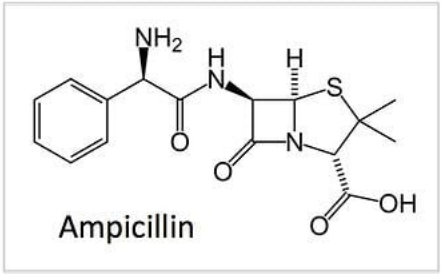
Plasmids 101: Antibiotic Resistance Genes

By Marcy Patrick



Resistance to antibiotics is a widely used tool in molecular biology, yet scientists rarely stop to think about how much easier it makes our lives. Plasmid transformation into *E. coli* is a fairly inefficient process– just 1 out of 10,000 cells on average! Without some means of quickly determining which cells successfully received the correct plasmid, scientists would spend hours to days trying find their correct clones. Additionally, the presence of a plasmid is disadvantageous from the bacterium's perspective – a plasmid-containing cell must replicate the plasmid in addition to its own chromosomal DNA, costing additional resources to maintain the plasmid. Adding an antibiotic resistance gene to the plasmid solves both problems at once – it allows a scientist to easily detect plasmid-containing bacteria when the cells are grown on selective media, and provides those bacteria with a pressure to keep your plasmid. Viva la (bacterial) resistance!

What are antibiotics?

Antibiotics are generally defined as agents that kill bacteria, or inhibit their growth. Although originally sourced from natural products, many common antibiotics used in labs today are semi-synthetic or fully synthetic compounds. Antibiotics can be categorized based on whether they directly kill bacteria (bactericidal) or slow growth/prevent cell division (bacteriostatic); however, the distinction between the two categories may be a bit of a gray area as some bacteriostatic reagents can kill bacteria when used at high concentrations (and vice versa). Looking around the lab, you'll likely find many of the antibiotics listed in the table below. Note, in this post we'll focus primarily on antibiotics against Gram negative bacteria. In future posts, we'll detail selection in non-bacterial cells such as yeast or mammalian cells.

Name	Class	Mode of Action*		Working Concentration**
Kanamycin	aminoglycoside	Binds 30S ribosomal subunit; causes mis-translation	Bactericidal	50-100 ug/mL
Spectinomycin	aminoglycoside	Binds 30S ribosomal subunit; interrupts protein synthesis	Bactericidal	7.5-50 ug/mL
Streptomycin	aminoglycoside	Inhibits initiation of protein synthesis	Bactericidal	25-100 ug/mL
Ampicillin	beta-lactam	Inhibits cell wall synthesis	Bactericidal	100-200 ug/mL
Carbenicillin	beta-lactam	Inhibits cell wall synthesis	Bactericidal	100 ug/mL
Bleomycin	glycopeptide	Induces DNA breaks	Bactericidal	5-100 ug/mL
Erythromycin	macrolide	Blocks 50S ribosomal subunit; inhibits aminoacyl translocation	Bacteriostatic	50-100 ug/mL in EtOH
Polymyxin B	polypeptide	Alters outer membrane permeability	Bactericidal	10-100 ug/mL
Tetracycline	tetracyclin	Binds 30S ribosomal subunit; inhibits protein synthesis (elongation step)	Bacteriostatic	10 ug/mL
Chloramphenicol		Binds 50S ribosomal subunit; inhibits peptidyl translocation	Bacteriostatic	5-25 ug/mL in EtOH

**In prokaryotes.* ***Dissolve in dH_2O and sterile filter unless otherwise specified.*

The above table lists some antibiotics commonly found in the lab, their mechanism for killing bacteria, and general working concentrations. For instructions on how to prepare antibiotic stocks, see Addgene's Reference Page.

How else can antibiotics be used in the lab?

Historically, antibiotics have also been used to disrupt genes at the chromosomal level. Scientists introduce an antibiotic resistance cassette within the coding region of the gene they are trying to disrupt or delete, which both inactivates the gene and acts as a marker for the mutation. When designing these types of experiments it is best practice not to use the same resistance cassette for the mutation and for plasmid selection. Additionally, scientists can use the loss of resistance as a marker for successful cloning. In these instances, the cloning vector typically has two separate resistance cassettes and your gene of interest is cloned into/inactivates or completely removes (in the case of Gateway cloning) one cassette. Counterselection allows the scientist to select bacteria that are only resistant to the antibiotic that remains intact.

Tips and tricks from the bench

- Use fresh stocks. Most antibiotics are stable in powder form, but quickly breakdown in solution. Storing aliquots at -20^{o}C and avoiding repeated freeze/thaw cycles will keep most antibiotics viable for at least 6 months.

- Ampicillin breaks down especially fast and plates should be used witin 1 month for optimal efficiency. Beware of satellite colonies!

- Carbenicillin is more stable than Ampicillin and can be used in place of Ampicillin in most applications.

- Antibiotics vary in their sensitivitly to heat and/or light – do not add them to media hotter than about 55^{o}C and store plates/stocks wrapped in foil if a light-sensitive antibiotic like Tetracycline is used.

- Keep in mind that some *E. coli* strains have natural antibiotic resistances, so make sure your plasmid and *E. coli* strain are compatible! Check out this list of common *E. coli* genotypes and their natural resistances.

Additional Resources on the Addgene Blog

- Read other Plasmids 101 Posts
- Learn How to Use Blue White Screening to Simplify Your Cloning Experiments
- See Where You Can Find Antibiotic Resistance on Our Plasmid Pages

Resources on Addgene.org

- Browse Our Molecular Biology Reference Pages
- View our Plasmid Protocols

- Find Your Perfect Plasmid Backbone

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Topics: Plasmid Elements, Plasmids 101, Plasmids

Leave a Comment

Marcy Patrick 2014/2/8 上午1:11:00

Hi Amir,

Thanks for your comment and for providing the review on marker-free plasmids. I agree that the development of plasmids that can be maintained without using antibiotics is an important advancement, especially for gene therapy and other large-scale biotech operations where biosafety and the emergence of antibiotic resistant strains is a real concern. I do not know whether these "non-traditional" methods of selection will be adopted into many areas of basic science research, but the technology is quickly making its way into the synthetic biology field and applied sciences--I am sure we will be hearing more about these types of plasmids in the future!

Reply to *Marcy Patrick*

AB 2015/11/19 上午4:38:39

How do you verify that the promoter driving the antibiotic resistance on a plasmid is expressed in the species of bacteria you are using (if not E. coli)? Also, why are the promoters not specified in plasmid diagrams?

Reply to *AB*

Marcy Patrick (Addgene) 2015/11/19 下午11:36:15

Dear AB,

Thanks for your question! Oftentimes the promoter for the antibiotic resistance cassette is omitted for simplicity in cartoon maps or may not be recognized as a "feature" with some mapping software even though they are required for the cassette to express. Many cloning plasmids utilize a promoter recognized by the E. coli sigma 70 RNAP (such as the endogenous cat, kan, or bla promoters) to constitutively express the resistance gene during exponential growth. If you are working with Eubacteria, it is likely these promoters will work. Otherwise you may need to check the literature and/or NCBI database to determine whether your bacteria have a comparable sigma factor that would recognize the promoter consensus sequences found at –10 and –35 upstream of the transcription start site.

I hope this helps!

Reply to *Marcy Patrick (Addgene)*

Marcy Patrick (Addgene) 2017/3/1 上午1:03:34

Dear Kajal,

Thanks for your question. Bacteria evolved to sense and adapt to their surroundings, and have developed receptors and signaling mechanisms to turn genes off or on as the environment requires. Resistance to antibiotics is an evolutionary response to exposure to these compound and likely stems from gene exchange and/or mutations that allow a bacterium to survive in the presence of antibiotics. This perspetive provides additional information and links to some of the early research into resistance plasmids: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1206476/pdf/ge13941465.pdf

I hope this helps!

Reply to *Marcy Patrick (Addgene)*

Jiya 2017/10/5 下午5:18:44

Are selectable markers already present on a plasmid or are they introduced on it? If introduced, then how?

Reply to *Jiya*

Jiya 2017/10/14 上午2:07:45

E.coli cells normally don't possess antibiotic resistance against ampicillin, chloramphenicol, tetracycline, or kanamycin... Still their plasmids are essential vectors... What acts as selectable markers in them?

Reply to *Jiya*

Marcy Patrick (Addgene) 2017/10/14 上午3:27:27

Hi Jiya,

Thanks for your question. Many bacteria, including E. coli, are or can become resistance to antibiotics, including possessing or gaining resistance to drugs that are commonly used in the lab like Amp, Kan, Tet, etc. In general the strains of E. coli used in the lab have been selected or specifically engineered to no longer have natural resistance to common lab antibiotics; however, some strains do still retain their natural resistance marker(s). Our post on common lab strains lists the natural resistance of some frequently used E. coli strains (http://blog.addgene.org/plasmids-101-common-lab-e-coli-strains).

Although drug selection is a common and fairly straightforward way to maintain a plasmid within a cell, it is by no means the only way. Generally a bacterium will keep a plasmid so long as the plasmid is providing some sort of advantage to the host cell. Our Plasmids 101 post on Environmental Plasmids may be useful to you: http://blog.addgene.org/plasmids-101-environmental-plasmids

Reply to *Marcy Patrick (Addgene)*

Suzan Ragheb 2019/7/22 下午1:34:46

I would like to ask if I want to use meropenem antibiotic (Carbapenem) in my transformation experiments, What is the suitable concentration to be used? Thanks

Reply to *Suzan Ragheb*

Jennifer Tsang (Addgene) 2019/7/22 下午7:51:10

Hi Suzan,

Thanks for your question. We don't have a specific answer for you in terms of concentration as it likely depends on what cells you're transforming and the plasmid you are using. You could try to empirically determine the best concentration for your experiment by growing on a selection curve of antibiotics as described here: https://www.addgene.org/protocols/over-agar-antibiotic-plating/

Reply to *Jennifer Tsang (Addgene)*

Jianxin Ma 2020/9/14 上午9:39:54

Does Addgene has plasmids carrying resistance to polymyxin?
Thanks.

Reply to *Jianxin Ma*

Andrew Hempstead (Addgene) 2020/9/15 下午9:52:52

Hi Jianxin,

Thanks for your question! I performed a search of our repository, and was able to find pGDP2 MCR-1 (https://www.addgene.org/118404/), a bacterial expression plasmid with an mcr-1 insert, which is a polymyxin-resistance gene. This plasmid is part of the Minimal Antibiotic Resistance Platform (ARP) (https://www.addgene.org/kits/wright-minimal-arp/) that includes a number of plasmids for expression of resistance genes.

I hope this helps!

Best,
Andrew

Reply to *Andrew Hempstead (Addgene)*

Xingdi Ma 2020/9/23 上午2:53:46

If a vector has two bacterial antibiotic resistance genes, should I add both antibiotics onto LB plates or just one when I propagate the vector plasmid?

Reply to *Xingdi Ma*

Andrew Hempstead (Addgene) 2020/9/23 下午9:20:56

Hi Xingdi,

Thanks for your question! The use of multiple antibiotics for selection is often dependent on the specific plasmid. On our plasmid pages, the recommended antibiotic(s) for growth in E. coli are found under the Growth in Bacteria section under "Bacterial Resistance(s)". There are some cases where two antibiotics will be recommended, such as to limit plasmid recombination or negative selection against a plasmid feature like ccdB. In other cases, a second antibiotic resistance may not be under a promoter that is functional in E. coli and, as a result, you would not want to use this antibiotic for selection in this host. But it could potentially be used in mammalian cells or another bacterial strain.

I hope this helps!

Best,
Andrew

Reply to *Andrew Hempstead (Addgene)*

Inga Zhu 2021/4/7 上午3:00:14

Hello:) thanks for the super nice article! I have a question....are there standard expression levels of beta lactamase for example and what is the concentration of it in periplasm? Or is it promoter dependent? And to which expression levels then correspond the recommended ampicillin concentration?

Reply to *Inga Zhu*

Nyla Naim (Addgene) 2021/4/7 下午9:57:27

Hello,

The expression/efficacy of beta lactamase depends on several factors like concentration in the periplasm (depends on synthesis from promoter, plasmid copy number, and protein turn-over) and the type of bacteria (gram-positive bacteria have an additional wall that the antibiotic must get through).
For a more detailed answer about the correlation, these publications may be helpful:

https://www.sciencedirect.com/science/article/pii/S1198743X1465031X
https://academic.oup.com/femsle/article/176/1/11/528493

For most plasmid and cloning applications, you can gauge how much Ampicillin to use based off of the plasmid copy number. Typically you can use 100ug/mL for high copy plasmids and 50ug/mL for low copy plasmids.

Best regards,
Nyla

Reply to *Nyla Naim (Addgene)*

Serysh Ghaus 2021/4/7 下午10:05:21

On a technical level, it's possible to shorten the 1-hour incubation time post-heat shock to couple of minutes if an Ampicillin resistance is used, but not possible if a Kanamycin resistance is used. Why?

Reply to *Serysh Ghaus*

Jennifer Tsang (Addgene) 2021/4/7 下午11:08:09

Hi Serysh,

Thanks for your question. This is because kanamycin inhibits translation meaning that if you plate them immediately, the cells don't have a chance to express the resistance gene.

Jennifer

Reply to *Jennifer Tsang (Addgene)*

Şeyma Polat 2021/6/3 下午5:49:07

I want to ask about using plasmids containing different antibiotic resistance genes. For example, I try to produce lentiviral vectors but one of my plasmids contains the Amphicilin resistance gene, and the other one contains Kanamycin resistance gene. Does that cause any problems for my study?

Reply to *Şeyma Polat*

Nyla Naim (Addgene) 2021/6/4 上午1:27:52

Hello Şeyma,

Usually there isn't a problem using lentiviral vectors that have different bacterial resistance genes. However, most researchers test different mammalian resistance genes like Puromycin and Hygromycin when preparing lentiviral particles. Please see our post on mammalian vectors or our Viral Vector Guide:
https://blog.addgene.org/plasmids-101-mammalian-vectors
https://www.addgene.org/viral-vectors/

~Nyla

Reply to *Nyla Naim (Addgene)*

Şeyma Polat 2021/6/4 上午3:05:52

Thanks for your quick reply :) I'll look at the links.

Reply to *Şeyma Polat*

Ruben Claus 2021/6/9 下午11:46:30

Imagine you are trying to bring a gene X to expression. After cloning the cDNA of your gene X in a compatible vector and transfection of bacteria, you discover you forgot to add the antibiotic. what would happen in that case?

Reply to *Ruben Claus*

Chrislyn Mujan 2021/6/26 下午3:58:20

what is the used of ampicillin resistance gene in cloning vector .

Reply to *Chrislyn Mujan*

Jennifer Tsang (Addgene) 2021/6/28 下午7:43:07

Hi Chrislyn,

You can use the ampicillin resistance gene to select for bacteria that contain your plasmid. You can read more about this in paragraph 1 on this page or in this article (https://blog.addgene.org/plasmids-101-positive-and-negative-selection-for-plasmid-cloning).

Jennifer

Reply to *Jennifer Tsang (Addgene)*

Nasrin 2021/7/14 下午7:19:49

Hello. Thanks for you informative article.
I'm planning on producing a 28 aa in pET 28 in Lb21 e.coli. I have never worked with expression vectors before. I'll be glad if answer some of my questions.
The resistance gene is kanamycin but i dont understand the process which colonies will be picked up? Our media must contain kanamycin right?
And when i want to design my vector with vectorbuilder, it offers to choose a ORF which are some fluorescent proteins. I dont get the necessity of it.
Would you mind clarifying

Reply to *Nasrin*

Andrew Hempstead (Addgene) 2021/7/14 下午9:56:49

Dear Nasrin,

Thank you for your questions!

For plasmids with a kanamycin resistance gene, kanamycin should be included in both the liquid medium as well as LB agar plates for selection of bacteria containing the plasmid. Please see our Recipient Instructions (https://www.addgene.org/recipient-instructions/myplasmid/) for information on handling bacteria harboring a plasmid.

At Addgene, we unfortunately do not perform any cloning experiments, but I would recommend reviewing our Molecular Cloning Techniques (https://www.addgene.org/mol-bio-reference/cloning/) for a few methods you could consider for cloning in your gene of interest encoding for a 28 aa protein.

I hope this helps!

Best,
Andrew

Reply to *Andrew Hempstead (Addgene)*

Nasrin 2021/7/15 上午1:17:48

That was very helpful. Thanks

Reply to *Nasrin*

Thu Ha Nguyen 2021/7/15 下午1:27:40

Hello, I receive a plasmid sequence also one plasmid vial.
But I don't know the antibiotics can use for screening. Could you introduce some method to determine my plasmod resistance region?

Reply to *Thu Ha Nguyen*

Jennifer Tsang (Addgene) 2021/7/15 下午8:05:21

Hi Thu,
Is this a plasmid from Addgene? If so. you can see the selectable marker for the plasmid on the Addgene's plasmid page in the "backbone" section.

Otherwise, you can BLAST the plasmid sequence to identify the antibiotic resistance gene. For more details on BLAST, check out this article: https://blog.addgene.org/tips-for-using-blast-to-verify-plasmids

Jennifer

Reply to *Jennifer Tsang (Addgene)*

SHIREESH APTE 2021/10/7 下午11:44:05

A DIY crisper-cas9 kit from The ODIN, provides agar in powdered form to be reconstituted with water and microwaved. My question is, will not microwaving decompose streptomycin (that is included in the agar powder). I tried contacting ODIN but have received no reply.

Reply to *SHIREESH APTE*

Andrew Hempstead (Addgene) 2021/10/8 下午9:10:44

Dear Shireesh,

Thank you for your question. From a review of the literature, I was able to find the following publication (https://pubmed.ncbi.nlm.nih.gov/11355358/) in which the antibiotics are added prior to heating in a microwave. I would recommend reviewing this for additional information.

I hope this helps!

Best,
Andrew

Reply to *Andrew Hempstead (Addgene)*

Add Comment

Marcy Patrick

January 30, 2014

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