

# Submitting Samples for FastTrack Sequencing Services

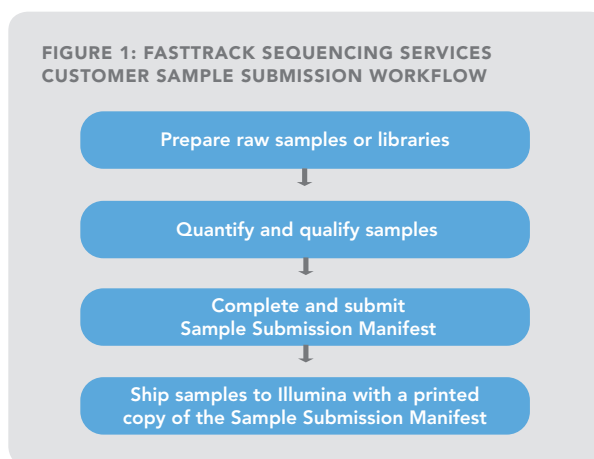
Guidelines for preparing, qualifying, and submitting samples for Illumina FastTrack Sequencing Services.

## INTRODUCTION

Illumina's FastTrack Sequencing Services provide affordable next-generation sequencing for custom genome-scale projects. Using Illumina Sequencing technology and the Genome Analyzer, FastTrack Services support both DNA and RNA applications. DNA sequencing options include sequencing or resequencing using single-read, paired-read, or mate pair library preparations with up to 12 indexed/multiplexed libraries pooled for a single deliverable. DNA libraries can be prepared using PCR products (minimum fragment size of 2.5 kb), DNA resulting from targeted selection using a hybridization enrichment method, restriction digest fragments, or immunoprecipitated DNA. RNA sequencing applications include mRNA sequencing, mRNA Expression Tag Profiling, and Small RNA Discovery and Analysis. The number of applications performed on the Genome Analyzer is growing every day. For the most current list of FastTrack Sequencing Services, please contact Technical Support or your local Account Manager.

This document offers guidelines for preparing, qualifying, and submitting samples to FastTrack Sequencing Services (Figure 1). For every application, researchers have the option of submitting DNA or RNA samples, or prepared libraries. Experience demonstrates that samples and libraries conforming to the requirements outlined in this

FIGURE 1: FASTTRACK SEQUENCING SERVICES CUSTOMER SAMPLE SUBMISSION WORKFLOW



document are more likely to provide the optimal amount of informative data.

## PREPARING SAMPLES FOR DNA SEQUENCING APPLICATIONS

DNA can be submitted as:

- Genomic DNA
- A pool of PCR products (minimum fragment size of 2.5 kb)
- A pool of products isolated via hybridization for targeted enrichment (method depends upon whether hybrid capture occurs before or after library preparation)
- Fragments from a restriction digest (in the case of reduced representation libraries)
- Immunoprecipitated DNA for ChIP-Seq

Indexes (adapter “barcodes”) can be added to genomic DNA or PCR products for sequencing multiple samples (multiplexing up to 12) in a single sequencing deliverable. Customers can choose to prepare their own libraries using Illumina’s sample prep kits or sample preparation can be completed at Illumina. Unprepared samples are typically referred to as “DNA Samples” or “Samples”, while prepared samples prep are referred to as “Libraries”. Table 1 summarizes sample and library submission requirements for various applications.

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## INPUT REQUIREMENTS FOR FASTTRACK SEQUENCING SERVICES APPLICATIONS

TABLE 1: APPLICATION INPUT\* BUFFER QC METHODS

	APPLICATION	INPUT*	BUFFER	QC METHODS
D N A	Whole-Genome Sequencing (single- and paired-read) PCR Products (minimum fragment length of 2.5 kb)	10 $\mu$ l at 500 ng/ $\mu$ l (5 $\mu$ g minimum)	TE Buffer	UV spectrophotometry
	Indexed Sequencing	20 $\mu$ l at 500 ng/ $\mu$ l (10 $\mu$ g minimum)	TE buffer	UV spectrophotometry
	ChIP Sequencing	10 $\mu$ l at 2 ng/ $\mu$ l (20 ng minimum)	TE buffer	PicoGreen assay
	Mate Pair Sequencing (with a gap between mate pairs up to 5 kb in length)	40 $\mu$ l at 500 ng/ $\mu$ l (20 $\mu$ g minimum)	TE buffer	UV spectrophotometry
R N A	Tag Profiling (Digital Gene Expression)	10 $\mu$ l at 500 ng/ $\mu$ l (5 $\mu$ g minimum)	DEPC-treated water	UV spectrophotometry and Agilent 2100 Bioanalyzer
	Small RNA Discovery and Analysis	40 $\mu$ l at 500 ng/ $\mu$ l (20 $\mu$ g minimum)	DEPC-treated water	UV spectrophotometry and Agilent 2100 Bioanalyzer
	mRNA Sequencing	40 $\mu$ l at 500 ng/ $\mu$ l (20 $\mu$ g minimum)	DEPC-treated water	UV spectrophotometry and Agilent 2100 Bioanalyzer

\* Minimum requirement. Customer should add 10% to the input requirements for Illumina to perform QC on the supplied samples.

**DNA Sample Quantification and Qualification**

- (1) PCR PRODUCTS: Submit purified and equimolarly pooled PCR products at least 2.5 kb in size to ensure that random shearing will be efficient.
- (2) ChIP-SEQUENCING SAMPLES: Quantify samples intended for ChIP Sequencing using the PicoGreen assay, a fluorometric DNA-specific quantification method. Samples should have a minimum concentration of 2 ng/ $\mu$ l and total input requirements as outlined in Table 1. Fragmented DNA should have a size distribution between 200 and 400 bp with the size and end-repair status declared in the Sample Submission Manifest.
- (3) RESTRICTION FRAGMENTS (reduced representation libraries): Contact your project manager to discuss appropriate input requirements. These are dependent upon the protocol and yield expected. Declare the end-repair status (dependent upon restriction enzyme used) in the Sample Submission Manifest.
- (4) NON-IMMUNOPRECIPITATED DNA: Quantify non-immunoprecipitated DNA samples using a UV spectrophotometric method such as the NanoDrop system. Illumina recommends that samples have  $OD_{260/280}$  ratios between 1.8 and 2.0. Sample concentration should be a minimum of 500 ng/ $\mu$ l, with the total input requirement as outlined in Table 1.
- (5) Submit sample DNA in TE buffer at a minimum concentration of 500 ng/ $\mu$ l in a clearly labeled 1.5–2.0 ml microcentrifuge tube sealed with parafilm.
- (6) Include a brief description of the DNA extraction and/or enrichment protocol(s) used in the “Additional Comments” field of the Sample Submission Manifest (Figure 2).
- (7) CARRIERS TO FACILITATE PRECIPITATION AND PELLET PAINT: Certain carriers and pellet paint types are not compatible with aspects of our sequencing technology. Non-robust results have been observed from samples where Lithium Chloride (LiCl), fluorescent pellet paint, or tRNA were used prior to precipitation and resuspension for sample preparation input.

**PREPARING SAMPLES FOR RNA SEQUENCING APPLICATIONS**

Total RNA can be submitted for RNA sequencing applications such as Tag Profiling, mRNA sequencing or Small RNA Discovery and Analysis. Customers can prepare their own libraries using Illumina's sample prep kits or sample preparation can be completed at Illumina. Unprepared samples are typically referred to as "RNA Samples" or "Samples", while prepared samples are referred to as "Libraries". Table 1 summarizes sample and library submission requirements for various applications.

**RNA Sample Quantification and Qualification**

- (1) Quantify total RNA samples using a UV spectrophotometric method such as the NanoDrop system. Illumina recommends that samples have  $OD_{260/280}$  ratios between 1.8 and 2.0. Sample concentration should be a minimum of 500 ng/μl, with the total input requirement as outlined in Table 1.
- (2) Evaluate samples using an Agilent Technologies 2100 Bioanalyzer and paste a copy of the electropherogram images into a worksheet included with the Sample Submission Manifest. Check that the size, purity, and concentration of the sample are within expectations or evaluate samples using gel electrophoresis. A denaturing 1% agarose gel can be run against a 1 kb ladder and the RNA integrity assessed upon staining with ethidium bromide. High-quality RNA shows a 28S rRNA band at 4.5 kb of twice the intensity of the 18S rRNA band at 1.9 kb. Messenger RNA appears as a faint smear from 0.1 to 12 kb.

- (3) Submit total RNA in DEPC-treated water at a minimum concentration of 500 ng/μl in a clearly labeled 1.5–2.0 ml microcentrifuge tube sealed with parafilm.
- (4) Include a brief description of the RNA extraction protocol used in the "Additional Comments" field of the Sample Submission Manifest (Figure 2).
- (5) CARRIERS TO FACILITATE PRECIPITATION AND PELLET PAINT: Certain carriers and pellet paint types are not compatible with aspects of our sequencing technology. Non-robust results have been observed from samples where Lithium Chloride (LiCl), fluorescent pellet paint, or tRNA were used prior to precipitation for RNA sequencing.

**PREPARING LIBRARIES FOR RNA OR DNA SEQUENCING APPLICATIONS**

These steps summarize the recommendations described in the Validate the Library section of Illumina's *Preparing Samples for Sequencing Genomic DNA* user guide.

**Library Quantification and Qualification**

- (1) Quantify libraries using a UV spectrophotometric method such as the NanoDrop system. The DNA yield using Illumina's sample preparation protocol should be between 500 and 1000 ng. The library  $OD_{260/280}$  ratio should be between 1.8 and 2.0.
- (2) Evaluate library concentration, size, and quality using an Agilent Technologies 2100 Bioanalyzer. Check that the size, purity, and concentration of the library are within expectations.

**HERE'S WHAT CUSTOMERS HAVE TO SAY**

"The Illumina FastTrack sequencing group offers exceptional services at a very reasonable price point. They provide a broad selection of essential upstream library preparation capabilities, several different options for sequence read length and configuration (single reads vs. paired ends), and flexibility in delivery of data back to the customer. The quantity of data we receive invariably exceeds our expectations, the turnaround times are remarkably fast, and the overall read quality is consistently very high. In our view, the FastTrack sequencing group provides a "virtual sequencing laboratory" that allows customers to access extraordinary sequencing data without having to make prohibitive expenditures in sequencing infrastructure. This group provides exceptional value, especially to first-time users that are eager to incorporate sequencing data into their research pipeline."

Chris Raymond, PhD.  
Senior Research Fellow and  
Head of the Molecular Informatics Research Laboratory  
Seattle, WA

- (3) Verify that insert sequences are from the genomic source DNA by cloning 4% of the library volume into a sequencing vector and conventionally sequencing 48 individual clones.
- (4) Estimate the molar concentration of the sample library by examining the median size of the library fragment. This is generally about 170 bp for a short-insert library. Multiply the estimated size of your insert by 650 (molecular mass of a base pair) to obtain the molecular weight of the library. Use this number to calculate the molar concentration of the library entered on the Sample Submission Manifest.
- (5) Submit half the volume of the final purified PCR product to Illumina for sequencing in a clearly labeled 1.5–2.0 ml microcentrifuge tube sealed with parafilm.

#### COMPLETING THE SAMPLE SUBMISSION MANIFEST

A Sample Submission Manifest must be completed and submitted for each FastTrack Sequencing Services project. The Manifest will be electronically sent to you by your Project Manager. Use the following instructions to fill out the information requested in each column of the Sample Submission Manifest (\*.xls) (Figure 2). Submit the Manifest via email to your Project Manager prior to shipping samples or libraries to Illumina. Include a printed copy of the Sample Submission Manifest in the shipment.

**Sample Name**—Enter a name for each sample. Illumina will use the Sample Name as a reference at delivery.

**Tube Identifier**—Enter the assigned barcodes into the “Tube Identifier” field. If your Project Manager has not

provided you with barcodes in advance, barcodes will be assigned upon arrival of the samples or libraries at Illumina. Until that time, the Tube Identifier should be used to cross-reference tubes to sample names.

**Application**—Select the sequencing project application from the dropdown menu. If the appropriate application is not available, identify the project as “research and development” and describe the application in the “Additional Comments” field.

**Application Units**—Enter the number of units for the application. For example, to request a 500 Mb targeted resequencing project, select “Targeted ReSeq 250Mb” from the “Application” column, and enter “2” in the “Application Units” column.

**Read Length**—Select the appropriate read length for the deliverable. If read length is not available, leave this field blank and explain in the “Additional Comments” field.

**Sample Type**—Select the type of sample from the dropdown menu. If the sample is a Customer Prepped Library, use “Customer Prepped Library” rather than “PCR Products”. “PCR Products” should only be chosen if the sample is a pool of PCR products intended for sample preparation.

**Sample Buffer**—Select the sample buffer from the dropdown menu. DNA samples must be submitted in TE buffer. RNA samples must be submitted in DEPC-treated water.

**Species**—Enter the genus and species name for the sample. If unknown, enter an organism descriptor.

#### HERE'S WHAT CUSTOMERS HAVE TO SAY

“We have submitted over 20 samples to Illumina’s FastTrack service for RNA-Seq and in every case we received a rapid turnaround (recently under 4 weeks) and a high quality dataset. A major attraction for us is the hands-off part; we submit total RNA and they return a 1–2 GB dataset at a cost that is not substantially more than one would have to pay in-house. The RNA-Seq data we have received has produced remarkable results for several projects in my lab and we are convinced that high-throughput sequencing is the technology of choice for transcriptome analyses. Illumina has a highly professional staff and it has been a pleasure working with this company.”

Ben Blencowe  
Principal Investigator  
University of Toronto  
Ontario, Canada

FIGURE 2: EXAMPLE OF A COMPLETED SAMPLE SUBMISSION MANIFEST (COLUMNS B–N)

B	C	D	E	F	G	H	I	J	K	L	M	N
Row	Sample Name	Tube Identifier*	Application	Application Units	Read Length	Sample Type	Sample Buffer	Species	Method of QC	260/280 ratio	Concentration	Units of Concentration
1	RF1	SS1001200-CSS	5 Gb Sequencing	1	75 nt	Genomic DNA	TE	Sebastes atrovirens	Multiple Methods (comments section)	1.8	550	ng/µl
2	ADP1	SS1001201-CSS	Research and Development	1	75 nt	PCR Products	TE	Arabidopsis	Multiple Methods (comments section)	1.8	550	ng/µl
3	RF2_brain	SS1001202-CSS	2 million tags DGE: N1a11	1	min. 20 nt (DGE: Tag Profiling)	Customer-Prepped Library	TE	Sebastes atrovirens	Multiple Methods (comments section)	1.8	10	nM

Additional Comments:  
 QC was completed using the NanoDrop method for quantity estimation and the Agilent Bioanalyzer for size distribution of PCR products of the customer-prepped library.  
 Agilent electropherograms are included on a worksheet in the Sample Submission Manifest.

FastTrack Sequencing Services customers must complete and submit this form with supporting sample quality and quantity validation data to their FastTrack Project Manager before shipping their samples. Use one row per sample or intended library for generating clusters. One Sample Submission Manifest can be used for multiple samples and projects.  
 \* It is very important that the unique barcode identifier obtained from the Project Manager (one per sample and application) be entered in this column. This is how your sample will be identified and tracked by the Services lab.

FIGURE 2 (CONTINUED): EXAMPLE OF A COMPLETED SAMPLE SUBMISSION MANIFEST (COLUMNS O–V)

O	P	Q	R	S	T	U	V
Row	Volume (µl)	Alignment Reference	GC Content (of Reference)	Approximate Avg Fragment Size (Applicable if Not Total RNA or gDNA)	Fragments Blunted (yes, no, or n/a)?	Custom Primer	Output File Requirements
1	20	No reference	50%	n/a	n/a	n/a	Standard Output with quality scores via FTP
2	15	Public-arabidopsis	50%	n/a	yes	n/a	Standard Output with quality scores via FTP
3	10	No reference	50%	90	yes	n/a	Standard Output with quality scores via FTP

**Method(s) of QC**—Select the method used for sample quantification from the dropdown menu. If multiple QC methods were performed, describe your supplementary analyses in the “Additional Comments” field. Submit data from sample quantification (e.g., gel images or Agilent Bioanalyzer reports).

**OD<sub>260/280</sub> Ratio**—Enter the OD value or “n/a” if not applicable.

**Concentration**—Enter the numeric value for sample concentration. See *Table 1* for minimum concentration requirements.

**Units for Concentration**—Enter ng/μl if submitting a sample. Enter nM if submitting a customer-prepared library.

**Volume (μl)**—Enter the total sample volume.

**Pooling**—Select individual or multiple samples from the dropdown menu. Enter the number of individuals pooled in each sample in the “Additional Comments” field. Pooled samples should contain equimolar amounts of each individual.

**Alignment to Reference**—Enter the appropriate reference or list of references to which the resulting sequence data should be aligned. Links to appropriate references can be entered here. If the reference sequence is proprietary, submit it with the Sample Submission Manifest via email or ftp. If a reference sequence is not available, enter “no reference.”

**GC Content**—Enter the estimated GC content of the reference genome. Note: If the GC content for the reference genome is not between 30% and 70%, expectations for reference alignment are lower.

**Approximate Average Fragment Size**—Enter the estimated fragment size range of your sequencing inserts if size selection was completed. Enter “n/a” if not applicable.

**Fragments Blunted (yes, no, or n/a)**—Enter “yes” if your samples are blunt-ended. Enter “no” if your samples are not blunt-ended. Enter “n/a” if not applicable. Applications where this is relevant include submission of restriction fragments (reduced representation of genomic DNA) and immunoprecipitated DNA.

**Custom Primer**—Indicate the name of the custom primer (if applicable) and submit a tube of the primer clearly labeled with its name and concentration with the library to be sequenced.

**Output File Requirements**—Select output file requirement from the dropdown menu. Output files are standard and are delivered via an ftp site.

**Additional Comments**—Enter additional comments on the extraction, PCR, quantification, and other methods that apply to sample submission.

**SHIPPING SAMPLES TO ILLUMINA**

All samples and libraries must be shipped in solution on dry ice. Please note that samples mailed from outside the U.S.A. may be subject to import permit requirements. Please contact your Project Manager or local Account Manager to discuss international shipments.

- (1) To ship your samples, submit each sample or library in a clearly labeled screwtop 1.5–2.0 ml microcentrifuge tube sealed with parafilm.
- (2) Place individual microcentrifuge tubes in a 50 ml disposable screw cap centrifuge tube for additional insulation during shipment. To prevent sample tubes from moving during shipment, pack any remaining space in the 50 ml tube with clean tissue paper prior to sealing.
- (3) Freeze samples prior to placement on dry ice to minimize opportunities for the samples to move in the tube. To keep samples and libraries frozen throughout the shipment, use enough dry ice to last up to one week.
- (4) Ship samples to:

**Illumina, Inc.**  
**ATTN: FastTrack Sequencing Services**  
**25861 Industrial Blvd.**  
**Hayward, CA 94545, USA**

For domestic shipments, ship samples using express next-day shipping (Monday through Wednesday). For international shipments, use priority international shipping, and confirm that the carrier can facilitate the importation of nucleic acid samples into the United States. Please arrange to have all international shipments arrive at Illumina on Tuesday, Wednesday, or Thursday.

- (5) Notify your Project Manager once the package has left your facility and send tracking information via email to [seqservices@illumina.com](mailto:seqservices@illumina.com).

**SAMPLE SUBMISSION CHECKLIST**

- Electronic Sample Submission Manifest template received.
- DNA samples quantified using UV method with OD<sub>260/280</sub> verified and entered into the Sample Submission Manifest.
- Agilent 2100 Bioanalyzer data collected.
- Library quality evaluation completed.
- Electronic Sample Submission Manifest prepared and sent to Illumina Project Manager.
- Email received from Illumina confirming the approval of Sample Submission Manifest.
- Samples shipped on dry ice to:
  - Illumina, Inc.**
  - ATTN: FastTrack Sequencing Services**
  - 25861 Industrial Blvd.**
  - Hayward, CA 94545, USA**
- Tracking number sent to Illumina Project Manager.

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