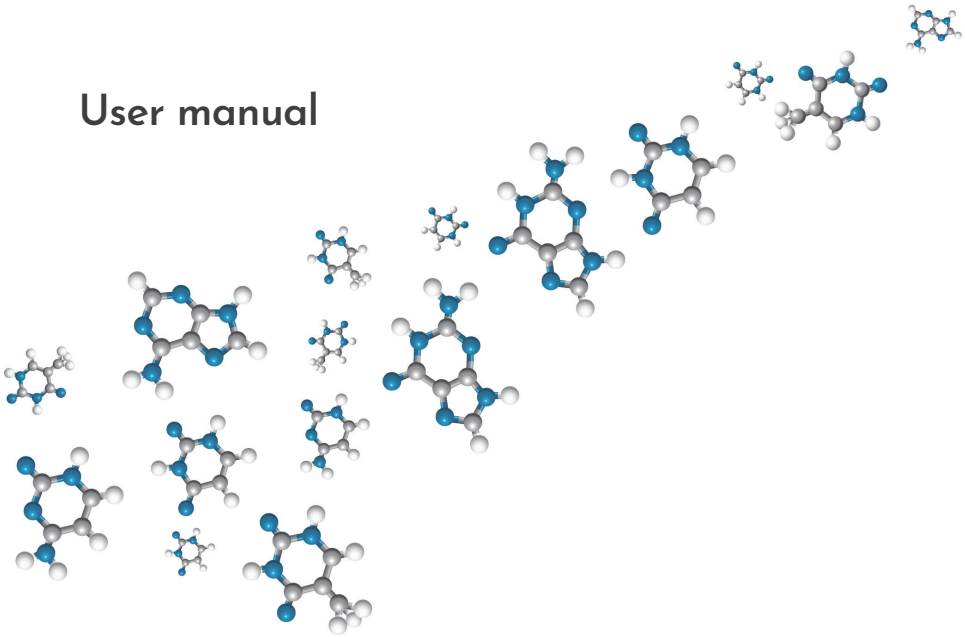
User manual



NGS
Library Quantification Kit
Version 1.0 - May 2019

Order #:

NGS Library Quantification Kit: TA20-NGSQ

tataabiocenter

Table of contents

Background

This kit is based on absolute quantification of your NGS libraries using Illumina adaptor primers and qPCR. The amplifiable fraction of the library will get amplified, and the quantity will be estimated using a standard curve. The goal of the analysis is to find the optimum concentration for the NGS library, since over- or underestimation of the concentration may compromise the sequencing capacity.

-Underestimation of the concentration → Overloading of the flow cell → Poor cluster resolution

-Overestimation of the concentration → Insufficient template amount → Low cluster density

Six DNA standards of known concentrations are part of the kit for generation of a robust standard curve. They will generate PCR products of 415 bp. After diluting your NGS library of unknown concentration 10 000 times and performing qPCR analysis, the library concentration is assessed. PCR based library preps yield mostly concentrations of 1-50 nM.

Contents

- NGS library assay primers (V = 250 µl primer mix, c = 10 µM per primer, Blue cap)
- TATAA SYBR GrandMaster Mix LowROX (V = 2 · 1250 µl, c = 2x)
- NGS library standards: 10-fold diluted Standard 1-6, dsDNA in TE buffer (V = 40 µl per standard)

Storage

Store the kit components at -20˚C through the expiration date printed on the labels. Avoid repeated freeze-thaw cycles.

Additional material and equipment requirements

- qPCR instrumentation with SYBR channel
- qPCR compatible plates with foil applicable for the PCR-cycler in use
- Pipettes and tips
- Tubes for preparing PCR mixes and dilutions
- Vortex and centrifuge

Protocol in brief:

1. Dilute your NGS libraries 10 000 times.
2. Make a qPCR mix using TATAA SYBR GrandMaster Mix LowROX 2x and the provided primers. Dispense to a qPCR plate.
3. Add the provided standard and your diluted NGS library to the plate.
4. Perform a qPCR run.
5. Evaluate the Cq values and calculate the standard curve and the concentrations of your NGS libraries.

Protocol

Sample setup

Plan your sample setup on the reaction plate. Consider that the sample measurement consists of:

- Six standard points in triplicates
- Three negative controls (NTCs)
- A variable number of NGS library samples to be analysed in triplicates.

On a 96-reaction plate there will therefore be space for a maximum of 25 NGS library samples, and on a 384-reaction plate a maximum 121 library samples.

Cycling protocol

Program the PCR instrument according to the following protocol:

	Temperature	Time	Cycles
Polymerase activation:	95°C	60 sec	1
Amplification:			
Denaturation	95°C	5 sec	35
Annealing	60°C	30 sec	
Extension (fluorescence acquisition)	72°C	10 sec	

Table 1 Cycling protocol

The amplification of DNA is analysed in the SYBR channel.

Preparation of PCR master mix

Proceed as described below to prepare the PCR master mix

- Thaw the solutions and briefly spin tubes in a micro centrifuge.
- Vortex tubes and spin again.
- Prepare the PCR master mix in a reaction tube.

The volumes are based on a single 10-µl reaction. Prepare the PCR master mix by multiplying the amount in the "Volume" column by the number of reactions to be cycled plus one or two additional reactions to cover pipetting losses.

Component	Volume (one 10-µl reaction)
TATAA SYBR GrandMaster mix (2x)	5 µl
H_2O (PCR grade)	2.6 µl
NGS assay primers	0.4 µl

- Vortex and spin master mixes.

Dilution of libraries

Typically, NGS libraries have a concentration between 1 - 50 nM, which is too high for qPCR quantification. Dilute your library 10 000 times to reach approximately 0.1 - 5 pM, respresenting the centre to upper part of this kit's dynamic range. Perform the dilution in two steps:

- Add 2 µl NGS library to 198 µl H_2O or TE-buffer in a reaction tube (100-fold dilution).
- Vortex and spin.
- Add 10 µl of the 100-fold diluted NGS library to 990 µl H_2O or TE-buffer in a second reaction tube.
- (10 000-fold dilution)
- Vortex and spin.

PCR run

- Pipet 8 µl PCR master mix into each PCR well.
- For the standards, add 2 µl standard.
- For the library samples of interest, add 2 µl of 10 000-fold diluted library sample DNA..
- For the negative controls, add 2 µl H_2O.
- Seal the PCR plates thoroughly with foil.
- Vortex and spin the plate.

Evaluation

Pre-treatment of data

- Check that all spectra look fine and that the replicates are not too scattered. Outliers should be excluded from the analysis. If two or more points are outliers , the measurement of that sample should be repeated.

Calculation of standard curve and concentration of library samples

For most qPCR instruments the standard curve and the concentartions of unknown samples are calculated automatically. This requires that the sample type and the standard concentrations (copies per µl, Table 1) were defined in the plate layout in the instrument's software. If the standard curve is not calculated automatically, the data may be exported and analysed externally by a qPCR analysis software such as GenEx (MultiD).

- Check that the efficiency of the standard curve is between 80% and 110%.
- Note the mean concentration of each library sample.
- Multiply the library concentrations by 10 000 to obtain the concentrations in pM of the original samples (this is done in the equation below). Since the standards and samples underwent same dilution in the qPCR workflow it is not necessary to account for the dilution with the master mix.
- Convert the concentration from the standard curve (equivalent to 415 bp), c', to the concentration corresponding to your average library fragment length, c, by the following formula:

$$c[pM] = c'[pM] \cdot \frac{415}{Avg.\,fragment\ length} \cdot\ 10\,000$$

where Avg. fragment length is the average fragment length of your primer library, and 415 is the product length of the standards. 10 000 is dilution fold.

Troubleshooting

I do not get any amplification/signal

The instrument may not have been programmed correctly or there may be a problem with your master mix. Evaluate if the problem is in the detection or the amplification by running the PCR-product on a gel or in capillary electrophoresis. Run a new test using the reference DNA provided with the kit.

Check that the adaptor sequences of your library are compatible with the provided primers.

My negative controls are amplified

The reagents are probably contaminated.

My replicates are not very tight

With good quality DNA and good pipetting technique, very high reproducibility is expected. Low amounts of DNA can lead to higher variation. Also, low quality DNA can lead to differences between replicates. Check the accuracy and reproducibility of your pipettes.

The calculated concentrations of my library are outside the dynamic range of the standard curve

The dilution of the library was not optimal. Estimate which dilution would make the concentrations fall within the dynamic range of the standard curve and repeat the measurement.

Contact

For more information about the product please contact us at order@tataa.com or +46 31 761 57 00.

qPCR training courses at TATAA Biocenter

TATAA Biocenter is a leading organizer of hands-on training in qPCR and related technologies. For comprehensive training program please visit www.tataa.com.

Other products from TATAA

ALU-assays

Alu elements are short stretches of about 300 bp that are unique to the human genomic DNA (gDNA). There are over one million Alu elements interspersed throughout the genome constituting about 10.7% of the bases. The TATAA Alu Assays are specific, wet-lab validated qPCR assays targeting consensus Alu sequences making them exceptionally sensitive for human gDNA. Alu assays are available that produce different amplicon lengths, which makes it possible to assess also the length distribution of the gDNA present.

TATAA GrandScript cDNA SuperMix

One-tube format

The SuperMix provides a sensitive and easy to-use solution for two-step RT-PCR. It is optimized for reliable cDNA synthesis over a wide dynamic range of input RNA and works exceptionally well with a wide variety of targets. The SuperMix produces excellent results in both real-time and conventional RT-PCR and is ideal for high-throughput applications or low copy samples.

TATAA PreAmp GrandMaster® Mix

Dynamic and sensitive

TATAA PreAmp GrandMaster® Mix is a 2x concentrated ready to use mix for PCR based pre-amplification of small samples, when looking at low expressed genes or when highly concentrated RNA is needed.

TATAA SYBR® GrandMaster® Mix

Efficiency, sensitivity and specificity

TATAA SYBR® GrandMaster® Mix is a 2x concentrated ready to use fastmix. With its unique combination of optimized components it delivers maximum PCR efficiency, sensitivity, specificity and robust fluorescent signal using fast, or conventional, cycling protocols with SYBR® Green qPCR.

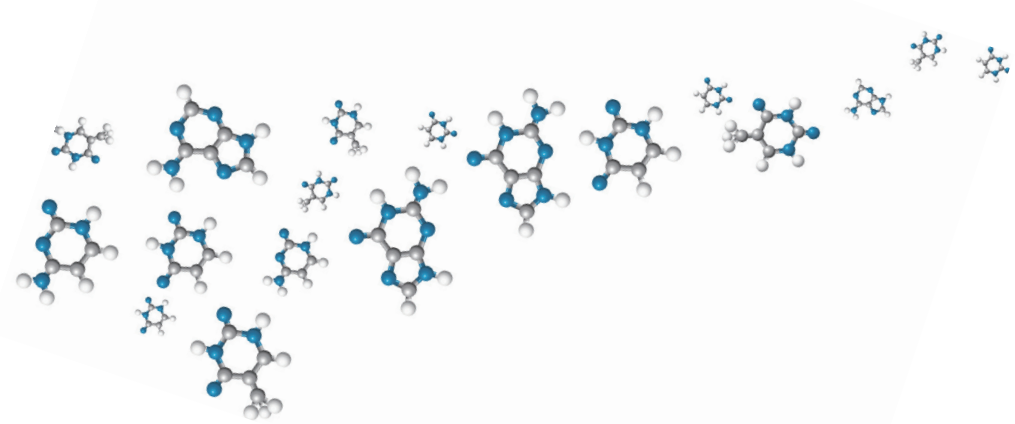
ValidPrime®

The most time and cost saving control for gDNA contamination of large RT-qPCR experiments. ValidPrime® enables you to look for gDNA in all samples separately and also reduces the costs compared to the traditional approach with RT(-) controls. By replacing the RT(-) controls with the ValidPrime® assay and a gDNA control sample with known concentration you can detect and even compensate for the contamination when up to 50% of the signal comes from gDNA.

About TATAA Biocenter

Founded in 2001 by pioneers in qPCR, TATAA Biocenter is Europe's leading provider of gene expression profiling services and a prime organizer of hands-on training in quantitative real-time PCR (qPCR). Our offer comprises the entire field of qPCR services, including commissioned services and research projects, hands-on training and highest quality products.

TATAA Biocenter develops high-quality products for quantitative real-time PCR applications which are available in our webshop or from any of our distributors.



Express your genius

TATAA Biocenter, with offices in Gothenburg, Sweden, and Prague, Czech Republic, is the leading provider of quantitative real-time PCR and NGS services and the prime organiser of quantitative real-time PCR and NGS workshops globally. TATAA Biocenter conducts commissioned research and training within the field of molecular diagnostics and gene expression analysis, along with developing real-time PCR expression panels. TATAA Biocenter has great experience and expertise in high resolution gene expression profiling, pathogen detection, and small sample/single cell analysis.

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