City of Hope
Sample Requirements
Sample Submission Guidelines

You are encouraged to set up a meeting to consult for experimental design with the core director, Dr. Xiwei Wu, to determine the optimal platform, library preparation protocol, and sample size. Please fill out the service request form at the time of sample submission. Typical turnaround time for microarray service is one - two weeks, and three - four weeks for sequencing, one - four weeks for data analysis. Please note that actual turnaround time might vary depending on the work load and complexity of the project.

Sample Requirement for Microarray preparation

Quality check is required for all samples by the core using Agilent Bioanalyzer 2100, NanaDrop1000 or Qubit 2.0. Samples that fail the quality analysis will be returned to researcher and ask for replacement.

RNA Submissions

All RNA must be clean and free from protein and DNA contamination and meet the following minimum requirements:

260/280 ratios must be between 1.9 and 2.1

260/230 ratios must be above 1.8 (if < 1.8, samples has organic contaminants)
We recommend using the QIAGEN RNeasy columns for total RNA purification. When working with Qiagen RNeasy columns for use with the Affymetrix system, please do not use β-mercaptoethanol; it is unnecessary and can cause high background in your GeneChips.

For small RNA: Perform total RNA purification that retains the small RNA such as Qiagen miRNeasy Kit. Total RNA must be submitted, and please do not do the enrichment step. miRNA samples will require two quality control (QC) steps: one for total RNA and one for small RNA.

Usually, 500 ng -1 µg RNA is good enough for QC and sample preparation. But it is varied depending on the technologies of your choice. Please check with our core staff for specific requirement.

DNA Submissions

260/280 ratios must be between 1.8 and 2.0

260/230 ratios must be above 2.0 (if < 2, samples has organic contaminants)
Genomic DNA can be extracted using any method that generated high quality DNA.

Amplified DNA must be purified, and we recommend using the Qiagen QiaQuick PCR purification protocol with samples eluted in nuclease-free water or the elution buffer provided by in the kit.

1-2 µg DNA is required for success analysis. But it is varied depending on the technologies of your choice. Please check with our lab staff for specific requirement.

Sample Requirements for NGS Sample Preparation

Please see appropriate sample requirements below if you would like core staff to prepare sequencing libraries for your samples.

- **DNA Sequencing**
  100 ng or more for genome DNA, PCR products, BAC, YAC etc suspended in EB buffer or H2O in a volume not to exceed 50ul. DNA should be as intact as possible, with an OD260/280 ratio of 1.8-2. The DNA amount should be over 250ng for PCR free sequencing library preparation protocol.

- **Targeted Exome Capture (Agilent Sure Select Protocol)**
  1 – 2 µg of purified DNA suspended in EB buffer in a volume not to exceed 50ul. DNA should be as intact as possible, with an OD260/280 ratio of 1.8-2.

- **Targeted Exome Capture (Life Technologies AmpliSeq Exome Protocol)**
  100 ng of purified DNA (or 250 ng for FFPE DNA) suspended in EB buffer in a volume not to exceed 20 µl. DNA should be as intact as possible, with an OD260/280 ratio of 1.8-2.

- **Whole Transcriptome (mRNA-Seq) Profiling**
  0.5-10µg of Purified RNA (DNA free) suspended in nuclease-free H2O in a volume less than, or equal to 50µl. RNA should be as intact as possible, with OD260/280 ratio of 1.8-2 and an OD260/230 ratio over 1.5. Core lab will verify RNA integrity via an Agilent Technologies 2100 Bioanalyzer. RIN ≥8.0 for polyA protocol.

- **Small RNA Sequencing**
  1 - 10µg of Purified RNA suspended in nuclease-free H2O, concentration >100ng/µl. RNA should be isolated using a method that preserves small RNAs. RNA should be as intact as possible, with OD260/280 ratio of 1.8-2 and an OD260/230 ratio over 1.5.

- **ChIP-Enriched DNA Sequencing**
  10ng of purified, ChIP-enriched, qPCR-verified, PicoGreen quantified DNA suspended in 30ul EB Buffer should be submitted.

A high-quality library is critical to obtaining high quality data. Protocols used by the Core have been optimized. We also offer library preparation services.

Users interested in making their own libraries to expedite their studies should download and read the current protocols for different kits offered by Illumina. Ordering information for kits is available upon request.

Sanger Sequencing Sample Requirements:

Log on the LIMS system at dnatools.coh.org

Concentrations:

- Template - PCR Product:
  Concentration at least 0.5 ng per 100 base pairs for fragments less than 500 bp.
- Dilute PCR sample to 1 ng/ul if PCR fragment is less than 500 bp.
  If your PCR sample is greater than 500bp then dilute PCR to 3ng/ 100 base pairs in 2 ul volume.

  For example: If your PCR size is 600 base pairs, multiply 600 X 0.03 which will give you the total amount of DNA necessary (18ng) for sequencing. Then dilute your PCR sample to the correct working concentration of 9ng/ul in 2ul total volume.
- Template – BAC Clones
  1ug/ ul concentration for BAC DNA

  Template – Plasmid
- Concentration at 100 ng/ul
  2.0 ul needed per reaction
- Custom Primers
  Concentration at 1pmol/ul
  10 pmol/ul for BAC sequencing primers
  4ul needed per reaction

Entry Form (After you have clicked Enter DNA Sequencing Requests, and entered the number of reactions)

- For City of Hope users, leave the PO Number field blank. This is for outside users only.
- Under the Grant Code Num. field please enter your grant code.
- The account number you enter must use the subaccount of 8028. Do not change this. Enter your entire account number (20/30 – 8028 – 6 digit account number) Ex. 30-8028-xxxxxx
- Enter the PI name “last name, first name” and in all capitals. Ex: SMITH, JOHN

Chemistry

- BigDye_V3.1 – For basic sequencing. Can read out to approximately 900 base pairs
- BigDye_V1.1 – Used only for short (less than 500 base pairs) PCR products when high resolution near the priming site is needed
- • BigDye_3.0-dGTP – Used only for templates that are likely to form strong secondary structures, hairpin loops, or with high GC content (greater than 75%) or bisulfite-treated samples. When using this chemistry, please include the Tm of your primer in the comments box
  Make sure after submitting the entry form, you print 2 copies of the table that appears. One of these copies must be placed in the inbox to inform us that the samples are ready to go. If we do not receive a copy of this form, your samples will never be sequenced. The other copy is for your reference.

  Please label your samples with less than 8 characters if possible. Write the labels on the cap of the tubes you are submitting. **Make sure that the labels on your tubes matches identically to the labels on the order form.**

  If you need a Chromatograph viewer, you can download one from Life Technologies.