FFPE)	350-450bp	>150bp	>150bp

QC GATES	MOHCCN CONSORTIA					
	BC2C	**PM2C**	**MOH-Q**	**PR2C**	**ACC**	**Consensus**
WGS % Duplication Rate	≤50% (we don't fail on dups but using this metric makes sense)	≤50%	≤50% (Lucigen usually give 5-10% dup, but we don't fail on dups)	None (using PCR-free almost exclusively, so not really an issue)	<50%	≤50%
WTS Clusters per sample	>=80,000,000	>80,000,000	>80,000,000	80,000,000	>80,000,000	>=80,000,000
WTS rRNA contamination	<10%	<35%	<10% <35% (FFPE)	<20%	<10%	<35%
WTS % mapped to coding	>25% exonic	>5%	>25% *expression efficiency* as reported by RNA-SeQC	Not set	>5%	>5%
WTS Mean Insert Size	>150bp	>150	>150bp >100bp (FFPE)	180-200bp	>150bp	>150bp

7. Data Approval

Common set of data deliverables: alignments and variant cells.

QC GATES	MOHCCN CONSORTIA					
	BC2C	**PM2C**	**MOH-Q**	**PR2C**	**ACC**	**Consensus**
Informatics pipeline & variant interpretation						
Callability (exonic space)	Not yet applicable	≥75% of target bases above 30x T, 30x N	Not yet applicable	95% of "mappable genome" at 14x in N		
Inferred Tumour Purity	>35% to report genomic findings and full report. RNA expression outliers are always reported	≥30%	≥30%	>=30%	>=30%	≥30%
Trimming	NA	Minimum base quality Q>20	Minimum base quality Q>25	OEM software adapter trimming during BCL conversion, Soft clipping only in mapping phase (Dragen feature), reads clipped to < 20nt are replaced with token 10Ns.	Minimum base quality Q>20	Minimum base quality Q>20

Source	Deliverables	Formats
WGS	Read alignments	BAM files aligned to GRCh38
	Somatic CNVs	Seg files
	Somatic SNVs and in/dels	VCF, MAF
	Structural variants	cBioPortal structural variant format, MAVIS somatic filtered DNA annotated file
	Ploidy estimation	Single value
RNA-seq	Read alignments	BAM files aligned to GRCh38
	Fusions	cBioPortal structural variant format, MAVIS somatic filtered RNA annotated file
	Gene expression level	Percentile, TPM, FPKM, Z-score

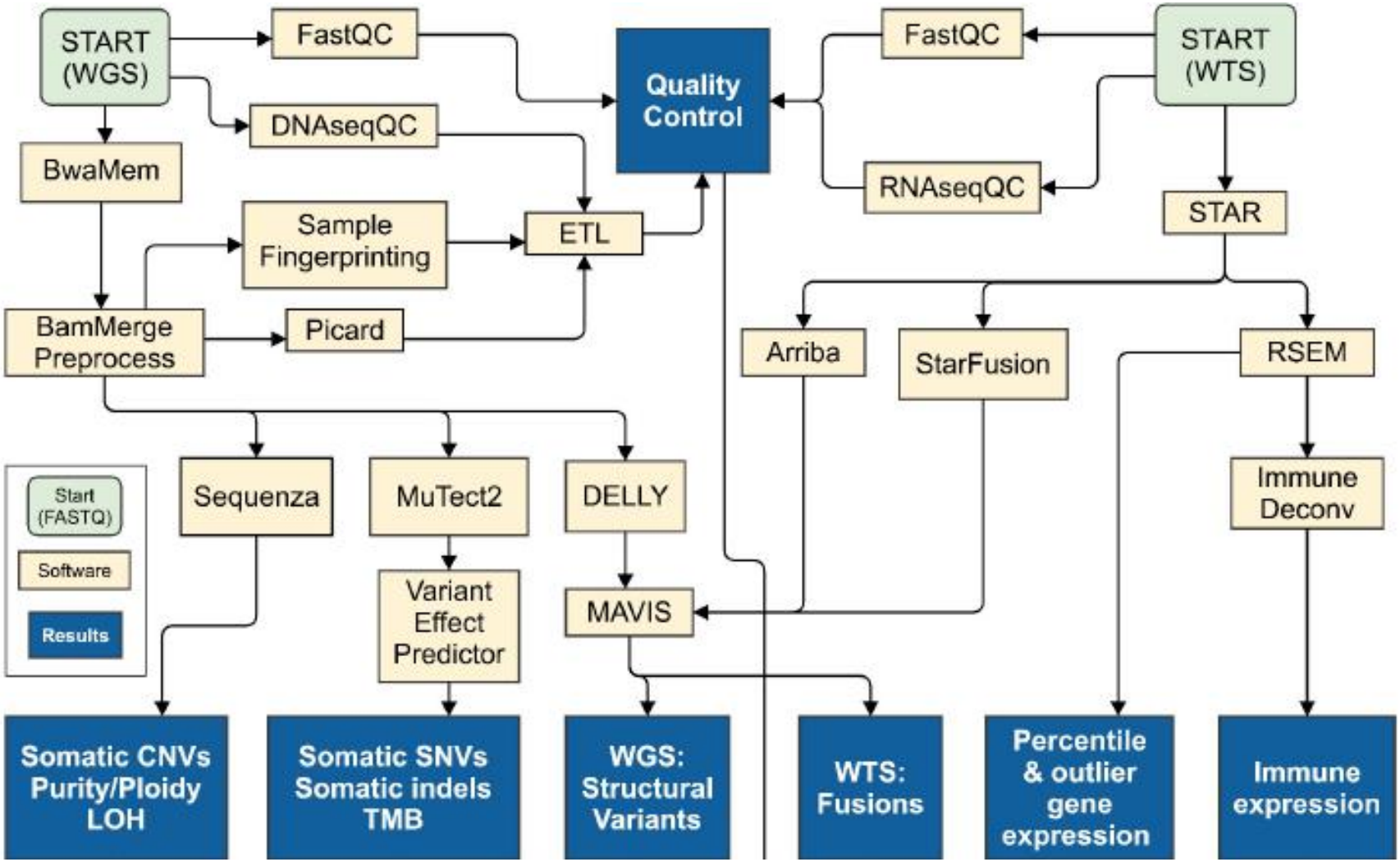

Figure 3. PM2C pathway example

8. Data Release (Reporting and Sign-out)

QC GATES	MOHCCN CONSORTIA					
	BC2C	PM2C	MOH-Q	PR2C	ACC	Consensus
Final report generation						
QC Passed at all stages	Yes for clinical BAM/VCF (Clinical director delegate)	Yes (Geneticist)	Yes	Yes	Yes	Yes
Calls are accurate/logical	Not yet applicable	Yes (Geneticist)	Not yet applicable	Not yet applicable	Not yet applicable	None

Document revision history

Developed by	Reviewed by	Endorsed by	Effective Date	Policy Version	Summary of revisions
TWG	Steering Committee	Network Council	December 7, 2023	1	

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