

# eLucidations™ Application Note

## High Efficiency *E. coli* Strains for Phage Display Combinatorial Peptide Libraries

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### ABSTRACT

Phage display technology allows expression of foreign peptides and proteins on the surface of filamentous bacteriophages. This methodology is used to identify peptide ligands for a wide variety of targets by screening for the ability to bind with high affinity and specificity. Phage display 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.

*E. coli* strains manufactured using optimized methods designed to enhance DNA uptake can improve the transformation efficiency over existing methods by approximately ten-fold ( $2-4 \times 10^{10}$  cfu/ $\mu$ g). These improvements can dramatically increase the absolute number of recombinants for 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 10 pg of pUC19 DNA into 25  $\mu$ L of cells. A 1.00 mm gap electroporation cuvette was used in a Bio-Rad Micro Pulser #165-2100 with settings of 10  $\mu$ F, 600 Ohms, 1800 Volts. Following the pulse, 975  $\mu$ L of Recovery Medium was added to the cuvette and the cells resuspended by pipetting 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** (Cat # 60522-1 & 60522-2)  
*F'*[*proA*<sup>+</sup>*B*<sup>+</sup> *lacI*<sup>q</sup>  $\Delta$ (*lacZ*)*M15* *zzf::Tn10*(*Tet*<sup>R</sup>)]/*fhuA2*  
*glnV*  $\Delta$ (*lac-proAB*) *thi-1*  $\Delta$ (*hsdS-mcrB*)5

**MC1061 F-** (Cat # 60514-1 & 60514-2)  
*araD139*  $\Delta$ (*araA-leu*)7697  $\Delta$ (*lac*)X74 *galK16*  
*galE15*(*Gal*<sup>S</sup>)  $\lambda$ -*e14-mcrA0* *relA1* *rpsL150* (*Str*<sup>R</sup>)  
*spoT1* *mcrB1* *hsdR20020*

**SS320 (MC1061F')** (Cat # 60512-1 & 60512-2)  
*F'*[*proAB* *lacI*<sup>q</sup>Z  $\Delta$ *M15* *Tn10* (*Tet*<sup>R</sup>)] *araD139*  $\Delta$ (*ara-leu*)7696 *galE15* *galK16*  $\Delta$ (*lac*)X74 *rpsL* (*Str*<sup>R</sup>) *hsdR2*  
(*r<sub>K</sub>*- *m<sub>K</sub>*+) *mcrA* *mcrB1*

**TG1** (Cat # 60502-1 & 60502-2)  
[*F'* *traD36* *proAB* *lacI*<sup>q</sup>Z  $\Delta$ *M15*] *supE* *thi-1*  $\Delta$ (*lac-proAB*)  $\Delta$ (*mcrB-hsdSM*)5(*rK* - *mK* -)

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## RESULTS

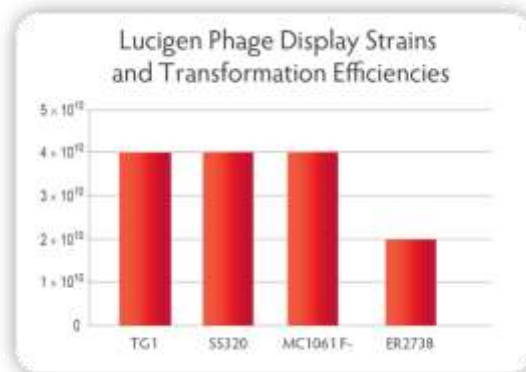
### Method Comparison for the Preparation of SS320 Cells



Method of Preparation	Transformation Efficiency
Lucigen	4 × 10 <sup>10</sup> cfu/μg
Traditional	6 × 10 <sup>9</sup> cfu/μg

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

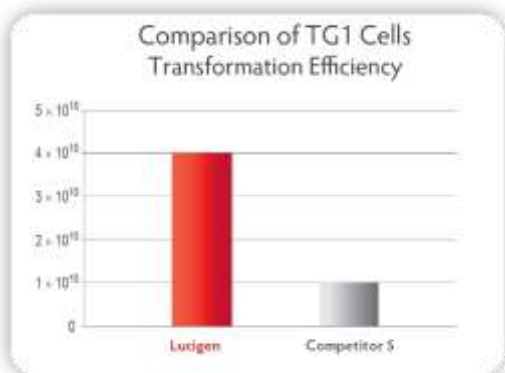
### Comparison of Phage Display Strains



Cell Line	Transformation Efficiency
TG1	4 × 10 <sup>10</sup> cfu/μg
SS320	4 × 10 <sup>10</sup> cfu/μg
MC1061 F-	4 × 10 <sup>10</sup> cfu/μg
ER2738	2 × 10 <sup>10</sup> cfu/μg

Transformation efficiency of Lucigen electrocompetent cells for phage display using pUC DNA.

### Comparison of Transformation Efficiency of TG1 Cells



Company	Transformation Efficiency
Lucigen	4 × 10 <sup>10</sup> cfu/μg
Competitor S	1 × 10 <sup>10</sup> cfu/μg

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

## SUMMARY

- The proprietary protocol developed by Lucigen allows greater *E. coli* host strain transformation efficiencies.
- Competent cells produced by Lucigen outperform competitor cells by up to 5-fold.
- Several strains of Lucigen's phage display competent cells are available with exceptional transformation efficiencies ( $\geq 2 \times 10^{10}$  cfu/μg with pUC DNA).