ExCyto™ PCR DNA Amplification from a Single Bacterial Colony

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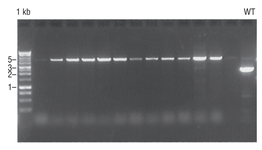
Abstract

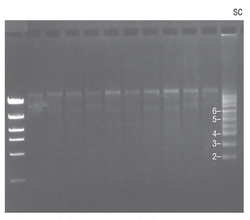
PCR amplification plays a critical role in many molecular biology procedures. A DNA template typically is purified and amplified using highly purified thermostable DNA polymerase. Alternatively, colony PCR only requires the addition of purified enzyme to a lysed cell preparation, omitting the step of template purification. ExCyto PCR is an advancement over colony PCR. In this system, the host *E. coli* cells express a chromosomally integrated gene for a thermostable DNA polymerase, allowing robust PCR amplification without the addition of exogenous enzyme. The resulting PCR products provide optimum substrates for plasmid analysis and sequencing. This approach will significantly reduce the cost of DNA amplification, while removing major bottlenecks in template purification.

Introduction

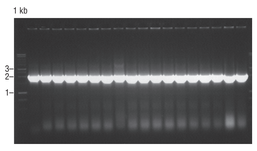
ExCyto PCR cells express a novel thermostable DNA polymerase. The thermostable DNA polymerase construct was integrated into the *E. coli* genome using the bacteriophage lambda RED recombinase system developed by Datsenko. The lambda Red gene products Gam, Bet and Exo are efficient at homologous recombination and are supplied on a low copy, temperature sensitive replicon. The gene to be moved into the chromosome was PCR amplified, along with a selectable gentamycin marker, with 500 bp of flanking sequence homologous to the chromosomal integration site. The amplified DNA was gel purified and transformed into the lambda RED containing host. Transformants were selected on gentamycin plates and the presence of the chromosomal integrant was confirmed by PCR analysis of the junctions involved and the expression of the thermostable DNA polymerase. The recombinase plasmid was eliminated from the host by growing at 42ºC and were checked for the absence of the plasmid.



PCR of MelA gene from Chromosome of the Integrants: From 2 µL of overnight grown cells from the integrants PCR was performed with primers flanking the integration site. Integrants show a band at 4.5 kb, WT has a band at 2 kb.



Plasmid Analysis of the Chromosomal Integrants: The chromosomal integrants were grown overnight and were checked for the plasmid by isolating DNA by standard method.



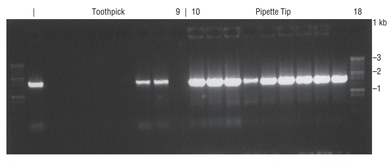
Expression of polymerase by the Chromosomal Integrants: A 2 kb insert from a low-copy plasmid was amplified by the colonies expressing the thermostable DNAP.

Methods

Proprietary formulations of cell lysis and single colony amplification were developed at Lucigen. Standard methods are used for vector+insert ligation and transformation of the ExCyto PCR Competent Cells. Following transformation and plating, pick a single colony with a pipette tip (do not use tooth picks as they may cause inhibition) and add 23 µL of the ExCyto PCR premix plus 1 µL of the each of the amplification primers. The size of the colony is critical and should be about 1 mm in diameter. Allow 15 hours of overnight incubation of plates. If the colony needs to be out grown for other purposes, place the tip into a culture tube containing media and antibiotics. Alternatively, 2µL of cells from overnight growth can be used instead of a colony sample. PCR is performed using the following cycling conditions:

The thermocycler was preheated to 94ºC.

Cycling step	Temperature	Time	# of Cycles
Initial Denaturation	94ºC	2 min	1
Denaturation	94ºC	15 sec	30
Annealing*	62ºC	15 sec	
Extension	72ºC	1 min/kb	
Final Extension	72ºC	10 min	1
Hold	4ºC	Indefinitely	1



Picking colonies with a toothpick may inhibit the PCR

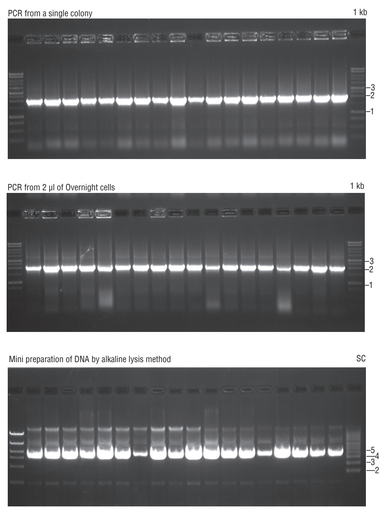
Comparison between the methods used to pick the colonies (Toothpick vs Pipette tip): The plasmid DNA was transformed into ExCyto PCR Electrocompetent cells and cells were plated on YT plates containing the appropriate antibiotics. Single colony PCR was performed as described in the methods. The colonies were picked with the toothpick (1-9) and with the pipette tip (10-18). It very clear that PCR worked from the colonies picked with the tip.

Legend For The Figures

Single colony PCR was performed as described in the methods and the colony was outgrown by placing the tip in the media containing the antibiotics. Using 2 µL of overnight grown cells, PCR was performed as described in the methods. The plasmids were isolated from cells using a standard alkaline lysis method.

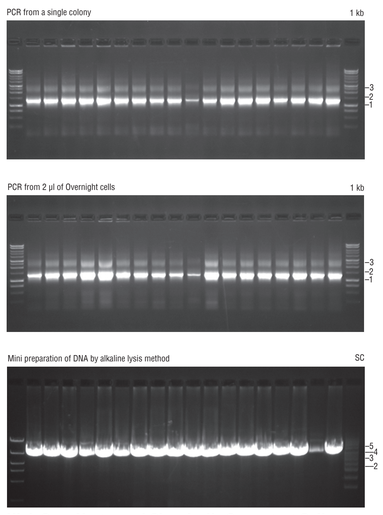
ExCyto PCR Chemically Competent Cells

Plasmid DNA



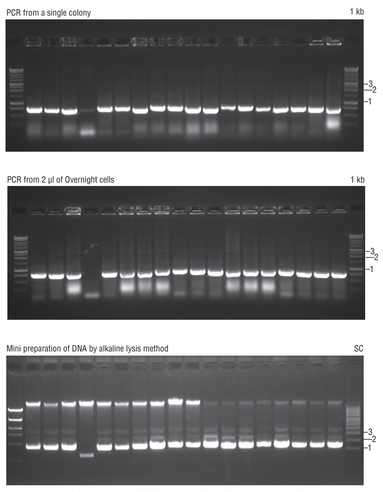
Plasmid DNA and Chemically Competent Cells: The plasmid DNA was transformed into ExCyto PCR Chemically Competent cells that were plated on YT plates containing the appropriate antibiotics.

High copy vector



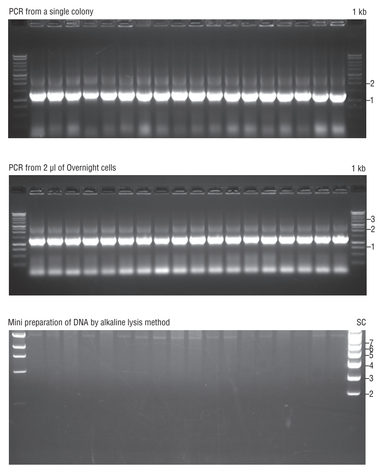
High Copy Vector and Chemically Competent Cells: The high copy empty vector DNA was transformed into ExCyto PCR Chemically Competent cells and were plated on YT plates containing the appropriate antibiotics.

Low copy vector



Low Copy Vector and Chemically Competent Cells: The Mel B gene was cloned into our low copy vector and the ligated product was transformed into ExCyto PCR Chemically Competent cells and were plated on YT plates containing the appropriate antibiotics.

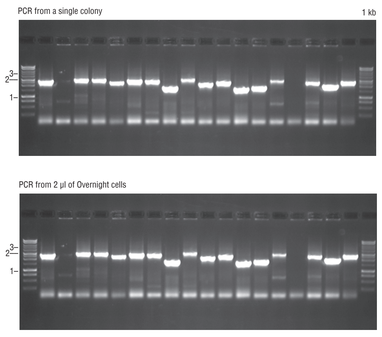
Single copy vector



Single Copy Vector and Chemically Competent Cells: The single copy vector DNA was transformed into ExCyto PCR Chemically Competent cells and were plated on YT plates containing the appropriate antibiotics.

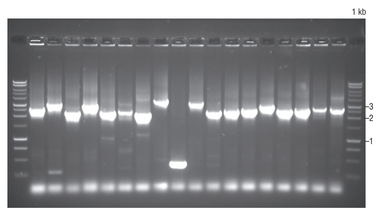
ExCyto PCR Electrocompetent Cells

High copy vector



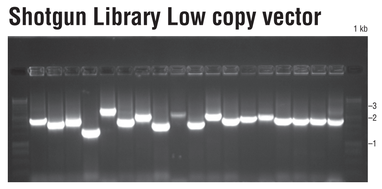
High Copy Vector and Electrocompetent Cells: A random shotgun library in a high copy vector was transformed into ExCyto PCR Electrocompetent cells and plated on YT plates containing the appropriate antibiotics.

Shotgun Library High copy vector



High Copy and Random Library: A random shotgun library in a high copy pEZSEQ vector was transformed into ExCyto PCR Electrocompetent cells and were plated on YT plates containing the appropriate antibiotics.

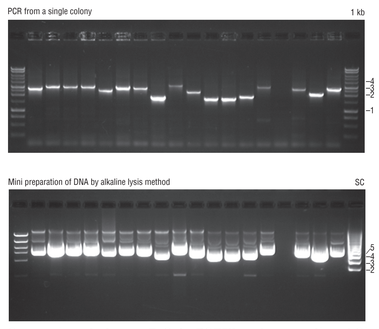
Shotgun Library Low copy vector



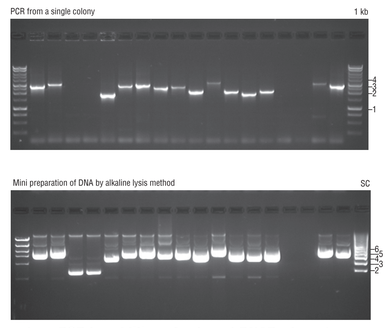
Low Copy and Random Library: A random shotgun library in a GC low copy vector was transformed into ExCyto PCR Electrocompetent cells and were plated on YT plates containing the appropriate antibiotics.

Comparison

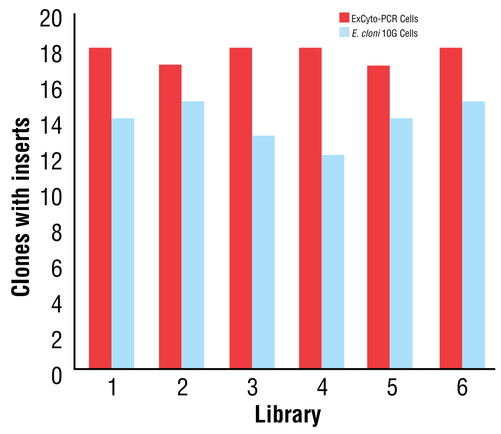
Single colony PCR from ExCyto PCR Electrocompetent cells



Conventional single colony PCR by addition of DNAP externally.



Comparison between ExCyto PCR™ and conventional Single colony PCR by addition of external DNAP: A random shotgun library in a high copy vector was transformed into ExCyto PCR Electrocompetent cells and were plated on YT plates containing the appropriate antibiotics. Single colony PCR was performed as described in the methods and the colony was outgrown by placing the tip in the media containing the antibiotics. The plasmids were isolated from these cells using a standard alkaline lysis method. Conventional single colony PCR performed by addition of 2.5 units of DNAP externally.



Higher efficiency of amplification from a single colony in ExCyto PCR cells: Six different shotgun libraries were transformed into ExCyto PCR electrocompetent cells and *E.cloni* 10G electrocompetent cells. Single colony PCR was performed as described in methods. Higher efficiency of amplification from a single colony in ExCyto PCR cells was observed compared to the conventional single colony PCR with addition of thermostable DNAP externally.

Summary

- Single colony PCR from the ExCyto PCR Competent Cells expressing the thermostable DNA polymerase was very efficient from high, low and single copy number plasmids and also from the chromosome. In case of single copy vectors the PCR yields were high compared to the plasmid DNA isolated by alkaline lysis method.
- ExCyto PCR from a single colony was more accurate and robust compared to the conventional single colony PCR using the externally added polymerase.
- ExCyto PCR from a single colony is very efficient from the colonies picked with the pipette tip compared to the colonies picked with the toothpick.
- ExCyto PCR method is user friendly, simple, time saving and much faster than the conventional minipreparation of the plasmid DNA. The DNA amplified from the single ExCyto PCR colony or overnight cells can be readily sequenced using the PCR primers.

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