

TITLE: Whole Exome Sequencing

SOP Number: D-DEZ-PRO-026

Revision Number: 0

Effective Date: 07 Apr 2015

4. Prepare each Sample Tube by adding 198µl of working solution and 2µl of stock DNA to a Qubit assay tube.
5. Vortex all tubes for 2-3 seconds.
6. Incubate tubes at room temperature for at least 2 minutes and less than 4 hours.
7. On the home screen of the Qubit, select DNA. Select “Yes” to read new calibration standards.
8. Insert Standard #1 Tube into chamber, press “Read”
9. Insert Standard #2 Tube into chamber, press “Read”
10. Select “Sample” on the Qubit display.
11. Insert Sample Tube into chamber, press “Read”
12. Press “Calculate Stock Conc.”. Enter 2µl for “Volume of Sample Used”
13. Record Stock concentration.
14. Repeat steps 11-13 until all samples are complete.
15. Discard sample and standard assay tubes.

F. LOAD COVARIS TUBES FOR SUBMISSION

16. Label Covaris microTUBE with sample identifier on side of metal cap. Markings on the glass will be removed by the sonication.
17. Based on stock concentration, calculate volume of stock DNA needed to obtain 3µg of DNA.
18. Pipette 3µg of stock DNA into microTUBE. To prevent blowback, make sure pipette tip does not create a seal with the cap.
19. Add sufficient volume of 1x TE Buffer (USB Corp) to microTUBE to bring total volume to 103µl.

F. SAMPLE QC, LIBRARY PREP, CAPTURE, AND SEQUENCING

20. Submit microTUBE to the IIHG-CDD for Agilent SureSelect V5 Sequencing at 4 samples per lane.

G. BIOINFORMATIC ANALYSIS

21. FASTQ files will be transferred to neon.hpc.uiowa.edu/Shared/IVR/ following sequencing.
22. Place files in `/Shared/IVR/NGS/raw_data/<YYYYMMDD>_DTVR_exome/`.
23. Create analysis directories using command:

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```
perl /Shared/IVR/NGS/fq_linker_2.pl \  
  --name <YYYYMMDD>_v214 \  
  --base /Shared/IVR/NGS/analysis/exome/DTVR/ \  
  --PE /Shared/IVR/NGS/raw_data/<YYYYMMDD>_DTVExome/ \  
  --copy /Shared/IVR/NGS/214/config.neon.v214 \  
  --copy /Shared/IVR/NGS/214/process_NGS.v214.pl
```

24. In analysis directory
(/Shared/IVR/NGS/analysis/exome/DTVR/<SampleID>/<YYYYMMDD>_v214) Execute the following command:

```
qsub -N <SampleID> process_NGS.v214.pl config.neon.v214 <SampleID> CLINICAL
```

25. Transfer resulting .xlsx file to DTVR shared folder on WIVR-file1.eng.uiowa.edu.

I. HISTORY

Effective Date	Revision	Change
07 Apr 2015	0	Initial issue of SOP