Ministry of National Guard Health Affairs
King Abdullah International Medical Research Center

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Sample Submission Specification and Guidelines for NGS and Array-CGH

1. DNA samples sequencing and array applications

DNA quality and quantity

Using OD260/280 ratio is a good indicator for the DNA sample purity where the value is between 1.8-2 is a good indicator for the DNA samples, Gel electrophoresis could be used to evaluate the condition of DNA sample, impure DNA sample appeared as a smear in 1% gel.

2. RNA samples sequencing and array applications

RNA quality and quantity

- RNase free condition is mandatory when handling RNA samples, we encourage client to use commercially certified reagent or kit for RNA extraction.
- Quality control of RNA samples need to assist using highly sensitive application such as 2100 Bioanalyzer or equivalent to be able to detect RNA integrity number (RIN) which should be 8.

3. Samples submission

3.1 DNA samples

- DNA samples should be submitted in **Elution buffer or distilled water**, this information should be included in the request form.
- Brief description of DNA extraction method.
- Sample should be transferred in **room temperature or cold ice packet**.

3.2 RNA samples

- RNA sample should be submitted in DEPC treated water in 1.5-2ul tube, clearly labeled, cap should be tight and wrapped by paraffin film.
- Brief description of RNA extraction protocol.
- Sample should be shipped in **DRY ice** to preserve RNA integrity

4. Platforms

In addition of the general guidelines we recommend that you read specific platform requirement before sample preparation.

4.1 Next Generation sequencing (NGS)

4.1.1 Human Whole Genome Sequencing

- Genomic DNA with concentration **not less than 100ng** in a total of 100µl volume.
- The OD260/280 ratio of regular samples should be within the optimum range (1.8-2).
- The purity of the extracted DNA samples from FFPE (or such forensic resources) should be mentioned with **gel picture** if possible.

4.1.2 Bacterial Whole Genome Sequencing

- Genomic DNA with concentration **not less than 20ng** in a total of 100µl volume.

4.1.3 Whole Exome Sequencing

- Genomic DNA with concentration **not less than 50ng** in a total of 100µl volume.

4.1.4 Target Sequencing

- Genomic DNA with concentration **not less than 20ng** in a total of 100µl volume.

4.1.5 Transcriptome Sequencing

- Total RNA samples concentration **not less than 20ng** minimum in a total of 50µl volume.

4.2 Sanger sequencing

Acceptance Criteria for the PCR Reaction

- DNA should be stored at **4 or -20** degree until being used.
- Samples will be rejected if the PI doesn't fill out and sign the service request form (9834/CR/D01V1/F1V3).
- Samples will be rejected if it's at low concentration, volume or purity.

Samples Specifications for PCR Reaction:

For each reaction we need:

- Concentration for the DNA between **50-100 ng/µl.**
- Volume is **2-3 µl.**
- Ratio **(1.8 – 2.1).**
- Primer concentration is **10 Pmol** for each forward and reverse.

Acceptance Criteria for Cycle Sequencing Reaction

- PCR products should be stored at **4 or -20** degree.
- Samples are rejected if the PI doesn't fill out and sign the service request form (9834/CR/D01V1/F1V3).
- Plate map and PCR picture MUST be included.
- Samples will be rejected if it's at low concentration, volume or purity.

Samples Specifications for Cycle Sequencing Reaction:

Each reaction requires:

- **20 ng/µl** of PCR products yield.
- Minimum **1 µl** volume.
- **5 Pmol** for each forward and reverse primer.

4.3 Array-CGH

Acceptance Criteria

- Freshly extracted DNA/RNA is preferred
- DNA should be saved at **4 or -20** degree until being used (no more than one week).
- RNA should be saved at **-20 or -80** degree until being used (no more than one week).
- Samples are rejected if the PI doesn't fill out and sign the service request form (9834/CR/D01V1/F1V3).
- Samples are rejected if the PI does not provide original nanodrop profile (concentration, purity… etc).
- Samples are rejected if it's at low concentration, volume or purity.

Type and volume For CytoScan HD

Genomic DNA must be:

- Double-stranded
- Free of PCR inhibitors
- Not contaminated and not degraded.
- Genomic DNA should be **5µl** in volume and concentration of **50 ng/µl** and ratio (**1.8 – 2.1**).

Type and volume For Human Exon ST and 3' IVT.

Genomic RNA must be:

- Free of RNase.
- Not contaminated and not degraded and free of PCR inhibitors.
- Genomic RNA should have a volume of **3µl** and concentration of **167ng/µl,** which is **500ng** of RNA template in total and ratio (**1.8 – 2.1**).

APPROVALS:

Prepared by:	Sequencing Team		
Reviewed by:	Dr. Bader Almuzaini **Team Leader, Sequencing Platform**	**(Signature)**	16-10-17 **(Date)**
Approved by:	Dr. Saleh AlGhamdi **Chairman, MGRD**	**(Signature)**	17/10/2017 **(Date)**

9834/CR/D01V1/F1V3

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Service Request Form

Code	Time	Date	Research Protocol Name	Research Protocol No.
PI Name		Contact No	Sample Reference No.	Number of samples
Badge	Pager	Delivered by	Received by	Type of sample
Consent form signed by patient: ☐ YES ☐ NO ☐ NA			PI signature:	

EXPERIMENT	TECHNOLOGY
Extraction ○ Human ○ Cell line ○ Other _____	○ DNA ○ RNA
PCR ○ Gene(s) Name ○ Primers Provided ☐ YES ☐ NO	○ Standard PCR ○ qPCR (Taqman) ○ Multiplex PCR ○ qPCR (syberGreen)
DNA Sequencing (PCR Gel Picture needed) ○ Gene(s) Name: ○ PCR (Primers Provided) ○ Ready Plate ◊ YES ◊ NO ◊ YES ◊ NO	○ 3730 xL DNA Analyzer
Next Generation Sequencing ○ Whole Genome ○ Transcriptome ○ Whole Exome ○ Gene Panels (Specify) ○ tDNA ○ Other _____	○ SOLiD 5500 ○ Ion Proton ○ Ion Torrent (PGM)
MicroArray ○ DNA ○ RNA (Gene panels)	○ CytoScan HD ○ 3'IVT ○ Human exon ○ miRNA ○ Human Gene
Cell Culture and Cell Storage: ○ Type of Cells ○ Western Blot ○ Cell Line ○ ELISA	○ Flask T25 cm ○ Flask T150 cm ○ Flask T75 cm ○ CryoFreeze Tube ○ Other _____

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PI approval	BN	Date	Signature
Chairman approval	**BN**	**Date**	**Signature**