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Quick-Start Protocol

QIAcuity® NGS Library Quantification Protocol

This protocol is optimized for the quantification of Illumina NGS libraries using the Illumina sequencing primers P5 forward and P7 reverse (Table 1) together with the QIAcuity EG PCR Kit (cat. nos. 250111, 250112 and 250113) and QIAGEN's QIAcuity instruments for digital PCR (dPCR).

The QIAcuity EG PCR Kit should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light. The QIAcuity EG PCR master mix can also be stored protected from light at 2–8°C. Unless otherwise indicated on the label, the components are stable for 12 months without showing any reduction in performance under these conditions.

Table 1. P5- and P7-specific primers used for Illumina NGS library quantification

Primer Name	Sequence	Reference
P5 forward	5′-AAT GAT ACG GCG ACC ACC GA-3′	Please consult support documents on Illumina's webpage
P7 reverse	5′-CAA GCA GAA GAC GGC ATA CGA-3′	

Further information

- *QIAcuity Application Note*: **www.qiagen.com/HB-2839**
- *QIAcuity User Manual*: **www.qiagen.com/HB-2717**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- A fluorescent reference dye is provided as a component of the QIAcuity EVA Green PCR Master Mix, for reliable detection of proper partition filling in the QIAcuity Nanoplates.
- Always start with the cycling conditions and primer concentrations specified in this protocol.
- The PCR must start with an initial incubation step of 2 min at 95°C to activate the QuantiNova® DNA Polymerase in the master mix.
- For ease of use, we recommend preparing a 20x concentrated primer mix containing the target-specific primers for each of your targets. The 20x primer mix consists of 8 µM forward primer and 8 µM reverse primer in TE buffer with low EDTA (0.1 mM). The performance of other assays might benefit from different concentrations of primer and probes.

Procedure

Template dilution

1. Prepare a dilution series of your library samples according to Table 2.

Table 2. Template dilution scheme

Dilution	Dilution factor	How to Dilute
D1	1:10	180 µL TE with 20 µL Stock
D2	1:100	180 µL TE with 20 µL D1
D3	1:1000	180 µL TE with 20 µL D2
D4	1:10,000	180 µL TE with 20 µL D3
D5	1:100,000	180 µL TE with 20 µL D4
D6	1:1,000,000	180 µL TE with 20 µL D5
D7	1:10,000,000	180 µL TE with 20 µL D6

* For libraries with an expected concentration larger than 10nM use dilution D7. For lower concentrated libraries use dilution D6 for optimal quantification results.

Reaction setup

2. Thaw the QIAcuity EG PCR master mix, template DNA, primers and RNase-free water (contained in the kit, cat. no. 1012888).
3. Vigorously mix the QIAcuity EG PCR master mix and the individual solutions.
4. Centrifuge briefly to collect liquids at the bottom of the tubes.
5. Prepare a reaction mix for the number of reactions needed according to Table 3. Due to the hot-start, it is not necessary to keep samples on ice during reaction setup or while programming the QIAcuity instrument.

Table 3. Reaction setup

	Volume/reaction		
Component	**Nanoplate 8.5k (24-well, 96-well)**	**Nanoplate 26k (24-well)**	**Final concentration**
3x QIAcuity EG Master Mix	4 µL	13.3 µL	1x
20x Primer-mix	0.6 µL	2 µL	0.4 µM forward primer 0.4 µM reverse primer
RNase-free Water	Variable	Variable	
Template DNA (added at step 7)	Variable*	Variable*	

* Appropriate template amount depends on the concentration of library fragments in the sample that might require running a dilution series following the scheme in Table 1.

6. Vortex the reaction mix.
7. Dispense appropriate volumes of the reaction mix, which contains all components except the template, into the wells of a standard PCR plate. Then, add template DNA into each well that contains the reaction mix.

   Note: The appropriate amount of template DNA depends on the concentration of library fragments in the sample and might require running a dilution series following the scheme in Table 3. Please also refer to the *QIAcuity User Manual Extension: Application Guide* for further details on additional parameters affecting template input amounts.
8. Transfer the content of each well from the standard PCR plate to the wells of the nanoplate.

9. Seal the nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.

   Note: For exact sealing procedure, please see the *QIAcuity System User Manual*.

Thermal cycling conditions

1. Program the cycler of the QIAcuity instrument according to Table 4.

Table 4. Cycling conditions

Step	Time	Temperature (°C)
PCR initial heat activation	2 min	95
3-step cycling (40 cycles)		
Denaturation	15 s	95
Annealing	15 s	55*
Extension	15 s	72
Cooling down	5 min	40

* Temperature during annealing/extension and number of cycles might vary depending on assay type the length of the Sample

2. Use the Imaging Setting of 200 ms and Gain 3.
3. Place the nanoplate into the QIAcuity instrument and start the dPCR program.

Document Revision History

Date	Changes
02/2023	Initial release
07/2023	Update Table 1. Deleted link and replaced with "Please consult support documents on Illumina's webpage"

Scan QR code for the *QIAcuity User Manual Extension: Application Guide*.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.