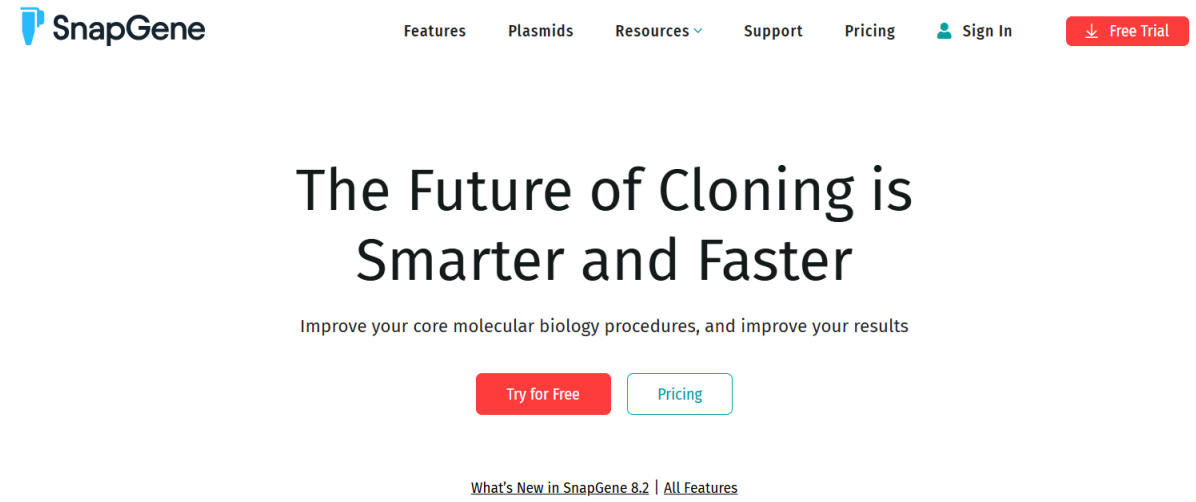
https://www.snapgene.com/



SnapGene is a widely used, user-friendly tool for planning, simulating, and documenting molecular cloning experiments. SnapGene was developed by Benjamin Glick and his team at GSL Biotech in Chicago, which he founded in 2004 after becoming frustrated with inefficiencies in his own molecular biology lab at the University of Chicago. The company has since been acquired by GraphPad Software (now operating as Insightful Science) and is a part of Dotmatics.

Here's a **concise summary** of the announcement regarding **SnapGene 7.0 by Dotmatics**:

🚀 Dotmatics Releases SnapGene 7.0

Date: July 12, 2023
Location: Boston, MA
Link: What's New in SnapGene 7.0

Dotmatics has announced the launch of **SnapGene 7.0**, a major update to its popular molecular biology software. The new version enhances the **user interface**, **data management**, and **feature functionality** to streamline molecular biology workflows.

🔍 Key Features in SnapGene 7.0

- **Modern Tabbed Interface:** Easy navigation between documents; supports pop-out windows for comparisons.
- **Improved Folder Panel:** Users can manage files/folders more efficiently and designate any folder as a project.

- **Advanced File Search:** Locate files by name, type, sequence content, or annotations using a sleek new interface.
- **Primer Annotations:** New insertion annotations for enzymes, codons, and motifs; auto-annotations for primer design elements like Golden Gate and Gateway sites.
- **Feature Preview:** Enhanced sequence map lets users preview detected features and customize annotations.
- **"Get Started" Pane:** Quick access to recent files and helpful resources including SnapGene Academy, tutorials, and guides.

🧬 About SnapGene

SnapGene, a Dotmatics company, is a widely used software for planning, visualizing, and documenting molecular cloning procedures, supporting researchers at top institutions globally.

🌐 Availability

- Free upgrade for current subscribers
  Purchase or upgrade available via www.snapgene.com
  Contact: **info@snapgene.com**

🏢 About Dotmatics

Dotmatics is a global leader in R&D software for the life sciences, with over 2 million users in 180+ countries. Its portfolio includes SnapGene, GraphPad Prism, Geneious, and others. Backed by Insight Partners, Dotmatics is focused on advancing data-driven research and innovation.

Media Contact:
📧 Maggie Quale – maggie.quale@dotmatics.com
📞 +1 (831) 325-7943

Let me know if you want this turned into a press release, newsletter snippet, or slide.

Key Benefits

- Plan, visualize, and document your cloning workflows
  Reduce errors, improve speed and accuracy

Ideal for both routine and complex cloning tasks

Core Features

Molecular Cloning Techniques
Supports major methods:

- Restriction Cloning (https://www.snapgene.com/series/simulate-restriction-cloning)
- Gateway Cloning
- Gibson Assembly
- In-Fusion Cloning
- TA & TOPO Cloning
- NEBuilder HiFi
- Golden Gate Assembly

Primer Design & PCR

- Design primers manually or automatically
- Simulate standard & complex PCR (mutagenesis, overlap extension)

Sequence Verification

- Align your construct to reference sequences
- Use trusted tools (Clustal Omega, MAFFT, MUSCLE, T-Coffee)
- Assemble Sanger reads into contigs (CAP3)

Visualization

- Interactive plasmid maps
- Sequence views with full annotation controls
- Realistic agarose gel simulations
- Multi-sequence alignment viewer

Automatic Documentation

- Tracks full cloning history step-by-step
  Embed visual history into files
  Supports undo and version tracking

Data Management

- Import/export from all major formats (GenBank, Vector NTI, etc.)
  Organize sequences into collections
  Share via local drives, servers, or cloud
  Batch processing and advanced search tools

Platform Support

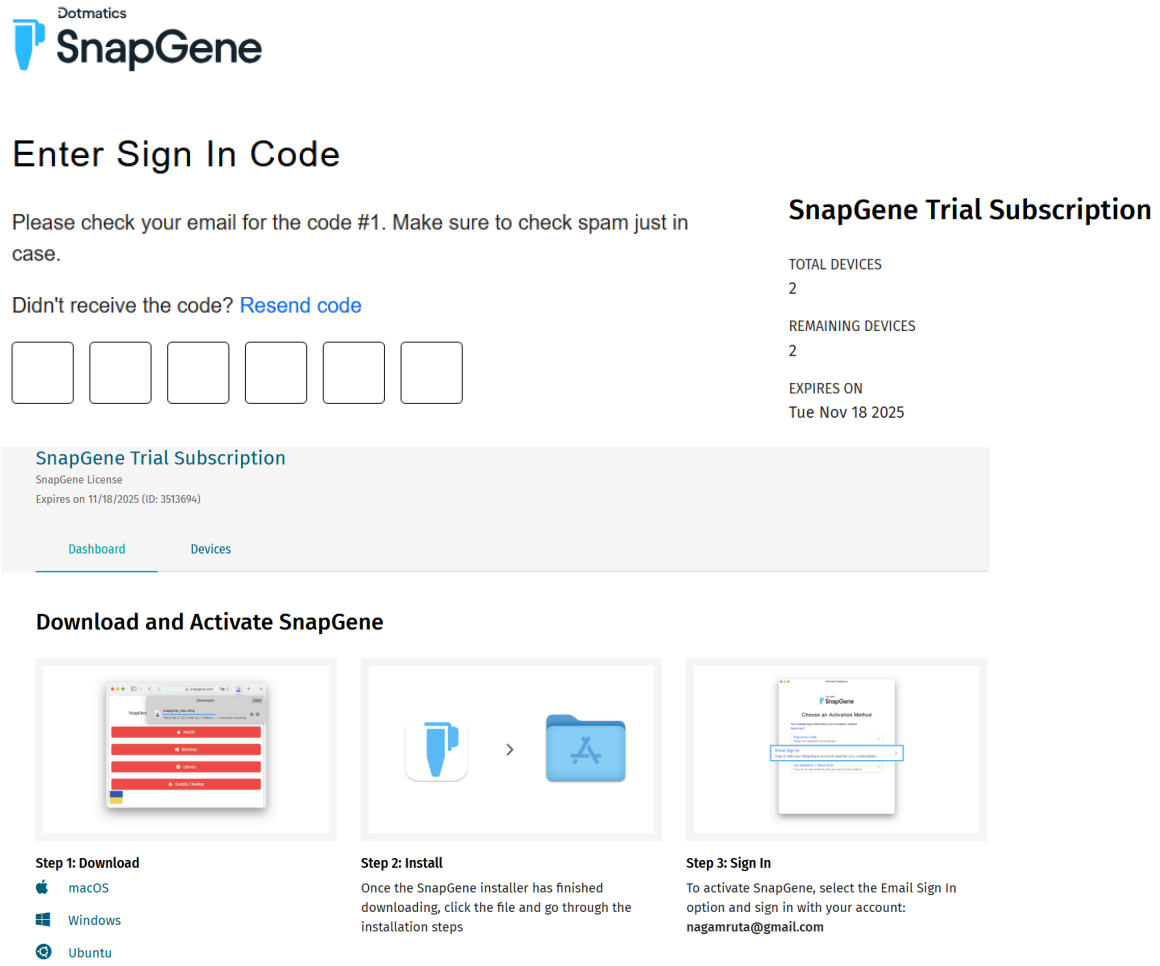
- Available on Windows, macOS, Linux
  Free SnapGene Viewer available

User Praise

Researchers highlight its ease of use, powerful visualization, and time-saving features as game-changing for modern molecular biology labs.

Get Started

- Free trial available
  Options for individual, institutional, or educational licenses
  Full documentation and tutorials through SnapGene Academy



https://www.snapgene.com/series/getting-started

https://www.addgene.org/
Addgene, the non-profit plasmid repository, is headquartered in **Cambridge, Massachusetts, USA**. It serves as a global resource, making it easier for academic and non-profit researchers to share and access plasmids and associated cloning data for their experiments.

pBR322: An Early Cloning Vector

pBR322 was one of the first **artificial cloning vectors** developed in **1977** by **Francisco Bolivar** and **Raymond L. Rodriguez**. It remains a fundamental tool in gene cloning and recombinant DNA research.

Key Features and Functions of pBR322:

1. **Cloning Vector:**
    - pBR322 acts as a carrier or vehicle for foreign DNA. The foreign DNA can be inserted into the plasmid and replicated inside a host bacterium (e.g., *E. coli*), which facilitates its amplification.
2. **Selectable Markers:**
    - **Ampicillin Resistance (AmpR)** and **Tetracycline Resistance (TetR)** genes are present.
    - When the gene of interest is inserted into the plasmid, it can disrupt one of these resistance genes, allowing scientists to select transformed bacteria based on their antibiotic resistance profile.
3. **Origin of Replication (Ori):**
    - pBR322 contains an **origin of replication** that allows the plasmid to replicate independently of the bacterial chromosome, ensuring that both the plasmid and the inserted DNA are copied within the host cell.
4. **Restriction Sites:**
    - pBR322 has multiple **restriction enzyme sites** where specific enzymes can cut the plasmid, allowing for the insertion of foreign DNA at precise locations.
5. **Origin of Name:**
    - The name "pBR322" comes from **"p"** for plasmid, **"BR"** for the developers Bolivar and Rodriguez, and **322** is a unique identifier. https://www.addgene.org/103056/

pUC18: A Derived Cloning Vector

pUC18 is a **derived cloning vector** based on pBR322, developed at the **University of California**. It incorporates several enhancements that make it a widely used tool for molecular cloning.

Key Features and Functions of pUC18:

1. **High Copy Number:**
    - pUC18 has a **high-copy number**, meaning that it replicates many times in the bacterial cell, leading to a higher yield of plasmid DNA. This is important for amplifying cloned genes in large quantities.
2. **Small Size:**

- It is a **small plasmid** (~2.7 kb), which is ideal for cloning smaller DNA fragments.

3. **Ampicillin Resistance:**
    - It contains a **gene for ampicillin resistance** (AmpR), making it possible to select bacterial cells that have successfully taken up the plasmid.
4. **Multiple Cloning Site (MCS):**
    - The plasmid has an **MCS**, a region containing several unique **restriction sites** for various enzymes. This allows easy insertion of foreign DNA into the plasmid.
    - The MCS is located within the **lacZ gene**, which plays a key role in the **blue/white screening** method.
5. **Blue/White Screening:**
    - In this method, when foreign DNA is inserted into the MCS, the **lacZ gene** is disrupted, preventing the formation of the enzyme **β-galactosidase**.
    - The disruption results in **white colonies** (recombinant plasmids), while **blue colonies** (non-recombinant plasmids) remain due to the functional β-galactosidase enzyme (which metabolizes X-Gal to create a blue color).

Applications of pUC18 and pBR322:

1. **Molecular Cloning:**
    - Both plasmids are crucial for **molecular cloning**, enabling researchers to insert and replicate foreign DNA in bacterial cells (e.g., *E. coli*).
2. **Teaching Tool:**
    - Their ease of use, especially with **blue/white screening**, makes them valuable for teaching **plasmid isolation**, **restriction digestion**, and **cloning** techniques in molecular biology.
3. **Subcloning and Sequencing:**
    - The **high-copy number** of pUC18 is particularly advantageous for **subcloning** or preparing plasmid DNA for **sequencing** and other downstream applications.

4. **Gene Expression:**
    - They can be used for expressing recombinant proteins, especially when combined with appropriate promoters and regulatory elements.

https://www.addgene.org/50004/

What is a Restriction Enzyme?

Restriction enzymes, also known as **restriction endonucleases**, are proteins that **cut DNA at specific sequences**. They serve as a natural defense mechanism for bacteria against viral infections by cleaving foreign DNA. In molecular biology, these enzymes are indispensable tools for gene cloning, DNA analysis, and recombinant DNA technology. Based on their properties, restriction enzymes are traditionally classified into **four main types**: **Type I**, **Type II**, **Type III**, and **Type IV**, each with unique characteristics in terms of their **structure**, **function**, and **mechanism of action**.

Comparison of Type I vs Type II Restriction Enzymes

Feature	Type I Restriction Enzymes	Type II Restriction Enzymes
Cleavage Site	Distant from recognition site (random)	At or near the recognition site (precise)
Co-factors Required	ATP, S-adenosyl-L-methionine	Magnesium ions (Mg^{2+})
Function	Restriction (cleaving) and modification (methylation)	Restriction only (cleaving)
Specificity	Low, cuts randomly	High, cuts at defined sites
Use in Molecular Biology	Limited, due to randomness	Essential for cloning, gene mapping, and DNA sequencing

Feature	Type I	Type II	Type III	Type IV
Structure	Complex, multi-subunit	Smaller, monomeric or dimeric	Large, multi-subunit	Varies, typically multi-subunit
Cofactors	ATP, S-adenosyl-L-methionine	Magnesium ions (Mg^{2+})	Requires two recognition sites	Recognizes modified DNA (e.g.,

				methylated DNA)
Cleavage	Random, far from recognition site	Defined positions near recognition site	Outside recognition sequence, two sites required	Cleaves modified DNA
Activity	Restriction + Modification	Restriction only	Restriction + Modification	Recognizes modified DNA
Examples	Rarely used in the lab	HindIII, EcoRI, NotI, FokI, BglI	Rarely used, e.g., McrBC	McrBC, Mrr
Use in Molecular Biology	Limited (due to random cleavage)	Widely used (cloning, mapping, sequencing)	Less common (incomplete digests)	Protects bacteria against modified DNA

A **Multiple Cloning Site (MCS)**, also referred to as a **polylinker**, is a key feature in many **cloning vectors**, such as plasmids, used in molecular biology. It is a short, strategically engineered region within the vector that contains several **unique recognition sites** for various **restriction enzymes**. The MCS is designed to facilitate the insertion of foreign DNA into the vector, making it easier for scientists to clone genes and perform experiments.

Key Features of MCS:

- **Function**:
  The main function of the MCS is to provide multiple options for inserting a gene of interest into the vector. This is achieved by providing various **restriction enzyme recognition sites**, enabling flexibility in choosing the most appropriate restriction enzymes for cutting and ligating the DNA.

- **Enzyme Recognition Sites**:
  The MCS contains a **cluster of unique recognition sites** for different **restriction enzymes**, such as **EcoRI**, **BamHI**, **HindIII**, **NotI**, and others. These sites are designed to be unique in the vector, meaning no other part of the vector can be cleaved by the same enzyme, ensuring that the insertion of foreign DNA occurs only at the MCS.

- **Location**:
  The MCS is usually located within a non-essential region of the plasmid or vector, so it does not interfere with the essential functions of the vector, such as **replication** or **antibiotic resistance**. In some cases, the MCS may be positioned in a **reporter gene** (like **lacZ**) to facilitate easy screening of recombinant clones.

- **Flexibility**:
  The presence of multiple restriction sites in the MCS gives researchers

flexibility in choosing the most convenient or compatible enzymes for cutting and pasting the desired gene. This is particularly useful when the foreign DNA is already prepared with certain enzymes that are compatible with the MCS.

- **Alternative Name**:
  The MCS is sometimes referred to as a **polylinker** because it links several different **restriction sites** in a single, convenient location.

Advantages of Using MCS in Cloning:

1. **Multiple Cloning Options**:
   The MCS gives researchers a range of restriction enzymes to choose from, increasing the likelihood of a successful ligation.

2. **Streamlined Cloning Process**:
   The availability of multiple enzyme sites simplifies the **cloning process** by allowing the use of enzymes that generate compatible ends for easy insertion of DNA.

3. **Simplified Screening**:
   In plasmids with an MCS located within a **selectable marker** or **reporter gene** (e.g., **lacZ**), insertion of the target gene can disrupt the function of the marker, allowing easy screening of recombinant clones via **blue/white screening**.

4. **Increased Flexibility**:
   The MCS allows you to **tailor** your cloning approach to the specific needs of your experiment, whether you need to ligate your gene into a vector with one enzyme or choose another based on the type of insert.

Example: Common Restriction Enzymes in MCS

- **EcoRI**: Recognizes the sequence **G-AATTC** and produces sticky ends for easier ligation.

- **BamHI**: Recognizes the sequence **GG-ATCC** and cuts between the G and A.

- **HindIII**: Recognizes the sequence **AAGCTT** and cuts between the two A's.

- **NotI**: Recognizes the sequence **GCGGCCGC** and generates sticky ends.

🧬 Project Title

Cloning of GFP Gene into pUC19 Vector Using EcoRI and HindIII Restriction Sites

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1. Objective

To clone the **Green Fluorescent Protein (GFP)** coding sequence into the **pUC19 plasmid vector** using the **restriction enzymes EcoRI and HindIII**, and to simulate the cloning process digitally using **SnapGene** software.

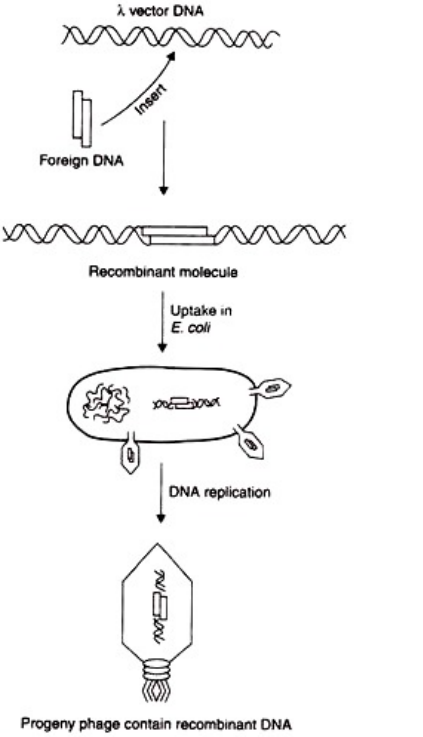
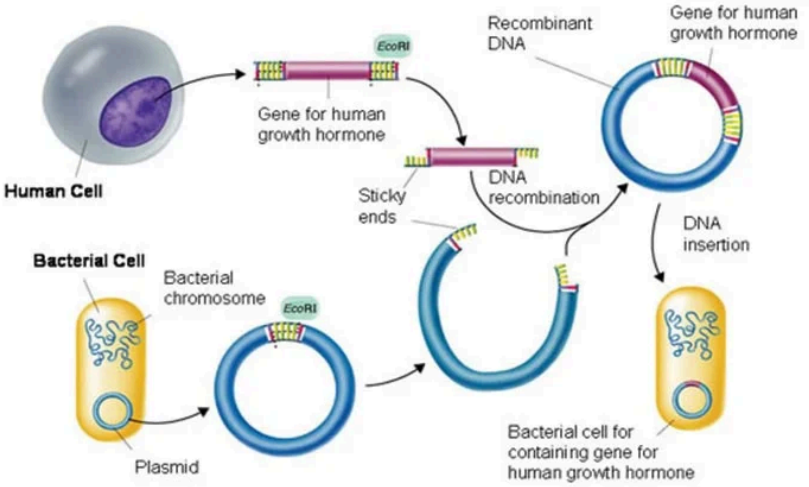


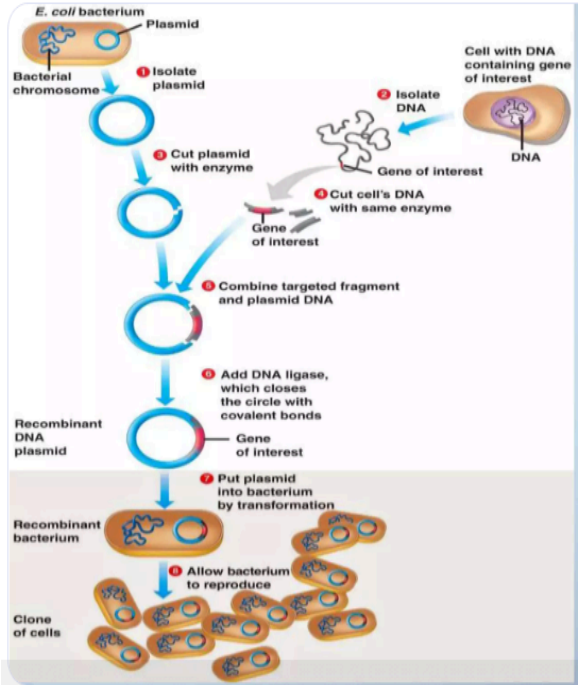
Fig. 23.5 Method for generation of a recombinant DNA molecule.

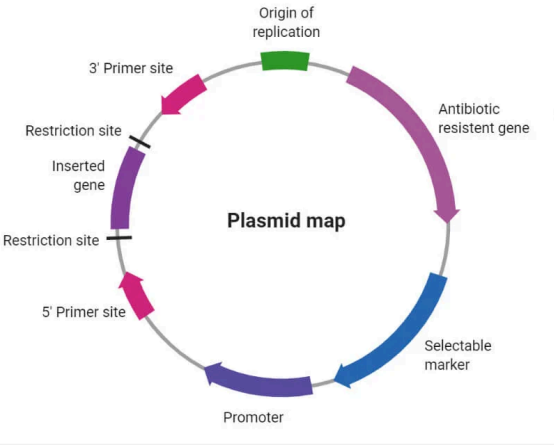
🌱 Why Clone the GFP Gene?

1. GFP Is a Visual Reporter — You Can *See* Gene Expression

- GFP fluoresces bright **green under UV or blue light** without any extra cofactors or substrates.
  That means you can literally *see* whether your gene is being expressed, without needing complex assays.
  In a plasmid like **pUC19**, you can visualize successful transformation by glowing colonies — making GFP a **built-in confirmation tool**.
- 💡 *In short:* Cloning GFP lets you *see* molecular biology happen in real time.







Properties of an Ideal Vector

An **ideal vector** is a DNA molecule used to carry foreign genetic material into a host cell for cloning or expression. To function efficiently, it must possess the following key properties:

1. **Origin of Replication (Ori)**:
   Contains specific sequences required to initiate and sustain replication within the host cell, ensuring multiple copies of the vector are produced.

2. **Selectable Marker Genes**:
   Includes genes (e.g., antibiotic resistance genes) that allow for the identification of host cells that have successfully taken up the vector.

3. **Multiple Cloning Site (MCS)**:
   A short DNA region containing several unique restriction enzyme recognition sites, enabling the easy insertion of foreign DNA fragments.

4. **Small Size**:
   A smaller vector is easier to manipulate, handle, and isolate, while allowing space for larger DNA inserts.

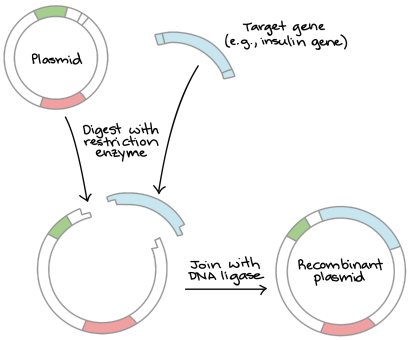
5. **Easy Isolation and Purification**:
   The vector should be simple to extract and purify from the host organism after transformation or cloning.

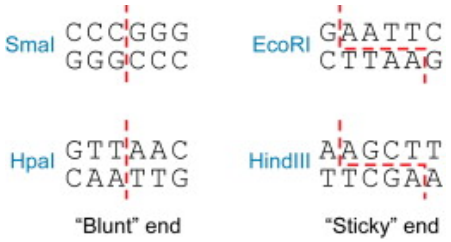
6. **Efficient Introduction into Host Cells**:
   The vector must be readily taken up by host cells through processes such as transformation, transfection, or conjugation.

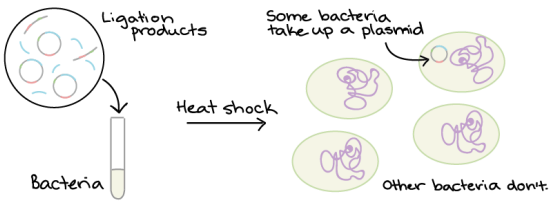
7. **Non-Toxic to Host Cells**:
   The vector should not harm or interfere with normal host cell metabolism or growth, ensuring stability and viability.

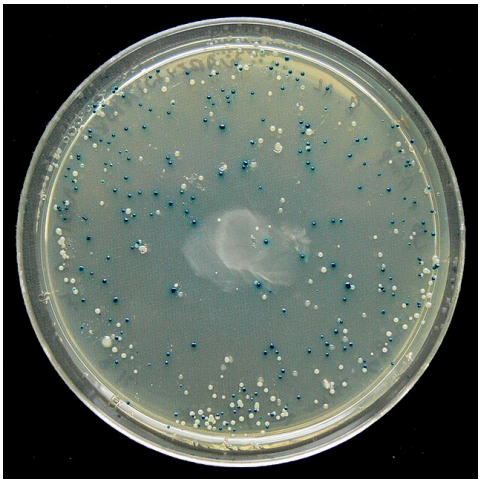
8. **Self-Replicating**:
   The vector must be capable of autonomous replication within the host cell to generate multiple copies of itself and the inserted DNA.

Step	Process	Main Enzyme/Tool	Purpose
1	Gene isolation	PCR	Obtain insert
2	Vector preparation	Restriction enzymes	Open vector
3	Digestion	Restriction enzymes	Create compatible ends
4	Ligation	DNA ligase	Join insert + vector
5	Transformation	Heat shock / Electroporation	Introduce plasmid into host
6	Selection	Antibiotic, screening	Identify recombinant colonies
7	Verification	Restriction map, sequencing	Confirm correctness
8	Expression	Promoter-driven expression	Produce protein
9	Analysis	Gel, fluorescence, assays	Test function









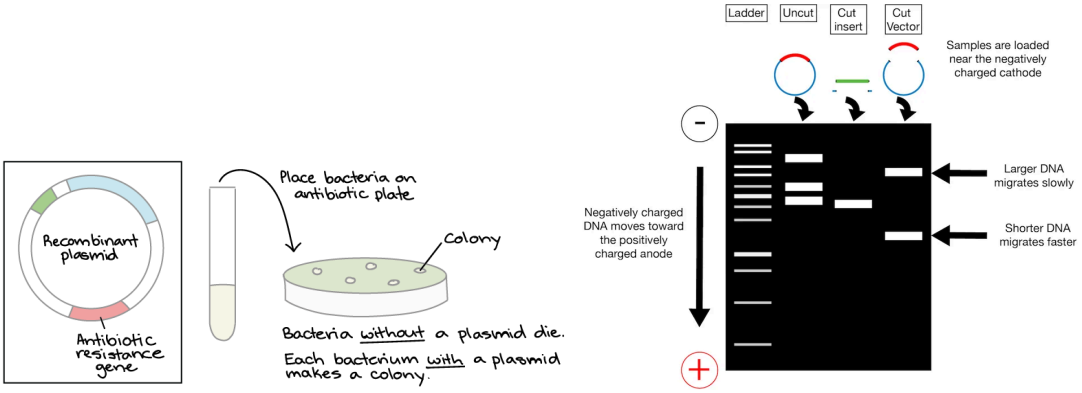
Blue–white screening is a quick method used in molecular cloning to identify bacteria that contain recombinant DNA.

In this technique:

- A **DNA fragment** is inserted into a **plasmid vector** and introduced into **bacterial cells** (usually *E. coli*).
- The bacteria are grown on plates containing **X-gal**.
- **If the plasmid has the DNA insert**, it disrupts the **β-galactosidase (lacZ) gene**, and the colonies appear **white**.
- **If the plasmid has no insert**, the lacZ gene remains functional, producing a **blue** color.

Thus, **white colonies** indicate successful insertion of foreign DNA, while **blue colonies** indicate non-recombinant plasmids.

This method works on the principle of **α-complementation** of the **β-galactosidase gene** and was popularized using the **pUC series of plasmid vectors**.



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2. It's Safe, Non-toxic, and Easy to Handle

- GFP is derived from the jellyfish *Aequorea victoria* — it's **non-pathogenic** and **non-toxic** to cells.
  That makes it ideal for **student projects**, **teaching labs**, and **biosafety level 1** environments.

---

3. It's a Proof-of-Concept for Any Gene Expression Workflow

Cloning GFP is often a **model experiment** for:

- Learning **restriction digestion**, **ligation**, and **transformation**.
  Testing **vector functionality** and **promoter activity**.
  Verifying **expression control** — if GFP expresses properly, you know your system works.
  Once you master this, you can replace GFP with **any gene of interest** (like a therapeutic protein, enzyme, or CRISPR component).

---

4. It's Useful in Research as a Reporter Tag

In advanced experiments, GFP can be:

- **Fused to another protein** (as a fluorescent tag) to track its localization in cells.
  Used in **promoter studies** — for example, linking GFP to a stress-response promoter to study gene regulation.
  Employed in **biosensors**, **gene silencing studies**, or **cell tracking**.
  So, cloning GFP is often the **first step** toward designing more complex constructs.

---

5. It Teaches You Core Genetic Engineering Concepts

Through GFP cloning, you learn:

- **Restriction enzyme compatibility** (sticky vs blunt ends)
  Directional cloning
  Reading frame maintenance
  Verification techniques (gel electrophoresis, sequencing)
  Vector selection and antibiotic screening
  These are **fundamental skills** for anyone entering molecular biology, biotechnology, or synthetic biology fields.

---

6. Perfect for Demonstration and Presentation

In project reports or viva presentations, GFP provides:

- A **visually appealing output** (glowing colonies or fluorescence images)
  Easy-to-understand logic (insert → express → glow)
  A safe, clear example of successful cloning

---

2. Background

Restriction cloning is a classical molecular biology technique used to insert a gene of interest into a plasmid vector. The **pUC19** vector contains a **multiple cloning site (MCS)** within the *lacZα* region, which allows blue-white screening and expression of recombinant genes.

pUC18 vs pUC19

Both **pUC18** and **pUC19** are part of the same plasmid family — small, high-copy-number cloning vectors derived from *E. coli*. They're nearly **identical in sequence (2686 bp)**, except for **one small but crucial difference** — the **orientation of the multiple cloning site (MCS)**.

🧩 The Key Difference

Feature	pUC18	pUC19
Orientation of MCS	Clockwise	Anticlockwise
Position of MCS relative to lacZα	Opposite direction	Reversed
Promoter direction	lac promoter transcribes left to right	lac promoter transcribes right to left
Sequencing primer site	M13 Forward primer site	M13 Reverse primer site
Effect on insert expression	Gene must match MCS direction	Gene must match opposite orientation

---

🧬 Why Choose pUC19 for GFP Cloning?

1. Direction Matches GFP Orientation

In pUC19, the **lac promoter** drives transcription **toward the MCS** in the same direction most coding sequences (like GFP) are written.
This means your **GFP gene will be expressed directly** after insertion — perfect for visible confirmation (fluorescence).

> ✅ pUC19 orientation = easier gene expression for inserts like GFP.

---

2. Easier Screening and Expression

- The MCS is within the **lacZα gene**, which enables **blue-white screening** (white colonies indicate successful insert).
- The **lac promoter** in pUC19 is strong and often sufficient to drive expression of small genes like GFP without needing an additional promoter.
  So, you can get both **cloning confirmation (white colonies)** and **expression (green fluorescence)** — double verification!

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3. Compatibility with Common Primers

- pUC19 includes the **M13 Reverse primer binding site**, widely used for sequencing cloned inserts.

- Many sequencing services and labs default to pUC19-based primer sites, making downstream analysis simpler.

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4. Same Size, Same Antibiotic Resistance

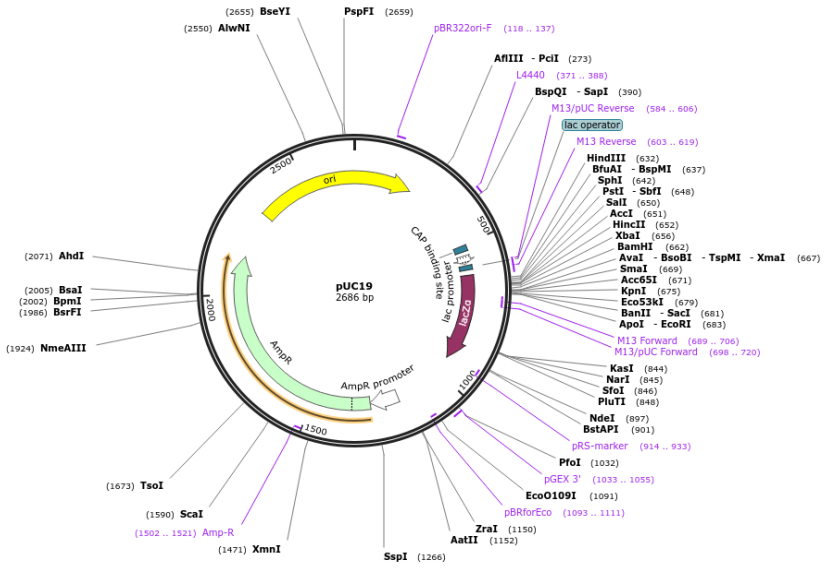
Both pUC18 and pUC19: https://www.addgene.org/50005/

- Are 2686 bp
- Carry **Ampicillin resistance**
- Have **high copy number (~100–500 copies/cell)**
  So using pUC19 doesn't increase complexity or size — just gives better orientation for certain inserts.

The **GFP gene** from *Aequorea victoria* is widely used as a reporter gene due to its intrinsic fluorescence under UV light. By inserting GFP into the pUC19 MCS between EcoRI and HindIII sites, we can visualize successful cloning and downstream expression studies.

3. Materials & Tools

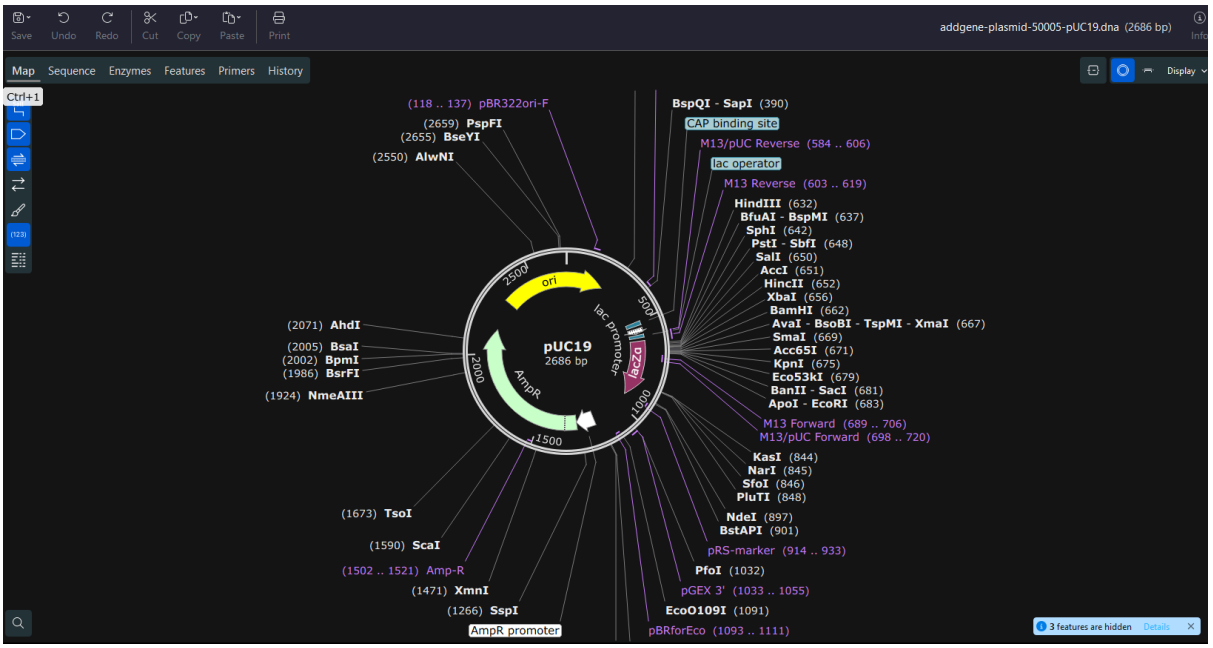
Component	Details
Vector	pUC19 (2686 bp, high-copy E. coli plasmid with Ampicillin resistance)
Insert	GFP coding sequence (~720 bp)
Restriction Enzymes	**EcoRI (GAATTC) and HindIII (AAGCTT)**
Software	SnapGene 8.4
Host organism (for reference)	*E. coli* DH5α

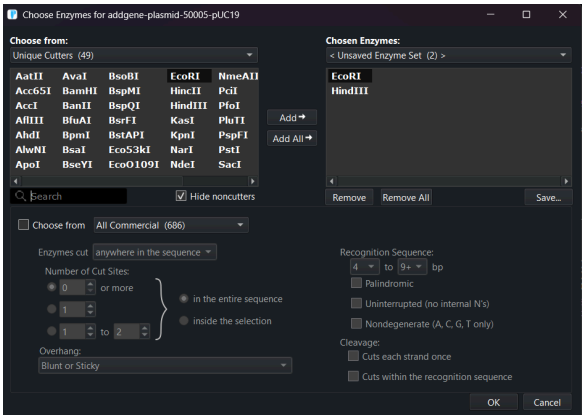


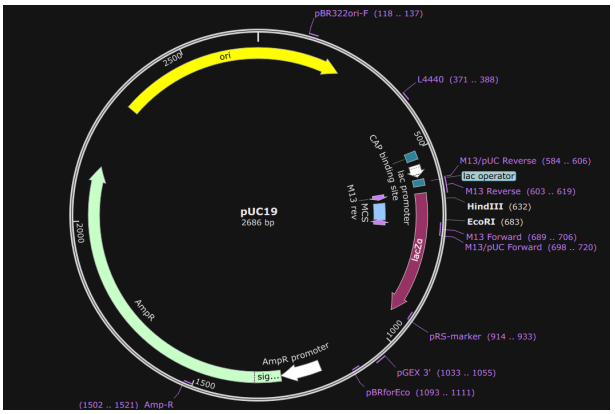
4. Methodology

Step 1: Vector Preparation

The **pUC19 plasmid** was opened in SnapGene.
Using **Enzymes → Choose Enzymes**, the MCS region was inspected for suitable restriction sites.
Unique sites **EcoRI** and **HindIII** were selected, which flank the MCS region and cut only once in the plasmid.







Step 2: Insert Sequence Preparation

https://www.ncbi.nlm.nih.gov/nuccore/L29345.1/

Aequorea victoria green-fluorescent protein (GFP) mRNA, complete cds

GenBank: L29345.1

GenBank Graphics

```
>L29345.1 Aequorea victoria green-fluorescent protein (GFP) mRNA, complete cds
TACACACGAATAAAAGATAACAAAGATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTT
GTTGAATTAGATGGCGATGTTAATGGGCAAAAATTCTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACAT
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CACACAATCTGCCCTTTCCAAAGATCCCAACGAAAAGAGAGATCACATGATCCTTCTTGAGTTTGTAACA
GCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAAATGTCCAGACTTCCAATTGACACTAAAG
TGTCCGAACAATTACTAAATTCTCAGGGTTCCTGGTTAAATTCAGGCTGAGACTTTATTTATATATTTAT
AGATTCATTAAAATTTTATGAATAATTTATTGATGTTATTAATAGGGGCTATTTTCTTATTAAATAGGCT
ACTGGAGTGTAT
```
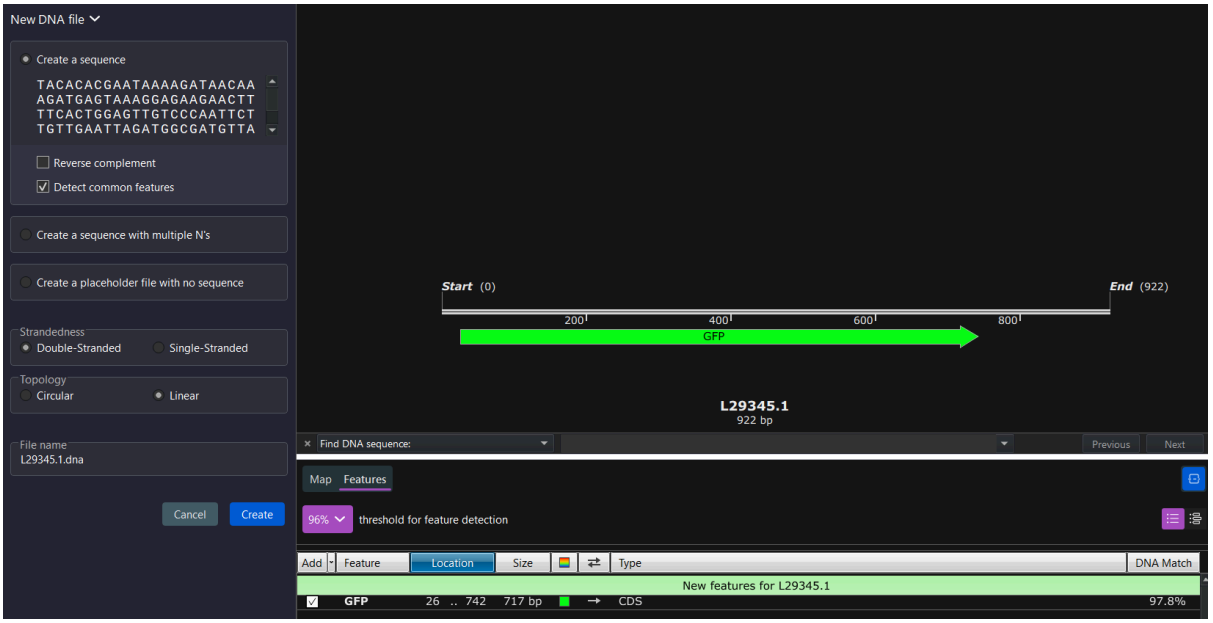

>L29345.1 Aequorea victoria green-fluorescent protein (GFP) mRNA, complete cds

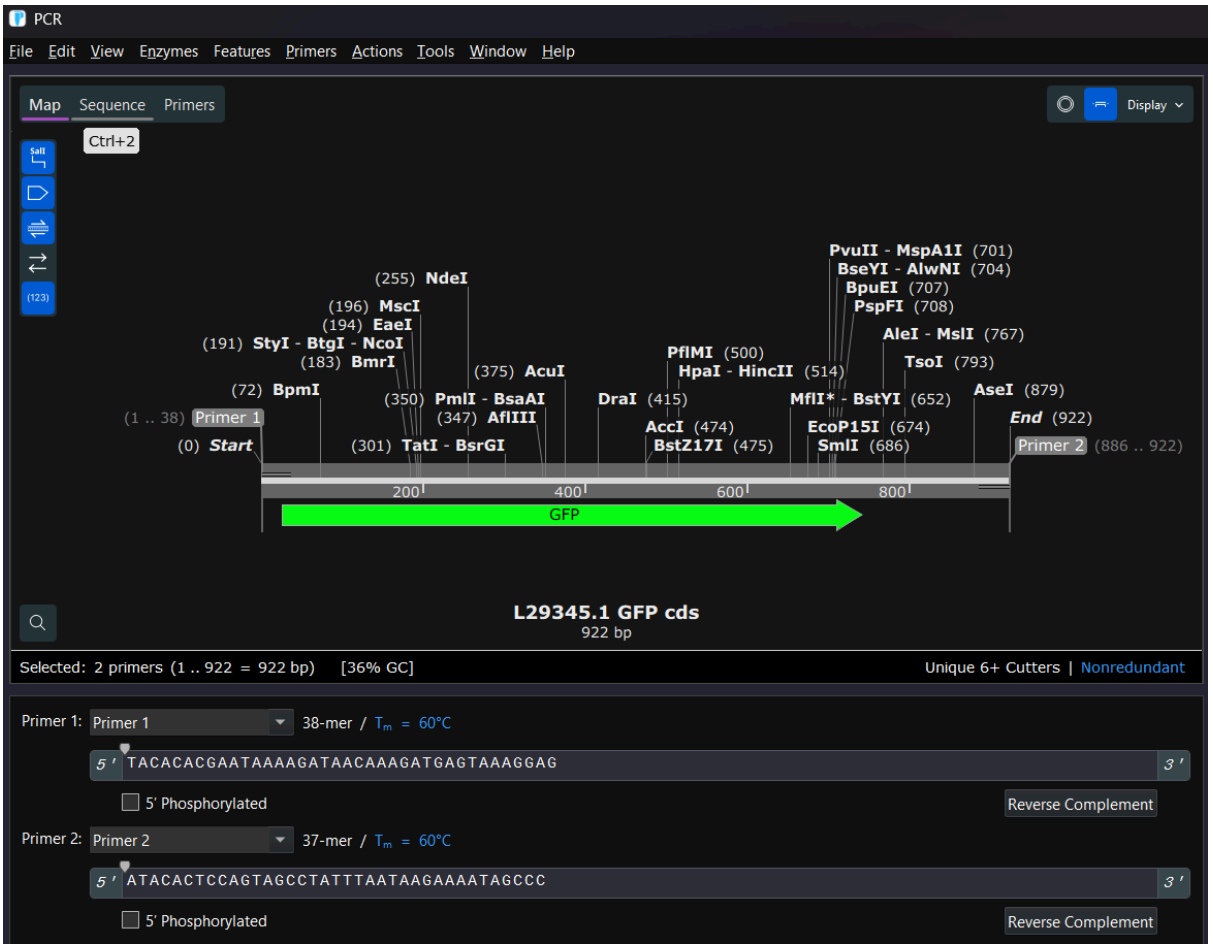
TACACACGAATAAAAGATAACAAAG**ATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTC**
TTGTTGAATTAGATGGCGATGTTAATGGGCAAAAATTCTCTGTCAGTGGAGAGGGTGAAGGTGATGCA
ACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGGAAGCTACCTGTTCCATGGCCAACACT
TGTCACTACTTTCTCTTATGGTGTTCAATGCTTTTCAAGATACCCAGATCATATGAAACAGCATGACT
TTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTACAAAGATGACGGGAAC
TACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTAT
TGATTTTAAAGAAGATGGAAACATTCTTGGACACAAAATGGAATACAACTATAACTCACATAATGTAT
ACATCATGGCAGACAAACCAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTAAAGAT
GGAAGCGTTCAATTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACC
AGACAACCATTACCTGTCCACACAATCTGCCCTTTCCAAAGATCCCAACGAAAAGAGAGATCACATGA
TCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAAATGTCC
AGACTTCCAATTGACACTAAAGTGTCCGAACAATTACTAAATTCTCAGGGTTCCTGGTTAAATTCAGG
CTGAGACTTTATTTATATATTTATAGATTCATTAAAATTTTATGAATAATTTATTGATGTTATTAATA
GGGGCTATTTTCTTATTAAATAGGCTACTGGAGTGTAT



Primers should generally have the following properties:

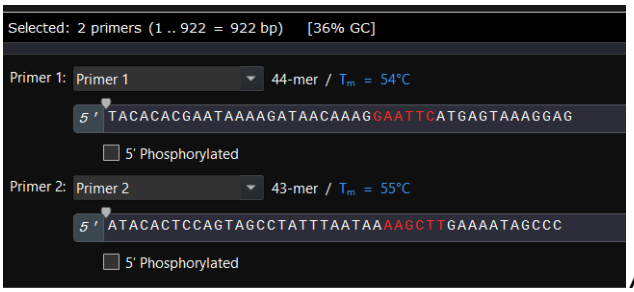
- Length of 18-24 bases
- 40-60% G/C content
- Start and end with 1-2 G/C pairs
- Melting temperature (Tm) of 50-60°C
- Primer pairs should have a Tm within 5°C of each other (55 Taq)
- Primer pairs should not have complementary regions

`Actions → PCR → Choose PCR Primers`



convert your L29345.1 GFP mRNA sequence into a synthetic cloning-ready insert with:

- A 5′ EcoRI site (GAATTC)
  A 3′ HindIII site (AAGCTT)
  Clean start and stop codons
  Possibly codon-optimized for *E. coli* or another organism

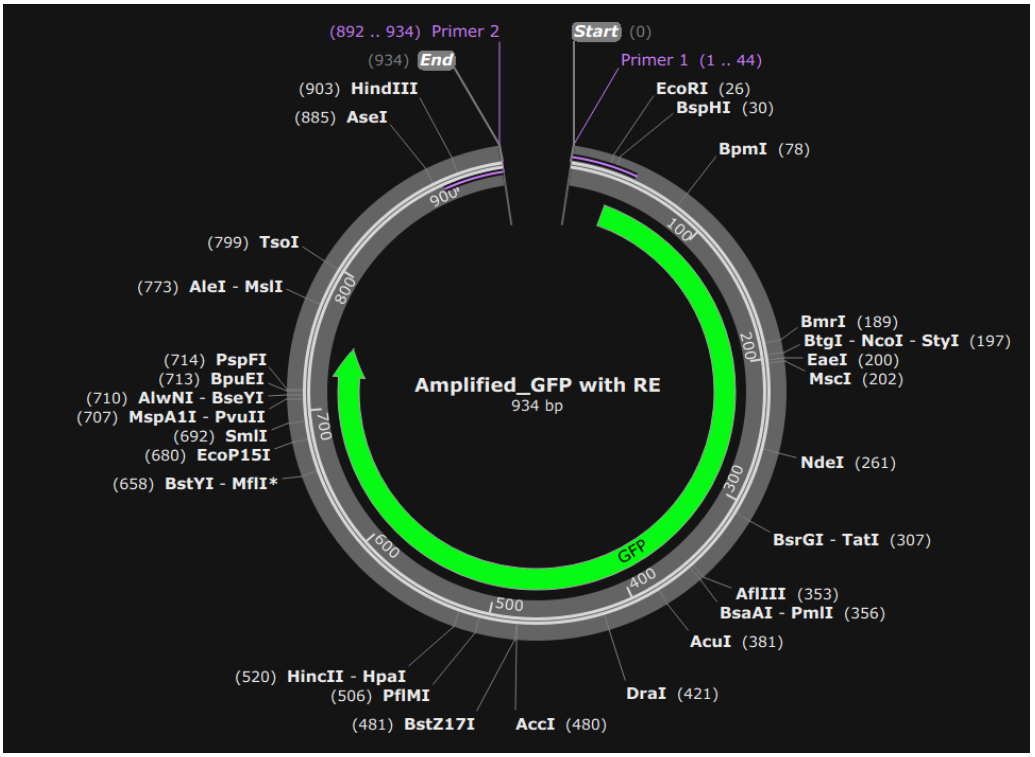


A synthetic GFP coding sequence was designed with compatible ends for cloning:

>GFP_coding_sequence_with_EcoRI_HindIII

TACACACGAATAAAAGATAACAAAGGAATTCATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCC
CAATTCTTGTTGAATTAGATGGCGATGTTAATGGGCAAAAATTCTCTGTCAGTGGAGAGGGTGAAGGT
GATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGGAAGCTACCTGTTCCATGGCC
AACACTTGTCACTACTTTCTCTTATGGTGTTCAATGCTTTTCAAGATACCCAGATCATATGAAACAGC
ATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTACAAAGATGAC
GGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAA
AGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAAATGGAATACAACTATAACTCACATA
ATGTATACATCATGGCAGACAAACCAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATT
AAAGATGGAAGCGTTCAATTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCT
TTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCCAAAGATCCCAACGAAAAGAGAGATC
ACATGATCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAA
ATGTCCAGACTTCCAATTGACACTAAAGTGTCCGAACAATTACTAAATTCTCAGGGTTCCTGGTTAAA
TTCAGGCTGAGACTTTATTTATATATTTATAGATTCATTAAAATTTTATGAATAATTTATTGATGTTA
TTAATAGGGGCTATTTTCAAGCTTTTATTAAATAGGCTACTGGAGTGTAT

- The sequence begins with an **EcoRI site (GAATTC)** and ends with a **HindIII site (AAGCTT)** for directional cloning.
  The ORF begins with a start codon (ATG) and ends with a stop codon before the HindIII site.

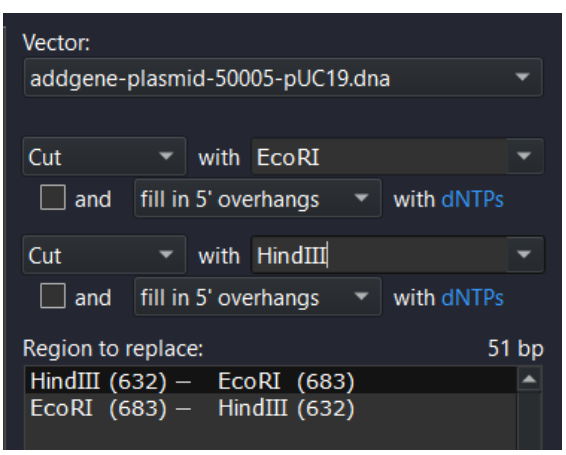


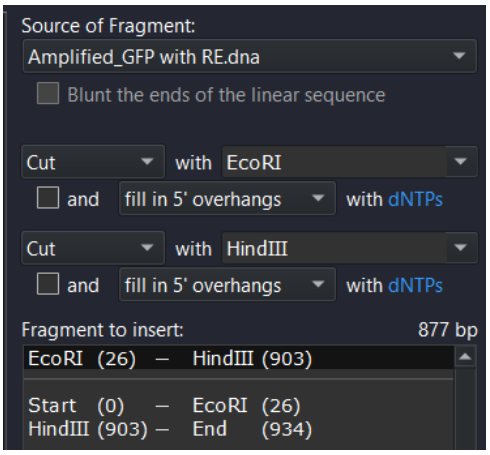
Step 3: Restriction Digestion Simulation

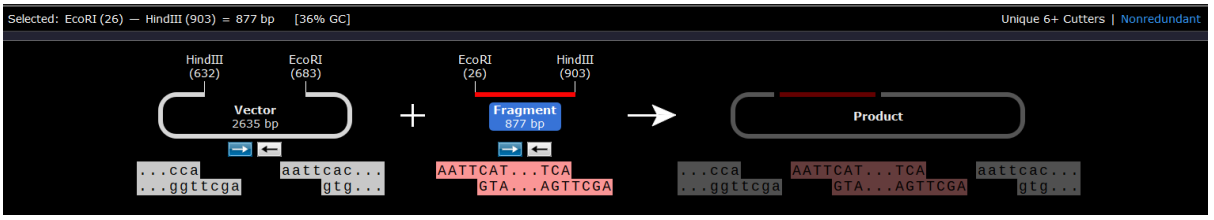
Both **pUC19 vector** and **GFP insert** sequences were digested with *EcoRI* and *HindIII* using:

`Actions → Restriction Digestion and Insert Cloning → Digest with Enzymes`

- SnapGene generated linear fragments showing compatible sticky ends.







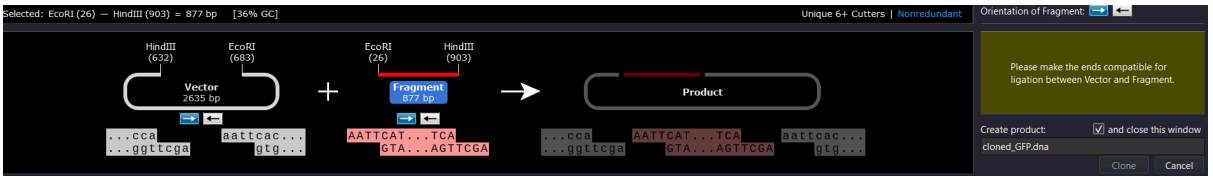
Why choose *E. coli* DH5α for transformation

Cloning strain (not expression strain)

DH5α is designed for **DNA propagation and storage**, not protein production.
It's *the* most common *E. coli* strain used for:

- **Plasmid amplification**
  Stable maintenance of recombinant DNA
  Blue–white screening (with pUC19, which carries the lacZα gene)
  High transformation efficiency
  So, when you're cloning your **GFP insert into pUC19**, the goal is to:

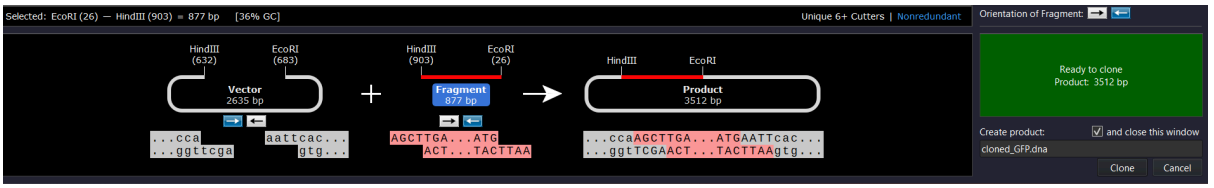
  "Make many copies of the recombinant plasmid, verify the insert, and then — if needed — transfer it into an expression strain later." DH5α is perfect for that step. So pUC19 + DH5α = standard **blue-white selection system** for confirming successful insertions.



Step 4: Ligation (Insertion Cloning)

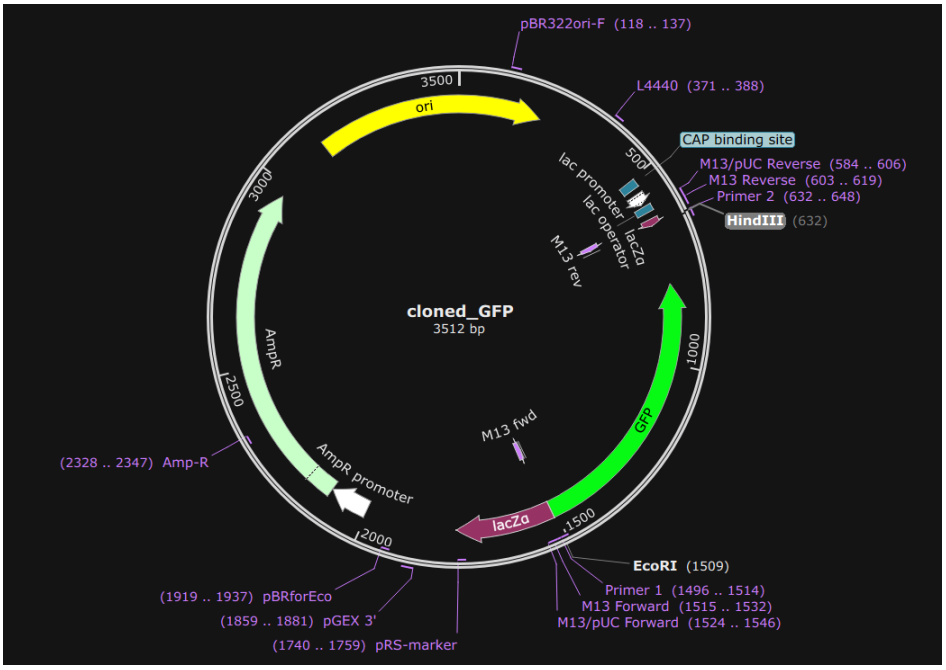
- In the **pUC19 (digested)** file:
  Selected **Actions → Restriction Cloning → Insert Fragment...**
  Chose the digested GFP insert file.
  Verified correct **5' to 3' orientation** and **reading frame**.

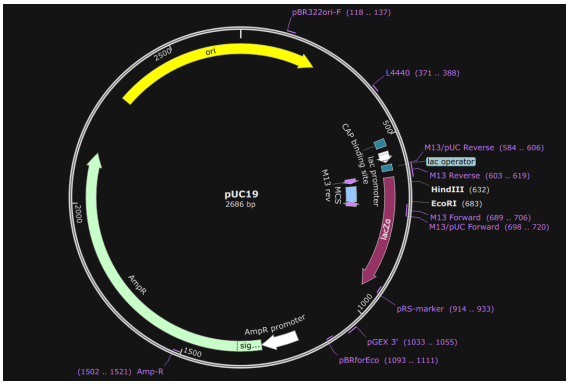
Performed digital ligation to generate **pUC19-GFP** recombinant plasmid.

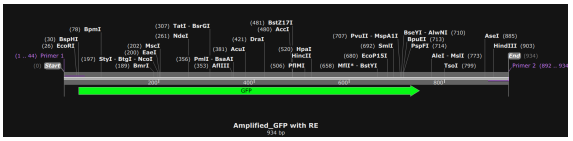


Step 5: Verification

- The new construct (**pUC19-GFP**) was analyzed using:
  Map view to confirm insert position within MCS.
  Sequence view to check junctions and confirm EcoRI–HindIII flanking sequences.
  Features panel to annotate the GFP gene and Ampicillin resistance gene.
  No internal EcoRI/HindIII sites were found within GFP — confirming successful in silico ligation.







5. Result

A recombinant plasmid **pUC19-GFP** (total size ≈ 3406 bp) was generated in SnapGene.
The GFP insert was successfully integrated between the EcoRI and HindIII restriction sites in the MCS region.

Construct summary: Vector backbone: pUC19 (Amp^R)
Insert: GFP (877 bp)
Total plasmid size: 2635 + 877 = ~3512 bp
Orientation: Correct (5'–3')
Enzyme sites: EcoRI and HindIII

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6. Discussion

This simulation demonstrates the efficiency of restriction enzyme-based cloning and how **SnapGene** can be used to visualize, verify, and document each step before performing actual wet-lab cloning. The resulting construct can serve as a **reporter plasmid**, allowing the expression of GFP under control of the *lac* promoter in *E. coli*. This workflow ensures accuracy in cloning design and prevents downstream cloning errors.

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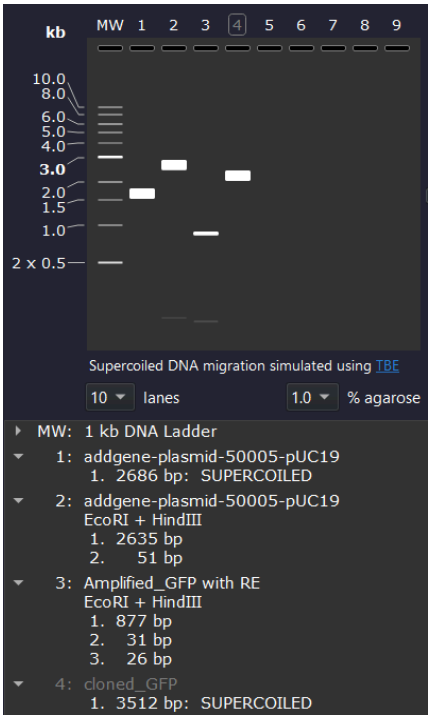
7. SnapGene Outputs (for report inclusion)



- Plasmid Map: `pUC19-GFP.dna`

Sequence Report: showing inserted GFP sequence and MCS annotation
Gel Simulation: verification of digestion fragments (~2.7 kb vector + 0.87 kb insert)