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FastTrack™ Microarray Services Sample Submission Guidelines

Achieve your research goals sooner with Illumina microarray study guidelines.

Project Orientation

Before the start of your project, a project manager from Illumina FastTrack Services (FTS) will call you to review the following:

- DNA preparation and shipping requirements
- Microarray Test Phase
- Microarray Production Phase

DNA Preparation Requirements

Based on our experience processing over 1,000,000 samples, we find that samples conforming to the following requirements are more likely to provide excellent microarray data.

- Quantify DNA using a dsDNA-specific method such as the PicoGreen method (Invitrogen, Catalog No. P7581, www.invitrogen.com). **Optical density (OD) quantification is considered suboptimal**. A PicoGreen DNA Quantification SOP is included at the end of this document.
- DNA samples must be shipped to Illumina in the midi deep well, barcoded plates provided by Illumina. Midi deep well plates are more robust and preferable to ensure the success of your study. In special cases, we can offer an alternative plate model (TCY plates) that requires less volume. However, TCY plates are more fragile and require an automated heat sealer. Ask your project manager for more information.
- The total amount of DNA required per sample depends on the requested assay and plate format (Table 1). Although both SNP genotyping and methylation studies use the Infinium Assay, more input DNA is required for the methylation assay because it starts with an extra bisulfite conversion step.
- We do not recommend normalizing samples to the required minimum concentration as there is no upper concentration limit for success. However, if samples must be diluted, the DNA solution buffer must be **10 mM Tris/1 mM EDTA**.
- The total volume required per sample also depends on assay and plate type (Table 1). All DNA samples **must have identical volumes**.
- DNA must be **pure, intact,** and of high molecular weight. DNA must be free of contaminating nucleic acids from other individuals or species.
- OD 260/280 must be between **1.8–2.0**.
- Submit a brief description of the DNA extraction protocols used along with the DNA samples.
- Any genetically identical samples (eg, tumor-normal sets, monozygotic twins, replicate samples from the same individual) must be disclosed to your project manager and annotated in the **Matched Sample ID** column of the manifest (Table 2).

Table 1: DNA Sample Concentration and Volume Requirements

	Minimum Concentration per sample[a]	Minimum Volume per sample	Total DNA per sample
Infinium Genotyping Assay			
Midi Plate	30 ng/µl	35 µl	1.05 µg
TCY Plate	30 ng/µl	20 µl	0.60 µg
Infinium Methylation Assay			
Midi Plate	40 ng/µl	40 µl	1.60 µg
TCY Plate	40 ng/µl	30 µl	1.20 µg

a. Higher concentrations are recommended. The success of samples on our platform is highly dependent on DNA concentration. We have seen no drop in call frequency related to DNA samples with concentrations higher than the requested amounts. However, we see a steep drop-off in performance for samples at concentrations below the requirements.

DNA Manifest and Illumina Barcoded Plates

Preparation of the DNA Manifest

To make sure that our production facility provides the highest-quality data possible, we require a sample manifest detailing the DNA sample information listed in Table 2. Illumina FTS uses standard operating procedures, and the manifest allows the identification of any variation in sample preparation as early as possible. After your FastTrack Services project is finalized with a signed quote and processed order, your Illumina FTS project manager will send a production DNA manifest, barcoded plates, and seals. After the Illumina project manager receives and approves the completed manifest files, you can ship the corresponding barcoded plates to Illumina FTS.

Throughout the process and within the data, we refer to your test or study DNA samples by the concatenation of the plate barcode and well position (eg, WG0XXXXXX-DNA_A01). This enables a seamless interface with our robotic processes and retains sample anonymity.

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Using Illumina Barcoded Plates

After receiving the plates and seals from your Illumina project manager, use the following guidelines to load your samples and seal the plates. These guidelines apply to both test and production DNA samples.

1. Leave wells A01 and A12 empty. They will be used later for Illumina control DNA.
2. Midi lids must be sealed tightly and completely. Illumina recommends first securing the seals by hand before using the mat applicator (Corning® Storage Mat Applicator, Catalog No. 3081, www.corning.com/lifesciences/worldwide.aspx) to make sure that each divet securely seals each well.
3. Seals for TCY plates must be applied using a heat sealer (Abgene, AB-0563/1000 96-well PCR plate carrier).
4. You are responsible for making sure the DNA samples in each well match what was declared in the manifest. The data we provide will only reference the barcode and well position of each DNA sample.

Test Phase Procedures

Before working with your study DNA samples, we will request test DNA samples. These test DNA samples will be run through our genotyping system and will serve as an opportunity to identify any potential DNA processing or shipping issues ahead of time. We request a minimum of 23 test DNA samples representative of your study DNA samples. (If you cannot send the requested number of test samples, contact your project manager.) If you have different DNA preparations or different sources of DNA, we request 6–8 representative samples from each source or preparation. A summary of test sample submission guidelines is listed below:

1. Quantify DNA using a dsDNA-specific method such as the PicoGreen method. **OD quantification is considered suboptimal**.
2. Illumina FTS will send a manifest, barcoded plates and lids, and a shipping container.
3. Required DNA concentrations are outlined in Table 1. Unlike production samples, test samples require a total volume of 40 µl per sample. All DNA samples must have identical volumes.
4. Prepare the DNA manifest as described in Table 2 and email the completed file to your Illumina project manager.
5. After your Illumina FTS project manager reviews and approves the completed DNA manifest, you can send the barcoded plates.
6. Place the test DNA samples in wells B01-H01, A02-H02, and A03-H03.

After we receive the samples, we will quantify them in our lab and run them through a test genotyping procedure. We will provide quantitative results for DNA concentration at our lab and qualitative results for the sample performance in our genotyping system.

NOTE: We do not provide quantitative results from the test DNA samples. The data from the test DNA samples are analyzed only for DNA performance, not for genotype or methylation results. ***After testing, any remaining DNA will be discarded.***

We expect to have this quantitative/qualitative feedback to you within 2 weeks of receiving your test DNA. If we note something of concern, we will work with you to correct any issues in DNA preparation and shipping before moving forward with your production DNA samples.

Table 2: Manifest Columns

Columns Specific to All Studies
DNA-plate barcode (eg, WG0XXXXXX-DNA)
Well position of the sample (eg, A05)
Customer Sample ID (eg, WG0XXXXXX-DNA_A05_SampleID)
Species of the sample (eg, Homo sapiens)
Gender of the individual (F: female, M: male, U: unknown)
Volume in the well (eg, 40 µl)
DNA concentration (eg, 30 ng/µl or higher)
Tissue source (eg, cell line)
Extraction Method (eg, phenol/chloroform)
Is Control (always set to 0)
Comments
Columns Specific to Genotyping Studies Only
If applicable, WGA method (eg, REPLI-g)
If applicable, mass of DNA used in WGA (eg, 50 ng)
Well position containing DNA from the mother (eg, WG0000777-DNA_A06_Name1)
Well position containing DNA from the father (eg, WG0000777-DNA_A07_Name2)
Well positions containing DNA replicates (eg, WG0000777-DNA_A08_Name3)
Columns Specific to Methylation Studies Only
Sample group

Production Phase Procedures

After the Test Phase, Illumina FTS will send the DNA manifest, barcoded plates and lids, and a shipping container for the Production Phase. The same sample preparation and shipping procedures are required for the production DNA samples as were required for the test DNA samples:

1. Quantify DNA using a dsDNA-specific method such as the PicoGreen method. **OD quantification is considered suboptimal.**
2. Required DNA concentrations and volumes are outlined in Table 1.
3. Email the completed DNA manifest file to your Illumina project manager. After your Illumina FTS project manager approves the completed DNA manifest, you can send the barcoded plates.
4. After shipping the plates, send an email notification with your express mail tracking number to your Illumina project manager. This will allow us to watch for your shipment.

We will notify you when we receive your DNA samples. After their receipt, we will quantify the DNA samples in our lab and begin the genotyping process.

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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.

A Note on Whole Genome Amplification

We strongly discourage customers from submitting whole-genome amplified (WGA) samples. **WGA samples have an unpredictable success rate.** However, if you must submit WGA samples, the following observations may be helpful:

- Unlike gDNA samples, the final concentration of WGA samples does not correlate to sample quality. After WGA, it is possible to have high DNA concentration that is not representative of the original DNA sample—particularly if the starting concentration was low or if contaminating DNA was present.
- We have found that the best metric correlating to WGA sample quality is the final quality/quantity of the sample *before amplification*. Furthermore, Illumina recommends using a minimum of 10 ng of gDNA for the WGA reaction. (Better results have been observed with ≥ 50 ng of gDNA.)
- You must clearly mark which samples are WGA on the electronic manifest, indicate the WGA method, and report the pre-WGA amplification amount (in ngs, as quantified using PicoGreen methods).

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 (Table 1)
- ❒ Electronic manifest prepared and sent to Illumina FTS (Table 2)
- ❒ 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

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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.

Reference

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

Materials and Equipment

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

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).

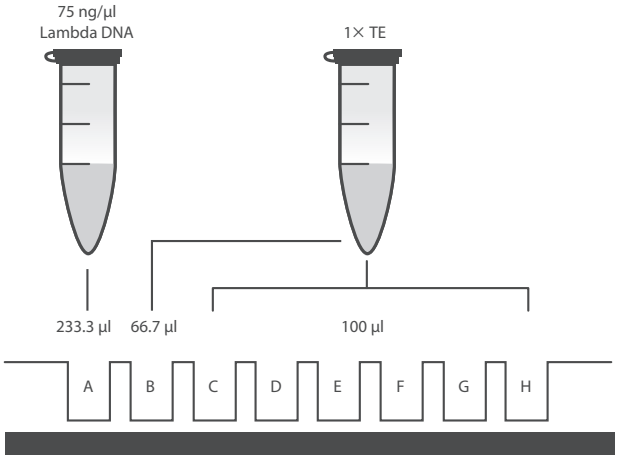

Figure 1: Workflow Standards—Preparation of Lambda DNA

   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 1.

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

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 4 outlines the volumes needed of each reagent.

   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 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.

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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."

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.

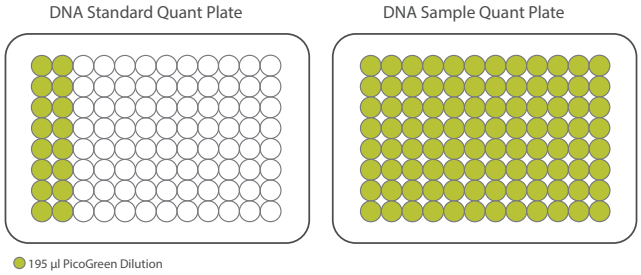

Figure 2: Plates—DNA Sample and Standard Quant

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

Table 4: PicoGreen Dilutions

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

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