FastTrack® Sequencing Services Sample Submission Guidelines

Step-by-step instructions for faster, better sequencing results, tested by Illumina scientists.

DNA Preparation Requirements

Based on our experience processing over 2,000,000 samples, we find that samples conforming to the following requirements are more likely to provide excellent whole-genome sequencing (WGS) data.

- Quantify DNA using a dsDNA-specific method such as the PicoGreen method (Invitrogen catalog number P7581, www.invitrogen.com). **Optical density (OD) quantification is considered suboptimal**.
- Submit minimum of 2 µg DNA per sample (minimum concentration 20 ng/µl, in a total volume of 100 µl).
- DNA samples must be shipped to Illumina in barcoded plates provided by Illumina.
- The DNA solution buffer must be **10 mM Tris/1 mM EDTA.**
- DNA must be **pure, intact**, and of high molecular weight.
- DNA must be free from contaminating nucleic acids from other individuals or species.
- OD 260/280 must be between **1.8–2.0.**
- **Samples must not be PCR-amplified.**
- Include a brief description of the DNA extraction protocol followed for each DNA sample.
- Genetically identical samples (eg, tumor/normal pairs, monozygotic twins, experimental replicates from the same individual) must be annotated on the manifest (Table 1) in the Matched Sample ID column and disclosed to your project manager.

Project samples are processed from 96-well barcoded plates provided by Illumina. Incomplete or partial plate batches will be accepted on a case-by-case basis. Please speak with your project manager for additional information. Illumina FastTrack Services will quantify all customer samples with PicoGreen. Samples failing our minimum concentration requirement may be replaced once by the customer with future batches.

DNA Manifest and Illumina Barcoded Plates

To ensure our production facility provides high-quality data, FastTrack customers must provide an electronic manifest detailing several sample characteristics (Table 1). Barcoded 96-well plates will be provided and include a unique DNA identification number describing the plate barcode and well position (eg, LP6002511-DNA_A01). This enables a seamless interface with our robotic processes and retains sample anonymity. Your Illumina sequencing services project manager will send you a production DNA manifest with the associated barcodes for your project.

Table 1: DNA Manifest Columns

Sample Characteristics
Group ID
Sample ID
Plate barcode (eg, LP6002511-DNA)
Well (eg, A01)
Sample barcode (eg, LP6002511-DNA_A01)
Species (eg, *Homo sapiens*)
Gender (F: female, M: male, U: unknown)
Volume in the well, in µl (eg, 200)
Concentration in ng/µl (eg, 30)
OD 260/280
Tissue source (eg, cell line, WBC)
Extraction method (eg, phenol/chloroform, QIAGEN)
Ethnicity
Parent 1 ID: barcode for sample DNA from the mother (eg, LP6002511-DNA_E03
Parent 2 ID: barcode for sample DNA from the father (eg, LP6002511-DNA_G04)
Replicate(s) ID: barcode for sample containing DNA replicate(s) (eg, LP6002511-DNA_B08)
Cancer sample, potentially cytogenetically abnormal (Y/N)
Matched sample ID(s): barcode for sample DNA from a matched sample (eg, LP6002511-DNA_B01)
Matched sample type (eg, twin, tumor-normal pair)
Comment
Coverage requested

An overview of FastTrack Project Procedures is described below.

Procedures

1. After your order is finalized with a **signed quote and processed order**, a project manager will contact you and send you barcoded plates and a corresponding sample manifest to complete (Table 1).
2. Quantify your samples using the procedures described in the enclosed *DNA Preparation Requirements* and *PicoGreen DNA Quantification SOP*.
3. Prepare the DNA manifest as described above and send the completed file electronically to your Illumina project manager

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before starting to prepare sample batches. It is important that you fill out the manifest completely and accurately. This manifest allows us to initiate a project in our database and track those samples through our processes. **We cannot accept DNA samples from you until we have received and approved your manifest.** You are responsible for maintaining the relationship of DNA positions on each DNA plate as it was declared in the DNA manifest. The data will refer to the barcode and well position of each DNA.

4. At the time of shipping, send an email notification with your express mail tracking number to your Illumina project manager. This will allow us to watch for your shipment.
5. We will notify you when we receive your DNA. After their receipt, we will quantify the DNA in our lab and begin the WGS process.

DNA Packaging and Shipping Requirements

Packaging Instructions

Illumina FTS provides barcoded DNA plates, Ziploc bags to protect the plates, boxes to hold the plates, and a thermal cooler for return shipping on dry ice.

1. Make sure that the DNA is placed only in the provided barcoded plates. Lids must be sealed tightly and completely.
2. DNA must be frozen solid before shipment.
3. Slide each plate into a small Ziploc bag then place them in the corrugated boxes. The box protects the DNA plates from direct exposure to the dry ice.
4. Place boxes containing DNA plates into the shipping cooler box.
5. Plates must be shipped on sufficient dry ice to make sure that the samples remain frozen. This avoids the possibility of cross-contamination or degradation. Illumina recommends completely filling the shipping container with dry ice to minimize air space. **We also recommend using dry ice pellets, not blocks, to avoid damaging the plates during shipment.** We cannot accept damaged or thawed DNA plates.
6. Remove your address label from the shipping container and attach our shipping label with the address below, along with the dry ice label, import permit, and customs forms (if necessary), to the outside of the shipping container. Be sure to include the date, amount of dry ice, and total weight, as well as your signature and contact information.

Shipping Instructions

Direct all shipments to:

Attn: (Name of Illumina project manager)

Illumina FastTrack Services
5200 Illumina Way
San Diego, CA 92122
USA

Domestic Shipping

1. To avoid extra transit time over the weekend, ship your samples at the beginning of the week.
2. Ship samples using **overnight express**.
3. After shipping, send an email notification with your **express mail tracking number** to your Illumina project manager. This will allow us to track and prepare for your shipment. We will notify you when we receive your test or production DNA samples.

International Shipping

Discuss international shipments with your Illumina project manager as early as possible.

1. **Permits**—Shipping DNA of some species can require obtaining an import permit. Permit request processing can take a minimum of 4 weeks depending on species and country of origin. The earlier we are aware of international shipping needs, the better equipped we will be to prevent delays. Human DNA samples do not require permits for most countries. Ask your project manager for more information.
2. **Customs Documents**—Descriptive forms must be shipped with the samples, whether or not a permit is required. Ask your project manager for more information.
3. **Courier Services**—Illumina recommends using full-service couriers to ensure safe transit of international dry-ice shipments. These couriers will provide reicing as needed if the shipment is delayed in customs. They will also present documentation directly to customs agents to facilitate the clearance process. Ask your project manager for more information.
4. **Departure Days**—To ensure a minimum time of transit, the best day for an international shipment to depart is either Monday or Friday.

Customer Test/Production Phase Checklist

- ❒ Electronic manifest, barcoded plates, lids, and shipping container received
- ❒ DNA samples quantified using PicoGreen protocols
- ❒ Electronic manifest prepared and sent to Illumina FTS (Table 1)
- ❒ Email from Illumina confirms that the manifest is approved
- ❒ DNA-containing barcoded plates are sealed and frozen solid, then shipped with sufficient dry ice pellets by express mail
- ❒ Email with express mail tracking number is sent to Illumina FTS
- ❒ Email from Illumina confirms that DNA was received
- ❒ Email results from Illumina DNA quantification/qualification received

PicoGreen DNA Quantification SOP

Illumina strongly recommends quantification of DNA samples using the PicoGreen method, which is specific for detecting dsDNA in solution, before submitting them for microarray analysis. Submission of inadequate amounts of starting material can lead to poor results or project delays.

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Reference

PicoGreen dsDNA Quantification Reagent and Kits.
(Molecular Probes, Catalog No. P7581, www.lifetechnologies.com)

Materials/Equipment Needed

Disposables

96-well plates as defined by customer equipment

Aluminum adhesive seals

50 ml serological pipette

50 ml conical tube

Aluminum foil

Multichannel pipette troughs

Reagents

PicoGreen dsDNA quantification reagent

1× TE (10 mM Tris/1 mM EDTA)

Lambda DNA (Invitrogen, Catalog No. 25250-028, www.lifetechnologies.com)

Equipment

Vortexer

Spectrofluorometer specific for PicoGreen

Centrifuge

Multichannel Pipettes

Procedure for Preparing DNA Sample

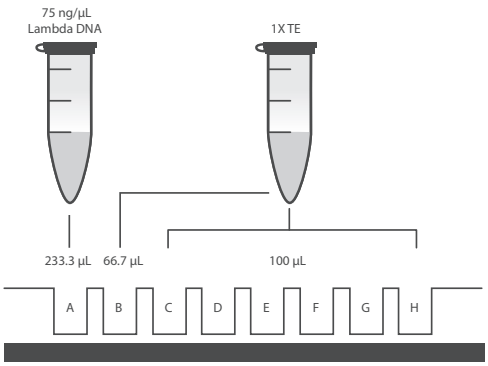

Figure 1: Workflow Standards—Preparation of Lambda DNA

Procedure

Setup

1. Prepare Lambda DNA Standards.

   a. Transfer 233.3 µl of 75 ng/µl lambda DNA to well A1 of a 96-well 0.65 ml midi plate (Figure 1).

   b. Transfer 66.7 µl of 1× TE to well B of column 1 of the same 96-well 0.65 ml midi plate (Figure 1).

   c. Transfer 100 µl of 1× TE to wells C, D, E, F, G, and H of column 1 of the same 96-well 0.65 ml midi plate (Figure 1).

   d. Serially dilute lambda DNA by transferring 133.3 µl of lambda DNA from well A1 into well B1 (Figure 1). Pipette mix contents of well B1 five times, then transfer 100 µl from well B1 into well C1. Pipette mix contents of well C1 five times, then transfer 100 µl from well C1 into well D1. Pipette mix contents of well D1 five times, then transfer 100 µl from well D1 into well E1. Pipette mix contents of well E1 five times, then transfer 100 µl from well E1 into well F1. Pipette mix contents of well F1 five times, then transfer 100 µl from well F1 into well G1. Pipette mix contents of well G1 five times. **Do not transfer solution from well G1 to well H1. WellH1 serves as the blank (0 ng/µl DNA).**

   e. Concentrations of lambda DNA standards are given in Table 2.

   f. Securely seal the lambda standards plate with cap, label as "Lambda DNA Standard," and store at 4ºC for future use.

Table 3: Concentration of Lambda DNA Standards

Row-Column	Concentration	Final Volume
A1	75 ng/µl	100 µl
B1	50 ng/µl	100 µl
C1	25 ng/µl	100 µl
D1	12.5 ng/µl	100 µl
E1	6.25 ng/µl	100 µl
F1	3.125 ng/µl	100 µl
G1	1.5262 ng/µl	100 µl
H1	0	100 µl

2. Prepare PicoGreen Spectrofluorometer Plates.
   Caution: PicoGreen is sensitive to photodegradation.

   a. Remove PicoGreen reagent from freezer and thaw at room temperature for 60 minutes in a light-impermeable container.

   b. Wrap aluminum foil around a 50 ml conical tube to prevent light penetration.

   c. Make a 1:200 dilution of PicoGreen to 1× TE in the 50 ml conical tube. Dilutions will be made for a maximum of 2 sample plates at a time. Table 3 outlines the volumes needed of each reagent.

Table 4: PicoGreen Dilutions

No. QDNA	Volume PicoGreen	Volume 1 × TE
1	115 µl	23 ml
2	215 µl	43 ml

   d. Cap the 50 ml dilution tube and mix with vortexer.

   e. Pour PicoGreen dilution into a clean multichannel pipette trough.

   f. Using a multichannel pipette, transfer 195 µl PicoGreen

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dilution to all 96 wells of the spectrofluorometer plates (Figure 2). Immediately cover plates with aluminum adhesive seal. This is the DNA Sample Quant Plate.

g. Repeat Steps 2c to 2f so that there is 1 DNA Sample Quant Plate for each DNA plate to be assayed.

h. Using a multichannel pipette, transfer 195 µl PicoGreen dilution to rows A to H of columns 1 and 2 of a new 96-well spectrofluorometer plate (Figure 2). Immediately cover plate with aluminum adhesive seal and label "DNA Standard Quant Plate."

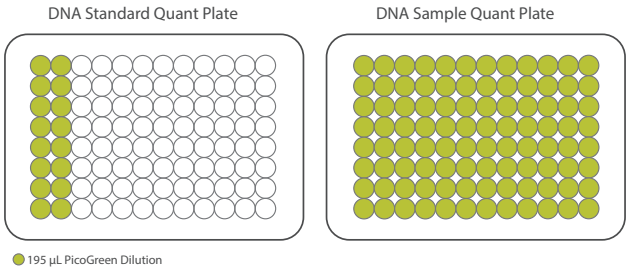

Figure 2: Plates—DNA Sample and Standard Quant

3. Dilute DNA in PicoGreen.

   a. These dilutions assume a sample DNA concentration between 0–50 ng/µl. Prepare your DNA accordingly before adding to the PicoGreen. This protocol is accurate for final concentrations between 0–50 ng/µl. Therefore, if you plan on making a subsequent dilution for samples that quant between 50 ng/µl and 75 ng/µl using this protocol, Illumina recommends diluting conservatively and rechecking the final concentration using PicoGreen.

   Using a multichannel pipette, transfer 2 µl of the Lambda DNA Standard to the DNA Standard Plate made in Step 2h.

Note: Illumina does not recommend diluting the samples. Send your Illumina project manager samples with concentrations ≥ 30 ng/µl or 40 ng/µl depending on assay type. We have seen no drop in call rate related to DNA samples with concentrations more than the stated requirements.

   b. Mix contents of Lambda DNA Standard plates into the DNA Standard Quant Plate with a multichannel pipette by pipetting up and down with at least 150 µl of the volume. Change tips between columns.

   c. Using a multichannel pipette, transfer 2 µl of each DNA to be assayed into the DNA Sample Plates made in Steps 2f to 2g.

   d. Mix contents of DNA Sample Quant Plates with a multichannel pipette by pipetting up and down with at least 150 µl of the volume. Change tips between columns.

Measuring Fluorescence

Fluorescence measurement depends upon the equipment available. Consult the manufacturer recommendations.

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