Evaluation by Real-time PCR:

DNA adsorption test with the FastGene® 96-well PCR plate

Purpose: The FastGene® 96-well PCR plate was tested for its DNA adsorption under different conditions

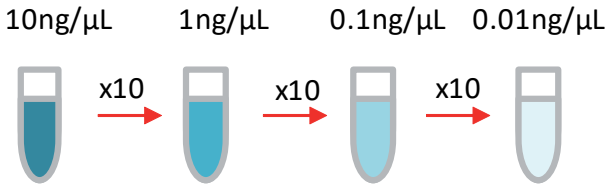
Cat. No. FG-1702

Material

Real-Time PCR system:	StepOnePlus Life Technologies - for detection
Thermal cycler:	Life Eco (Bioer) - for incubation
Plate:	FastGene® 96-well PCR plate (FG-1702)
Template DNA:	Mouse Genomic DNA (Clontech Cat. No. #636402)
Primer for qPCR:	HRPT-gDNA-F: CCACTTGTGACGAAAGCACC (HPLC grade) HRPT-gDNA-R: GTTGTCTACGCTCTGGCAGT (HPLC grade) (Target: single copy gene)

Method

1.) Preparation of a DNA serial solution: Mouse Genomic DNA was diluted 1:10 in 5 mM Tris-HCl with the start concentration of 10 ng/µl was diluted four times 1:10. The lowest DNA concentration correlates with the lowest input in a NGS application.



2.) 4 x 50 µl of the different concentrated DNA solutions were transferred, aligned side by side, in the top row of a FastGene® 96-well PCR plate. The DNA solutions were incubated according to standard enzyme incubation steps:

Low temperature:	4 °C, 30 min (as typical condition of reaction mixture storage)
Middle temperature:	37 °C, 30 min (as typical condition of enzyme reaction)
High temperature:	65 °C, 30 min (as typical condition of enzyme denaturation)

After these incubation steps, the DNA solutions were transferred to the second row and the incubation was repeated, totalling in 4 incubation rows and 120 min total incubation time.

3.) After the last transferation and incubation, the DNA concentration in the last row was compared to the control DNA concentration of the initial solution by qPCR quantification.

• Reaction components

KAPA SYBR Fast qPCR Master Mix (2x)	10 µl
Forward Primer (10 µM)	0.4 µl
Reverse Primer (10 µM)	0.4 µl
Template (incubated DNA or Control DNA)	2 µl
Nuclease Free PCR water	7.2 µl
Total amount	20 µl/rxn

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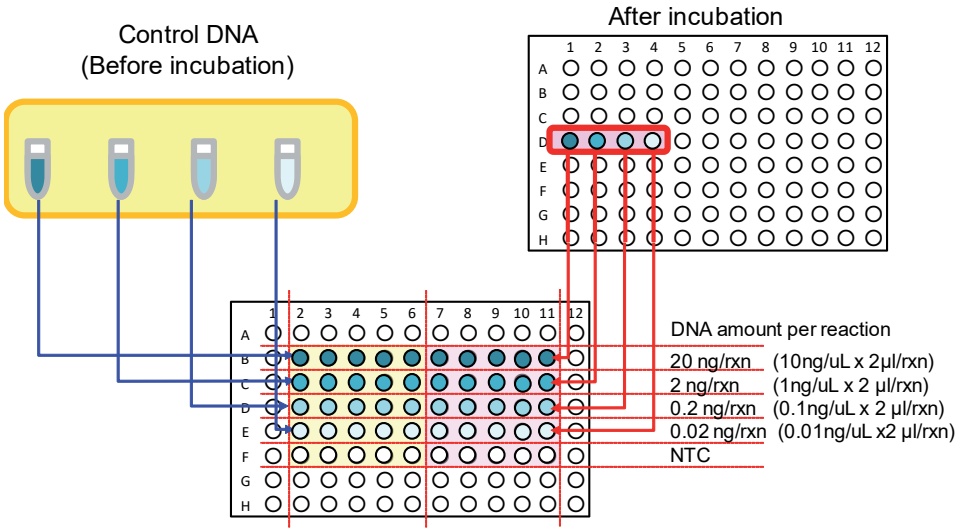


• qPCR cycle program

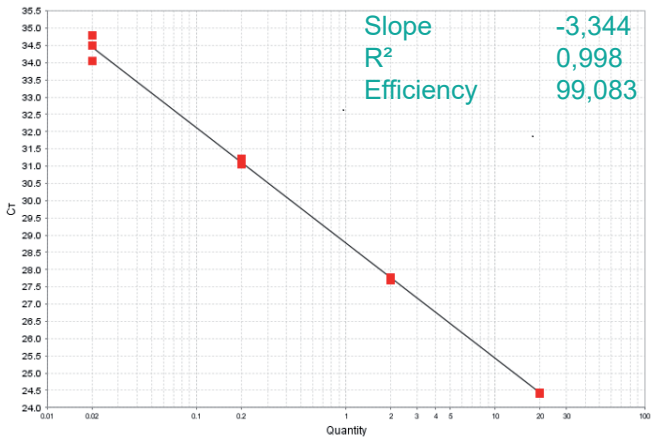
Step	Temp.	Duration	Cycles
Enzyme Activation	95 °C	3 min	
Denature Anneal/Extended	95 °C 62 °C	5 s 40 s }	40 cycles
Dissociation*	95 °C 60 °C 95 °C	15 s 1 min 15 s }	+0.3 °C stepwise

*default melt curve setting of StepOne Plus

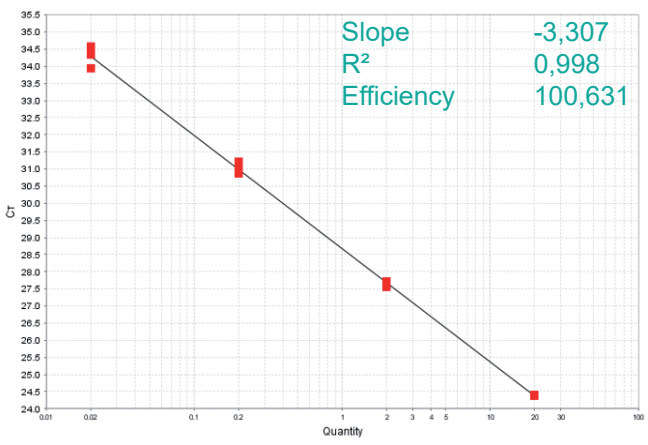
Results

Incubation temperature: 4°C

Before incubation (control)



After incubation



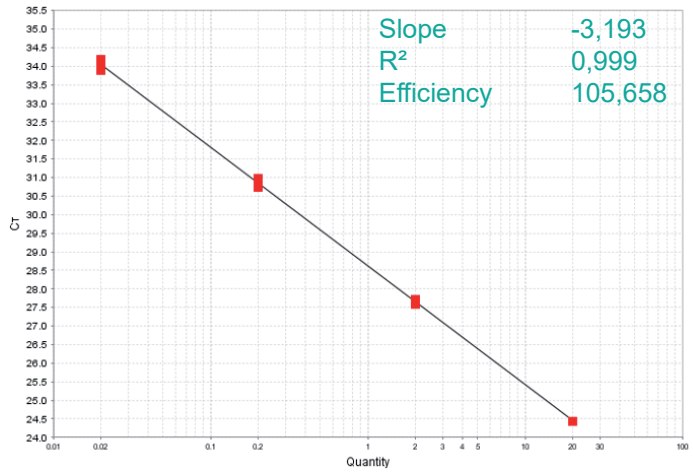
	Ct value (before incubation)	Ct SD	Ct value (after incubation)	Ct SD	Difference*
20 ng / rxn	24.43	0.017	24.39	0.018	-0.04
2 ng / rxn	27.74	0.036	27.64	0.078	-0.10
0.2 ng / rxn	31.13	0.079	31.02	0.181	-0.11
0.02 ng / rxn	34.45	0.380	34.28	0.318	-0.16

*Difference=(Ct value after incubation) - (Ct value before incubation)

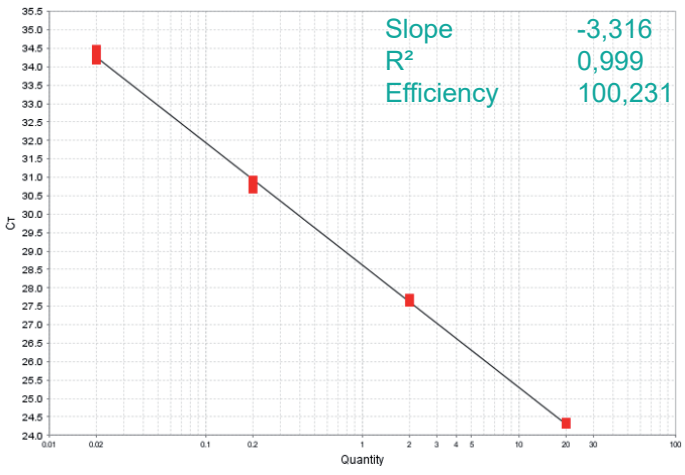
There could not be found a significant difference between the Ct value before and after the incubation by 4 °C. Based on these results, it can be assumed that no binding of DNA to the plate has occured.

Incubation temperature: 37°C

Before incubation (control)



After incubation



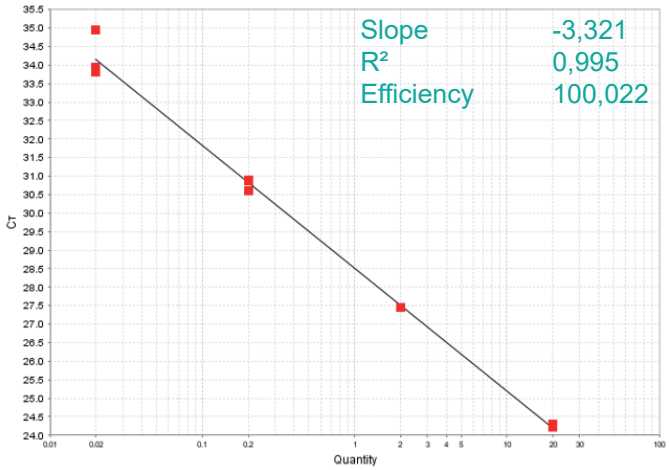
	Ct value (before incubation)	Ct SD	Ct value (after incubation)	Ct SD	Difference*
20 ng / rxn	24.43	0.010	24.32	0.027	-0.11
2 ng / rxn	27.66	0.062	27.68	0.046	0.01
0.2 ng / rxn	30.89	0.131	30.77	0.132	-0.12
0.02 ng / rxn	34.00	0.150	34.35	0.158	0.34

*Difference=(Ct value after incubation) - (Ct value before incubation)

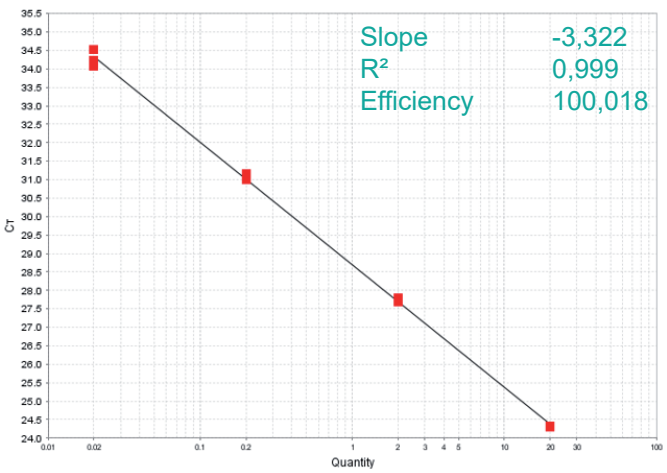
There could not be found a significant difference between the Ct value before and after the incubation by 37 °C. Based on these results, it can be assumed that no binding of DNA to the plate has occured.

Incubation temperature: 65°C

Before incubation (control)



After incubation



	Ct value (before incubation)	Ct SD	Ct value (after incubation)	Ct SD	Difference*
20 ng / rxn	24.26	0.037	24.32	0.012	0.05
2 ng / rxn	27.46	0.007	27.75	0.053	0.29
0.2 ng / rxn	30.79	0.154	31.08	0.087	0.29
0.02 ng / rxn	34.23	0.619	34.28	0.235	0.05

*Difference=(Ct value after incubation) - (Ct value before incubation)

There could not be found a significant difference between the Ct value before and after the incubation by 65 °C. Based on these results, it can be assumed that no binding of DNA to the plate has occured.

Conclusion

The incubation tests under all three conditions (4 °C, 37 °C, 65 °C) showed no significant decrease of DNA concentration after incubation in comparison with the control DNA amount before incubation. Thus no DNA binding to the FastGene® plate (FG-1702) was observed by qPCR detection. This means however, there are no difficulties to perform qPCR quantification even with the lowest input of DNA amount in NGS applications, since there is no measureable effect of the plate on the results.