SIGNATURE APPROVALS:

Laboratory Director: Must be signed by a minimum of one of those listed at the right:	Biswajit Das, Ph.D. Chris Karlovich, Ph.D. Mickey Williams, Ph.D.	*Signature / Date:* Biswajit Das -S (Affiliate) Digitally signed by Biswajit Das -S (Affiliate) Date: 2023.02.03 09:27:37 -05'00'
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REVISION HISTORY:

Document No.	Version	Description of Revision	Effective Date
MCCRD-SOP0064	1.0	Original Release	02/03/2023

1.0 PURPOSE/SCOPE

This Standing Operating Procedure (SOP) describes procedures for detection of Human Leukocyte Antigen (HLA) typing using whole exome sequencing (WES) data for reporting in the NCI Patient-Derived Models database as performed by the Molecular Characterization Laboratory (MoCha) at the Frederick National Laboratory for Cancer Research.
This SOP is for research purposes only and no clinical samples will be processed using this SOP.

2.0 REFERENCE DOCUMENTATION

Number	Title/Link
[1]	Szolek, A, Schubert, B, Mohr, C, Sturm, M, Feldhahn, M, and Kohlbacher, O (2014). OptiType: precision HLA typing from next-generation sequencing data Bioinformatics, 30(23):3310-6.
[2]	https://github.com/FRED-2/OptiType

3.0 RELATED SOPS

Available on the PDMR website: https://pdmr.cancer.gov/sops

Document Number	Title
MCCRD-SOP0011	Whole Exome Sequencing Data Analysis Pipeline and Specifications

Approved For Use

4.0 DESCRIPTION OF HLA Typing DETECTION

4.1 The mouse reads are first filtered out from whole exome sequence (WES) data following the WES data analysis pipeline in the SOP MCCRD_SOP0011.

4.2 HLA reads are extracted from paired-end human only sequencing data.

4.3 HLA type is estimated using OptiType[1,2].

5.0 CODE DESCRIPTION

5.1 HLA reads are extracted from paired-end sequencing data

```
razers3 -i 95 -m 1 -dr 0 -tc 20 -o ${sample}_1.bam
$OPTITYPE_HOME/data/hla_reference_dna.fasta ${sample}_hg19_R1.fastq.gz

razers3 -i 95 -m 1 -dr 0 -tc 20 -o ${sample}_2.bam
$OPTITYPE_HOME/data/hla_reference_dna.fasta ${sample}_hg19_R2.fastq.gz

samtools bam2fq ${sample}_1.bam >${sample}_1.fastq

samtools bam2fq ${sample}_2.bam >${sample}_2.fastq
```

5.2 OptiType is used to detect HLA type for PDX sample

```
OptiTypePipeline.py -i {sample}_1.fastq ${sample}_2.fastq  --dna -v -o
${sample} -c $OPTITYPE_HOME/config.ini
```

5.3 HLA type is reported at model level with #concodance/#total PDX specimens in the model. The data for PDC and PDOrg is reported separately.

6.0 Example HLA Profile Reporting in the NCI PDMR database:

6.1 All PDX tumors sequenced (n=5) are in concordance

HLA Profile(s)

A1	A2	B1	B2	C1	C2	PDX Concordance / PDM Type
A*02:01	A*25:01	B*08:01	B*14:02	C*07:01	C*08:02	5/5

6.2 In a small fraction of models, intra-model HLA variation in sequenced PDX tumors is observed.

HLA Profile(s)

A1	A2	B1	B2	C1	C2	PDX Concordance / PDM Type
A*01:01	A*32:01	B*08:01	B*14:01	C*05:01	C*07:01	1/6
A*01:01	A*32:01	B*08:01	B*14:01	C*07:01	C*08:02	3/6
A*01:01	A*32:01	B*08:01	B*14:01	C*07:01	C*08:04	2/6