

Dear Colleague,

Thank you for using Illumina FastTrack Services. We are committed to your success. Project success will be determined by DNA preparation, sample shipping and good communication between our organizations. We will guide you through this process by sharing our best practices - which have been developed through hundreds of service projects.

We have learned that the most important criterion for success is ensuring the accurate quantification of input DNA. While this may sound relatively simple, accurate DNA quantification across large sample sets can be quite difficult. Before you send us the samples for your custom study, we will work with you to help you understand and utilize our procedures using test DNAs (see enclosed *Guidelines for the Preparation and Shipment of Test DNAs*). This “testing” will familiarize you with the procedures for preparing and sending us your actual study DNAs.

The enclosed documentation provides comprehensive information on the best practices for preparation, quantification and shipment of the DNAs for your methylation project. If you have any questions, please contact either your Illumina project manager or your sales representative. Please carefully review all of the following:

- I. DNA Preparation Requirements
- II. Processing Test DNAs
- III. DNA Shipping Requirements
- IV. Customer DNA Checklist
- V. PicoGreen® DNA Quantification SOP

We look forward to working with you and helping you to reach your study goals.

Best Regards,

Your Illumina Genomic Services Team

## I. DNA Preparation Requirements

Our experience shows that samples conforming to the following requirements are more likely to provide excellent data.

### A) Important DNA criteria

- **DNA should be quantified using a DNA-specific method** such as the PicoGreen method (Molecular Probes catalog number P7581; <http://www.molecularprobes.com>)
- **All DNAs must have a minimum concentration of 70 ng/μL.** Because the Infinium assay which follows the bisulfite conversion requires a concentration of 50ng/ul, we need at least 70ng/ul input DNA into the conversion reaction. Higher concentrations are fine, as we have no known upper limit in concentration. The total amount of DNA required is dependent on the plate format that you will select.

Plate Type	Min. μg per sample	μL per sample
Midi (deep well plate with simple cap mat)	2.8	40
TCY (thinner plate that requires a heat sealer)	2.1	30

- **We do not recommend diluting your samples. If you must dilute down to 70ng/ul, we suggest that DNA be diluted in 10mM Tris/1mM EDTA.**
- **A brief description of the DNA extraction protocol(s)** that were used should be submitted with your DNAs.
- **As a preliminary test, we will ask for a few representative samples.** We will evaluate these sample DNAs for performance quality on the Illumina Methylation platform (see *Processing Test DNAs*).

### B) DNA manifest and Illumina bar-coded plates

To ensure that our production facility provides the highest quality data possible, we will ask for you to provide a manifest detailing several sample characteristics. Illumina provides bar-coded plates and we reference your study DNAs by the plate number and well position. This allows for a seamless interface with our robotic processes and retains sample anonymity. Your Illumina Services project manager will send you a test DNA manifest and later on a production DNA manifest with barcodes that will be used for your project. **This manifest needs to be filled out and returned to your project manager before the plates will be shipped to you.** The Illumina lab utilizes standard operating procedures, and it is important for us to identify any variation in sample preparation as early as possible.

#### 1) Manifest Columns:

- DNA-plate barcode (e.g., WG0888888-DNA)
- Well position of the sample (e.g., A05)

- Customer Sample ID (e.g., WG0888888-DNAA05\_Name)
- Sample group (taken into account for analysis)
- Species of the sample (e.g., Homo sapiens)
- Gender of the individual (F: female, M: male, U: unknown)
- Comments
- Volume in the well (e.g., 40  $\mu$ L).
- DNA concentration (e.g., 70ng/ $\mu$ L or greater)
- Tissue source (e.g., cell line)
- Extraction Method (e.g., Phenol/Chloroform)

## 2) Using Illumina bar-coded DNA plates:

- Illumina will provide bar-coded plates (MIDI or TCY) with corresponding lids or seals once the DNA manifest has been returned to your project manager.
- **For both test and production DNAs, wells A01 and A12 must remain empty for Illumina DNA controls.**
- Lids for MIDI plates must be sealed tightly and completely (ABGene cat. AB-0566/SP). We recommend that you first secure the cap mats by hand prior to using the mat applicator to ensure that each divet securely seals each well.
- Seals for TCY plates must be applied using a heat sealer ( e.g. AB-0563/1000 96-well PCR plate Carrier from Abgene)
- The customer is responsible for maintaining the relationship of DNA positions on each DNA plate as it was declared in the DNA manifest. The data will reference the barcode and well position of each DNA.

## II. Processing Test DNAs

Prior to having you send your study DNAs, we will request test DNAs from you. These test DNAs will be run through our genotyping system and will serve as an opportunity to practice DNA preparation and shipping. Typically, we ask that you send us a minimum of 23 test DNAs (up to 47) that are representative of your study DNAs. *(If you have needs that require you to send fewer test samples, please contact your project manager).* If you have different DNA preparations or different sources of DNA, we ask that you include 6-8 representatives of each in your test DNAs. We will use the same preparation and shipping procedures for the test DNAs as we will use for your study DNAs.

1. Please prepare the DNA manifest as described in the enclosed *DNA Preparation Requirements* and send the completed file electronically to your Illumina project manager. It is very important to fill out that 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 from you until we have received and approved your manifest.**

2. We will send you a bar-coded, Illumina-supplied plate and shipping container once we receive and approve the DNA manifest.
3. Please quantify your samples using the procedures described in the enclosed DNA *Preparation Requirements* and *PicoGreen DNA Quantification SOP*.
4. Please place the test DNAs in wells B01-H01, A02-H02 (*Please note that for both test and production DNA samples, we will ask you to leave wells A01 and A12 empty*).
5. Please seal, secure the lid and freeze the DNA plate as described in the enclosed *DNA Shipping Requirements*. Secure the seal by hand prior to using a mat applicator to prevent damage to the divets in the cap mats.
6. At the time of shipping, please send an email notification with your express mail tracking number to your Illumina project manager. This will allow us to watch for your shipment.
7. We will notify you when we receive your DNAs. After their receipt, we will quantify the DNAs in our lab and run the samples through a test procedure. We will provide you with quantitative results for DNA concentration at our lab and qualitative results for the sample's performance on our platform. Once tested, any remaining DNA will be destroyed.

*NOTE: We do not provide quantitative results from the test DNAs. The methylation data from the test DNAs are analyzed only for DNA performance.*

8. Depending upon our production queue, we expect to have this feedback to you within two weeks of receiving your DNA. If we note something of concern from our tests, we will work with you to correct any issues in DNA preparation and shipping before we receive your production DNAs.

### III. DNA Shipping Requirements

#### A ) DNA Packaging Instructions

1. Illumina will supply you with all the materials you need to ship your DNA to us.
2. Ensure that the DNA is placed in bar-coded plates (provided) and that plates are sealed with the cap mats or aluminum seals (also provided).
3. **DNA must be solidly frozen prior to shipment and remain frozen**
4. We provide you with shipping material to protect the plates. Please place each plate in a small Ziploc bag (provided) prior to place them back in the small or large corrugated carton box (provided).

5. **Plates must be shipped on sufficient dry ice to ensure that the samples remain frozen. This is to avoid the possibility of cross-contamination or degradation.** We recommend that you completely fill the shipping container with dry ice to minimize air space. We also recommend that you use dry ice pellets and not blocks to avoid damaging the plates during shipment. We cannot accept DNA for any study that has thawed or was not shipped frozen.
6. Remove your address label from the shipping container.
7. Attach OUR shipping label, which was included with your plate shipment and customs forms, to the outside of the shipping container. Please, be sure to include the date, amount of dry ice, total weight, and your signature and contact information.

#### **B) Package Shipment Instructions**

1. Domestic shipment (within the U.S): Illumina will provide the FedEx return forms. To avoid extra transit time over the weekend, please ship your samples at the beginning of the week. DNA should be shipped overnight express. After FedEx has picked up the box(s), please email your project manager with tracking and delivery information.
2. Special requirements for international shipments: Discuss international shipments early with your project manager. Shipping DNA of some species may require that we obtain a permit. Permit requirements can take a minimum of 4 weeks depending on species and country of origin. The earlier we are aware of your needs, the better equipped we will be to prevent delays in the forward progress of your project. Human DNAs do not require permits for most countries but descriptive forms need to be shipped with the samples. Ask your project manager for more information. Illumina uses different international couriers depending of the country. Your project manager will arrange for a pick up at your convenience.

**All shipments must be directed to:**

**Attn: (Name of your project manager)  
Illumina Sequencing Services  
5200 Illumina Way  
San Diego, CA 92122  
USA**

#### IV. Customer DNA Checklist

- Electronic manifest template received
- DNA samples quantified using PicoGreen protocols to 70ng/ $\mu$ L or greater
- Electronic manifest prepared and sent to Illumina project manager
- Email confirmation received stating that Illumina has received and approved my electronic manifest
- Bar-coded Illumina-supplied plate(s) and shipping container received
- DNA samples aliquotted at **constant volume** - all DNA samples have identical volume (please refer to the table under *DNA Preparation Requirements* for minimum requirements)
- DNA-containing bar-coded Illumina-supplied plate(s) sealed and frozen.
- Frozen DNA-containing bar-coded Illumina-supplied plate(s) shipped with sufficient dry ice pellets by express mail
- Email confirmation of Illumina's receipt of DNA received
- Email results from Illumina DNA quantification/qualification received

## V. PicoGreen® DNA Quantification SOP

### 1.0 REFERENCES

- 1.1 PicoGreen dsDNA Quantification Reagent and Kits. Molecular Probes P7581

### 2.0 MATERIALS / EQUIPMENT REQUIRED

#### 2.1 Disposables

- 2.1.1 96 well-plates as defined by customer's equipment
- 2.1.2 Aluminum adhesive seals
- 2.1.3 50 mL serological pipette
- 2.1.4 50 mL conical tube
- 2.1.5 Aluminum foil
- 2.1.6 Multichannel pipette trough

#### 2.2 Reagents

- 2.2.1 PicoGreen dsDNA quantification reagent (Molecular Probes Cat # P7581)
- 2.2.2 1x TE (10mMTris/1mM EDTA)
- 2.2.3 Invitrogen Lambda DNA (Cat# 25250-028)

#### 2.3 Equipment

- 2.3.1 Vortexer
- 2.3.2 Spectrofluorometer specific for PicoGreen
- 2.3.3 Centrifuge
- 2.3.4 Multitichannel Pipettor

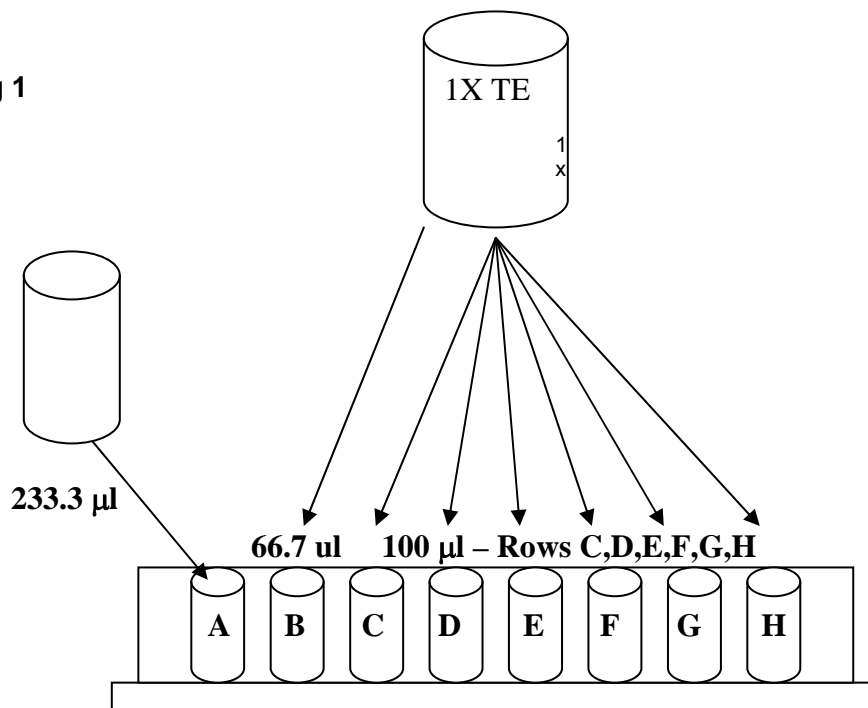
### 3.0 PROCEDURE

#### 3.1 Set up

##### 3.1.1 Prepare lambda DNA Standards

- 3.1.1.1 Transfer 233.3  $\mu\text{L}$  of 75 ng/ $\mu\text{L}$  lambda DNA to well A1 of a 96 well plate (Fig 1).
- 3.1.1.2 Transfer 66.7  $\mu\text{L}$  of 1X TE to well B of column 1 of the same 96-well 0.65 MIDI plate (Fig 1).
- 3.1.1.3 Transfer 100  $\mu\text{L}$  of 1X TE to wells C, D, E, F, G, and H of column 1 of the same 96-well 0.65 mL MIDI plate (Fig 1).
- 3.1.1.4 Serially dilute (Fig 1) lambda DNA by transferring 133.3  $\mu\text{L}$  of lambda DNA from well A1 into well B1. Pipette mix contents of well B1 five times, then transfer 100  $\mu\text{L}$  from well B1 into well C1. Pipette mix contents of well C1 five times, then transfer 100  $\mu\text{L}$  from well C1 into well D1. Pipette mix contents of well D1 five times, then transfer 100 $\mu\text{L}$  from well D1 into well E1. Pipette mix contents of well E1 five times, then transfer 100  $\mu\text{L}$  from well E1 into well F1. Pipette mix contents of well F1 five times, then transfer 100  $\mu\text{L}$  from well F1 into well G1. Pipette mix contents of well G1 five times. **Do not transfer solution from well G1 to well H1. Well H1 serves as the blank (0 ng/ $\mu\text{L}$  DNA).**

Fig 1



3.1.1.5 The concentration of lambda DNA standards is given in Table 1.

**Table 1 – Concentration of lambda DNA Standards**

Row-Column	Conc. (ng/µL)	Final Volume
A1	75	100
B1	50	100
C1	25	100
D1	12.5	100
E1	6.25	100
F1	3.125	100
G1	1.5262	200
H1	0	100

3.1.1.6 Securely seal the Lambda standards plate with cap, label as “Lambda DNA Standard”, and store at 4°C for future use.

**3.1.2 Prepare PicoGreen Spectrofluorometer Plates**

Caution: PicoGreen is sensitive to photodegradation.

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

3.1.2.2 Wrap aluminum foil around a 50 mL conical tube to prevent light penetration.

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

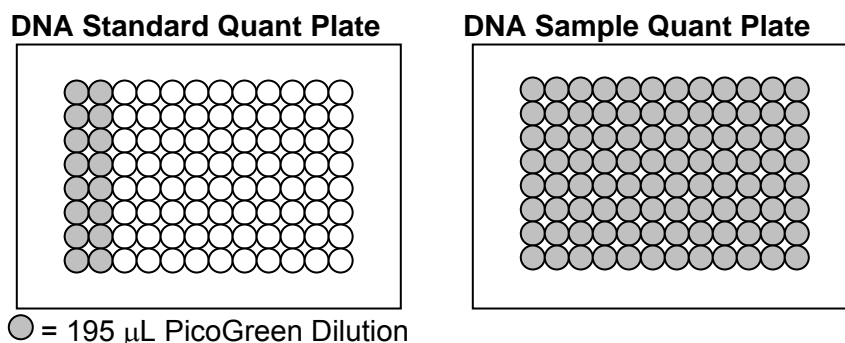
**Table 2 – PicoGreen dilution**

No. QDNA Plates	Volume PicoGreen (µL)	Volume 1x TE (mL)
1	115	23
2	215	43



- 3.1.2.4 Cap the 50 mL dilution tube and mix with vortexer.
- 3.1.2.5 Pour PicoGreen dilution into a clean multichannel pipette trough.
- 3.1.2.6 Using a multichannel pipette, transfer 195  $\mu\text{L}$  PicoGreen dilution to all 96 wells of the spectrofluorometer plate(s) (Fig 2). Immediately cover plate(s) with aluminum adhesive seal. This is the “DNA Sample Quant Plate”.
- 3.1.2.7 Repeat steps 3.1.2.3 to 3.1.2.6 so that there is one DNA Sample Quant plate for each DNA plate to be assayed.
- 3.1.2.8 Using a multichannel pipette, transfer 195  $\mu\text{L}$  PicoGreen dilution to rows A to H of columns 1 and 2 of a new 96 well spectrofluorometer plate (Fig 2). Immediately cover plate with aluminum adhesive seal and label “DNA Standard Quant Plate.”

Fig 2



### 3.1.3 Dilute DNA in PicoGreen

- 3.1.3.1 These dilutions assume a sample DNA concentration between 0 and 75  $\text{ng}/\mu\text{L}$ . Prepare your DNA accordingly before adding to the PicoGreen. This protocol is quite accurate for final concentrations between 0 and 75 $\text{ng}/\mu\text{L}$ . Thus, if you plan on making a subsequent dilution for samples that quant between 75 $\text{ng}/\mu\text{L}$  and 100 $\text{ng}/\mu\text{L}$  using this protocol, we recommend that you dilute conservatively and recheck your final concentration using PicoGreen. Using a multichannel pipettor transfer 2  $\mu\text{L}$  of the Lambda DNA Standard to the DNA Standard Plate made in steps 3.1.2.8.

Note: Illumina does not recommend that you dilute your samples. Send your project manager samples with concentration  $\geq 70\text{ng}/\mu\text{L}$ , we will return left over DNA at the end of the project.

- 3.1.3.2 Mix contents of “Lambda DNA Standard” plates into the “DNA Standard Quant Plate” with a multichannel pipettor by pipetting up and down with at least 150  $\mu\text{L}$  of the volume. Change tips between columns.
- 3.1.3.3 Using a multichannel pipettor transfer 2  $\mu\text{L}$  of each DNA to be assayed into the DNA Sample Plate(s) made in steps 3.1.2.6 to 3.1.2.7.
- 3.1.3.4 Mix contents of DNA Sample Quant Plate(s) with a multichannel pipettor by pipetting up and down with at least 150  $\mu\text{L}$  of the volume. Change tips between columns.

## 3.2 Measuring Fluorescence

Depends upon equipment available. Consult manufacturer’s recommendations.