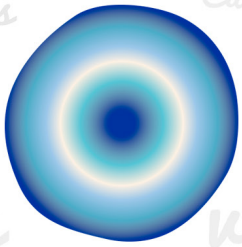

Competent Cells Selection Guide

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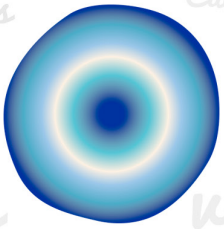

Competent Cells Selection Guide

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Competent Cells Selection Guide

1.0 Introduction

Of the hundreds of *E. coli* strains available, which one should you select for a cloning or protein expression project? Choosing the most suitable *E. coli* strain and the relevant protocol for making the cells competent are key to the success of your experiment. By carefully selecting the right strain for your specific needs, you can increase the chances of experimental success by focusing on bacterial strains containing attributes required for your experiment. In addition, the protocol you use to make a strain competent can affect the transformation efficiency of the competent cells. Despite the seemingly daunting prospect, the selection of the best competent strain for a particular application can be straightforward. The purpose of this guide is to help you choose an appropriate protocol for making competent cells as well as the proper strain for a particular experiment.

1.1 What is Transformation?

Transformation is the process by which exogenous DNA such as a plasmid is introduced into a cell. The ability to transform bacteria with plasmids underpins molecular biology. This is because apart from enabling you to study a gene of choice, bacterial transformation also provides a means to store and replicate plasmids. A cell that has been processed and made ready for the uptake of foreign DNA is described as competent. A plasmid is a circular double-stranded extrachromosomal element that is capable of independent replication within a cell. Plasmids carry both a bacterial origin of replication and an antibiotic resistance gene for use as a selectable marker in bacteria. Insertion of DNA fragments or genes into a plasmid creates a recombinant plasmid vector, which can be transformed into competent cells (Fig 1).

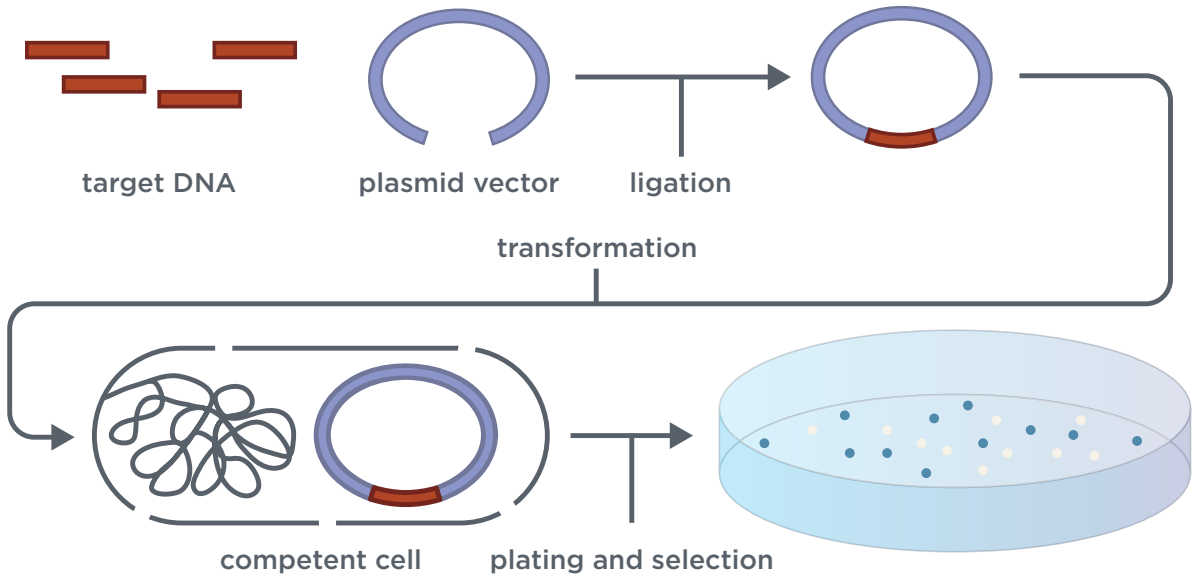
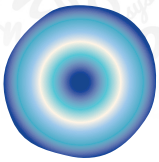


Figure 1. Transformation into a competent cell line of choice.



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Apart from traditional cloning, competent cells are used for high-throughput cloning and protein expression studies. Transformation of a recombinant plasmid vector carrying your gene of interest into a competent *E. coli* expression strain remains a popular method for producing recombinant proteins. The two methods for making competent cells are via chemical treatment (e.g., the $CaCl_2$ method) or by the application of an electric field (electroporation). Each method has its own pros and cons, which will be discussed in greater detail below.

1.2 Chemically Competent *E. coli* Cells

When *E. coli* cells are resuspended in a $CaCl_2$ solution at 0°C for about 30 minutes to an hour, it is thought that the Ca^{2+} ions puncture the cell membrane thereby creating pores in them. The positively charged calcium ions also mask the negatively charged supercoiled DNA thus enhancing membrane-DNA binding. A heat-shock treatment at 42°C forces the DNA into the competent cells. You can use the following protocol to make chemically competent cells:

- Pick a single colony of bacteria and inoculate it into 5.0 ml of LB media overnight at 37°C.
- Transfer the overnight culture (50 µl) into 20 ml of LB media in a 50-ml falcon tube.
- Grow with aeration at 37°C till the OD_{600} is between 0.2 – 0.5.
- Centrifuge the cells at 5000xg for 5 minutes at 4°C.
- Resuspend the pellet in 5.0 ml of ice-cold 0.1 M $CaCl_2$ and incubate on ice for 45 minutes followed by centrifugation.
- Resuspend the pellet in 2.0 ml of resuspension solution (7% glycerol and 50 mM $CaCl_2$).
- Store the competent cells on an ice-water bath at 4°C and use it for transformation within a week.

Tips for Success

Optimizing Chemical Competency

- To avoid contamination, follow good lab practices such as autoclaving all glassware and growth media.
- Use a freshly streaked colony and harvest the cells at OD_{600} 0.2 – 0.5.
- Keep the cells and all the materials (pipettes, tips, chemical solutions, centrifuges) chilled.
- Treat the cells gently by centrifuging at low speeds and minimize pipetting.

1.3 Electrocompetent *E. coli* Cells

The application of an electric current to electrocompetent cells also punctures their cell membranes and forces the plasmid DNA into the cells. You can use the following protocol to make electrocompetent cells:

1. Pick a single colony of bacteria and inoculate it into 10.0 ml of LB media overnight at 37°C.
2. Transfer 8.0 ml of the inoculum into 800 ml of LB media in a 2-l flask and grow with aeration to an OD_{600} between 0.7 to 1.0 at 37°C.
3. Keep the culture on ice for an hour.
4. Harvest the cells by centrifugation (5000xg for 5 minutes at 4°C) using 8 pre-chilled centrifuge pots or 50-ml falcon tubes.
5. Discard the supernatant and gently resuspend the cells in 10 ml of chilled 10% glycerol.
6. After resuspension, increase the volume of each of the resuspended cells to 25 ml with 10% glycerol.
7. Centrifuge the cells at 5000xg for 5 minutes at 4°C and discard the supernatant.

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8. Wash the cells twice more (steps 5 to 7). Resuspend and pool all the cells in a final volume of 6 ml 10% glycerol.
9. Keep the cells on ice for 10 minutes before aliquoting 200 µl each into cryovials.
10. Flash freeze the vials in liquid nitrogen and store at -80°C.

Tips for Success

Optimizing Electrocompetency

In addition to the tips mentioned above for optimizing the preparation of chemically competent cells:

- Wash the *E. coli* cells thoroughly in order to rid them of salts from the LB growth media so that the electric charge is not diminished via conduction through the growth medium during electroporation
- To reduce the adverse effects of heat on the fragile cells, perform electroporation at 0°C.

Chemically Competent vs. Electrocompetent Cells

Although electroporation produces higher transformation efficiency and the process is not as tedious as chemical transformation, it is relatively more expensive because you need an electroporation equipment. For frequent and routine transformations (e.g., ligation reactions, transforming plasmid DNA for the purposes of storage and amplification) in which you are just interested in obtaining colonies, it is more cost effective to make your own competent cells. For cDNA library preparation or high-throughput cloning, electroporation or buying ultracompetent cells is recommended.

1.4 Transformation Efficiency

In order to measure the optimum amount of DNA that can be transformed into the competent cells, you will need to determine their transformation efficiency. The efficiency of transformation is the number of transformants per microgram of supercoiled DNA. You can calculate this value by transforming cells with a known amount of supercoiled DNA. Next, divide the number of colonies formed on the agar plate by the amount of DNA used. The level of competency you expect would depend on the method of preparation (Table 1).

$$\text{Transformation efficiency} = \frac{\text{no. of transformants (colonies) X final volume at recovery (ml)}}{\text{µg of plasmid DNA X volume plated (ml)}}$$

Table 1. Typical Efficiency of Transformation by Competent Cell Preparation Method

Type of Competent Cell	Transformation Efficiency
Chemical (in-house)	10^6 to 10^7
Chemical (commercial)	10^9
Electroporation (in-house)	10^8 to 10^9
Electroporation (commercial)	10^{10}

Transformation efficiency cannot be improved by simply inundating the competent cells with DNA because of the dose-response relationship (Fig 2). Regardless of the source of the component cells, you will keep getting more transformants untill you hit a plateau after which the addition of more DNA will have no effect.

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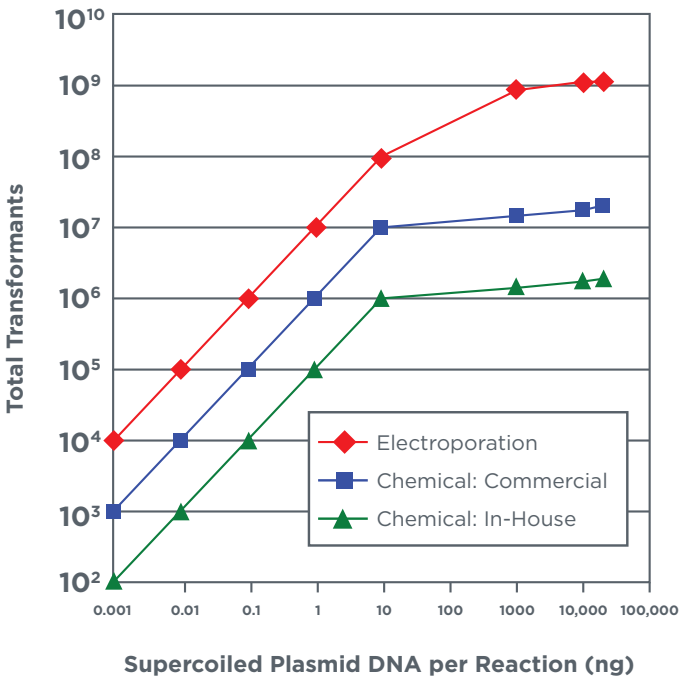

Figure 2. Plasmid DNA dose-response of transformation. The linear part of the curve determines the transformation efficiency.

1.5 Genotypes of Competent Cells

The convention for writing the genotype of a strain is to list only the known mutations with the assumption that everything else is wildtype. For example, the genotype of DH5α *(F⁻ Φ80lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17 (rk-, mk+) phoAsupE44 λ⁻thi 1 gyrA96 relA1)* will just be written as *endA*. A delta symbol before a mutant allele denotes a gene deletion. A mutant allele is not written with the minus sign hence *endA* simply describes the *endA* null phenotype.

Table 2. Some Genetic Markers of Commonly Used Competent Cells

Genetic Marker	Description
lacZΔM15	This is a partial deletion of the lacZ gene, which allows for α-complementation. Used for blue/white color selection.
lacI^q	Any strain carrying the *lacI^q* marker overproduces the lac repressor, which negatively regulates transcription from the lac promoter. The addition of IPTG removes this repression.
endA1	Produces good quality DNA because of the strain's endonuclease I deficiency.
recA1	Provides insert stability and helps prevent unwanted recombination between insert and host.
tonA	This mutation prevents T1 and T5 phage infection, which protects your clones.
mcrA, mcrBC and mrr	These mutations confer the ability to clone methylated DNA (genomic or cDNA) without restricting it thus resulting in better represented libraries.
F´ episome	Strains carrying the F´ episome can produce ssDNA.

Competent Cells Selection Guide

2.0 Strains of Competent Cells

Competent *E. coli* cells fall into two categories: cloning and expression strains. Some of the common cloning strains are DH5α, HB101, and their numerous commercial derivatives. In general, an ideal cloning strain must be:

- Highly transformable
- Retain the transformed DNA without restricting it
- Replicate plasmid with structural and functional integrity
- Produce good yields of plasmid DNA from sample preparations

In Tables 3, 4, and other parts of this guide, we mention and discuss several commercially available *E. coli* strains and their corresponding suppliers. The inclusion of these proprietary *E. coli* strains and vendors is not intended to reflect their importance, nor is it intended as an endorsement by GENEWIZ. The *E. coli* strains and suppliers mentioned are provided for informational and non-commercial use only. Any reference to any vendor, process, service or strain by trade name, trademark, or manufacturer or otherwise does not constitute or imply the endorsement, recommendation, favoring or approval of GENEWIZ.

Table 3. *E. coli* Strains for Routine Cloning

Desired Application	Mutation/Feature	Representative Strain
Blue-white screening for recombinant cells	*lacZΔM15*	DH5α TOP10 (ThermoFisher Scientific) TOP10F' (ThermoFisher Scientific)
Allows for lower endonuclease degradation which ensures higher plasmid transfer rates	*endA1*	DH5α TOP10 TOP10F'
Reduces homologous recombination for a more stable insert	*recA1*	DH5α TOP10 TOP10F'
Efficient transformation of unmethylated DNA from PCR amplifications	*hsdR*	DH5α TOP10 TOP10F'
Efficient transformation of methylated DNA from genomic preparations	*mcrA*	DH5α TOP10 TOP10F'
The presence of the F' episome enables this strain to produce ssDNA	*F' episome*	TOP10F'
A very fast growth rate cuts down the time for transformation and cell growth	*lacZΔM15, hsdR, lacX74, recA, endA, tonA*	Mach1 (ThermoFisher Scientific)

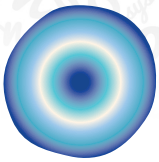

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Table 4. *E. coli* Strains for Cloning Unstable DNA, ccd Gene, and cDNA Libraries

Desired Application	Mutation/Feature	Representative Strain
Cloning of methylated genome sequences and unstable inserts such as retroviral sequences or direct repeats	*mcrA, mcrBC, hsdRMS, mrr, recA1*	MAX Efficiency® Stbl2™ (ThermoFisher Scientific)
Optimized for cloning direct repeats found in lentiviral expression vectors	*mcrA, mcrBC, hsdRMS, mrr, recA1, endA1*	One Shot® Stbl3™ (ThermoFisher Scientific)
Electrocompetent derivatives of Stbl2™ cells that can be transformed with large plasmids (e.g., 50 kb cosmids and 100–200 kb P1 clones)	*mcrA, mcrBC, hsdRMS, mrr, recA1*	ElectroMAX™ Stbl4™ (ThermoFisher Scientific)
Designed to reduce the copy number of most vectors thus making amenable for cloning unstable DNA	*mcrA, mrr, hsdRMS, mcrBC, endA1, recA1*	CopyCutter™ EPI400™ (EpicentreBio)
Suitable for routine cloning and the production of ssDNA	*endA, recA, hsdR, lacI^{q}ZΔM15, F$'$ episome*	Promega™ JM109 (ThermoFisher Scientific)
Engineered to accommodate eukaryotic genes with nonstandard secondary and tertiary structures, including cruciforms (caused by inverted repeats) and Z-DNA	*McrA, McrCB, McrF, Mrr, HsdR, endA, recB, recJ*	SURE 2 Supercompetent Cells (Agilent Technologies)
The product of the ccdB gene kills *E. coli* by targeting DNA gyrase. This technique has been used in the Gateway® Cloning System (ThermoFisher Scientific)	*ccdB*	One Shot® ccdB Survival™ 2 T1^R (ThermoFisher Scientific)
For cloning and sample preparation of pBR322 plasmids, plus cDNA library construction	*recA1, hsdS20*	HB101
cDNA library construction	*lacZΔM15, mcrA, mcrBC, mrr, hsdRMS, recA1*	DH10B™ (ThermoFisher Scientific)

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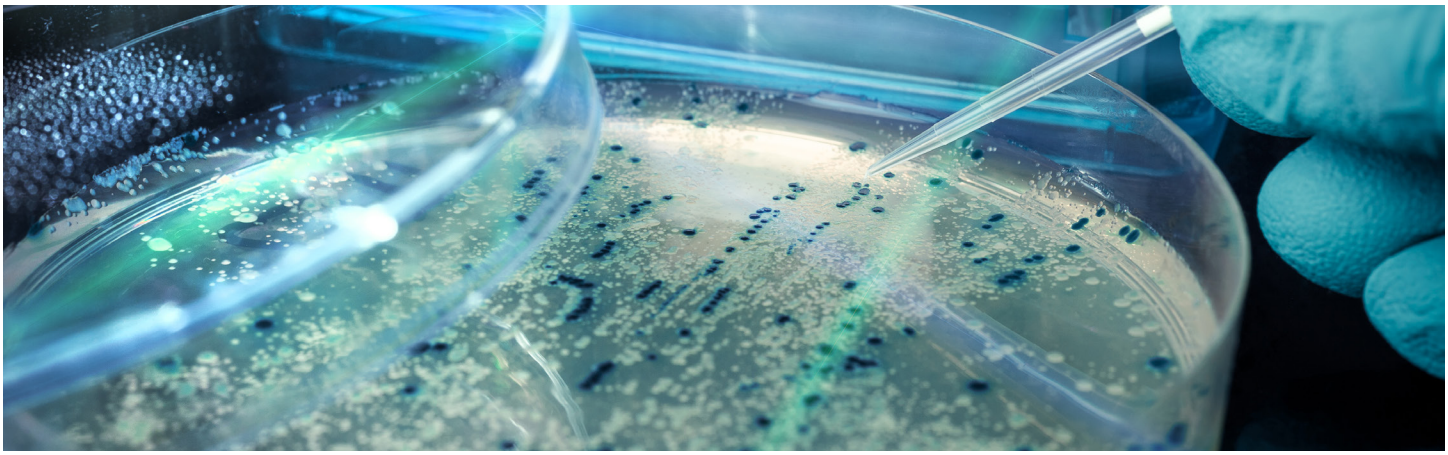


For routine recombinant protein expression in *E. coli,* the first step involves cloning of the target gene into an appropriate expression vector followed by the transformation of the recombinant plasmid into a suitable host system for subsequent protein expression analysis. A good expression system consists of (i) a strong promoter; (ii) a repressor of the strong promoter; and (iii) an inducer of expression. The T7 RNA Polymerase System is widely used for producing proteins in *E. coli.* In the next section, we will examine the properties of two of the most popular *E. coli* strains for protein expression.

2.1 Strains for Protein Expression

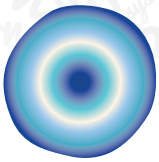
In general, an ideal host strain for protein expression must have the following properties:

- Transformable
- Protease deficient (lon, ompT)
- Expression system (e.g., T7 polymerase, araD)
- Promoter control

In practice, an expression vector (e.g., the pET series) containing your gene of interest cloned downstream of the T7 promoter is transformed into an *E. coli* expression host such as BL21(DE3) or BL21(DE3)pLysS (Table 5).

Table 5. Some *E. coli* Strains for Protein Expression

Desired Application	Mutation/Feature	Representative Strain
T7 promoter expression system for the overproduction of nontoxic proteins	T7 expression strain; Deficient in Lon and OmpT proteases; Resistant to T1 phage	BL21(DE3)
Tighter promoter control to minimize basal expression of proteins that may be toxic to the cell.	In addition to the features of BL21(DE3), this strain contains a plasmid that produces T7 lysozyme to control the basal expression of T7 RNA polymerase	BL21(DE3)pLysS



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Promoter control is vital in order to control both the timing and nature of recombinant protein induction. Basal expression may kill the cells if the protein is toxic. On the other hand, a wild uncontrolled expression of a gene of interest can have adverse effects on the cells. A *lacI*^{q} mutant strain makes enough repressor to keep the promoter in check until you are ready to induce protein expression. When you induce protein expression by the addition of IPTG to the culture, T7 RNA polymerase is expressed by the DE3 element in the chromosome. T7 RNA polymerase transcribes the gene of interest, followed by translation of the desired protein by the endogenous protein translation machinery. In the absence of IPTG, T7 polymerase expression is repressed by *lac I* gene.

3.0 Summary

E. coli competent cells are one of the workhorses of molecular biology hence it is important to know which strains to select for different applications. The decision whether to buy competent cells or make them yourself depends on several factors such as your budget, frequency of use, expertise, and the particular experiment. We hope that this guide has given you the knowledge to approach all facets of competent cell selection and use with ease and confidence.

Gene Synthesis is a Convenient way to Obtain Your Plasmid DNA for Transformation

Gene synthesis is the process of synthesizing a gene *in vitro* without the need for initial template. Contrary to the prevailing dogma, commercial gene synthesis service is the most cost effective alternative to traditional cloning and other molecular biology procedures. The main reasons include:

- **Time savings:** Traditional cloning involves a multi-step process that includes cloning strategy design, primer synthesis, PCR, gel extraction, bacteria transformation, and other complex steps. This process requires considerable amount of time and human resource that gene synthesis does not.
- **Cost savings:** In most cases, it costs less to order a synthetic gene than it does to order oligos, cloning kits, and DNA sequencing services.
- **Enhanced DNA performance:** Gene synthesis allows for codon optimization which has been proven to increase the efficiency of protein expression.
- **Convenience:** Without the need for a physical template and without design restrictions associated with the traditional cloning process, a researcher can get a gene of his/her choice by simply supplying the nucleotide sequence or amino acid sequence.

GENEWIZ is a global CRO that provides a wide range of DNA services. Our gene synthesis service has a quick turnaround of 5-10 business days plus expert technical and project management support. Learn more

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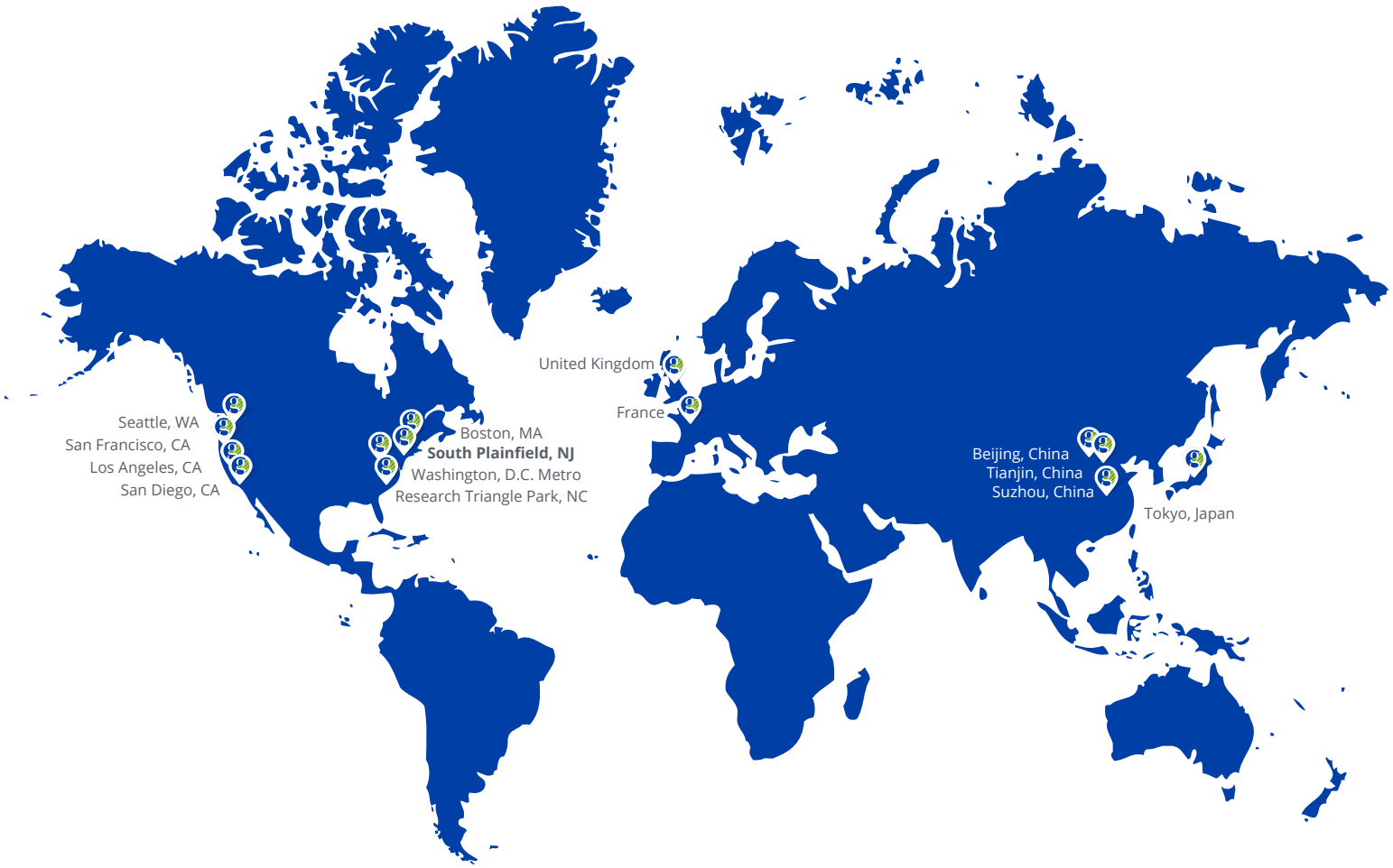

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