User Guide

Human Genomic DNA Quantification kit

qPCR quantification of Human genomic DNA

100 reactions

Version 1.3

Kit contents

Human Genomic DNA quantification kit

	Cap colour	Size
gDNA specific primer/probe*		0.5 mL
YouSeq qPCR 2x MasterMix		1 mL
5x Standards**		5 x 0.1 mL
DNase/RNase Free Water		1.5 mL
Template Resus. Buffer		1.5 mL

* FAM Labelled Probe
** Arrive lyophilised and require reconstituting

Other items to be supplied by the user

- qPCR instrument
- Plasticware suitable for your PCR machine
- Pipettes and general laboratory equipment

qPCR plate set up

Resuspension of Reagents

1. Resuspend the Primer/Probe mix with **110 µL** of DNase/RNase Free water
2. Resuspend each Standard (1-5) with **500 µL** of Template Resus. Buffer

qPCR Set-up

3. Load each well of your PCR plate/ PCR tubes with the following mix below
    i. Recommendation – Make a bulk mix and dispense this into all appropriate wells
    ii. e.g. 10 reactions (plus 1 rxn overage):
        1. 110 µL YouSeq qPCR MasterMix
        2. 55 µL Library specific primers
        3. 33 µL Water
    iii. Remember an additional 5 wells are required for the provided standards

10 µL YouSeq qPCR MasterMix
5 µL gDNA specific primers
3 µL Water

Samples for quantification

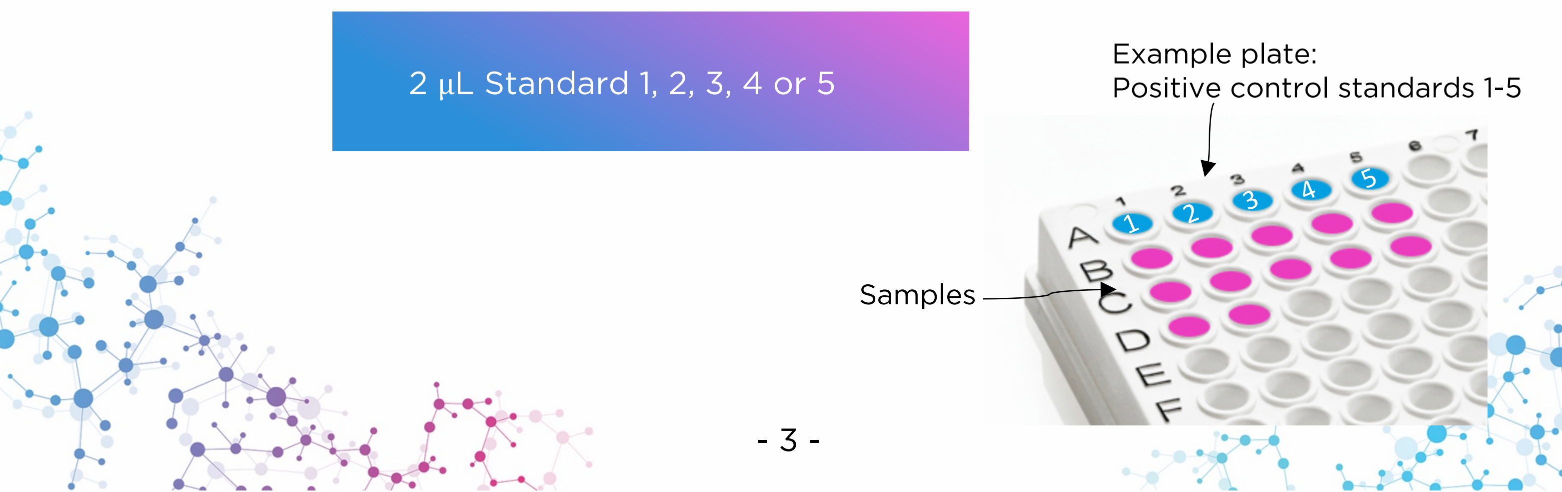
4. Add **2 µl** of your sample gDNA

2 µL sample gDNA

Positive control standards

5. Load 5 wells to create your positive control standard curve

2 µL Standard 1, 2, 3, 4 or 5



6. Seal the plate and load into your qPCR instruments.

Standard curve set up in qPCR instrumentation software

7. Your qPCR instrument will have a function to programme a ‘standard curve’. This is simply a matter of inputting the concentration of the 5 positive control standards supplied in the kit.

Standard no.	Concentration
1	100 ng/µl
2	50 ng/µl
3	10 ng/µl
4	1 ng/µl
5	0.1 ng/µl

exact setup on screen varies according to instrument and software

qPCR protocol set up

8. Run the following PCR protocol:

	Temp	Time	
	95°C	3 minutes	
40 cycles	95°C	10 seconds	
	60°C	60 seconds	Data Capture*

**Make sure to collect fluorogenic data through the FAM/SYBR (Green) channel*

Data interpretation

Pass Criteria:

The criteria need to be met to ensure the run was performed optimally and quantification values are as true as possible.

- PCR efficiency – 90 – 110% efficient

If this is not met, the run has failed and would deliver an incorrect gDNA quantification. We'd recommend repeating this run.

Quantification of Sample genomic DNA Concentrations

Your qPCR instrument software will automatically compare the Cq values obtained from your samples to those from the positive control standards in the kit. This calculation will deliver a 'calculated concentration' in ng/µl of each of your gDNA samples.

Refer to your qPCR instrument software for instructions.

If the sample concentration falls outside the dynamic range of the standards, dilute the sample and try re-running.