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Product Guide

For chemically competent cells

Version: 30072014

Product	Cat#	Package size
Chemically competent cells TZ101α (10e8); 20 x 0.1mL	M3434.0020	20 transformations
Chemically competent cells TZ102α (10e9); 10 x 0.1mL	M3435.0010	10 transformations
Chemically competent cells TZ102α (10e9); 10 x 0.1mL	M3435	Other pack sizes on request

Abbreviations

L, mL, µL	**Litres, milli litres, micro litres respectively**
g, µg, ng	**grams, micro grams, nano grams respectively**
LB	**Luria Bertani medium**
XGal	**5-Brom-4-Chlor-3-indolyl-ß-D-galactopyranoside**
TE	**Tris-EDTA (10 mM Tris-HCl, pH8.1, 1 mM EDTA)**
Amp	**Ampicillin**
RT	**Room temperature (18°C – 24°C)**

Related products / overview

• Genaxxon DNA Gelextraction Kit	S5344
• Genaxxon JustSpin Gel Extraction Columns	S5337
• Genaxxon SpinClean Spin Columns	S5304
• Genaxxon pMBL TA Cloning Kit	M3164
• Genaxxon Taq DNA Polymerase	M3001
• Genaxxon chemically competent cells TZ101α (10e8)	M3434
• Genaxxon chemically competent cells TZ102α (10e9)	M3435
• Genaxxon T4 DNA Ligase	M3027

Notes on Warranties and Disclaimer

Genaxxon is dedicated to your success and every batch of this product is tested with an extensive routine procedure to make sure that it meets all your needs. However, it has neither been developed nor tested for a specific application.

This product is for research use only. For *in vitro* use only

Genaxxon's liability with respect to any product is limited to the replacement of the product. No other warranties are provided by Genaxxon. Genaxxon is not liable to any direct, indirect, incidental or consequential damage arising out of or in connection with the use of any of Genaxxon products.

Manual Contents

No matter where you get the DNA from you put into your ligations, you had to work to get it. You want to make the most of it. Competent cells have to be reliable and as efficient as possible. On the other hand, there is no reason to waste money on cells that are overqualified for the job. This guide describes the efficiencies and strain properties that are available to make it easier to make the choice.

Introduction to competent *E. Coli*

Principally there are two ways to transform *E. coli:* chemical transformation and electroporation. Chemical transformation is more convenient and electroporation is more efficient.
Each method, and factors that affect them, are described in detail below.

Types of *E. coli*

The kind of *E. coli* used in research are harmless lab strains of *E. coli.* Many different strains of *E. coli* are available, and nearly all of them are derivatives of a single strain called K12. K12 was first isolated in 1922. BL21 comes from *E. coli* strain B, first described in 1946. Labs have passed around (mostly K12) strains and made mutants and shuffled around mutations until the average strain had a long list of genetic markers called a genotype. To most people, a genotype looks like it is written in Martian. Many markers are not relevant to most people. The following may help in the decision of what strain to use. Strains may be divided into three groups.

Cloning strains are efficiently transformed, offer blue/white screening, and do not restrict foreign DNA. These are the cells that are most often used in molecular biology experiments.

Expression strains are used to express a protein efficiently from a given construct.
Expression strains do not need to be efficiently transformed to be useful.

Specialty strains have particular properties that are occasionally used.

Drop dialysis for desalting of ligation reactions

For use in combination with Alligator ligated DNA and subsequent electroporation.

Material

- Sterile petri dish or microwell plate
- Sterile $H_2O_{bidest.}$
- Drop dialysis membrane, pore width 0.025µm, diameter about 1cm (e.g. Millipore)
- Tweezers

Procedure

1. Fill the sterile petri dish/microwell plate with sterile $H_2O_{bidest.}$
2. Remove the drop dialysis membrane from the packing with tweezers and place it on the water surface (**NOTE**: there should be no water on top of the membrane!) .
3. Pipet desired amount of ligation reaction (10-20µL) in the centre of the drop dialysis membrane (**NOTE:** Do not use more than 20µL!).
4. Dialyse for 20 min at room temperature.
5. Remove the dialysed ligation reaction carefully from the drop dialysis membrane with a pipet (the volume will probably have increased during dialysis).
1. The dialysed ligation reaction can now be used directly for electroporation.

References

Original Papers On Chemical Transformation of *E. coli*.

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2. Cohen, S.N., A.C.Y. Chang and L. Hsu (1972) Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. *Proc Natl Acad Sci USA. 69*: 2110-2114.

High Efficiency Transformation of *E. coli* Grown at 37°C.

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General Review and Methods of Preparing and Using Competent Cells.

Hanahan, D., J. Jessee, and F. Bloom (1991). Plasmid transformation of *Escherichia coli* and other bacteria. *Methods in Enzymology 204*: 63-113.

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There are many bacteria that are transformed naturally. *E. coli* is not one of them. Natural transformation is very different from electroporation, chemical transformation and protoplast transformation.

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2. Dower, W.J. and C.W. Ragscale (1988) *Nucleic Acids Research 16*: 6127-6145.

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1. Smith, M.D., J. Jessee, T. Landers and J. Jordon (1990) High Efficiency Bacterial Electroporation: 1 x 1010 *E. coli* Transformants/mg. *Focus 12*: 38-41.

2. Li, S.J., T.A. Landers, and M.D. Smith.
Electroporation of plasmids into plasmid-containing Escherichia coli. *Focus 12*: 72-75.

Everything About Molecular Cloning - Transformations, Too.

1. Sambrook, J., E. Fritsch and T. Maniatis (1989) *Molecular Cloning: A Laboratory Manual* Second Edition. Cold Spring Harbor Press, Cold Spring Harbor, NY.

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Properties of cloning strains

This section features the properties of cloning strains that people find most useful. The genotypes that are responsible for these features appear in parentheses.

Blue/white Screening (Δ(*lac*ZYA-*arg*F)U169 Φ80d*lac*Δ(*lac*Z)M15)

pUC19 and similar plasmids code for β-galactosidase (*lac*Z), which cleaves X-gal and turns colonies blue on an X-gal plate. Inserts cloned into the plasmid disrupt the β-galactosidase gene and the colonies are white. The plasmid only code for a small part of the β-galactosidase gene (called the α peptide), and the chromosome codes for the rest. Both parts are required for activity. Since the plasmid is complementing the chromosomal mutation, this effect is called "α complementation". More on blue/white screening is discussed in the “Special Technical Features” section on page 9.

Recombination Deficient (*rec*A)

E. coli has a repair system which will recombine homologous sequences. Many people fear that recombination can cause plasmids to rearrange or delete insertions because of recombination. For this reason, cloning strains are traditionally *rec*A mutants. *Rec*A strains have the advantage of having simple plasmid profiles (mostly CC monomer) whereas *Rec*+ strains have dimers, trimers, etc. and their nicked relatives that make uncut plasmid lanes complicated.

Genomic clones often have duplicated regions, but these duplications are usually rather short, tandem duplications. They are unstable, but this instability is not due to *rec*A function. If the length of the duplicated sequence is less than 200 bp, *rec*A has no effect whatsoever.

The *rec*A repair system is useful to *E. coli* and disabling it causes the cells to grow slower and be less healthy. For this reason, some expression strains (like BL21) are not *rec*A mutants.

Endonuclease Deficient (*end*A)

E. coli has a powerful endonuclease on the outside of the cell that degrades any type of DNA. *End*A mutants are devoid of this activity. The *end*A endonuclease has little or no effect on transformation efficiency, but can have a profound effect on the quality of plasmid DNA preparation. If plasmid DNA preps degrade when placed in magnesium containing buffers, it is usually because the DNA was made from an *End*A+ bug.

Restriction Deficient (*hsd*RK-)

Most lab strains are *E. coli* K12 derivatives. K12 strains methylate their DNA at K12 sites (AAC(N6)GTGC and GCAC(N6)GTT). In K12 strains, DNA that is not methylated at these sites is degraded by a restriction enzyme. Many, but not all, cloning strains of *E. coli* are mutated in the gene that codes for this restriction enzyme. BL21 does not methylate, nor does it restrict unmethylated DNA.

Reduction in plasmid copy number (*pcnB*)

Some genes cause problems if they are on high copy number plasmids. Cells that contain these plasmids will not grow anymore. A solution for this problem is to use cells that will reduce the copy number of widely used plasmids, like: pUC, Bluescript, etc..

Methyl Restriction Deficient (*mcr*A, *mcr*B, *mrr*)

E. coli has a system of enzymes that degrade DNA if it is methylated at the "wrong" sites. Genomic DNA from eukaryotic sources is methylated at all the wrong sites, as far as *E. coli* is concerned. When cloning genomic DNA from eukaryotic cells, it is essential to use a host that is deficient in all three of these methyl restriction systems. On the other hand, when cloning PCR fragments, cDNA, or fragments from previously made clones, there is no methyl restriction and it is not necessary to use a methyl restriction deficient host.

Single Strand Ability (F+ or F')

F is a huge plasmid (99 kb) that is naturally found in *E. coli* K12. There are derivatives of the F plasmid that also contain chromosomal DNA. These F derivatives with a bit of chromosomal DNA are called F' ("F prime") plasmid. *E. coli* with the F (or F') plasmid make special surface features that allow them to be infected with M13 and similar phage. This property is useful if one wants to make single-stranded DNA or generate phage display libraries.

Phage Resistance (*fhu*A, *ton*A or T1R)

Commercial cDNA banks are sometimes infected with T1 phage, and there are many other ways to get into trouble with T1 or a T1 relative. Unlike other phage, T1 is resistant to drying and subsequently is almost impossible to eliminate. Cells which are resistant to T1 are fast becoming standard in laboratories.

Lac Promoter Control (*Lac*IQ)

Even though gene expression is not being covered in this guide, it is important when making plasmid constructs to keep your expression promoter off until you are ready to turn it on. High level expression of many genes is bad for the host. When this happens, mutants that do not express the gene at a high level grow faster and take over the culture. Some expression systems use the *lac*, *tac*, or *trc* promoters to express cloned genes on high copy plasmids, but do not have the cognate repressors on the expression plasmid. Under these circumstances, there is not enough *lac* repressor to keep the promoters off. A mutant that produces more *lac* repressor (*Lac*IQ) can repress *lac*, *tac*, and *trc* promoters until IPTG is added to induce them.

Properties of Genaxxon Cloning Hosts

		TZ101a	TZ102a
Blue/white screening		✓	✓
Recombinant deficient		✓	✓
Endonuclease deficient		✓	✓
Restriction deficient		✓	✓
Single strand ability		✓	✓
Reduction in copy number		-	✓
Methyl restriction deficient		-	-
Phage resistance		-	-
Lac promoter control		-	-

Competent cells – Special technical features

T1 Phage and Its Relatives
Competent Cells with the T1 resistant marker protect your work and laboratory from infection by bacteriophage T1 or relatives.
The T1 resistance marker is nice to have. Here's why:
• T1 and its relatives kill *E. coli*.
• T1 is mainly present in some libraries of cDNA clones that get passed around between labs and genome centres.
• If you have experienced T1 phage contamination, you will never forget it. It's total devastation for your clones.
• The T1 resistance marker protects your clones. It does not impair them.

Blue/white Screening
Blue/white screening is a popular tool for quick and easy identification of recombinants. Genaxxon TZ101α and TZ102α strains carry the *lac*ZΔM15 marker that enables blue/white screening.

Genotypic markers
Δ*lac* - deletes ß–gal gene
ϕ80d*lac* - makes ß-□peptide
*lac*ZΔM15 - makes ß-□peptide

X-gal is a chromogenic substrate for ß-gal (*lac*Z).
When cleaved, a blue colour is produced.

How Does It Work?

Cells - Need to make the ß-□peptide, but not the whole ß-galactosidase (ß-gal) molecule.
Plasmids - Carry the ß-□peptide sequence for ß-gal in the multiple cloning site (MCS).
Host strains - Genotypes with the *lac*ZM15 marker lack the α-peptide sequence for α-gal.
The α-□peptide must be supplied by the plasmid.

When DNA is inserted into the MCS of the plasmid, the α-□gene is interrupted. There is no α-□peptide to complement the ß-gal protein and thus the ß-gal is not functional. When transformed, colonies containing the plasmid + insert (recombinants) are white. If the α-□gene is still intact (nonrecombinants), ß-gal is activated and the colonies are blue.

How much X-gal and IPTG should I use?

Prepare 4% X-gal in dimethylformamide (DMF) and 100mM IPTG in sterile water. Spread 30µL of each on the surface of a 30mL plate. Stocks can also be added directly to cooled medium prior to pouring plates.

Final concentration: 40µg/mL X-gal and 1mM IPTG.

My plate looks like the stars in the sky - big colonies down to tiny colonies, all sizes.

The selection is off. Streak cells that should grow on the selection, and cells that should not. The cells that should grow are struggling; you have too much antibiotic. If the cells that should not grow are growing where the streak is heaviest, you don't have enough antibiotic. If there is too much antibiotic, you are losing transformants. Try half as much. If there is not enough antibiotic, then you are getting "breakthrough" of nontransformed cells that are mutants to a low level of resistance. Try using twice as much antibiotic.

When I use the control and calculate transformation efficiency, I get a number that is 2 to 4 times as high as the specification.

The spec is a minimum. Count your blessings.

I left my cells in the ice bucket overnight. Can I still use them?

Don't waste your time unless you are desperate.

My freezer died but the temperature only went to -50°C before I transferred the cells. Can I still use them?

You're down but not out. Expect a 2- to 5-fold loss in efficiency.

People in my lab keep putting my cells on the bench while they root around for their samples in the -70°C. Can I still use them?

People don't mean any harm - they probably figure that if the cells are only out for a minute and don't thaw, they're OK. That's natural. Wrong, but natural. Your cells are losing potency over time because of this, but only slowly. Try putting the cells in a different place so they are not in people's way.

By the time I get through with my ligation, there is only an hour left before I have to go home. Is there any way to shorten the protocol?

You can, as described in Section 4, "What's Important in Chemical Transformation" on page 4. You will sacrifice some efficiency. If you need the most possible transformants, don't cut corners. If you usually get more colonies than you test anyway, start by cutting the expression time to 30 minutes, and thaw the tube with your fingers to save time.

Factors that affect transformation

Forms of DNA

Relaxed plasmids, which are formed in ligation reactions, transform *E. coli* with the same efficiency as supercoiled plasmids. Linear plasmids and single-stranded plasmids transform very poorly (< 1% compared to double strand DNA). A special host is needed to achieve chromosomal transformation, which is very inefficient. Usually, a transformation involves a mixture of linear (nontransforming) and circular (transforming) DNA.

Amount of DNA

It seems obvious that if you add more DNA to a transformation, you get more transformants. For chemically competent cells; however, adding more than 10ng of pUC19 DNA does not result in significantly more transformants. The point of diminishing returns is about 100ng of pUC19 for electrocompetent cells.

But what happens with ligations? A ligation mixture contains insert DNA, linear vector, re-circularized vector, and vector with insert (both circular and linear). The concentration of all these components together is usually about 50ng/µL. Even if everything is fine, the nontransforming DNA will be in the majority - will it compete out the transforming DNA? Not usually. With 20ng of total DNA per reaction, the nontransforming DNA will decrease the efficiency of the transforming DNA only about 2-fold for chemically competent cells, and not at all for electrocompetent cells.

If the ligation reaction is concentrated by precipitation and 500ng are added to a single reaction, the competition effects can drop the transformation efficiency 10-fold for chemically competent cells, but will still not affect electroporation.

Source of DNA

DNA from eukaryotes is heavily methylated in a way that drives *E. coli* wild. *E. coli* have restriction systems that restrict these types of methylation. When cloning any genomic DNA, it is wise to use an *mcr* mutant. DNA generated by PCR is unmethylated, so cloning a PCR fragment from genomic DNA does not require a *mcr* mutant. Therefore TZ101α is suitable to ligation of PCR fragments.

Other Chunk in the Donor DNA

Donor DNA should not have detergent, phenol, alcohol, PEG, or DNA-binding protein in it. For electroporation, donor DNA cannot have salt in it either. DNA in TE buffer works well. Ligase and PEG strongly inhibit transformation. A central problem in molecular biology is that both of these are components of most ligation reactions.

Most people just dilute the ligation mixture 3-fold and transform with 1µL, or just add 1µL and hope for the best.

However, the best thing to do is to precipitate the ligation mixture ("Protocols for Transformation" on page 9 for details). Another possibility is to perform a "drop dialysis" (see page 18).

Storage & Handling

Arrival. Competent cells arrive on dry ice and need to be kept at -70°C to keep them at their peak. You can keep your cells in peak form if you take a few precautions.

- First, clear out the space in the -70°C freezer where the cells are going to be stored.
- Unpack the box at the freezer, not on the other side of the lab.
- Place the cells in the -70°C freezer and shut the door. You don't have to rush, but please don't take the cells out for a stroll, or leave them on the bench while you rummage around trying to clear out a space for them. Even when they stay frozen, cells lose efficiency as they warm up.

Storage. We've all worked in labs, so we all know that -70°C space is shared, cold, cramped, and confusing. While the cells are stored in the freezer, people will need to get something that's behind them or under them; and, over time, the cells will spend some time in places warmer than -70°C. This is unavoidable but can be minimized by placing the cells where everybody can get to them but they aren't in the way of other things.

When defrosting freezers, making major re-arrangements, etc., take the time to grab some dry ice and place the cells in (not on) the dry ice while everything is shuffled around.

Refreezing cells. If you thaw out a tube of competent cells on purpose and want to refreeze the remainder, you can. Place the tube in crushed dry ice or in a dry ice-ethanol bath (best), buried in a bed of dry ice (second best), or by itself on a metal shelf at -70°C for an hour before putting it in the box (third best).
You'll lose about 2-fold in efficiency. If you simply put the tube back in the box and place it in the freezer, you could lose 5- to 10-fold in efficiency.

Chemical Transformation

Chemical transformation is achieved by suspending the cells in an ice-cold buffer that contains calcium chloride and other salts. Typically, these cells are stored frozen. When desired, the cells are thawed and DNA is added. Transformation occurs when the cells are warmed briefly. Transformed cells are diluted into media; and after a time, they are plated onto media that selects for transformants.
Transformation efficiencies vary from 10^8 to 10^9 transformants per microgram of pUC19 DNA with commercially prepared cells (10^6 to10^7 for most home-made cells).

What's Important in Chemical Transformation

Transformation protocols are described in detail under “Protocols for Transformation” on page 9. Let's face it, nobody does transformations EXACTLY like they are supposed to.
What's important?

Sample Calculation: colonies on control plate / ng of control DNA plated X 1000 ng/µg = T/µg

Let's say you added 0.1ng of control DNA (1µL of 0.1ng/µL, freshly diluted) to 100µL of competent cells. 900µL of SOC medium is added prior to expression. 100µL (equivalent to 0.01ng DNA) is then diluted in 900µL SOC and 100µL is plated (equivalent to 0.001ng DNA).

If 100 colonies are counted on the plate, calculate the transformation efficiency as follows:

100 cfu on control plate / 0.001ng of control DNA plated X 1000 ng/µg = T/µg

100 colonies / 0.001ng X 1000ng/µg = 1 x 10^8 T/µg.

Frequently asked questions

How come I didn't get any transformants (chemical transformation)?

• Try the control DNA to make sure the cells are competent.
• Does this donor work on any cells? Maybe the ligation did not work.
• Is the selection appropriate to the plasmid?
• If you concentrate the cells by centrifugation, be gentle. Try plating 0.1 ml before concentrating the rest of them.

How come I didn't get any transformants (electroporation)?

• Try the suggestions listed above.
• If the control does not work either, make sure everything is hooked up correctly. If it is, do a test pulse this way: Add 40µL of LB or SOC to a used cuvette and pulse it. It should arc and make a snapping sound. If it does not, you are not connected or your machine is dead. Don't panic. Perhaps a cable has broken. Get someone to test the cables and the cuvette chamber to make sure they conduct electricity (most common problem).
If they are OK, your electroporation machine may be dead. Call the manufacturer.

Why did my cells explode when I pulse them?

• You added too much to the reaction. Try adding less DNA or ethanol precipitating the DNA as described in the electroporation section.
• Check to make sure that the settings are right - 200 Ω, 2.0 kV, 25 µF capacitor.
• The cells have too much salt in them. If you make them yourself, wash them one more time.

I get a lot of little colonies around big colonies. What are they?

These are satellite colonies. They are not transformants. Incubate your plates for less time, use more antibiotic, or use fresher plates to get rid of them. If you plate 0.1mL of an electroporation, you are probably going to see them. Ignore them, they will not grow overnight if you use selection.

Substances that inhibit electroporation

"Impurity"	extracted by
Proteins	column purify or phenol extraction and ethanol precipitation
Detergents	ethanol precipitation
PEG	column purify or phenol extraction and ethanol precipitation
Ethanol	dry pellet before resuspending in TE
Salts	ethanol precipitation

Inhibition of Chemical Transformation by Ligation Mixture
Section 3, "Factors That Affect Transformation" on pages 2 and 3 covers this subject in detail. The best way is to ethanol precipitate the ligation and resuspend the pellet in an equal volume of TE. In this case, use 1 µl of resuspended DNA per 100 µl competent cells. Alternatively, dilute the ligation 3X and add 1 µl per 50 µl competent cells.

Inhibition of Electroporation by Ligation Mixture
The salts and the PEG are death to electroporation. Ethanol precipitate the ligation and resuspend the pellet in TE. You can concentrate the ligation up to 10-fold in this step. You can also add 1 µg of carrier (tRNA or total RNA). Add 1 µl of resuspension per 40 µl of competent cells.

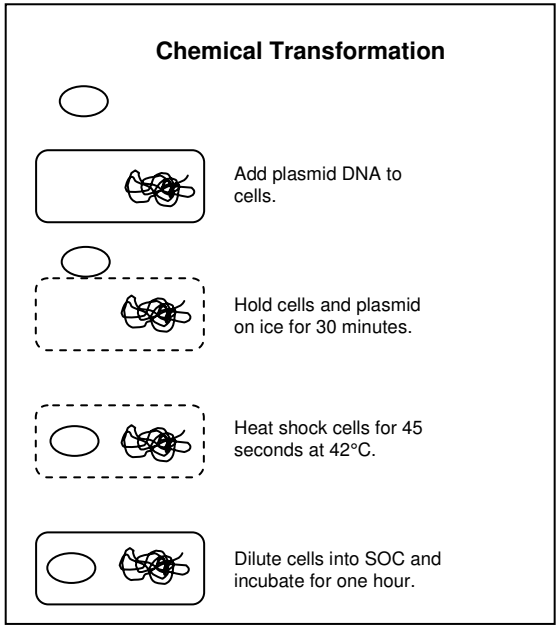
Adding too much Ligation Mixture to the Transformation
The most common mistake when transforming *E. coli* is to put too much ligation mix in the transformation. As suggested in the protocols, less than 1 µl of a ligation is sufficient for any type of transformation. Adding more LOWERS the number of transformants. For chemically competent cells, the ligase and PEG in the mix inhibits transformation. For electrotransformation, the added salt lowers efficiency or causes arcing. Sometimes you have to squeeze every possible transformant out of a ligation. To do this, there are two options:

1. Precipitate the ligation and resuspend it in TE as described in "Protocols for Transformation" on page 9.

2. Dilute the ligation 3-fold in TE and use 1 µL per transformation.

Transformation Efficiency: What It Means and How It Is Calculated
Transformation efficiency is a measure of the ability of cells to be transformed. Transformation efficiency is expressed as the number of transformants per microgram of pUC19. It can be written as transformants/µg, T/µg, as colony forming units per microgram, or cfu/µg. The higher the efficiency, the higher the fraction of cells in the reaction that are actually transformable, and the more transformants for the same DNA.

If you transform high efficiency competent cells (10^9 transformants/µg) with 1 µg of pUC19, you will not get 10^9 transformants because the cells are saturated at 0.01 µg of pUC19 in a 100 µl reaction. The efficiency is instead measured with 50 picograms of pUC19 in a reaction. An efficiency of 10^9 transformants/µg is the same as 10^3 transformants/pg; so 50 pg should generate 50 x 10^3 transformants. We recommend to dilute such a transformation 100-fold, plate 0.1 ml, and look for 50 colonies. Likewise, an efficiency of 10^{10} transformants per µg (10^4 transformants/pg) can be measured by putting 20 pg in a reaction and looking for 20 x 10^4 transformants, i.e., 200 colonies when 0.1 ml of a 100-fold dilution is plated.



What's important?

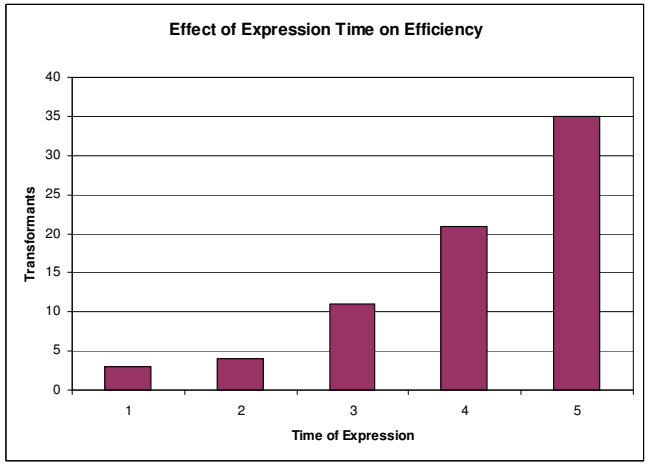
Thawing the cells
Thaw cells for 5 to 10 minutes directly on ice. If you are in a hurry, you can thaw the cells by rolling the tube in your fingers until the ice melts. DO NOT run water over the tube or put the tube in a water bath. If you do this, you can't stop when the cells reach 0°C. If you are busy and the cells stay in the ice bucket for an hour, that's fine. Beyond that, you'll start dropping in efficiency about 2-fold every hour or so. If you leave the cells in the ice bucket overnight, don't bother using them.

Incubating the DNA with the cells on ice
Incubating on ice is necessary for chemically competent cells. If you heat shock right away, your efficiences will be down 10-fold. If you incubate for only 15 minutes, you'll be down 3-fold.

The heat shock.
The heat shock works best in a Falcon tube with a 42°C bath. Lots of people have 37°C but not 42°C baths. A 45 second heat shock at 42°C gives the best results; but, one minute at 37°C works almost as well (down 2-fold).

Expression
The effect of the expression time depends on the plasmid and strain. With pUC19 and TZ101α, the efficiency is down 10-fold if you plate without any expression time at all, down 7-fold if you plate after 15 minutes, and down 3-fold if you plate after 30 minutes. SOC medium gives 2-fold better results than LB medium for chemically competent cells.

Plates
Some plates give better results than other plates; but, there are no magic plates. If you use plates that are less than six months old and are not too dry, you should have good results. The only plates to be careful about are tetracycline plates.
Tetracycline breaks down, particularly in the light, to toxic products that kill everything except contaminants. Put the tetracycline in when the agar's cooled down and ready to pour; and, throw the plates out after 3 months.



Electroporation

Electroporation of *E. coli* requires a high cell density and a non-ionic buffer. As with chemically competent cells, the cells are usually stored frozen. The cells are thawed, mixed with donor DNA, and placed in an electroporation chamber attached to an electroporation device. The apparatus delivers a 5 millisecond pulse of about 1,900 volts. Efficiencies of 10^{10} transformants per µg pUC19 DNA are expected from commercially prepared cells (10^{9} for home-made cells).

Ampicillin 10 mg/mL

1g	ampicillin
100mL	water

Dissolve ampicillin in water. Filter sterilize.
Store at 4°C.

LB + ampicillin

1mL	10mg/mL filtered ampicillin
1Liter	autoclaved, cooled LB

Store at room temperature.

Streptomycin 25mg/mL

25mg	streptomycin
100mL	water

Dissolve streptomycin in water. Filter sterilize.
Store at 4°C.

IPTG 100 mM

23.8mg	IPTG
1mL	water

Dissolve IPTG in water. Filter sterilize.
Aliquot in working volumes and store at -20°C.

X-gal 4%

40mg	X-gal
1mL	DMF

Dissolve X-gal in dimethyl formaide (DMF).
Store at -20°C. Protect from light. Note that X-gal and IPTG are described in “Special Technical Features” on page 16.

Troubleshouting

Transformation frequency is affected by the purity of the DNA, how the cells are handled, and how the transformation is actually performed.

Impurities in the DNA
Spin columns can be used to purify DNA from PCR reactions, ligations, endonuclease digestions, or other treatments. Its also possible to use phenol-chloroform extraction, followed by ethanol precipitation. The presence of salts is indicated if the donor DNA causes electrocompetent cells to explode.

Media and Formulations

If you use “ready-to-use” Media, you can skip this section.

LB Medium

10g	Tryptone
5g	Yeast Extract
10g	NaCl
to 1 Litre	water

Autoclave. Cool to room temperature before using.
Store at room temp.

LB Agar

10g	Tryptone
5g	Yeast Extract
10g	NaCl
15g	agar
to 1 Litre	water
add 1 stir bar	

Autoclave. Cool to 45-50°C in a water bath. Place on stir plate. Add antibiotics while stirring. Pour 25-35 ml per 87 mm plate. Let plates sit at room temperature overnight. Bag plates and store at 4°C.

SOC Medium

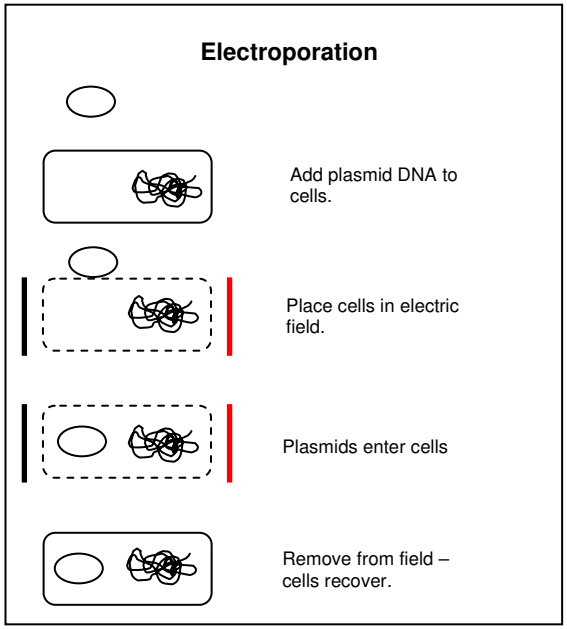
2g	Tryptone
0.5g	Yeast Extract
1mL	1 M NaCl
0.25mL	1 M KCl
1mL	1 M MgCl2
1mL	1 M MgSO4
1mL	2 M glucose
to 100mL	water

Add tryptone, yeast extract, NaCl, KCl to 97mL water. Stir, autoclave, and cool to room temperature. Prepare MgCl2, MgSO4, and glucose stocks and filter sterilize. Add magnesium and glucose solutions to cooled medium. Filter sterilize. pH should be 7.0.
Store at room temperature.

LB + 20% glycerol

80mL	LB
20mL	glycerol

Put 80 ml LB in a small flask. Add 20 ml glycerol. Run the LB up and down in the pipet a couple of times to dissolve the residual glycerol. Filter sterilize.
Store at room temperature.



What's Important in Electroporation

The most important factor in electroporation is the conductivity of the sample. It should be as close to zero as possible. The number one cause of exploding electrocompetent cells is putting too much ligation mix in the transformation reaction.

Thawing the cells
This is the same as for chemically competent cells on page 4.

Incubating the DNA with the cells on ice
For electrocompetent cells, incubation on ice is not necessary. You have an hour on ice if you want it. After that, efficiency starts to drop slowly, just like chemically competent cells.

The pulse
The heart of the electroporation process is the pulse. Instead of a heat shock, the cells are exposed to a very short and very intense electric field. The pulse has to be 4 to 5 msec at a field strength of 20 kV per cm. The field strength is usually achieved with a voltage of 2.0 kV and a 0.1 cm cuvette. With a 0.2 cm cuvette, it is impossible to reach this field strength because most machines can only deliver 2.5 kV. The length of the pulse is often achieved with a 25 µF capacitor and a 200 Ω shunt resistor.

The object of electroporation is to briefly expose the cells to a field, not to perform electrophoresis. Most of the charge runs through a shunt resistor. Conductivity in the sample causes the charge to run through the cell sample instead, often causing a spark (arcing) that blows the cells all over the inside of the cuvette. Increasing the size of the resistor causes the charge to take longer to dissipate, which increases the pulse time. Destabilizing the cell membrane for too long causes the cells to die. In addition, long pulse times promote ionization of the air in the cuvette, which leads to arcing. Decreasing the size of the resistor decreases the pulse time. Some protocols call for increasing the voltage and decreasing the pulse time.

Expression
The effect of the expression time depends on the plasmid and strain. Details have to be given by manufacturer.

Plates
Plates are no more an issue with electrocompetent cells than they are for chemically competent cells. Since electrocompetent cells are so concentrated, there can be more of a tendency to form satellites when high cell densities are plated. Satellites are untransformed cells that form small colonies in clusters around real transformants. Satellites do not grow when streaked on selective agar or inoculated into selective media.

Optimizing A New Strain

The best transformation uses pulses of 4 to 5 milliseconds, although some protocols call for as much as a 10 millisecond pulse, or as little as 2. To optimize for a new strain, start with a 4 to 5 msec pulse and vary the voltage so that the field strength varies from 15 kV/cm to as high as you can go without blowing the cells up (20 to 25 kV/cm, depending on many factors). You can vary the pulse from 2 msec to 10 msec by varying the value of the shunt resistor. Try combinations. If your cells always blow up, they have too much salt. Wash them one more time.

Protocols for Transformation

(from the Genaxxon product inserts)

Notes on Ligation Reactions:
The ligation reaction mixture inhibits transformation. Less transformants are observed from ligation reactions than from transformations with plasmid DNA.

Use of 0.5µL of a ligation reaction per 50µL of competent cells. For best results, either purify the ligation mixture by ethanol precipitation prior to transformation
or dilute the ligation reaction 3-fold in TE buffer and use 1µL per 50µL competent cells.

General Handling of Competent Cells

• Competent cells are very sensitive to any change in temperature. Cells must be thawed on ice. The transformation should be started immediately after the cells are thawed.

• Competent cells must be treated gently. Mix cells by swirling or gently tapping the reaction tube. Do not mix by pipetting or vortexing.

• Once thawed, the cells should be used. Refreezing thawed competent cells will result in a significant drop in transformation efficiency.

Transformation of Chemically Competent Cells

Advanced Preparations

- Equilibrate a non-shaking water bath to 42°C.
- Place SOC medium at room temperature.
- Prepare LB agar plates with the appropriate antibiotic. If blue/white screening for recombinants is desired, the plates should include 40µg/mL X-gal and 1mM IPTG.
- Agar plates should be placed in a 37°C incubator for about 30 minutes prior to plating.

Procedure

1. Remove competent cells from -70°C and place directly in ice. Thaw cells for 5 to 10 minutes.
2. Gently mix cells by tapping tube.
3. Add 1-50ng of DNA (or 1µL control DNA) into the 50µL competent cells. Swirl the pipettor tip through the cells while dispensing DNA. Gently tap tube to mix.
4. Place the tubes on ice for 30 minutes.
5. Heat-shock the cells for 45 seconds in a 42°C water bath. Do not shake.
6. Add 450µL of room temperature SOC medium to each transformation reaction.
7. Incubate at 37°C for one hour with shaking (225 to 250 rpm).
8. Spread on LB agar plates containing appropriate antibiotic (e.g., 100µg/mL ampicillin for control pUC19).
9. Incubate the plates at 37°C overnight (12 to 16 hours).

Transformation of Electrocompetent Cells

Notes on Ligation Reactions and Experimental DNA:

Ligation reactions inhibit transformation. Less transformants are observed from ligation reactions than from transformations with plasmid DNA.

Ligation reactions should be diluted 5-fold in TE buffer prior to transformation.
Use 1µL of the diluted ligation reaction per 40µL of electrocompetent cells.

Salts and buffers inhibit electroporation
Experimental DNA should be in a low ionic strength buffer, like TE. Samples containing too much salt will result in arcing at high voltage, which may harm the sample and the equipment.

Advanced Preparations

• Place the electroporation chamber on ice.
• Prepare LB agar plates with the appropriate antibiotic. If blue/white screening for recombinants is desired, the plates should include 40µg/mL X-gal and 1mM IPTG.
• Agar plates should be placed in a 37°C incubator for about 30 minutes prior to plating.