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# qPCR standard for library quantification V.1

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## Abstract

Protocol for the preparation of a standard for DNA library quantification by quantitative real-time PCR (Meyer et al. 2008; Gansauge et al. 2020).

## References

Meyer, M., Briggs, A. W., Maricic, T., Höber, B., Höffner, B., Krause, J., Weihmann, A., Pääbo, S., & Hofreiter, M. (2008). From micrograms to picograms: Quantitative PCR reduces the material demands of high-throughput sequencing. *Nucleic Acids Research*, 36(1): e5.

Gansauge, M.-T., Aximu-Petri, A., Nagel, S. *et al.* Manual and automated preparation of single-stranded DNA libraries for the sequencing of DNA from ancient biological remains and other sources of highly degraded DNA. *Nature Protocols*, 15, 2279-2300 (2020).

## Notes

The qPCR standard comprises of a 60 bp fragment of pUC19 sequence flanked by double-indexed Illumina adapters. Indices are qPCR specific and not used for the preparation of sample libraries. The standard is obtained through two successive PCRs. It is then purified, quantified and diluted to concentrations between  $10^8$  and  $10^2$  molecules per micro liter.

## Materials

|  | Reagent/consumable                                                                               | Supplier             | Catalogue number |
|--|--------------------------------------------------------------------------------------------------|----------------------|------------------|
|  | <b>Reagents</b>                                                                                  |                      |                  |
|  | Herculase II Fusion DNA Polymerase, including 5x Herculase buffer and dNTP mix (25 mM each dNTP) | Agilent Technologies | 600675           |
|  | MinElute PCR Purification Kit                                                                    | Qiagen               | 28006            |
|  | pUC19 plasmid DNA                                                                                | NEB                  | N3041S           |
|  | Primer CL105_makeSTD103.F *                                                                      | IDT                  | -                |
|  | Primer CL106_makeSTD103.R †                                                                      | IDT                  | -                |
|  | Primer P5_iPCR_qPCR_xxQ188 ‡                                                                     | IDT                  | -                |
|  | Primer P7_iPCR_qPCR_xxQ §                                                                        | IDT                  | -                |
|  | Agilent DNA 1000 Kit                                                                             | Agilent Technologies | 5067-1504        |
|  | TET buffer                                                                                       | self-made            |                  |
|  | TE buffer ¶                                                                                      | self-made            |                  |
|  | Water, HPLC-grade                                                                                |                      |                  |
|  | <b>Consumables</b>                                                                               |                      |                  |
|  | 0.2-ml PCR eight-tube strips                                                                     | Eppendorf®           | EP0030124359     |
|  | 1.5-ml Eppendorf LoBind Tubes                                                                    | Eppendorf®           | 0030108051       |

\* Order oligonucleotide CL105\_makeSTD103.F at 1µmol synthesis scale (Integrated DNA Technologies, desalted).

Dissolve in TE buffer at a concentration of 100 µM. Prepare a 10 µM working dilution in water. Sequence:

ACACTCTTTCCCTACACGACGCTCTTCCTCGTCGTTTGGTATGGCTTC

† Order oligonucleotide CL106\_makeSTD103.R at 1µmol synthesis scale (Integrated DNA Technologies, desalted).

Dissolve in TE buffer at a concentration of 100 µM. Prepare a 10 µM working dilution in water. Sequence:

GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCATGTAACCTCGCCTTGATCGT

‡ Order oligonucleotide P5\_iPCR\_qPCR\_xxQ188 at 1µmol synthesis scale (Integrated DNA Technologies, HPLC purified). Dissolve in TE buffer at a concentration of 100 µM. Prepare a 10 µM working dilution in water.

Sequence: AATGATACGGCGACCAACGAGATCTACACCAATTCAACACTCTTTCCCTACACGACGCTCTT

§ Order oligonucleotide P7\_iPCR\_qPCR\_xxQ at 1µmol synthesis scale (Integrated DNA Technologies, HPLC purified). Prepare a 10 µM working dilution in water. Dissolve in TE buffer at a concentration of 100 µM.

Sequence: AGCAGAAGACGGCATACGAGATACCACAGTGAAGTTCAGACGTGT

|| See document in the Appendix for the preparation of TET buffer.

¶ See document in the Appendix for the preparation of TE buffer.

## Equipment

- Thermal cycler for PCR strip tubes (e.g., Bio-Rad C1000 Touch Thermal Cycler, cat. no. 1840197)
- NanoDrop spectrophotometer (Model ND-1000 V3.8.1)
- Agilent 2100 Bioanalyser 2100 expert DNA 1000 Series II (cat. no. G2938C)
- Label printer (e.g. Brady M611, cat. no. M611-EU-LABS) and tube labels (e.g. labels for TLS2200/TLS PC Link/Polyester, cat. no. PTL-82-499)

## Protocol

1. In a 0.2-ml PCR eight-tube strip, prepare the following reaction mix using 1ng of pUC19 plasmid DNA as template and primers CL105 and CL106. Mix properly.

|  | Reagents                                | Volume       | Final concentration in reaction |
|--|-----------------------------------------|--------------|---------------------------------|
|  | 5x Herculase buffer                     | 20µl         | 1x                              |
|  | 25 mM each dNTP                         | 1µl          | 250µM                           |
|  | 10 µM CL105                             | 4µl          | 400nM                           |
|  | 10 µM CL106                             | 4µl          | 400nM                           |
|  | 400 U/µl Herculase II Fusion Polymerase | 1µl          | 4U/µl                           |
|  | Water                                   | 69µl         |                                 |
|  | 1 ng/µl pUC19 plasmid DNA               | 1µl          | 0.01 ng/µl                      |
|  | <i>sum</i>                              | <i>100µl</i> |                                 |

2. Place the strip tube into a thermal cycler and perform cycling with the following temperature profile.

| Step                 | Temperature | Duration | # of cycles |
|----------------------|-------------|----------|-------------|
| Initial denaturation | 95°C        | 2min     | 1           |
| Denaturation         | 95°C        | 30s      | 25          |
| Annealing            | 60°C        | 30s      |             |
| Extension            | 72°C        | 30s      |             |
| Final extension      | 72°C        | 5min     | 1           |
| Cooling              | 10°C        | forever  | hold        |

3. Purify the PCR product using the MinElute PCR Purification Kit following the manufacturer's instructions. Elute in 30 µl TE buffer.

4. Determine the concentration of the PCR product using the NanoDrop spectrophotometer. The expected concentration range is 50–100 ng/µl. Dilute PCR product to 1 ng / µl in TE buffer in a 1.5-ml Eppendorf LoBind Tube.

5. In a 0.2-ml PCR eight-tube strip, prepare the following reaction mix use 1ng of the purified PCR product as template for a second amplification with a combination of P5 and P7 indexing primers used only for preparing the qPCR standard. Mix properly.

| Reagent                                  | Volume       | Final concentration in reaction |
|------------------------------------------|--------------|---------------------------------|
| 5x Herculase buffer                      | 20µl         | 1x                              |
| 25 mM each dNTP                          | 1µl          | 250µM                           |
| 10 µM P7_iPCR-qPCR_xxQ                   | 4µl          | 400nM                           |
| 10 µM P5_iPCR-qPCR_xxQ188                | 4µl          | 400nM                           |
| 400 U/µl Herculase II Fusion Polymerase  | 1µl          |                                 |
| Water                                    | 69µl         |                                 |
| 1 ng/µl PCR product dilution from step 4 | 1µl          | 0.01 ng / µl                    |
| <i>sum</i>                               | <i>100µl</i> |                                 |

6. Place the strip tube into a thermal cycler and perform cycling with the following temperature profile.

|  | Step                 | Temperature | Duration | # of cycles |
|--|----------------------|-------------|----------|-------------|
|  | Initial denaturation | 95°C        | 2min     | 1           |
|  | Denaturation         | 95°C        | 30s      | 25          |
|  | Annealing            | 60°C        | 30s      |             |
|  | Extension            | 72°C        | 30s      |             |
|  | Final extension      | 72°C        | 5min     | 1           |
|  | Cooling              | 10°C        | forever  | hold        |

7. Purify the PCR product using the MinElute PCR Purification Kit. Elute in 30 µl TE buffer.

8. Measure the DNA concentration on a DNA-1000 chip using the Bioanalyzer 2100. Calculate the concentration of the PCR products in molecules per µl, e.g. using the Promega Biomath Calculator (<https://www.promega.de/en/resources/tools/biomath/>) and the Avogadro constant.

9. Dilute the purified PCR product to  $10^{10}$  copies/µl in TET buffer in a 1.5-ml Eppendorf LoBind Tubes.

10. Prepare a ten-fold dilution series in TET buffer ranging from  $10^9$  to  $10^2$  copies/µl as follows:

- Prepare a ten-fold dilution ( $10^9$  copies/µl) by combining 100µl of the  $10^{10}$  dilution with 900µl TET buffer in a 1.5-ml Eppendorf LoBind Tubes
- Use the same scheme for further dilutions until reaching  $10^2$  copies/µl.

11. Aliquot 100µl per dilution into one or more 0.2-ml PCR eight-tube strips to obtain one or more working aliquots of qPCR standard.

#### Note

##### [Labeling]

Label the 1.5-ml Eppendorf LoBind Tubes containing the stock dilutions as well as the eight-tube strips containing the working aliquots with the date of creation and your initials.

12. Store stock and working aliquots at  $-20^{\circ}\text{C}$  until used.

## Appendix



## Document

NAME

**TET buffer**

CREATED BY

**Sarah Nagel**

Preview

## Document

NAME

**TE buffer**

CREATED BY

**Anna Schmidt**

Preview