Genetics NEWS

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Products for PCR

efficiency
polymerase
convenience
proof-reading
engineered enzyme
archeal type B polymerase
endpoint PCR
incl. loading dye
ReadyMix
cDNA
direct PCR
incl. dNTPs
high GC content

FastGene® Optima

tissue
best results
HotStArt
ANTIBODY
low copy
long range
SNP
genotyping
gene knock out
multiplex PCR
routine PCR
mouse tail
very pure
optimized blend
high-thoughput
complex templates

FastGene® Optima
(#LS28, 250 units incl. dNTPs)

FastGene® Optima HotStart ReadyMix
(#LS29, 500 x 25 µl reactions)

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FastGene® Optima^HotStart ReadyMix for the

Genotyping of knock-out mice

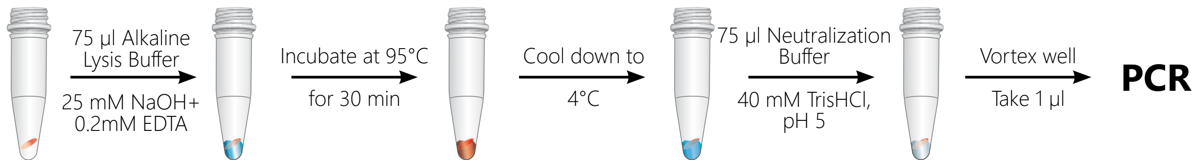
User's comment

„PCR is essential for us to detect gene-knock out. The FastGene® Optima^HotStart ReadyMix suppressed non-specific amplififcation and produced easy to analyze results. Inexperienced freshman students of the medical school could successfully perform genotyping without making any mistakes."

The following data were provided by the courtesy of Dr. Mamoru Aoto
Department of Circulatory Physiology, Graduate School of Medicine, Ehime University, Japan.

Experimental conditions

Extraction of DNA from a 2 mm mouse tail tissue:



PCR Reagents:
1. FastGene® Optima HotStart ReadyMix with loading dye (#LS29)
2. Competitor T's enzyme ready mix with loading dye

Template:
1 µl of DNA isolated from mouse tail tissue

PCR product size:
~163 bp

PCR Setup:

FastGene® Optima^HotStart ReadyMix	
Primer set (10 µM)	2.5 µl
Template: mouse tail DNA	1 µl
2 x Master Mix with enzymes and loading dye	12.5 µl
H_2O	9 µl
Total	25 µl

Competitor T's polymerase	
Primer set (10µM)	2.5 µl
Template: mouse tail DNA	1 µl
10 x Buffer	2.5 µl
MgCl (2.5 mM)	2 µl
dNTP (2.5 mM)	4 µl
PCR enzyme mixture	0.25 µl
H_2O	39.3 µl
Total	50 µl

PCR system:
T100 Thermal Cycler (BIO-RAD)

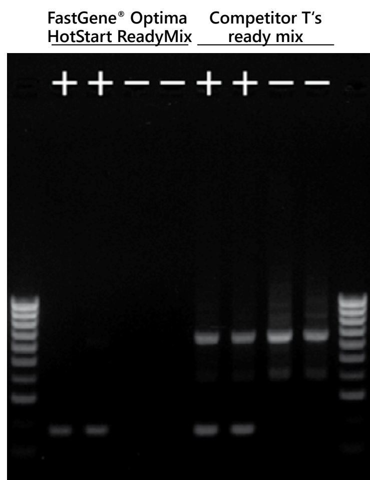
PCR cycling conditions:

Step	Temperature	Time	
Initial denaturation	95°C	3 min	
Denaturation	95°C	15 sec	35 cycles
Annealing	55°C	15 sec	35 cycles
Extension	72°C	15 sec	35 cycles
Final Extension	72°C	5 min	

Electrophoresis conditions:

Concentration of Agarose	1 %
Voltage	100 V
Time	30 min
Sample volume	10 µl

Results



The PCR specificity and easiness to use the kits were analyzed for *FastGene® Optima^HotStart ReadyMix* and a competitor. The results can be summarized as follows:

- **FastGene® Optima^HotStart ReadyMix did not show non-specific amplicons.**
- Competitor resulted in the presence of a large non-specific band.
- Amplifcation efficiency was equally high for both enzymes.
- PCR preparation was very easy with the ReadyMix.
- Electrophoresis was easily done since it already contained a loading dye.

FastGene® Optima for the

Amplification of GC-Rich DNA

User's comment

„We saw less of non-specific amplification with GC-Rich fragments when using the FastGene® Optima. The sequencing results were at least comparable to those of competitor T's long amplicon reagent mixture."

The following data were provided by the courtesy of Ms. Ryoko Nakayama
Department of Pathology, Tsurumi University School of Dental Medicine, Japan.

Experimental conditions

The comparison of FastGene® Optima was done with a polymerase mixture designed for long amplifications from competitor T. Amplification of two GC-rich amplicons were done using the following reaction mixture:

PCR Reagents:
1. Competitor T enzyme Mix
2. FastGene® Optima (#LS28)

Template:
cDNA was created by reverse transcribing RNA isolated from a cell line of human origin

PCR product size and GC-content:
1. 1839 bp and 60.7%
2. 1260 bp and 64,3%

PCR Setup:

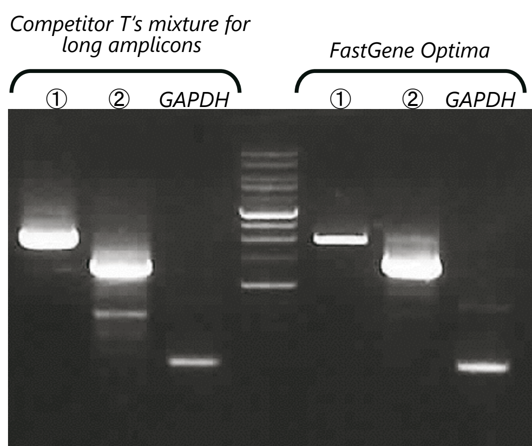
Competitor T's Polymerase mixture for large amplicons		
Primer set	1 µl	1 µl
Template: cDNA of a human cell line	2 µl	0.5 µl
2 x Buffer (GC-rich template)	12.5 µl	12.5 µl
dNTP	2 µl	2 µl
Enzyme mixture	0.2 µl	0.2 µl
H_2O	7.3 µl	8.8 µl
Total	25 µl	

FastGene® Optima		
Primer set	1 µl	1 µl
Template: cDNA of a human cell line	2 µl	0.5 µl
5 x Buffer (GC-rich template)	5 µl	5 µl
$MgCl_2$	2.5 µl	2.5 µl
dNTP	1 µl	1 µl
FastGene® Optima	0.1 µl	0.1 µl
H_2O	13.4 µl	14.9 µl
Total	25 µl	

PCR cycling conditions:

Step	Temperature	Time	
Initial denaturation	94°C	5 min	
Denaturation	94°C	30 sec	35 cycles
Annealing	58°C	30sec	
Extension	72°C	2 min	
Final Extension	72°C	7 min	

Results



Competitor T's reagent and FastGene® Optima successfully amplified fragment (1) and (2). The specificity of both enzyme mixtures was high for both amplicons.

Amplified fragments were inserted into T-easy vector and transformed into E. coli. A selection of clones were send for sequencing to determine the number of mutations.

	Sequence	Clone No.	Determined sequence (bp)	Number of mutation	Number of mutation per Kb
1	Optima	18	1338	0	0.00
		20	422	1	2.37
		22	720	1	1.39
	Competitor T	6	1358	3	2.21
		8	608	4	6.58
2	Optima	1	1264	0	0.00
	Competitor T	1	1264	0	0.00
		11	381	1	2.62

Mutations were found in both enzyme mixtures. We analyzed however only a limited number of clones. Therefore, it is difficult to evaluate the accuracy. Nonetheless, FastGene® Optima had less mutations in when compared to competitor T.

FastGene® Optima HotStart ReadyMix for the

Direct PCR from *Escherichia coli* colonies

User's comment

„The PCR products using the FastGene® Optima HotStart ReadyMix with loading dye resulted in a clearer electrophoresis pattern when compared to competitor T's ready mix, since no smearing was observed. This smearing observed with the competitor's ready mix made the determination of the presence of the insert quite difficult. This is interesting, since the starting material (1 colony) was very similar. The starting amount was probably inadequate for the competitor's enzyme. For the FastGene® Optima HotStart ReadyMix this was clearly not the case since one could clearly distinguish between colonies with insert (~800 bp) and without (~300 bp). Interestingly, good results were obtained using only 12.5 µl of reaction volume."

The following data were provided by an institutional customer in Japan.

Experimental conditions

PCR Reagents:

1. FastGene® Optima HotStart ReadyMix with loading dye (#LS29)
2. Competitor T's enzyme ready mix with loading dye

Template:

24 transformed Escherichia coli colonies for each ready mix

PCR product size:

~800 bp (with insert) or
~300 bp (without insert)

PCR system:

Thermal Cycler Dice (Takara)

PCR Setup *(for both polymerases)*:

FastGene® Optima *HotStart ReadyMix* with loading dye or Competitor T's ready mix with loading dye	
Primer set	0.5 µl (0.25µM)
Template: E. coli colony ***directly*** added to the reaction	1
2 x Master Mix with enzymes and loading dye	6.25 µl
H_2O	5.75 µl
Total	**12,5 µl**

PCR cycling conditions:

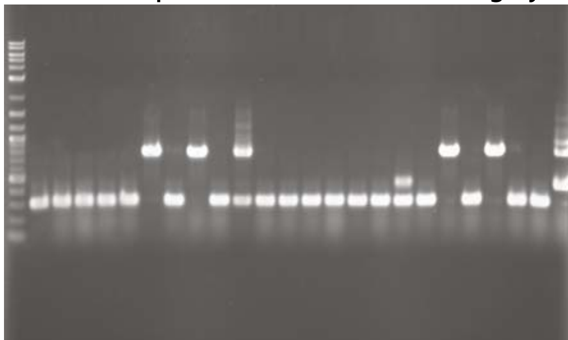
Step	Temperature	Time	
Initial denaturation	95°C	5 min	
Denaturation	95°C	30 sec	35 cycles
Annealing	55°C	30 sec	35 cycles
Extension	72°C	2 min	35 cycles
Final Extension	72°C	5 min	

Electrophoresis conditions:

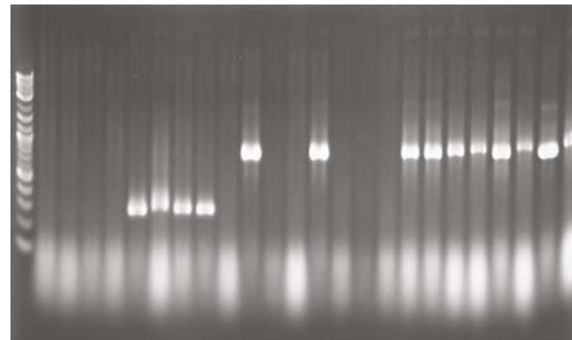
Parameter	Value
Concentration of TAE- Agarose	1.2 %
Voltage	135 V
Time	30 min
Sample volume	5 µl

Results

FastGene® Optima *HotStart ReadyMix* with loading dye



Competitor T's ready mix with loading dye



The electrophoresis pattern looked much better with FastGene® Optima HotStart ReadyMix, when compared to the results obtained using the competitor's ready mix. Additionally, more primer dimers were observed when using the competitor's ready mix.

FastGene® Optima for the

Amplification of complex DNA Templates

User's comment

„Almost no primer dimer were observed when using FastGene® Optima, especially when compared with competitor T's enzyme mixture. The amplification efficiency of the FastGene® Optima was high as well."

The following data were provided by an institutional customer in Japan.

Experimental conditions

Comparison of the FastGene® Optima and a polyermase mixture of a competitor T was performed using the following conditions:

PCR Reagents:
1. Competitor T enzyme Mix
2. FastGene® Optima (#LS28)

Template:
cDNA was created by reverse transcribing RNA isolated from catshark liver tissue.

PCR system:
GeneAmp PCR System 9700 (ABI)

PCR Setup:

Competitor T's polymerase mixture for complex templates	
Primer set	1 µl (0.5µM)
Template: complex catshark's liver cDNA	0.5 µl (5ng)
10 x Buffer	5 µl
dNTP	4 µl
PCR enzyme mixture	0.2 µl
H_2O	39.3 µl
Total	50 µl

FastGene® Optima	
Primer set	1 µl (0.5µM)
Template: complex catshark's liver cDNA	0.5 µl (5ng)
5 x Buffer	10 µl
$MgCl_2$	4 µl
dNTP	1.5 µl
FastGene® Optima	0.2 µl
H_2O	32.8 µl
Total	50 µl

PCR product size:
1030 bp

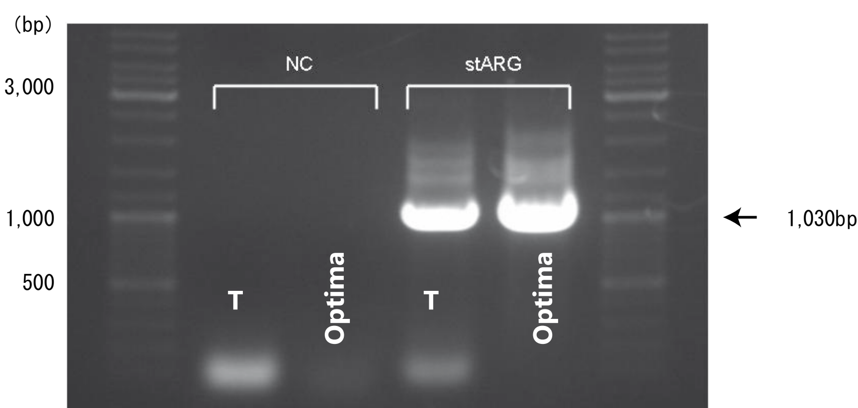
PCR cycling conditions:

Step	Temperature	Time	
Initial denaturation	94°C	2 min	
Denaturation	94°C	1 min	35 cycles
Annealing	59°C	45 sec	35 cycles
Extension	72°C	1 min	35 cycles
Final Extension	72°C	10 min	

Electrophoresis conditions:

Parameter	Value
Concentration of Agarose	1.2 %
Voltage	100 V
Time	35 min
Sample volume	20 µl

Results



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