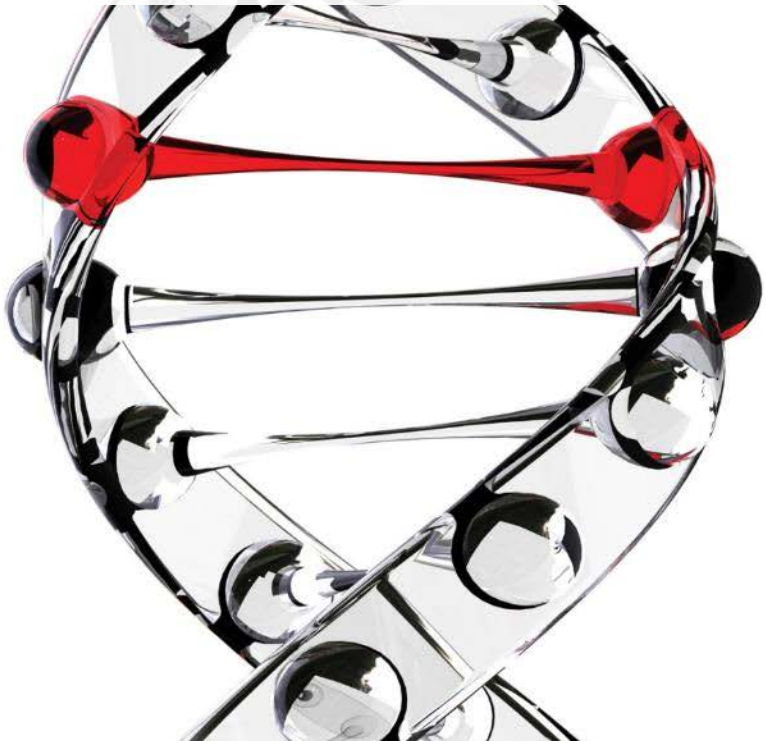
Competent Cells 101:

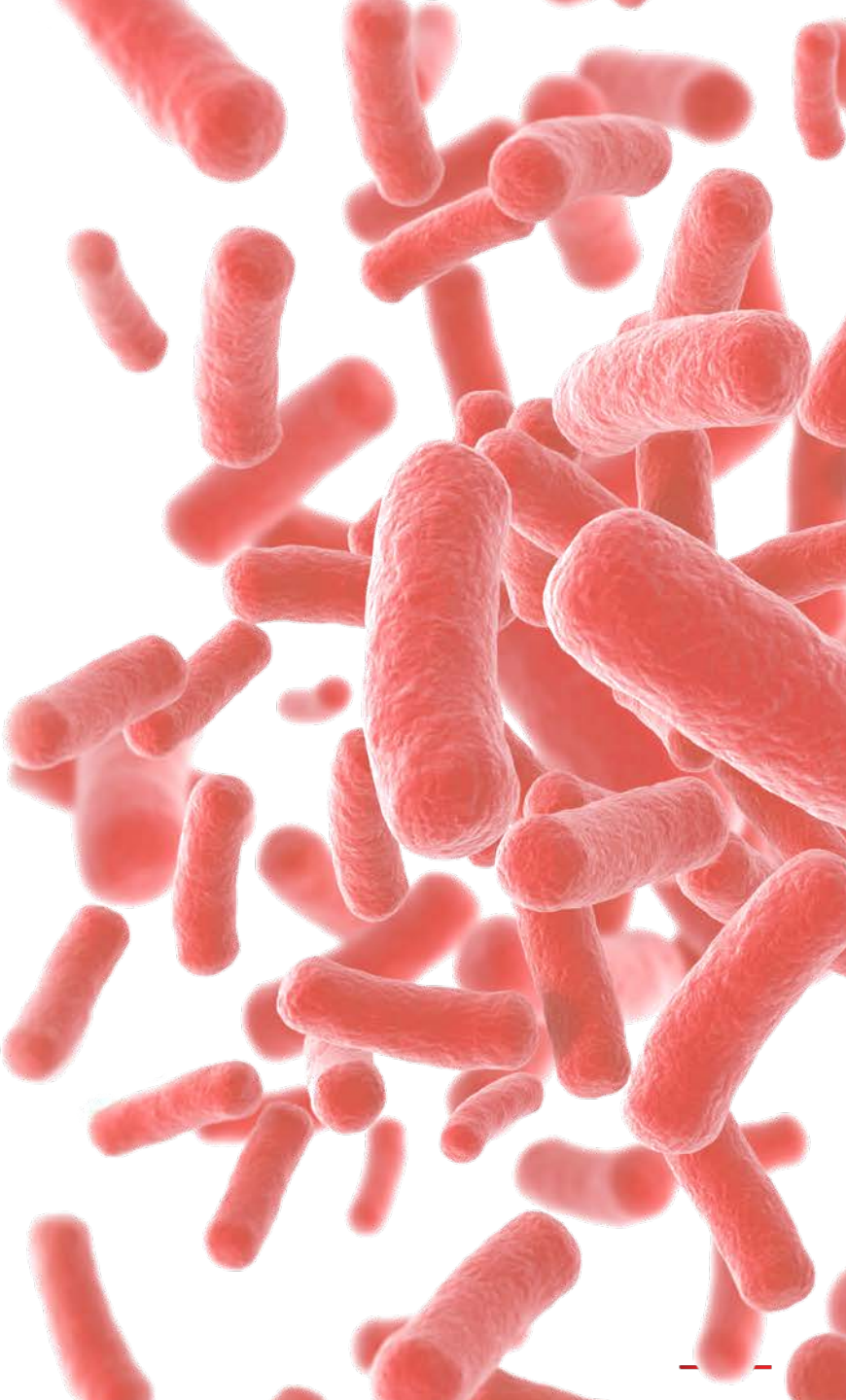
Maximizing Your Success in Cloning, Protein Expression, and Library Generation



Lucigen®
Simplifying Genomics

Agenda

- Selecting your competent cell format:
  - electrocompetent vs. chemically competent
- Maximizing transformation efficiency to create large libraries
- Choosing the right strain:
  - Strain genotypes and properties
  - Specialized strains for specific applications
- Engineered strains for low-endotoxin protein and plasmid preparation



Competent Cell Choices

Multiple differences between formats

Chemically Competent Cells:

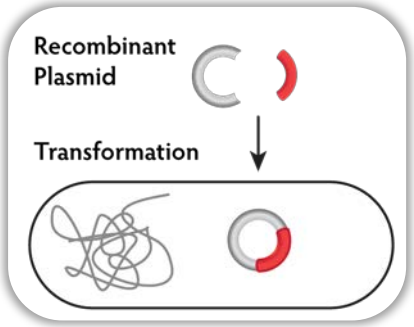
- Easy to work with, less expensive
- Do not require specialized equipment
- Accept larger DNA volumes
- Lower transformation efficiency than Electrocompetent Cells
- Used for routine cloning and plasmid propagation

Electrocompetent Cells:

- Shorter protocol, amenable to automation
- Highest transformation efficiency
- Require an electroporator instrument
- Require low DNA volumes
- Used for library construction and propagation

How Does Transformation Work?

Protocols are optimized for maximum cell viability and efficiency



Chemically Competent (Heat Shock) Transformation:

- Cells and DNA are incubated together on ice
- Salt masks the negative charge on DNA
- Heat shock (42°C) enhances DNA uptake
- Media is added and cells are “recovered” for 1 hour at 37°C

Electrocompetent Transformation:

- Cells and DNA are mixed immediately before electroporation
- Electrical current is applied, creating pores in the membrane
- Cold temperature is maintained to reduce cell damage
- Media is added and cells are “recovered” for 1 hour at 37°C

Transformation Workflow Comparison

Protocols have parallel steps, different time and equipment requirements

Chemically Competent Cells

1. Remove cells from -80°C and thaw on ice
2. Add DNA to cells
3. Incubate on ice 30 min
4. Heat shock 42°C 45 sec.
5. Incubate on ice 2 min
6. Add Recovery Medium
7. Incubate 1 hour at 37°C with shaking

~ 1 hour 45 minutes

Electrocompetent Cells

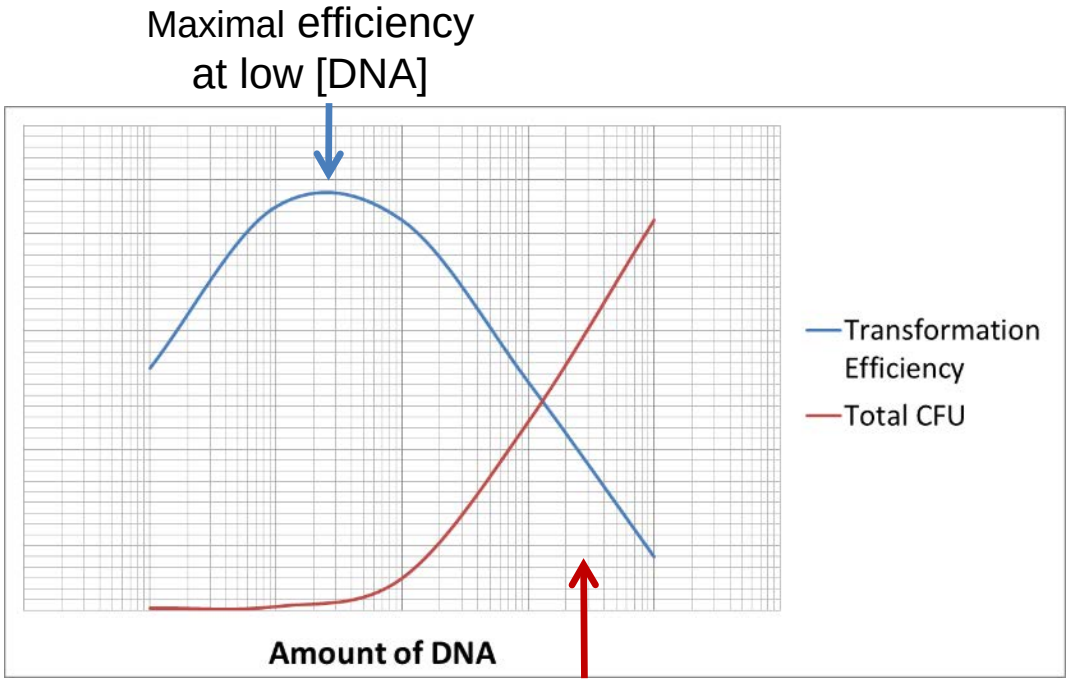
1. Remove cells from -80°C and thaw on ice
2. Add DNA to cells
3. Transfer cells + DNA to pre-chilled electroporation cuvette
4. Perform electroporation using recommended parameters
5. Immediately add Recovery Medium
6. Incubate 1 hour at 37°C with shaking

~ 1 hour 10 minutes

What is Transformation Efficiency?

Highest transformation efficiency is achieved with very low amounts of DNA

- Measures the rate at which cells take up plasmid and form colonies
- Reported as **C**olony **F**orming **U**nits per µg DNA (CFU/µg)
- Determined using intact, supercoiled plasmid DNA (10 pg of pUC19 plasmid is standard)



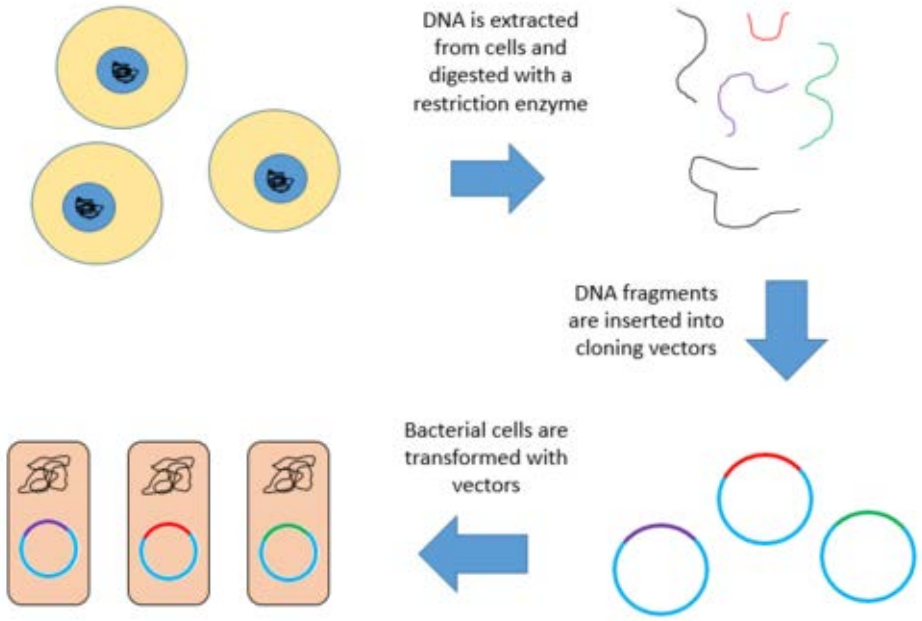
Why is Efficiency Important?

Higher efficiency = Higher Diversity = Better Library Screen = More Confidence in Results

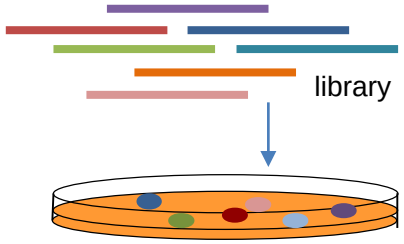
DNA libraries are **collections of DNA clones**

Biotech/pharma customers **require high efficiencies** for library construction

Customers need the highest efficiency cells possible to produce the **highest diversity libraries**



Tips for Library Transformations



- Direct electroporation of ligation reactions
  - *heat inactivate ligase as recommended by supplier*
- Clean up ligation reaction to remove ligase and buffer
- Recover DNA in water at 200 ng/µL or higher
- Use up to 200-300 ng ligated DNA in 1 µL H_2O per standard 25 µL electroporation reaction
- Scale-up: maintain 200-300 ng DNA in 1µL H_2O per 25 µL cells
  - *-test and optimize at small scale prior to scale-up*
- For cell volumes 50 µL to 300 µL, use 2 mm cuvettes
  - *-optimization of electroporation parameters may be needed*

Plating Medium Matters

Salt concentration is *critical* for optimal colony growth.

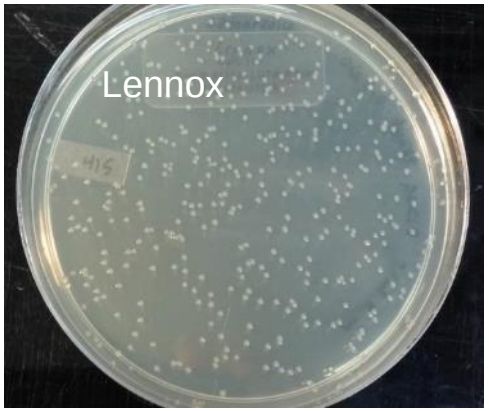
All LB Medium is not created equal.

LB-Lennox (per liter)

5 g NaCl, Low Salt

5 g Yeast Extract

10 g Tryptone

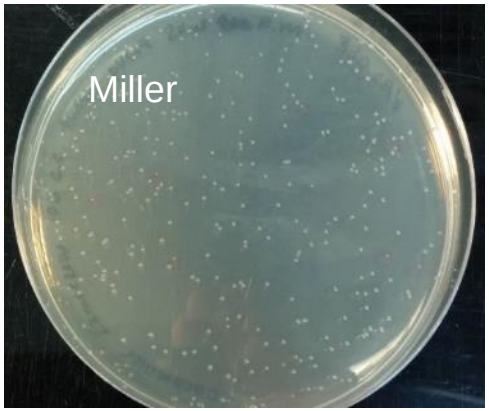


LB-Miller (per liter)

10 g NaCl, High Salt

5 g Yeast Extract

10 g Tryptone



E. Cloni® 10G cells overnight growth comparison (~16 hours).

Use Low-Salt LB-Lennox for most Lucigen cloning and protein expression strains

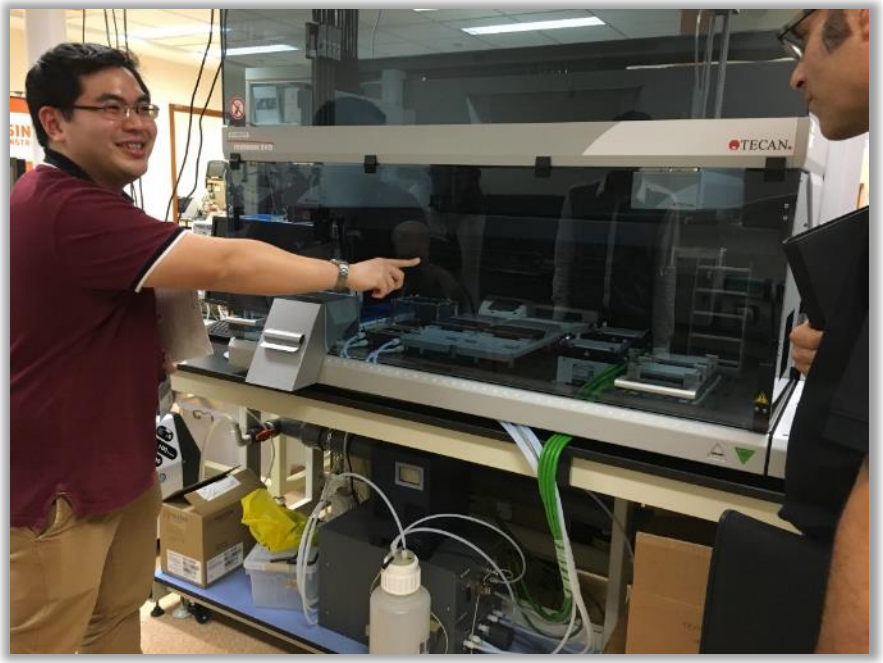
(**Only exception: ClearColi® competent cells should be plated using *High-Salt* LB-Miller).

Competent Cell Packaging Formats

Choose the best solution for your workflow

Routine cloning and library construction:

- **1 reaction** per tube (SOLO)
- **2 reactions** per tube (DUO)
- **6 reactions** per tube (6-Packs)



High-throughput cloning:

- **96-well** microarray plates

Custom formats for any workflow:

- Customer-specified vessel formats, aliquot sizes and strains

What competent cell strain(s) do you use in your lab?

Lucigen Competent Cell Categories

Cells for all applications

- General cloning and library construction
- Phage Display library construction
- Large/Difficult fragment cloning
- Protein Expression
- Low Endotoxin

General cloning and library construction

Strain	Supports cloning Methylated DNA	Contains F' Plasmid	Suitable for Blue/White Screening	IPTG needed for Blue/White Screening
E. cloni® 10G	Yes	No	Yes	No
E. cloni 10GF'	Yes	Yes	Yes	Yes
E. cloni 5-alpha	No	No	Yes	No

↑

Mammalian DNA
Plant DNA

Genotypes of General Cloning Strains

E. cloni*® 10G:** F- ***mcrA*Δ(*mrr-hsdRMS-mcrBC*) *endA1 recA1* Φ80 Δ(*lacZ*)M15** Δ*lacX74 araD139* Δ(*ara,leu*)*7697 galU galK rpsL nupG* λ- ***tonA (StrR)

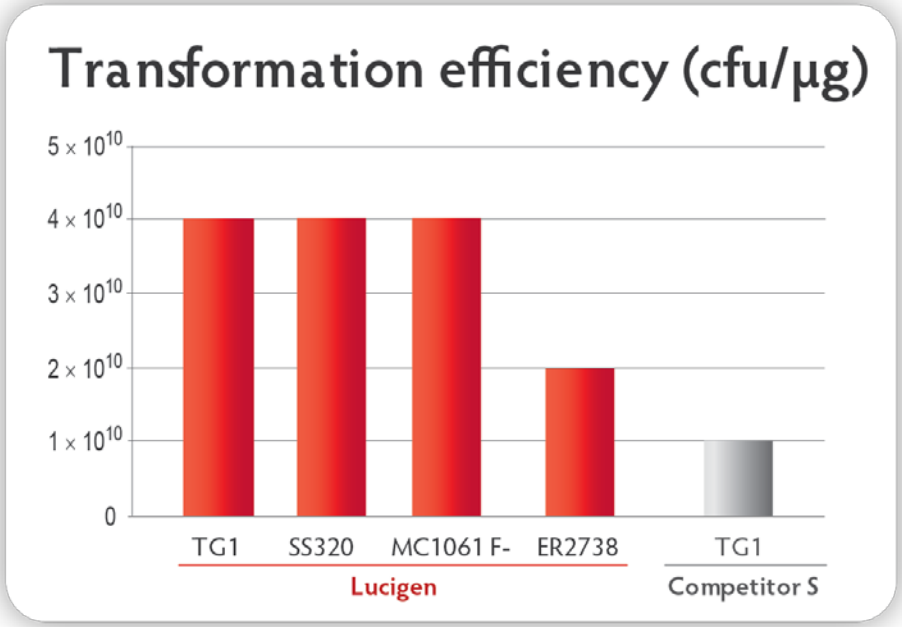
E. cloni* 10GF´: [F´** *proA+B+* ***lacI^{q}ZΔM15::Tn10* (TetR)] /*mcrA* Δ(*mrr-hsdRMS-mcrBC*) *endA1 recA1* Φ80 Δ(*lacZ*)M15** Δ*lacX74 araD139* Δ(*ara,leu*)*7697 galU galK rpsL nupG* λ- ***tonA (StrR)

E. cloni* 5-alpha:** ***fhuA2*Δ(argF-lacZ)U169 phoA glnV44* Φ*80* Δ*(lacZ)M15 gyrA96* ***recA1*** *relA1* ***endA1*** *thi-1 hsdR17*

Genotype	Function
endA1	Eliminates endonuclease that commonly contaminates DNA preps and reduces yield
recA1	Reduced recombination
tonA, fhuA2Δ	Resistance to T1 bacteriophage
F’	Allows M13 infection for ssDNA production; Carries *lacI^q*, overexpresses lac repressor
mcrA Δ(mrr-hsdRMS-mcrBC)	Eliminate restriction of methylated foreign DNA
Φ*80* Δ*(lacZ)M15*	β-galactosidase ω fragment – blue-white screening

Phage Display Strains

- SS320
- TG1
- ER2738
- MC1061 F-



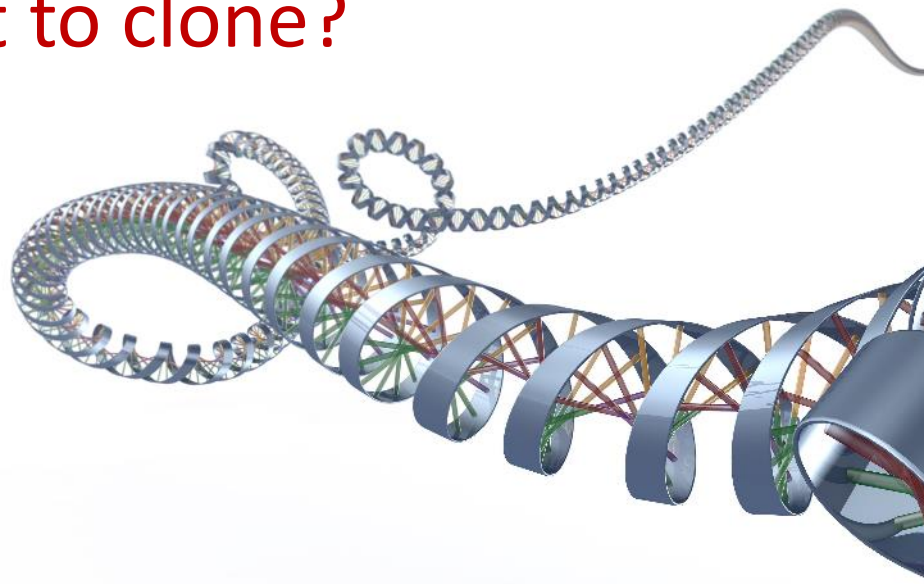
Phage Display Strains

Cell Strain	Uses	Amber Suppressor	Transformation Efficiency
TG1	Phage Display Library Screening Protein Expression	Yes	≥4 x 10^{10} cfu/µg
SS320 (MC1061F')	Phage Display Library Screening	No	≥4 x 10^{10} cfu/µg
MC1061 F- (same as SS320 but lacks F' episome)	General Cloning Phage Display	No	≥4 x 10^{10} cfu/µg
ER2738	Phage Display Library Screening	Yes	≥2 x 10^{10} cfu/µg

Cloning Large/Difficult DNA

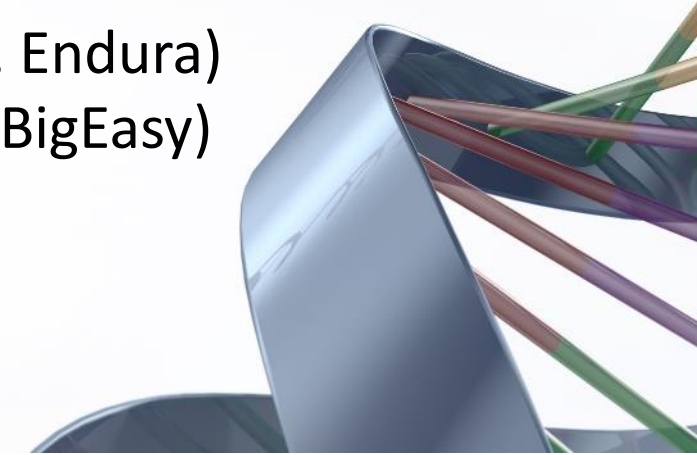
What makes DNA difficult to clone?

- Toxic coding sequences
- Promoters
- A-T Rich DNA
- Large fragments (>10 kb)
- Repetitive sequences



What are the solutions?

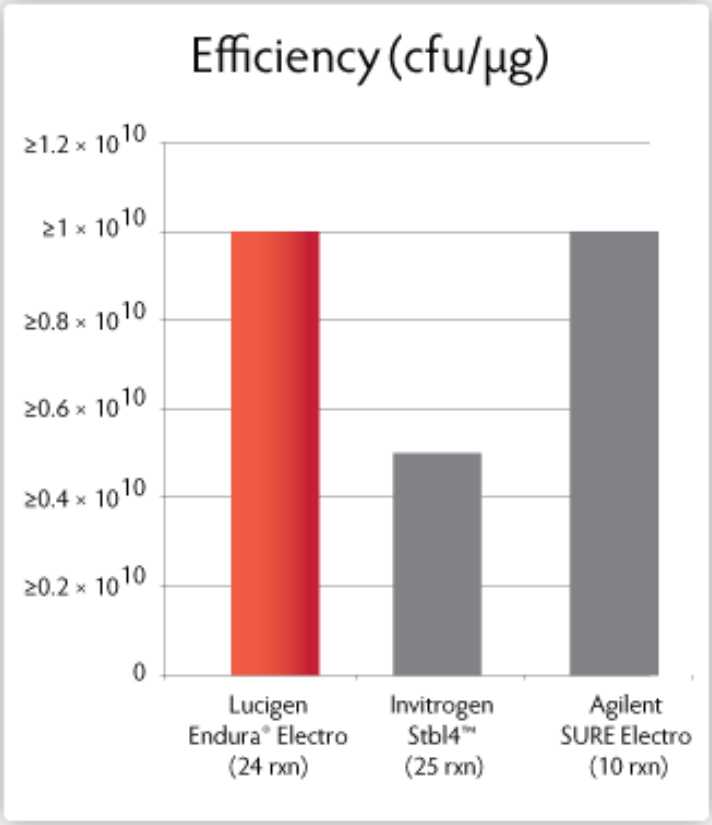
- Specialized competent cell strains (e.g. Endura)
- Vectors and Vector/Host systems (e.g. BigEasy)



Endura™ Competent Cells

Clone unstable sequences

- Ideal for construction of lentiviral vector libraries
- Reduce unwanted recombination
- Stabilize DNA repeats
- Obtain high DNA yields
- Direct replacements for Stbl3™ and SURE strains
- Available in electrocompetent or chemically competent formats



Endura™ Competent Cells

Recommended by the Broad Institute for lentiviral GeCKO CRISPR Libraries

GeCKO Genome-scale CRISPR Knock-Out | LENTIVIRAL CRISPR TOOLBOX

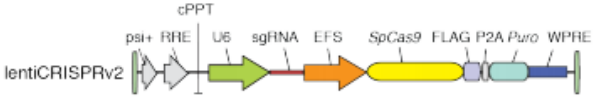
ZhangLab

Genome-scale CRISPR Knock-Out (GeCKO) v2.0 pooled libraries

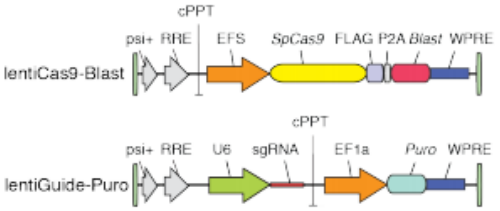
CRISPR (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats) is a microbial nuclease system containing a combination of CRISPR-associated (Cas) genes as well as non-coding RNA elements capable of programming the specificity of the CRISPR-mediated nucleic acid cleavage. Using lentivirus, we delivered the type II CRISPR nuclease system to facilitate genome editing in mammalian cells in a pooled library targeting early consecutive exons (Shalem*, Sanjana*, *et al.*, Science 2014). **Here we describe how to amplify GeCKO v2.0 DNA plasmids to have sufficient quantity to produce lentivirus, while maintaining full library representation**.

Library description: The GeCKO v2 libraries consist of specific sgRNA sequences for gene knock-out in either the human or mouse genome. Each species-specific library is delivered as two half-libraries (A and B). When used together, the A and B libraries contain 6 sgRNAs per gene (3 sgRNAs in each library). We recommend screening the entire library (A and B) when possible but if adequate representation cannot be obtained with the entire library, screening can be performed with one half-library. Since more cells are needed in the screen as number of constructs in the library increases, this design has the flexibility of screening with just the A library (3 sgRNAs per gene) at a smaller scale or screening the full library (6 sgRNAs per gene). Both A and B libraries contain 1000 control sgRNAs designed not to target in the genome. The A library also targets miRNAs (4 sgRNAs per miRNA). Each library is available in a 1 vector (lentiCRISPRv2) or 2 vector (lentiCas9-Blast and lentiGuide-Puro) format. The 2 vector format with the library in lentiGuide-Puro has the advantage of higher titer for the library virus but requires cells to already contain Cas9 (usually genomically integrated using lentiCas9-Blast). See p. 3 for full library specs.

One vector lentiviral GeCKO system



Two vector lentiviral GeCKO system



Visit: http://bit.ly/CRISPR-Endura

Bacterial Vector/Host Systems

Systems for Any Insert Size

Options available:

- CloneSmart® kits for <15 kb
- BigEasy® v2.0 kits for exceptionally difficult targets
- CopyRight® kits for Fosmid or BAC cloning

Convenient, fast, easy, and effective

- Pre-cut, dephosphorylated – ready to clone
- <1% Background
- Minimize cloning problems

See recent webinar on "Cloning the Unclonable"
http://www.lucigen.com/webinars.html

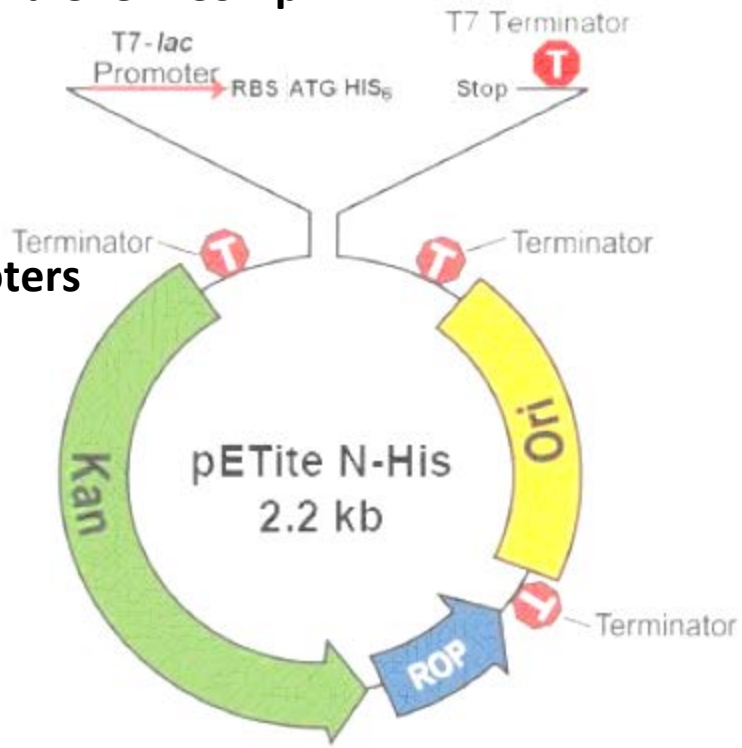
Lucigen Competent Cells for Protein Expression

T7 Expression hosts

- *E. cloni*® EXPRESS BL21(DE3): Electrocomp and Chem Comp
- HI-Control™ BL21(DE3) Chemically Competent Cells
- OverExpress™ C41(DE3) & C43(DE3): Electrocomp and Chem Comp
- ClearColi BL21(DE3): Electrocompetent Cells

Non-T7 hosts for expression from bacterial promoters

- *E. cloni* 10G
- HI-Control™ 10G
- Biotin XCell™ F'



Cloning and Expression of Toxic Targets

Background expression of toxic targets is problematic in cloning and expression

Problem:
Background expression of T7 RNA Polymerase

= Uninduced target gene expression

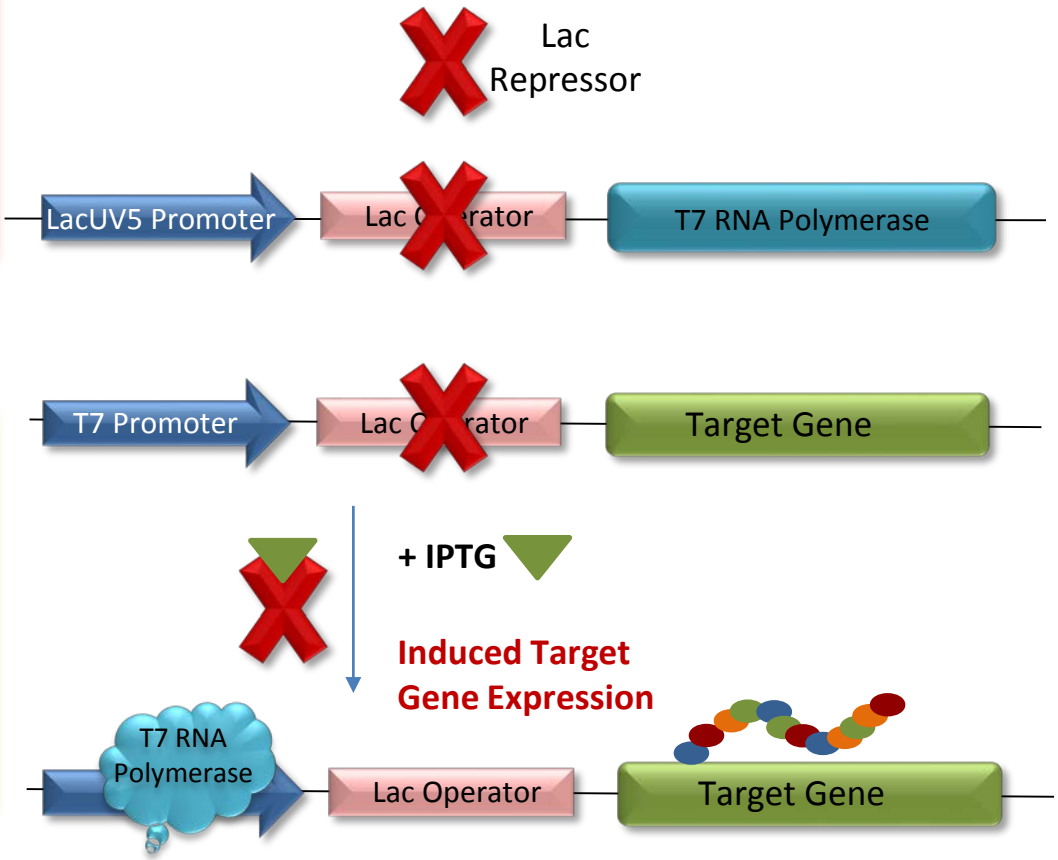
Decreased colony count and cell viability

Solutions:

HI-Control BL21(DE3)

OverExpress C41(DE3) and C43(DE3)

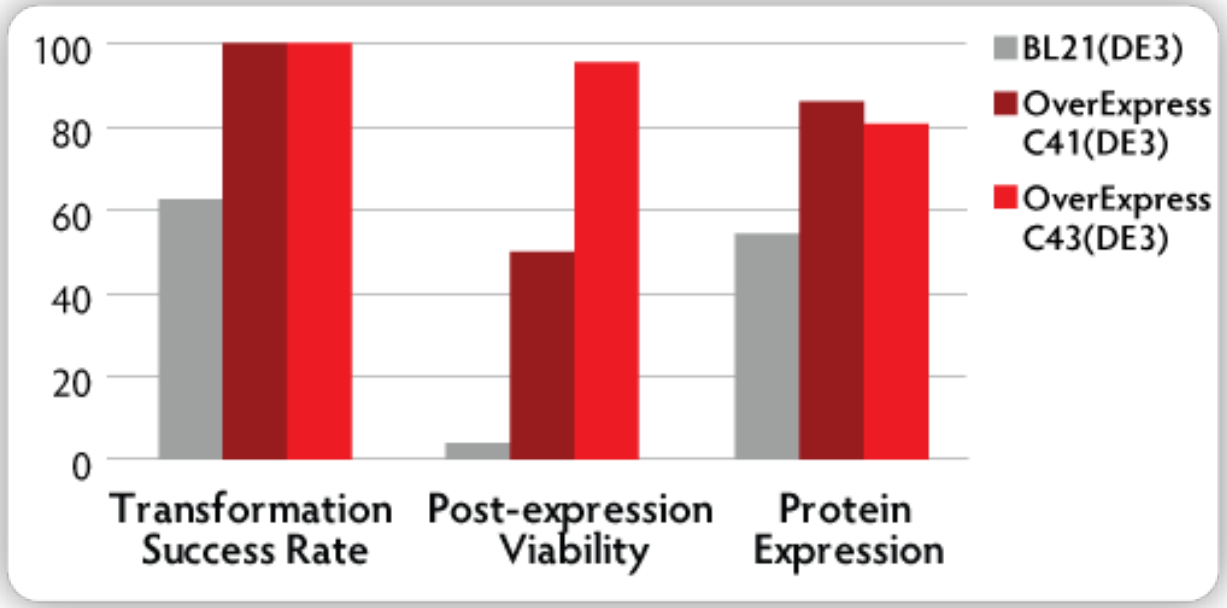
Improved control of T7 RNA polymerase



OverExpress® C41(DE3) & C43(DE3)

Better Viability and Expression of Toxic & Membrane Proteins

- BL21(DE3) derivatives
- Useful for expression from T7 promoters
- Cited in over 350 publications



Dumon-Seignovert, et al. , (2004). *Protein Expression and Purification* **37**, 203-206.

Competent Cells for Low Endotoxin

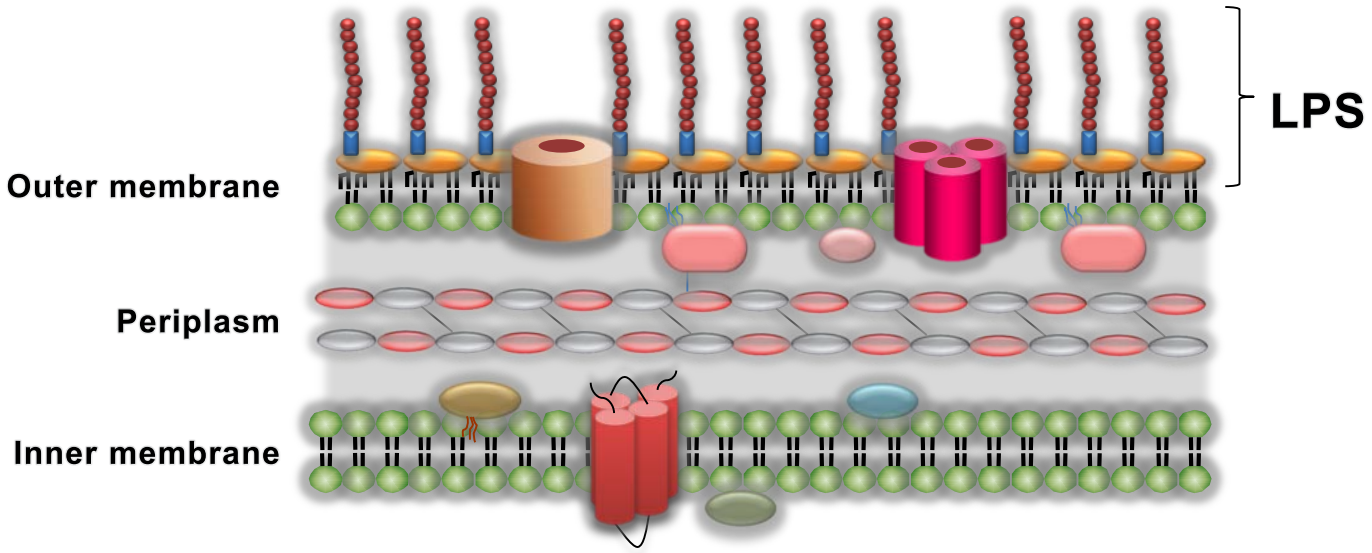
Protein and Plasmid Production

Do you currently need
endotoxin-free DNA (plasmid)
and/or protein preps
in your research?

What is Endotoxin Contamination?

Endotoxin is present in E. coli protein and DNA preps

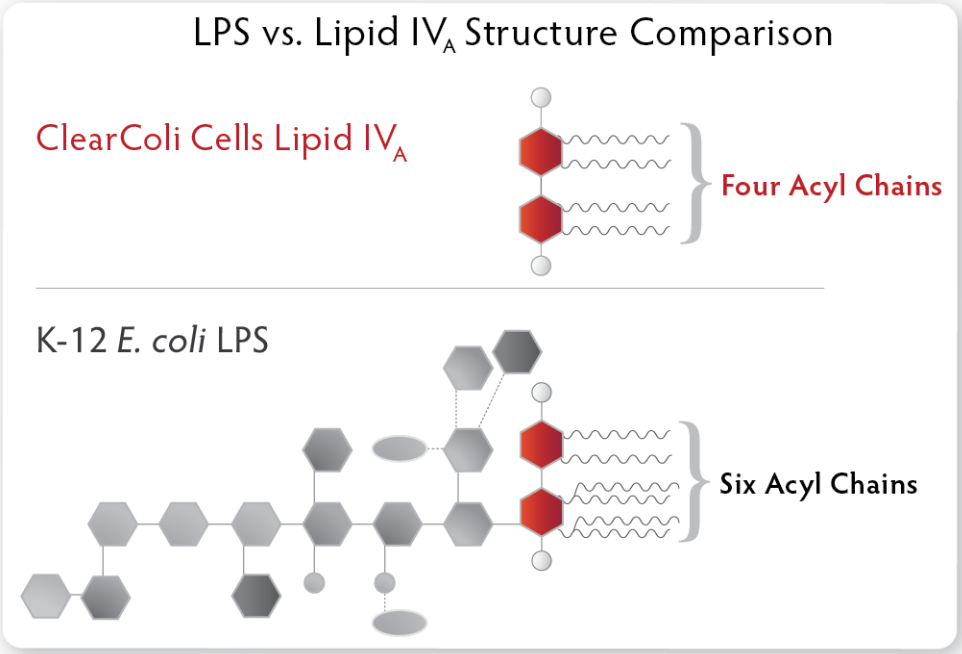
- **Endotoxin, or LPS (lipopolysaccharide),** is part of the bacterial outer membrane
- LPS is problematic:
  - Elicits an immune response in human cells
  - Affects cell viability and growth
- LPS **must** be removed from therapeutic proteins, cell-based assay ligands and cell culture additives
- **Endotoxin removal is difficult, costly and time-consuming**



ClearColi® Does Not Produce LPS

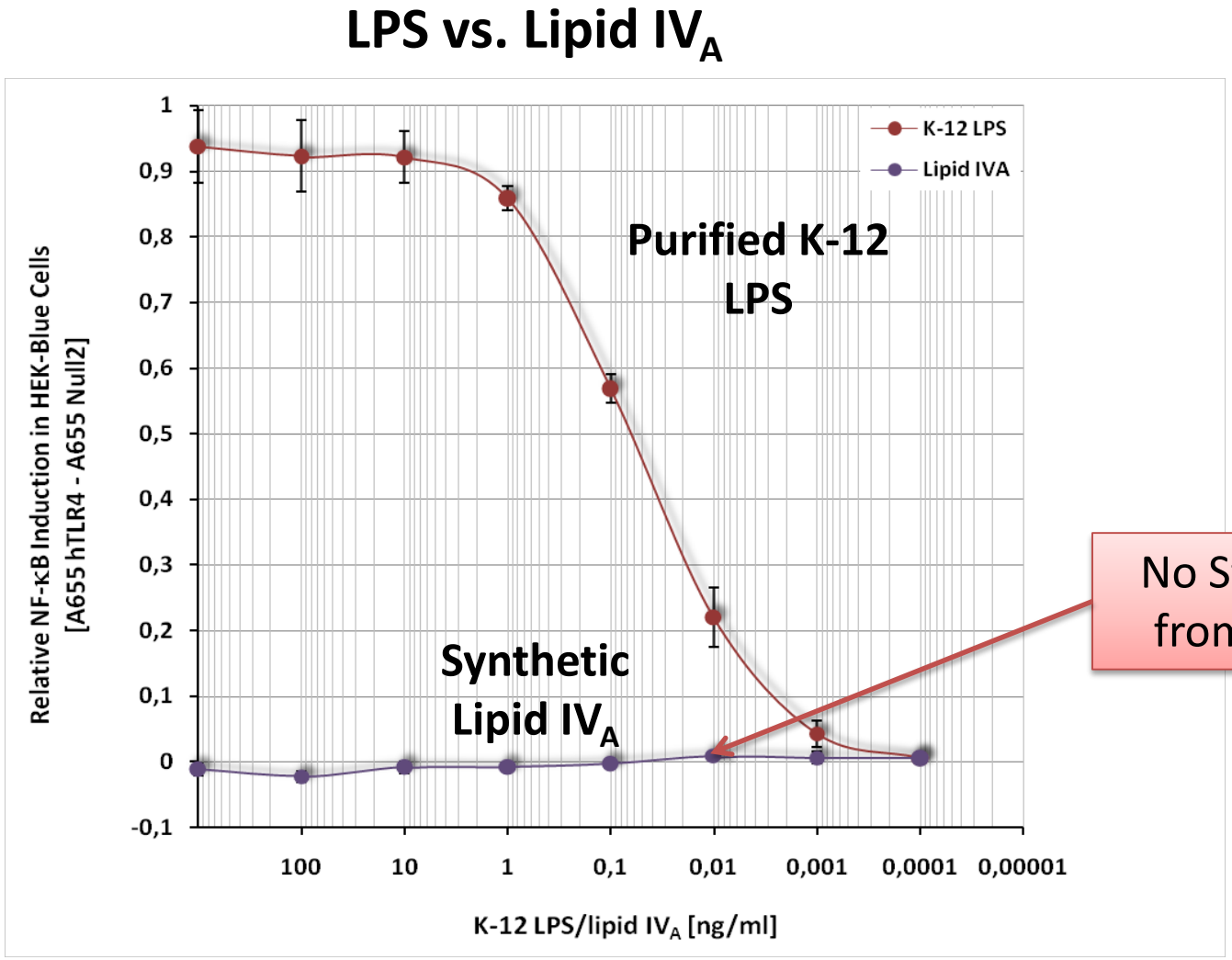
Save time and money by eliminating downstream LPS removal

- ClearColi is genetically engineered to produce Lipid IV_A instead of LPS
- Contains 7 deletion mutations for modifying LPS to Lipid IV_A and 1 compensating mutation
- Structure of Lipid IV_A *does not* trigger an immune response in human cells



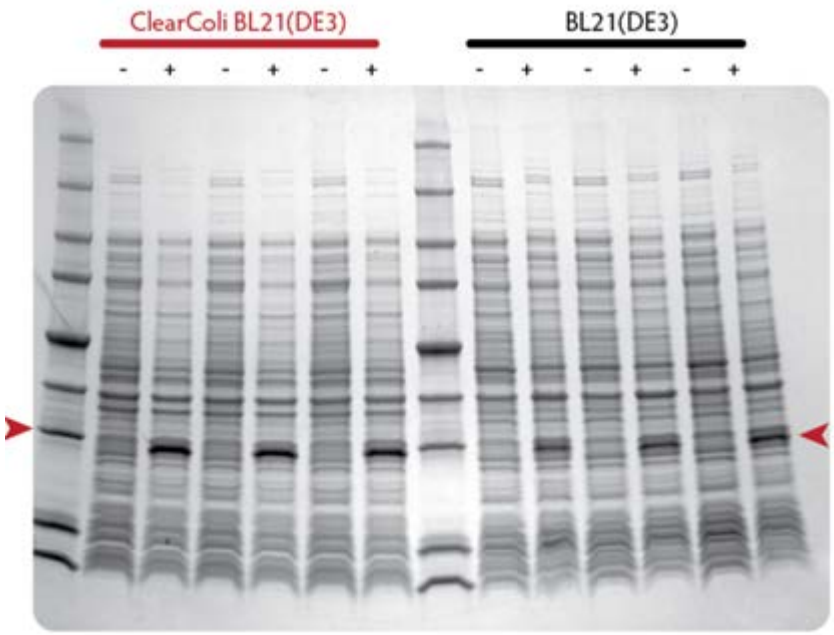
HEK-Blue™ hTLR4/MD-2 Stimulation Assay

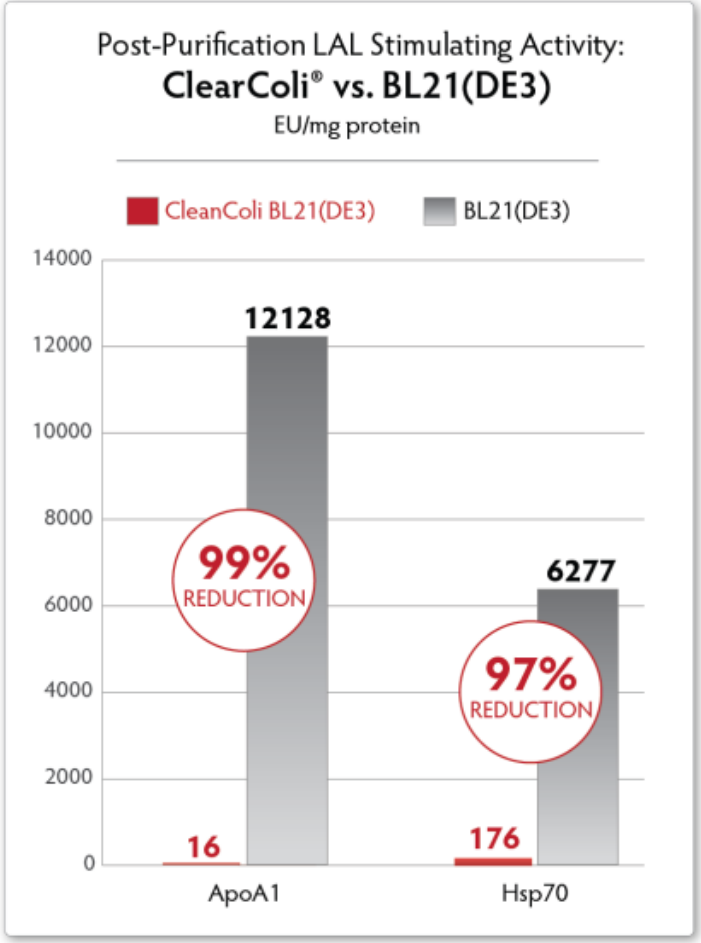
Lipid IV_A does not trigger the endotoxic response in human cells



ClearColi® BL21(DE3) Strain for Protein Production

- Achieve equivalent protein expression yield & profile
- Reduce endotoxin by > 97%
- Lipid IV_A can be removed during standard purification (ie, Ni^{2+} column)

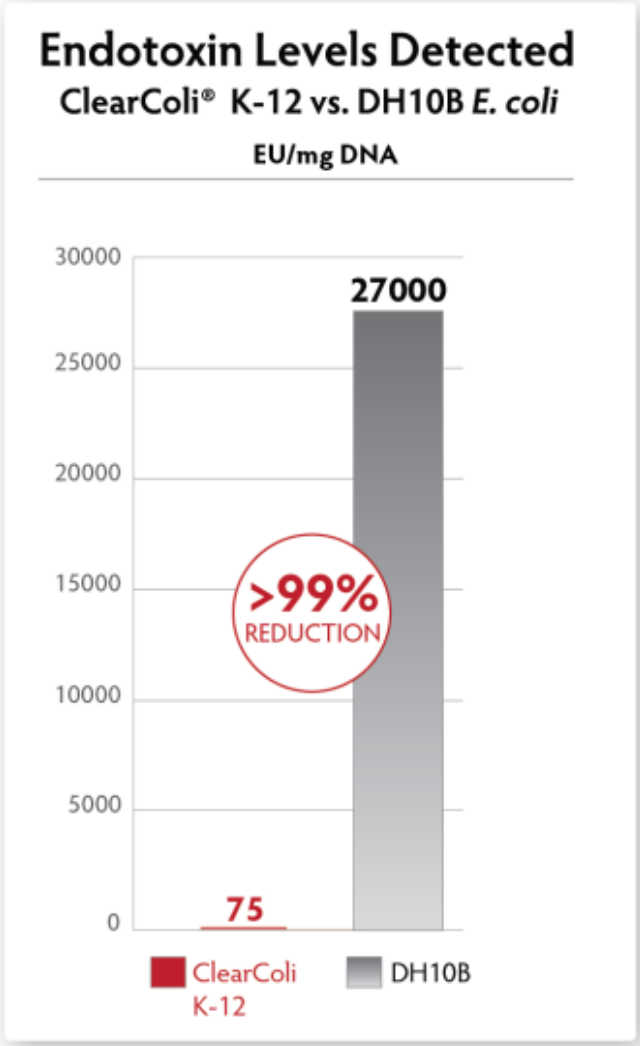




ClearColi® K12 Strain for Plasmid Production

- Produce low-endotoxin plasmid DNA
- Achieve equal or better plasmid DNA yield
- Reduce endotoxin by >99% using standard plasmid prep kits

	Plasmid Yield per 1 mL
ClearColi K-12	**4.83 µg**
***E. cloni*® 10G**	**3.75 µg**



Important ClearColi Facts

- ClearColi cells require higher NaCl than other Lucigen strains
  - –use LB Miller medium (10 g/L NaCl)
- ClearColi cells grow more slowly than standard strains
- LAL assay has some sensitivity to lipid IV_A, but human TLR4/MD2 does not
- Different mammalian systems have different sensitivities to lipid IV_A

http://www.lucigen.com/faq-clearcoli.html

Custom Competent Cells

Your strain of choice, made to the highest competency

- ✓ Made-to-order competent cells
- ✓ Lucigen's strains and customer-supplied strains
- ✓ Chemically competent or electrocompetent cells
- ✓ Custom dispense volumes and formats
- ✓ Customer-specified tubes and plate types
- ✓ Up to 4×10^{10} cfu/µg
- ✓ Lucigen is registered to the ISO13485 quality management standard for medical devices through BSI, Inc.

http://www.lucigen.com/Custom-Competent-Cells-/

Sample Lucigen Competent Cells

Free samples for the following strains:

E. cloni® 10G ElectroComp Cells
E. cloni 10G ChemComp Cells

Endura™ ElectroComp
Endura ChemComp

Phage Display ElectroCombo Pack
Includes TG1, SS320 (MC1061 F') and ER2738

http://lucigen.com/samplecells.html

Find the right competent cells for your application:
http://lucigen.com/compcellguide

Questions? www.lucigen.com

Thank You!

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