MAb test on PCR product as template.

When using genomic DNA as template in a PCR reaction an initial long (minutes) denaturation step is used before the cycling. To omit this denaturation step, which could also activate the hot-start taq, tests were performed with PCR product as template.

Hot start Taq was made by the following recipe.

Mab concentration				
	1 µg/ 5U Taq	0.5 µg/ 5U Taq	0.1 µg/ 5U Taq	
10xBuffer	4	4	4	µl
Taq 40U/µl	1	1	1	µl
MAb	9	4,5	0,1	µl
H2O	26	30,5	34,1	µl
Incubate for 10 min at room temp.				

1µl template was added to 250µl PCR master mix. The tube was thoroughly vortexed and 10µl PCR mix was added to 24 wells. This was done for the 4 different reaction conditions, 1µg MAb, 0,5µg MAb 0,1µg Mab or 0µg MAB (not hot-start Taq).

PCR product was diluted 1:1 000 000 and 1:100 000 000

PCR mix and cycling are presented below.

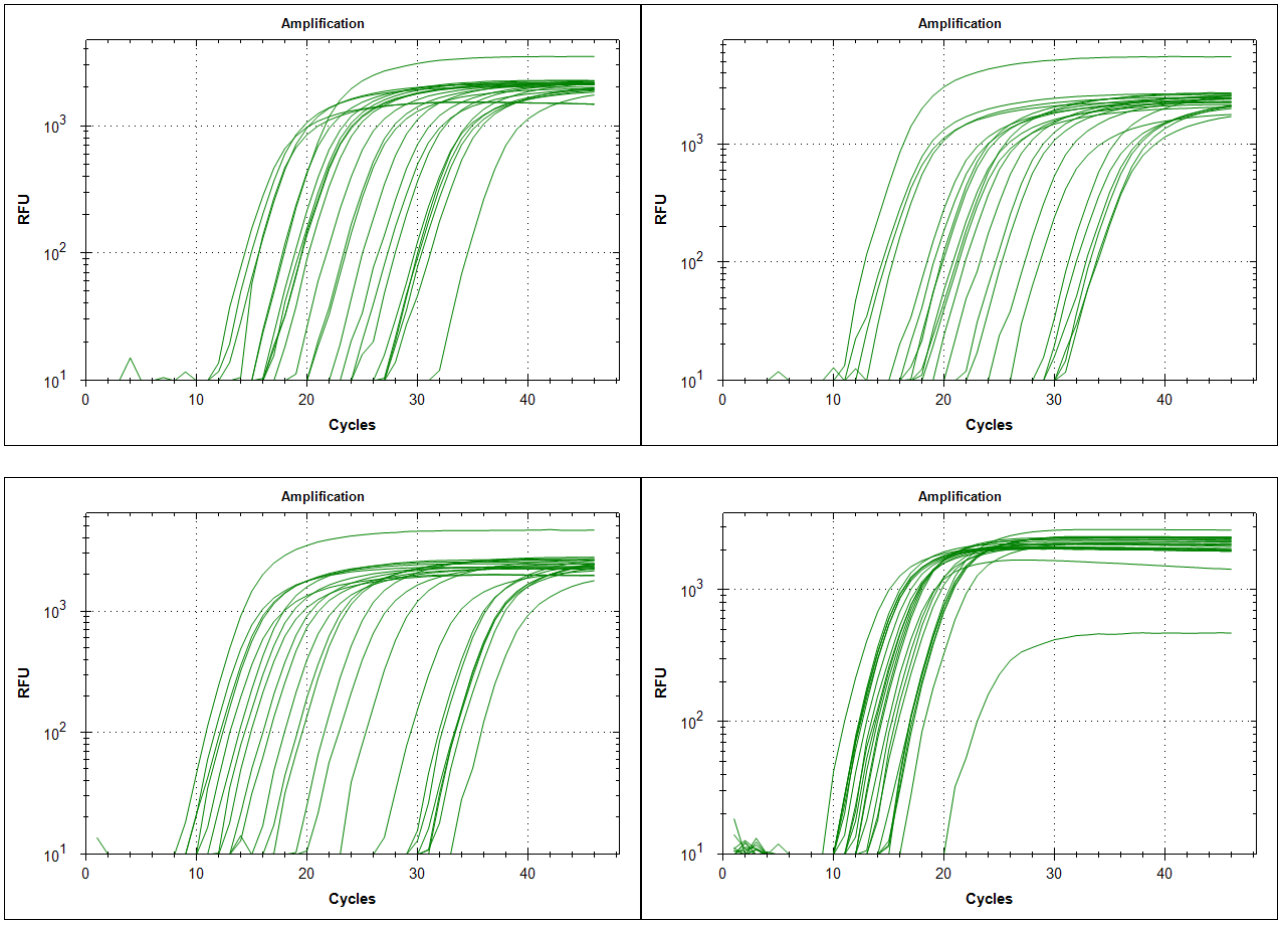
Fragment:	**Mito**		A56°C	Date	25.05.2021		
PCR volum, µl	10		# of reactions		25		
	Working						
	solutions		Total volume 250 µl		Desired		
	concentration		Volum		concentration		
H2O			187,75				
10X Thermopol #1	0	mM MgCl	25,00				
MgCl	200	mM	2,50		2,00001	mM	
Primer forward	100	µM	0,75		0,3	µM	94C-20sec
Primer reverse	100	µM	0,75		0,3	µM	A 56C-20sec 45cycles
Fam GC-Clamp	100	µM	0,00		0,00001	µM	72C-40sec
dNTP	100	mM	1,00		400	µM	Melting
PCR prod	250	ng	1,00		1	ng/µl	
EVA green 20X			10,00		0,8	X	
BSA	100	%	2,50		1	%	
Taq-Mab (1, 05, or 0,1)	1	U/µl	18,75		0,075	U/µl	
PFU (Paulo)	75	U/µl	0,00		0	U/µl	

Fragment:	**Mito**		A56°C	Date	25.05.2021		
PCR volum, µl	10		# of reactions		25		
	Working						
	solutions		Total volume 250 µl		Desired		
	concentration		Volum		concentration		
H2O			206,03				
10X Thermopol #1	0	mM MgCl	25,00				
MgCl	200	mM	2,50		2,00001	mM	
Primer forward	100	µM	0,75		0,3	µM	
Primer reverse	100	µM	0,75		0,3	µM	
Fam GC-Clamp	100	µM	0,00		0,00001	µM	
dNTP	100	mM	1,00		400	µM	
PCR prod	250	ng	1,00		1	ng/µl	
EVA green 20X			10,00		0,8	X	
BSA	100	%	2,50		1	%	
Taq	40	U/µl	0,47		0,075	U/µl	
PFU (Paulo)	75	U/µl	0,00		0	U/µl	

Amplification curves for 1, 0.5, 0.1 and 0 µg Mab. (10^{-6} dilution of template)

Cq values estimated by linear regression for the 24 technical replicates.

	Mean Cq	1 STD
1µg	22,8	5,92
0,5µg	23,5	6,50
0,1µg	21,7	8,60
0µg	14,6	2,39



Amplification curves for 1, 0.5, 0.1 and 0 µg Mab. (10^{-6} dilution of template).

Cq values estimated by linear regression for the 24 technical replicates.

	Mean Cq	1 STD
1µg	29,7	4,61
0,5µg	28,6	5,47
0,1µg	20,0	3,93
0µg	17,6	2,93