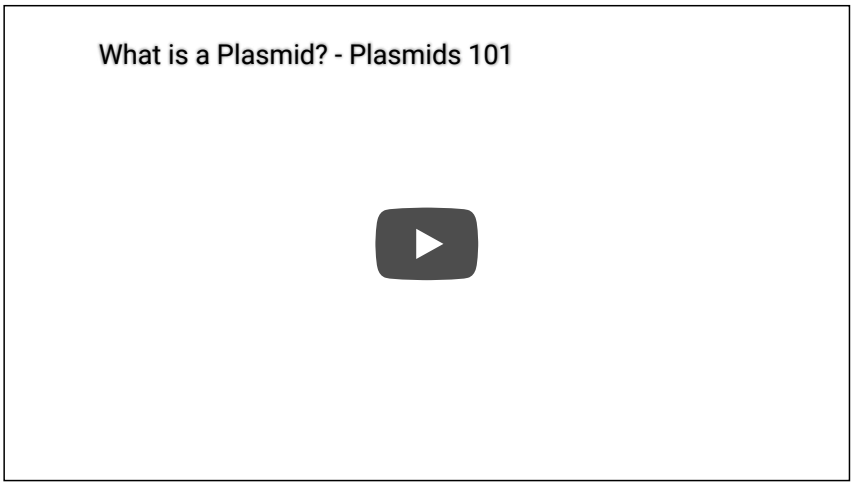
Plasmids 101: What is a plasmid?

By Margo R. Monroe

Originally published Jan. 14, 2014 and updated Apr. 14, 2020.

Any newcomer who joins a molecular biology lab will undoubtedly be asked to design, modify, or construct a plasmid. A plasmid is a small circular piece of DNA found in bacterial cells, and someone new to plasmids may need some extra guidance to understand the specific components that make up a plasmid and why each is important.

Our "Plasmids 101" series designed to educate all levels of scientists and plasmid lovers - serves as an introduction to plasmids. Plasmids 101 will provide you with an overview of general molecular biology knowledge and techniques, and empower you with a firm understanding of the fundamentals. Our mission is to curate a one-stop reference guide for plasmids, so that you can spend less time researching the basics and spend more time developing cleverly designed experiments and innovative solutions necessary for advancing the field.



What is a plasmid?

At their most basic level, plasmids are small circular pieces of DNA that replicate independently from the host's chromosomal DNA. They are mainly found in bacteria, but also exist naturally in archaea and eukaryotes such as yeast and plants. In nature, plasmids provide one or more functional benefits to the host such as resistance to antibiotics, degradative functions, and/or virulence. All natural plasmids contain an origin of replication (which controls the host range and copy number of the plasmid) and typically include a gene that is advantageous for survival, such as an antibiotic resistance gene.

In contrast, plasmids utilized in the lab are usually artificial and designed to introduce foreign DNA into another cell. Minimally, lab-created plasmids have an origin of replication, selection marker, and cloning site. The ease of modifying plasmids and the ability of plasmids to self-replicate within a cell make them attractive tools for the life scientist or bioengineer.

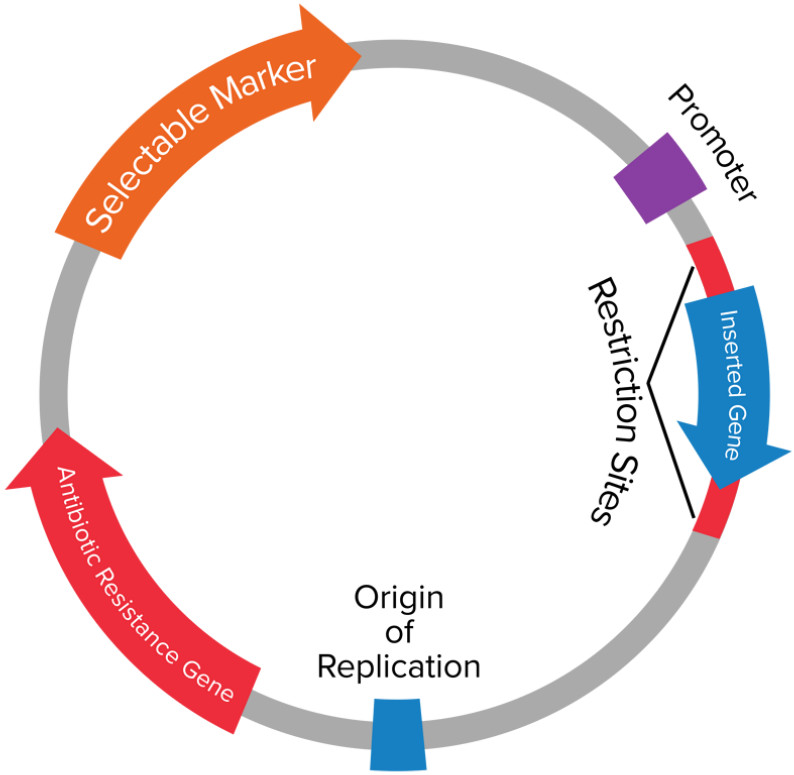


Figure 1: Map of a plasmid with its elements described below.

Vector Element	Description
Origin of Replication (ORI)	DNA sequence which allows initiation of replication within a plasmid by recruiting replication machinery proteins
Antibiotic Resistance Gene	Allows for selection of plasmid-containing bacteria.
Multiple Cloning Site (MCS)	Short segment of DNA which contains several restriction sites allowing for the easy insertion of DNA. In expression plasmids, the MCS is often downstream from a promoter.
Insert	Gene, promoter or other DNA fragment cloned into the MCS for further study.
Promoter Region	Drives transcription of the target gene. Vital component for expression vectors: determines which cell types the gene is expressed in and amount of recombinant protein obtained.
Selectable Marker	The antibiotic resistance gene allows for selection in bacteria. However, many plasmids also have selectable markers for use in other cell types.
Primer Binding Site	A short single-stranded DNA sequence used as an initiation point for PCR amplification or sequencing. Primers can be exploited for sequence verification of plasmids.

How is a plasmid constructed in the lab?

Due to their artificial nature, lab plasmids are commonly referred to as “vectors” or “constructs.” To insert a gene of interest into a vector, scientists may utilize one of a variety of cloning methods (restriction enzyme, ligation independent, Gateway, Gibson, etc). The cloning method is ultimately chosen based on the plasmid you want to clone into. Regardless, once the cloning steps are complete, the vector containing the newly inserted gene is transformed into bacterial cells and selectively grown on antibiotic plates.

Importantly, because the bacteria from which plasmids are isolated grow quickly and make more of the plasmids as they grow, scientists can easily make large amounts of plasmid to manipulate and use in later work.

How do scientists use plasmids?

Generally, scientists use plasmids to manipulate gene expression in target cells. Characteristics such as flexibility, versatility, safety, and cost-effectiveness enable molecular biologists to broadly utilize plasmids across a wide range of applications. Some common plasmid types include cloning plasmids, expression plasmids, gene knock-down plasmids, reporter plasmids, viral plasmids, and genome engineering plasmids.

Some of the many things that plasmids can be used to do include:

- Produce large amounts of a protein so that scientists can purify and study it in a controlled setting. Read more:
  - Plasmids 101: Protein Tags
- Produce proteins that glow so that scientists can track their location or quantity inside a cell
  - Plasmids 101: Green Fluorescent Protein (GFP)
  - Plasmids 101: Luciferase
- Monitor the level of a chemical in a particular environment
- Produce enzymes that will make specific, controlled changes to an organism's genome (genome engineering)
- Produce synthetic viruses that can be used in research or for therapeutics

Addgene has compiled various educational resources to facilitate plasmid use in the lab. Addgene's Molecular Biology Reference includes information about molecular cloning, how to choose a plasmid vector, molecular biology tools and references, and how to maintain your plasmid stocks. The guide also contains multiple protocols and troubleshooting tips to make plasmid usage as simple and straightforward as possible.

If you have a question about a specific plasmid element that you would like answered or any topic suggestions for our Plasmids 101 series, please let us know in the comments.

Note: Marcy Patrick contributed to the writing of this article.

Topics: Plasmids 101, Plasmids

Leave a Comment

Jungwoo Lee 2014/12/18 上午11:43:39

Learn about genetic engineering

Reply to *Jungwoo Lee*

Marcy Patrick (Addgene) 2014/12/19 下午10:36:03

Dear Jungwoo Lee,

Thank you for your suggestion! Addgene has a few blog posts related to genome engineering that can be found here: http://blog.addgene.org/topic/genome-engineering

Additionally, we host an educational resource describing ZFN, TALEN, and CRISPR technologies: http://www.addgene.org/genome-engineering/

We will also be posting some Plasmids 101 blogs that tie in certain plasmid elements to genome engineering processes, so be on the look out for those in early 2015!

Reply to *Marcy Patrick (Addgene)*

junaid bhat 2017/6/15 下午11:36:14

hi
my question is does ori recruit transcriptional machinery for replication

Reply to *junaid bhat*

Marcy Patrick (Addgene) 2017/6/16 下午9:02:25

Dear Junaid,

Thanks for your question! Plasmids use the host cell's replication machinery, but the mechanism varies depending on which ori is present. Our blogs on origins of replication provide more details as to how these elements work. I would recommend reviewing these posts and their associated references: http://blog.addgene.org/plasmid-101-origin-of-replication and http://blog.addgene.org/plasmids-101-stringent-regulation-of-replication

I hope this helps!

Reply to *Marcy Patrick (Addgene)*

Pernille Rasmussen 2018/2/7 下午8:52:37

Hello
Can I use a plasmid from E.coli in K. pneunomiae ? For instance can i use a pET plasmid in K. pneunomiae ?

Reply to *Pernille Rasmussen*

Tammy Ane 2018/10/1 下午7:07:00

Great blog posting. Thank you

Reply to *Tammy Ane*

Maliheh Parvanak 2018/10/24 下午9:29:06

nothing

Reply to *Maliheh Parvanak*

Chandrabhan Chauhan 2019/3/1 下午11:19:54

Very thanks ..... I am student of TD college jaunpur up.

Reply to *Chandrabhan Chauhan*

Rajkumar Ghuraiya 2019/6/8 下午4:45:14

Really very happy to say,your post is very interesting to read.I never stop myself to say something about it.You're doing a great job.Keep it up.

Reply to *Rajkumar Ghuraiya*

Deepika joshi 2019/9/13 下午2:57:16

nice blog thank you for sharing
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Reply to *Deepika joshi*

Sahraoui Dounia zed 2019/11/11 下午9:55:19

I want définition of plasmide col plaes

Reply to *Sahraoui Dounia zed*

zima zima 2019/12/3 下午6:53:06

what is difference between plasmid and and a chimera plasmid?

Reply to *zima zima*

Andrew Hempstead (Addgene) 2019/12/4 下午10:20:39

Dear Scientist,

Thank you for your question!
A chimeric plasmid is a plasmid in which DNA from another source has been cloned into the plasmid. I would recommend the following publication (https://www.ncbi.nlm.nih.gov/pubmed/807234), which describes a chimeric plasmid created from plasmid and Drosophila melanogaster DNA.

I hope this information helps!

Best,
Andrew

Reply to *Andrew Hempstead (Addgene)*

Nyla Naim (Addgene) 2020/4/22 下午9:49:55

Hello,

Interesting question. The '19' comes from the original vector that was used to make it, M13mp19. More details can be found in the associated publication (https://www.ncbi.nlm.nih.gov/pubmed/6323249), which explains how the numbering system was based on different restriction enzyme sites that were added to plasmid.

Best regards,
Nyla

Reply to *Nyla Naim (Addgene)*

IBRAHIM HASSAN 2020/5/1 下午8:44:53

Hi,

Do naturally occuring plasmids possess an expression promoter? Hypothetically, lets say i have an E. coli strain which carries a plasmid bearing a gene of interest naturally. Could i isolate the plasmid in question and transfect a mammalian cell in vivo, and expect the mammalian cell to express the gene of interest?

Thank you in anticipation of your kind response. Regards!

Reply to *IBRAHIM HASSAN*

Michael Lemieux (Addgene) 2020/5/4 下午9:45:53

Hi Ibrahim,

Thank you for your question! Natural plasmids sometimes carry genes that aid in the survival of the host organism. Those genes would need promoters to drive expression, but the promoters would again be specific to the host organism. So a natural plasmid isolated from bacteria, for example, would generally not be expected to express in a mammalian system. This is why scientists create artificial plasmids and typically need to insert an appropriate promoter before expressing in their target cells.

I hope this helps!
Mike

Reply to *Michael Lemieux (Addgene)*

Natalie 2020/9/27 上午9:22:42

Hi, thanks for putting together this resource! I just wanted to point out that there is a mistake in your table for the description of the ORI. It says that the transcription machinery is recruited, but it should be the replication machinery.

Reply to *Natalie*

Jennifer Tsang (Addgene) 2020/9/28 下午8:03:46

Thank you Natalie! You're correct. We've made this update.

Reply to *Jennifer Tsang (Addgene)*

Jack 2020/12/1 上午10:34:34

I have a question. How are plasmids protected from bacterial DNases during transformation? I read that bacterial DNAs are heavily methylated and this prevents them from being degraded but what about plasmids?

Reply to *Jack*

Jennifer Tsang (Addgene) 2020/12/2 下午9:00:56

Hi Jack,

Yes plasmid DNA can be methylated in cells or before the transformation, if needed. You can find more about methylation on our blog post: https://blog.addgene.org/plasmids-101-methylation-and-restriction-enzymes

This paper describes methylation in vitro for H. pylori transformation using cell-free extracts: https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1365-2958.2000.02036.x

Hope this helps.

Jennifer

Reply to *Jennifer Tsang (Addgene)*

A C 2021/1/2 上午11:43:01

What are the techniques for transferring desirable genes using plasmid?

Reply to *A C*

Jennifer Tsang (Addgene) 2021/1/4 下午11:28:52

There're so many! Are you looking to integrate genes into the chromosome using plasmids?

One way is to use lambda red recombineering: https://blog.addgene.org/lambda-red-a-homologous-recombination-based-technique-for-genetic-engineering

Here's our blog category with all about plasmid cloning which covers many techniques to clone genes into plasmids: https://blog.addgene.org/topic/plasmid-cloning

Reply to *Jennifer Tsang (Addgene)*

Dan Smith 2021/3/8 下午11:12:34

You also need to add a promoter infront of the Ab resistance gene

Reply to *Dan Smith*

Mateus Barros 2021/6/9 上午6:02:38

Helo! I'm building a plasmid vector of Sleeping Beauty transposon encoding anti-CD19 CAR and I'm going to electroporate this into gamma-delta T cells. I would like to know if this vector will need antibiotic resistance genes as they will be directly inserted into these T cells.

Reply to *Mateus Barros*

Ruby Santiago 2021/7/20 下午12:27:38

Where does this information come from?

Reply to *Ruby Santiago*

Tashfia Rahman 2021/7/27 上午2:31:03

If plasmids have already an antibiotic resistance gene why we need another selectable marker(antibiotic resistance)? Is it for differentiating within the transformed plant cells?

Reply to *Tashfia Rahman*

Nyla Naim (Addgene) 2021/7/28 下午9:51:48

Hello Tashfia,

There are a few reasons why having two antibiotic resistance genes could be helpful. For example, if you plan to express your plasmid in a mammalian cell line and want to select cells to make a stable cell line, your plasmid will need to have a mammalian selection marker as well as a bacterial selection marker to amplify the plasmid in E. coli.
In gateway cloning, two bacterial selection markers are often used. One marker is removed after cloning and serves as a useful indicator for a successful experiment.

I hope this helps!
~Nyla

Reply to *Nyla Naim (Addgene)*

lawra francisca 2021/11/4 下午6:54:52

i have a question. artificial plasmid like pUC18 is more likely to use in gene cloning compare to the natural plasmid extract direct from the cell. what is the reason scientist prefer in using artificial plasmid?

Reply to *lawra francisca*

Nyla Naim (Addgene) 2021/11/5 上午1:27:10

Hello,

"Artificial" or "recombinant" plasmids are very useful. The plasmids most researchers work with today have elements that were sourced from naturally occurring plasmids and genes from other organisms.

pUC18, for example, has been optimized for gene expression and has unique restriction enzyme sites in useful locations for cloning. For a more detailed description of plasmids and the history or recombinant DNA, check our our Molecular Biology Reference: https://www.addgene.org/mol-bio-reference/

Best regards,
Nyla

Reply to *Nyla Naim (Addgene)*

Add Comment

Margo R. Monroe
April 02, 2020

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