

## ABSTRACT

Phage display technology places foreign peptides and proteins on the surface of filamentous bacteriophages. This methodology is used to identify peptide ligands to a wide variety of targets by screening for the ability to bind with high affinity and specificity. It has become a cornerstone method to investigate molecular interactions involving protein surfaces.

Library construction using existing technology can generate  $3 \times 10^8$  recombinants, which is adequate for coverage of the hexapeptide sequence space ( $20^6 = 6.4 \times 10^7$ ). Although random peptide libraries with longer amino acid sequences have been constructed, they are of limited utility because libraries are not sufficiently large to completely explore the additional sequence space.

Other variations of phage display, such as antibody display and cDNA display, incorporate large proteins into the virion. The functional utility of these libraries is also limited by the number of transformants that can be generated using the technology available today.

New strains of E. coli selected for enhanced DNA uptake can improve the transformation efficiency over existing methods by approximately ten-fold ( $3\text{--}5 \times 10^{10}$  cfu/ $\mu\text{g}$ ). These improvements can dramatically increase the absolute number of recombinants for these challenging applications, significantly reducing the cost to produce and screen phage display libraries of peptides and proteins.

## Methods

The electrocompetent cells were made using a proprietary method of cell preparation developed by Lucigen. This method produces electrocompetent cells that have higher transformation efficiencies than that of cells produced using traditional methods.

The transformation efficiency was tested by transforming 10pg of pUC19 DNA into 25  $\mu\text{L}$  of cells. A 1.00mm gap electroporation cuvette was used in a Bio-Rad Micro Pulser #165-2100 with settings of 10  $\mu\text{F}$ , 600 Ohms, 1800 Volts. Following the pulse, 975  $\mu\text{L}$  of Recovery Medium was added to the cuvette and the cells resuspend by pipeting up and down three times.

The cells and Recovery Medium were transferred to a culture tube and placed in a shaking incubator at 250 rpm for 1 hour at 37°C. The transformed cells were diluted 1/100 and spread on YT agar plates containing carbenicillin. The plates were incubated overnight at 37°C.

## Genotypes

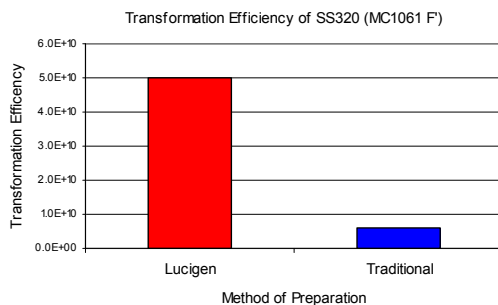
ER2738  
F' proA+B+ lacIq  $\Delta$ (lacZ)M15 zff::Tn10(TetR)/fluA2 glnV  $\Delta$ (lac-proAB) thi-1  $\Delta$ (hsdS-mcrB)5

TG1  
supE thi-1 D(lac-proAB)D(mcrB-hsdSM)5 (rK- mK-) [F' traD36 proAB lacIqZDM15]

SS320 (MC1061F)  
F' hsdR2 hsdM+ hsdS+ araD139  $\Delta$ (ara-leu)7697  $\Delta$ (lac)X74 galE15 galK16 rpsL (StrR) mcrA mcrB1

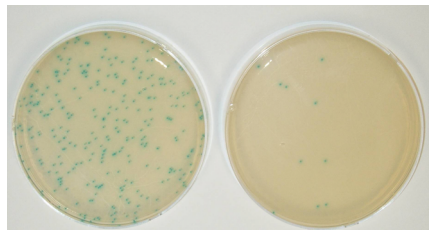
## Results

### Method Comparison for the Preparation of SS320 Cells



Method of Preparation	Transformation Efficiency
Lucigen	$5 \times 10^{10}$
Traditional	$6 \times 10^9$

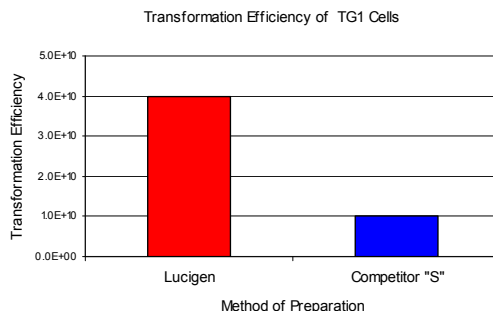
The Lucigen SS320 cells were compared with cells from prepared with traditional methods.



A Lucigen  
B Traditional  
Method of Preparation

Electrocompetent cells were prepared using the Lucigen proprietary protocol (A) and traditional protocol (B). Cells were transformed with pUC DNA and plated on YT plates containing carbenicillin, X-Gal and IPTG.

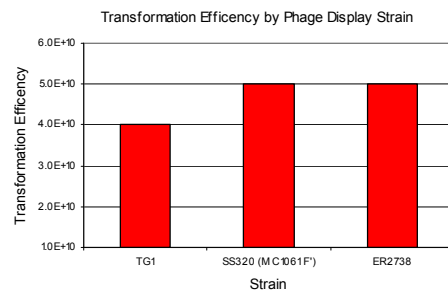
### Comparison of Transformation Efficiency of TG1 Cells



Company	Transformation Efficiency
Lucigen	$4 \times 10^{10}$
Competitor S	$1 \times 10^{10}$

The Lucigen TG1 cells were compared with cells from competitor "S" using pUC DNA.

### Phage Display Strains and Transformation Efficiencies



Cell Line	Transformation Efficiency
TG1	$4 \times 10^{10}$
SS320 (MC1061 F)	$5 \times 10^{10}$
ER2738	$5 \times 10^{10}$

Electrocompetent cells of various phage display strains were prepared using Lucigen's proprietary cell preparation method. The cells were transformed with pUC DNA.

## SUMMARY

- The proprietary protocol developed by Lucigen allows a greater transformation efficiency of E. coli strains to be achieved.

- Competent cells produced by Lucigen outperform the competition by up to five fold.

- The Lucigen method of preparation is transferable to a wide variety of E. coli strains.