Measuring the Ion Sphere™ Quality Control Kit Using the Quantus™ Fluorometer

Materials Used:

- Ion Sphere™ Quality Control Kit (for the Ion Torrent; Life Technologies Cat.# 446856)
- Unenriched and enriched Ion Sphere™ particles
- Quantus™ Fluorometer (Cat.# E6159)
- 0.5ml PCR tubes (Cat.# E4941)
- Qubit® Easy Calculator Microsoft® Excel® Spreadsheet for use with IonSphere™ Quality Control Kit (available at: http://ioncommunity.lifetechnologies.com)

This protocol was developed by Promega Applications Scientists and is intended for research use only. Users are responsible for determining the suitability of the protocol for their application.

Further information can be found in Technical Manual #TM396, available at: **www.promega.com/protocols**

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Here we describe a method using the Quantus™ Fluorometer with the Ion Sphere™ Quality Control Kit to assess the template efficiency of Ion Sphere™ particles (ISPs).

Introduction

The Ion Torrent™ PGM™ and Ion Proton™ systems sequence DNA templates that have been clonally amplified on primer-coated beads, known as Ion Sphere™ Particles (ISPs). Proper DNA-to-ISP ratio is critical; however, variations during library and template preparation, as well as enrichment steps may lead to inefficient template binding to the ISPs. Overloading of DNA prior to template preparation, for example, can result in polyclonal amplification, which will produce sequencing reads that are uninterpretable by the instrument and are discarded. Adding too little DNA will reduce the number of reads obtained for the sample due to untemplated ISPs. Therefore, quality control and confirmation of template-positive ISPs prior to sequencing are recommended.

One method for measuring the ISPs template efficiency is with the Ion Sphere™ Quality Control Kit. This assay labels the Ion Sphere™ Particles (ISPs) with two different fluorophores: Alexa Fluor® 488 and Alexa Fluor® 647. The probe labeled with Alexa Fluor® 488 anneals to all of the ISPs present, while the Alexa Fluor® 647 labeled probe anneals to only the ISPs with extended templates. The ratio of the Alexa Fluor® 647 fluorescence (templated ISPs) to the Alexa Fluor® 488 fluorescence (all ISPs present) yields the percent templated ISPs.

The Quantus™ Fluorometer allows quick and easy assessment of template-positive ISPs prior to performing a sequencing run. Here we describe a method using the Quantus™ Fluorometer with the Ion Sphere™ Quality Control Kit to assess the template efficiency of Ion Sphere™ particles (ISPs). This experiment created ISPs using the Ion AmpliSeq™ Cancer Hotspot Panel v2.

Method

Sample Type: Unenriched and enriched with Ion Sphere™ particles
Input: 2µl of unenriched and 10µl of enrinched sample

1. Transfer 2µl of the unenriched sample and 10µl of the enriched sample into separate microcentrifuge tubes.
2. Add 19µl of Annealing Buffer and 1µl of Ion Probes to each sample and mix by pipetting.
3. Place tubes into a 95°C heat block for 2 minutes. Then transfer the tubes to 37°C heat block for 2 minutes. (Alternatively, use a thermal cycler for this step.).

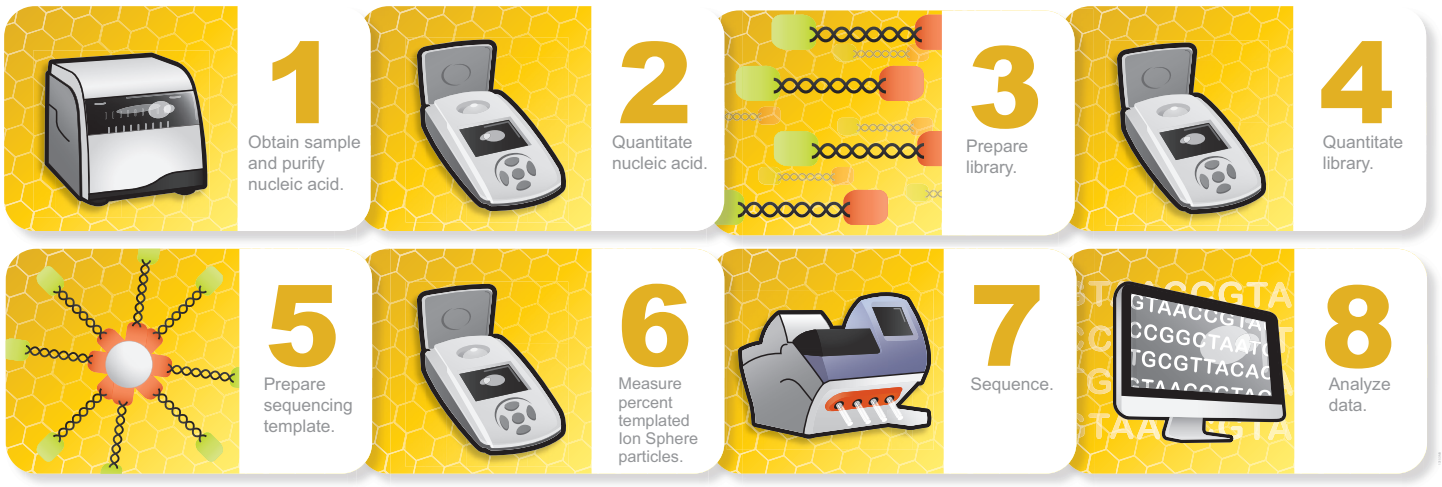

Figure 1. Schematic Workflow for using the Quantus™ Fluorometer throughout the Ion Torrent Sequencing workflow.

A.

Calibration Standard	RFU	Calibration Factor
Alexa Fluor® 488 Calibration Standard	24673.72	0.40
Alexa Fluor® 647 Calibration Standard	55790.25	

Percent Template ISPs

	Raw RFU Value		Background RFU (Negative Control Tube)			
Sample ID	AF 488	AF 647	AF 488	AF 647	Conversion Factor*	Percent Templated ISPs
Enriched 1	374.9	514.8	37.1	9.3	1.0	60%
Enriched 2	440.6	605.9	37.1	9.3	1.0	59%
Enriched 3	398.0	551.3	37.1	9.3	1.0	60%
Unenriched 1	1641.3	610.6	37.1	9.3	1.0	15%
Unenriched 2	1431.6	577.0	37.1	9.3	1.0	16%
Unenriched 3	1679.4	626.9	37.1	9.3	1.0	15%

Fluorophore	Acceptable RFU range
Alexa Fluor® 488	> 100 counts; no upper limit Samples with < 100 counts usually correlate with no or very few ISPs in the assay. Cells with < 100 counts will be highlighted in red.
Alexa Fluor® 647	Any value, with the condition that the Alexa Fluor® 488 RFU value is > 100 counts

B.

Calibration Standard	RFU	Calibration Factor
Alexa Fluor® 488 Calibration Standard	17261	0.67
Alexa Fluor® 647 Calibration Standard	23382	

Percent Template ISPs

	Raw RFU Value		Background RFU (Negative Control Tube)			
Sample ID	AF 488	AF 647	AF 488	AF 647	Conversion Factor*	Percent Templated ISPs
Enriched 1	258.0	220.0	23.0	3.0	1.0	62%
Enriched 2	299.0	260.0	23.0	3.0	1.0	62%
Enriched 3	264.0	233.0	23.0	3.0	1.0	64%
Unenriched 1	1116.0	260.0	23.0	3.0	1.0	16%
Unenriched 2	967.0	244.0	23.0	3.0	1.0	17%
Unenriched 3	1150.0	264.0	23.0	3.0	1.0	16%

Fluorophore	Acceptable RFU range
Alexa Fluor® 488	> 100 counts; no upper limit Samples with < 100 counts usually correlate with no or very few ISPs in the assay. Cells with < 100 counts will be highlighted in red.
Alexa Fluor® 647	Any value, with the condition that the Alexa Fluor® 488 RFU value is > 100 counts

Figure 2. Data generated for the Ion Sphere™ QC Assay using Quantus™ and Qubit® 3.0 fluorometers. IonSphere™ particles were templated using the Ion AmpliSeq™ Cancer Hotspot Panel v2. Enriched and unenriched Ion Sphere™ particles were assayed in triplicate on both fluorometers, and the resulting RFU values entered into the Qubit® Easy Calculator Microsoft® Excell Spreadshet. Acceptance Criteria for unenriched Ion Sphere™ particle samples is 10–30% and for enriched Ion Sphere™ particles samples is greater than 50%. Performance of the assay was identical on both instruments.

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4. Remove the tubes and wash samples three times to remove unbound probes by adding 200µl of Quality Control Wash Buffer; mix by vortexing and then collect by centrifugation at 15,500RCF for 90 seconds.
5. Collect the liquid, leaving 10µl in the bottom of the tube. When removing liquid do not disturb the pellet.
6. After the third wash, add 190µl of Quality Control Wash Buffer for a final volume of 200µl. Mix by quickly vortex mixing and transfer samples to 0.5ml tubes.
7. Turn on the Quantus™ Fluorometer and select the "Tools" tab followed by the "Raw Measurement" tab. The screen will display two tabs labeled "Blue" and "Red". Highlight the "Blue" tab; insert the AF488 standard, and close the lid.
8. Record your value and repeat for the AF647 standard with the "Red" tab.
9. Read all samples using both the blue and red channels and record your values.
10. Use 200µl of Quality Control Wash Buffer as a negative control.
11. Place your values into Qubit® Easy Calculator Microsoft® Excel® Spreadsheet ,and find the conversion factor value on the Ion Community website according to your kit and lot number.
12. The percent template ISPs will be calculated and displayed in the spreadsheet.

Summary

The Quantus™Fluorometer enables quick and easy measurement of the Ion Sphere™ QC Kit to assess quality control of templated libraries prior to sequencing. The assay takes advantage of two different fluorophores to label ISPs with bound and unbound templates, and the ratio between the two represntes the percentages of templated ISPs. Any fluorometer capable of reporting raw RFU can be used to read the assay. When the Qubit™ 3.0 was compared to the Quantus™ Fluorometer for this purpose, performance was identical (Figure 2).

Ordering Information

Product	Cat.#
Quantus™ Flurometer	E6159

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