TripleMaster® PCR System

Applications:
- very long targets (genomic > 20 kb; episomal up to 50 kb)
- long targets (genomic 10 - 20 kb, episomal 10 - 40 kb)
- 150 bp – 10 kb targets with high fidelity for cloning and sequencing purposes
- rare and long cDNA from RT reactions
- GC-rich and other difficult templates

Table 1: Buffer selection guide

Buffer Choice	Target size in kb						Template DNA
	< 0.5	0.5 - 1	1 - 5	5 - 10	10 - 20	> 20	
Tuning Buffer	-	+/-	+	++	++	+++	Plasmid
	+/-	+	++	+++	+++	+++	Phage
	+/-	+	++	+++	+++	+++	Genomic
HighFidelity Buffer	+++	+++	++	++	+	-	Plasmid
	+++	+++	++	++	+	-	Phage
	+++	+++	++	+	+/-	-	Genomic

+++	high yield/high specificity	++	good yield/high specificity
+	lower yield or reduced specificity	+/-	low yield and/or low specificity depending on target
-	not suitable		

Ansatz:

SAMPLE NUMBER COMPONENT	1X ANSATZ [ML]
Master Mix1	20
H₂	
Pr	
Pr	
Te	
fo	
fo	
fo	
M	
H₂	
10	
dN	
Tr	

Table 3: Program parameters optimized for the 40 kb control PCR

Cycle no	Cycle	Step	Temp.	Time	Description
		1	**93°C**	**3 min**	Initial template denaturation
1 - 10	**10x**	2	**93°C**	**15 sec**	Template denaturation
		3	62°C	**30 sec**	Primer annealing
		4	**68°C**	21 min	Primer extension/elongation
11 - 38	8x - 28x	5	**93°C**	**15 sec**	**Template denaturation**
		6	62°C	**30 sec**	Primer annealing
		7	**68°C**	21 min + **20 sec**	Primer extension/elongation

* Time increment of 20 seconds; for each elongation step the time is extended by 20 seconds (21 min 20 sec in 11th cycle; 21 min 40 sec in 12th cycle, etc).

Table 4: Variable cycling parameters optimized for various long targets

	Human genomic targets								Episomal targets				
	tPA					β globin		Factor VIII	λDNA				
Size (kb	15	18	22	24	27	23	29.8	29.7	10	20	30	35	40
Annealing temp (°C)	65	65	65	65	65	62	62	57	62	62	62	62	62
Elongation time (min)	10	15	20	21	27	18	18	24	8	15	20	21	21
Cycle no.	27	27	27	27	28	28	28	28	18	18	18	18	18
Total cycles	10+	10+	10+	10+	10+	10+	10+	10+	10+	10+	10+	10+	10+