Streamlining the Genotyping Workflow with the MultiGene OptiMax Thermal Cycler

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Common laboratory research practice utilises PCR to validate transgenic mouse lines. Validation of these lines typically involve multiple primer sets with various annealing temperatures leading to a very tedious and time consuming process.

To allow researchers the opportunity to evaluate multiple transgenes within one PCR reaction, Labnet offers the MultiGene OptiMax. Traditional thermal cyclers utilise a Peltier microchip block that is enabled for either homogeneous or gradient temperature mode. Additionally, with a traditional thermal cycler, a user can only utilise one annealing temperature per experiment. The new Labnet MultiGene OptiMax has six distinct Peltier microchip elements that allow users to select up to six different annealing temperatures. This allows for the possibility to evaluate multiple genes in one experiment.

The focus of this study was to evaluate the ability of the Labnet MultiGene OptiMax to perform multiple PCR reactions with various annealing temperatures within one experiment. The mouse line used in this application note is a transgenic PTD mouse. Typically, the validation for this line requires three separate PCR runs that are more than three hours each. The Labnet MultiGene OptiMax decreased the time required for these PCR experiments by completing the validation with one PCR run, which was performed in approximately three hours. The results depicted here demonstrate the ability to achieve similar product yields using the Labnet Multigene Optimax compared to a traditional thermal cycler. Taken together, the Labnet thermal cycler provides users with an option for a time saving programming mode to quickly validate transgenic mouse lines.

Materials and Methods

Transgenic Mouse

The three transgenes of interest in the PTD mouse line are the P (promoter), T (F1/F1 – floxed allele), and D (reporter allele) modified endogenous genes. The selected PCR primers for each have annealing temperatures of 51.7°C, 65.0°C, and 60.0°C, respectively.

DNA extraction

For the DNA extraction, 1mm of mouse tail was excised, added to 75µL of alkaline lysis buffer (25 mM NaOH, 0.2 mM EDTA, pH 12) and vortexed for 1 minute. The extract mixture was then incubated for 30 minutes at 37°C and vortexed briefly. Lastly, 75 µL of the neutralising solution (40 mM Tris-HCl, pH 5) was added to the DNA extraction tube and briefly vortexed again.

PCR set up

A master mix was prepared based on the amount of PCR reactions performed during each study. To prepare the mix, 62.5 µL of OneTaq® 2X Master Mix with Standard Buffer (New England Biolabs, Cat. No. M0482L), 5 µL of each primer, and 27.5 µL of H2O was added to a microcentrifuge tube. For each PCR reaction, 25 µL of the master mix was aliquoted into a 0.2mL PCR tube (BioExpress C-3310-1).

When programming the MultiGene OptiMax, Blocks 1 2, and 3 were assigned for the P, D, and T genes respectively. The ramp rate on the MultiGene OptiMax is 5°C /sec heating and 3.5°C /sec cooling.

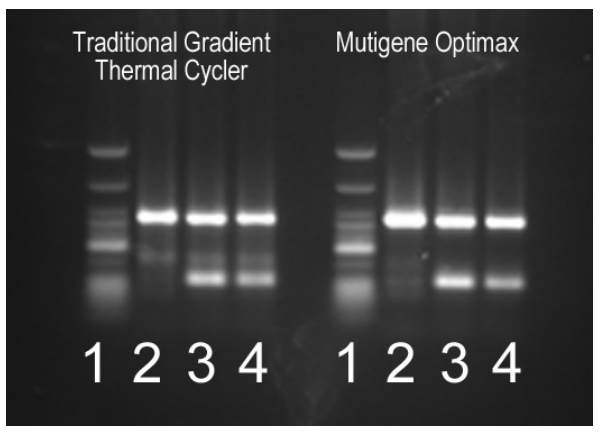
PCR Methods

P Gene	*D Gene*	*T Gene*
94°C for 3 mins	94°C for 3 mins	94°C for 3 mins
35 cycles of:	35 cycles of:	35 cycles of:
94°C for 30 secs	94°C for 30 secs	94°C for 30 secs
51.7°C for 1 min	61.0°C for 1 min	65.0°C for 1 min
68°C for 1 min	68°C for 1 min	68°C for 1 min
Then	Then	Then
68°C for 2 mins	68°C for 2 mins	68°C for 2 mins
4°C hold	4°C hold	4°C hold

Electrophoresis

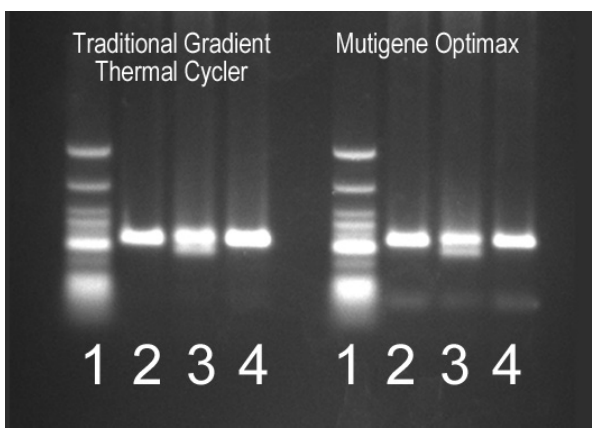
Following the PCR reaction, 12.5µL of the samples were loaded onto a 2% agarose gel (BioExpress, Cat No. 0710-100G) containing 10µL of Ethidium bromide (Fisher, Cat. No. BP902-1) in 1X TAE (Fisher, Cat. No. BP902-1). Additionally, 15µL of the Quick-Load® Low Molecular Weight DNA Ladder (New England Biolabs, Cat. No. N0474S) was also loaded onto the gel. The gel was transferred to an electrophoresis unit, which was operated under constant voltage at 100 volts for 1 hour. Following electrophoresis, the gel was imaged using an Enduro GDS (Labnet, Cat No. GDS-1302).

Results



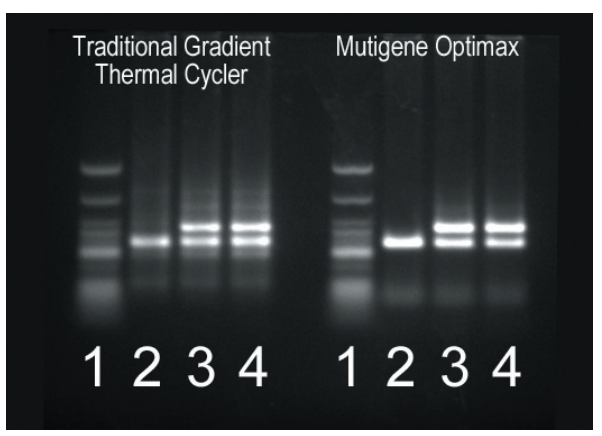
1- Ladder
2- WT (wild type)
3- P transgene + WT
4- P transgene +WT

Controls were from previously genotyped animals displaying either a P transgene or a Wild Type (WT) band. (Not depicted here)



1- Ladder
2- F1/F1 (homozygous of T gene)
3- Het (one copy of T gene + WT)
4- F1/F1 (homozygous of T gene)

Controls were from previously genotyped animals displaying either a WT or both WT gene bands. (Not depicted here)



1- Ladder
2- DR/DR (homozygous of D gene)
3- Het (one copy of D gene + WT)
4- HET

Controls were from previously genotyped animals displaying either homozygous for D gene or both WT and D gene bands. (Not depicted here).

Summary

Similar PCR product yields were obtained from both the thermal cycler and the MultiGene Optimax thermal cycler. When the traditional gradient cycler was used for three separate PCR experiments, the entire study was performed in approximately nine hours. In contrast, equivalent results were obtained with the MultiGene Optimax in approximately three hours.

Conclusion

With the ability to perform various PCR reactions with multiple annealing temperatures in a current manner, the MultiGene OptiMax is the ideal instrument for improved PCR efficiency in genotyping labs.

We wish to thank Labnet International for supplying the Enduro GDS imager, Enduro 300V power supply and the OptiMax Thermal Cycler. Furthermore, we would like to thank New England Biolabs, Inc and BioExpress for donating product for this application note.