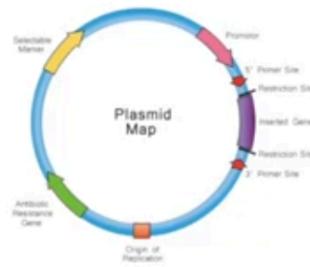


Molecular Biology (Digital) Tools: Benchling, SnapGene, Geneious, ApE

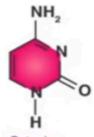
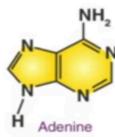
Design and simulate molecular biology experiments in silico (i.e. on the computer).



Design and Simulate Molecular Biology Experiments on a Tool

The formats include

1. FASTA Format
2. GenBank Format
3. GFF (General Feature Format)
4. BED (Browser Extensible Data) Format
5. PDB (Protein Data Bank) Format
6. VCF (Variant Call Format)
7. SAM/BAM (Sequence Alignment/Map)
8. SBML (Systems Biology Markup Language)
9. GTF (Gene Transfer Format)



FASTA Format:

- **Use:** Widely used for storing nucleotide or amino acid sequences.
- **Structure:**
 - A **header line** that starts with a ">" symbol, followed by a **sequence identifier** and **optional description**.
 - The sequence itself (either nucleotide or protein).

```
>sp|P68871|HBB_HUMAN Hemoglobin subunit beta OS=Homo sapiens OX=9606 GN=HBB PE=1 SV=2
MVHLTPEEKSAVTALWGKVNDEVGGEALGRLLVVPWTQRFFESFGDLSTPDAMGNPKVKAHGKKVLGAFSDGLAHLDNLKGTF
```

```
ATGAAATTTCGCAAGTAAAAAAAATAATCAAAAAAATTCAAGCAAAATGACGAGCGTTATAG
AGAATTAGATAATTAGTACAAGAAGGAAATGGCTCACGTTAGGTGGAGGTTCTGTCTTG
GTAATGTGCTCATGTGTTAACCTTATTTAAAGAGATTAAGGATAATATTTTATTTAT
ATTTAAAGTATTATTTATTAAGTGTATGTGAATGAATAAAATTTGCTAAAGAACCTT
AAACAAAATGGTAACTATAGTTGTAACATCCGAAACTCACAACCTTATTTGTATGATTA
TGTTCTTATTGTTATTCCTTATTGAAATAAAAGGGAAATTCAAAGAACGCCGA
AGCTTTAATTACAATTGGCTATACCATGTTAGATGCCGTTAGTCATTGGCCTC
ATAGGTCTTACAAGAACTACTGGAAATATCCAATCATTGTTCTCAATTAAAGTATCCAT
```

GenBank files are commonly used to store nucleotide sequences and their annotations (features like genes, promoters, coding regions, etc.). There are multiple ways to open and work with GenBank files, depending on your needs.

Structure:

- Header section with metadata (e.g., locus, source, definition).
- Feature table describing genes, CDS (coding sequences), promoters, and other annotations.
- The sequence at the bottom.

LOCUS SCU49845 5028 bp DNA linear PLN 21-JUN-1999 DEFINITION Saccharomyces cerevisiae TCP1-beta gene, partial and Axl2p. ORIGIN

2. Biological Sequence Analysis Tools: Specialized bioinformatics tools can open and visualize GenBank files in a more intuitive way. Here are some common tools:

1. SnapGene:

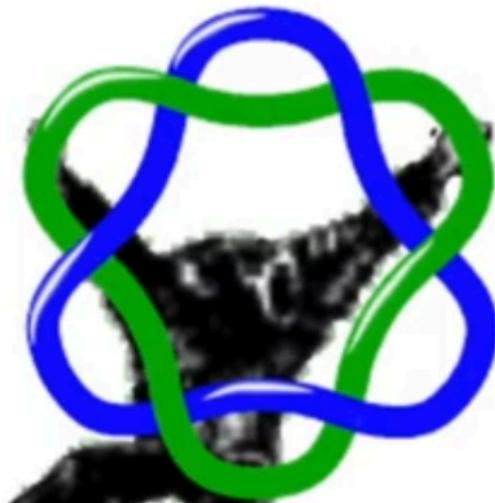
SnapGene is a popular DNA sequence visualization tool that supports GenBank files.

Features: It offers a graphical view of sequences, annotations, plasmid maps, and cloning simulation. How to Open: Open SnapGene → Go to File → Open → Select the GenBank (.gb/.gbk) file.



3. ApE (A Plasmid Editor):

- ApE is a free sequence editor designed for plasmid construction and manipulation. It can read GenBank files and provides a graphical view.
- How to Open: Open ApE → File → Open → Select your GenBank file.



5. UGENE:

- **UGENE** is an open-source bioinformatics software that supports a variety of sequence formats, including GenBank.
- **How to Open:** File → Open → Choose the GenBank file.



free open-source cross-platform bioinformatics software

4. Geneious:

- **Geneious** is a comprehensive bioinformatics tool for DNA, RNA, and protein sequence analysis. It supports a wide range of file formats, including GenBank.
- **How to Open:** Import the GenBank file into Geneious to view and analyze the sequence and annotations.



Command-Line Tools:

1. Biopython:

Biopython is a powerful Python library for bioinformatics that allows you to programmatically open, parse, and analyze GenBank files.

How to Use: [Install Biopython](#) (`pip install biopython`). Use the SeqIO module to read a GenBank file.

```
from Bio import SeqIO
for record in SeqIO.parse("example.gb", "genbank"):
    print(record.id)
    print(record.seq)
```

2. EMBOSS (European Molecular Biology Open Software Suite):

- **EMBOSS** includes command-line tools for sequence analysis that support
- GenBank format. You can use tools like **seqret** to convert and visualize GenBank sequences.

EMBOSS/Jemboss

European Molecular Biology Open Software Suite

Online Tools:

NCBI Viewer (Genome Data Viewer):

NCBI offers a web-based viewer that allows you to open and visualize GenBank files directly in your browser.

How to Use: Visit the NCBI Genome Data Viewer → [Upload or paste the GenBank sequence](#) → [View annotations, genes, and other features](#).

NB: it appears to be only useful for eukaryotic genomes (please, comment if otherwise)



Opening in a Text Editor Vs These Tools



Introduction to Molecular Cloning:

Molecular cloning involves isolating and manipulating a specific DNA fragment (insert) and introducing it into a plasmid (a small DNA molecule) to replicate it in a host organism, like bacteria. This process has transformed biological research and medicine. Key developments such as the discovery of restriction enzymes and advancements like Polymerase Chain Reaction (PCR) have greatly improved cloning methods.

Cloning Applications:

Molecular cloning is used in various fields like gene research, protein expression, developing vaccines, and creating genetically modified organisms.



Figure 1. Molecular cloning process.

Steps in the Plasmid Cloning Cycle:

The cloning process involves a series of steps summarized in the Plasmid Cloning Cycle:

- Manipulating DNA: Isolating the DNA insert, cutting the vector, and ligating them together.
- Transformation: Inserting the recombinant DNA into a bacterial host.
- Selection and Screening: Identifying successful recombinant molecules.
- Extraction: Isolating the recombinant DNA from bacteria.
- Analysis: Analyzing the cloned DNA to confirm its correctness.

In traditional cloning, restriction enzymes are used to cut both the insert and vector, allowing them to be ligated together. This method requires careful consideration of the vector, insert, host, and final application to ensure success.

Advancements in Cloning Methods:

There are now more efficient methods for cloning, such as Gibson Assembly, which uses exonucleases, polymerases, and ligases instead of restriction enzymes. Tools like SnapGene have also simplified cloning, offering software to visualize cloning strategies, generate maps, and analyze clones.

DNA Vectors (Plasmids):

Plasmids are the most common vectors used in cloning. They have important features, like:

- Restriction sites (for cutting DNA),
- Origin of replication (for replication in bacteria),
- Antibiotic resistance genes (for selecting successful clones),
- Multiple cloning sites (MCS) (for easier insertion of the DNA fragment).

Plasmid Maps:

Plasmids have maps that show the location of these features. For example, the pBR322 plasmid has antibiotic resistance genes, and the pUC19 plasmid has a useful MCS for flexibility in cloning.

Cloning Organisms:

Different model organisms (like mice, zebrafish, and yeast) require specific plasmids that can replicate and express genes in their cells. The features needed in the plasmid vary depending on the organism being studied.

Plasmid Cloning Cycle Steps:

The plasmid cloning cycle involves five main steps:

- DNA Manipulation: Isolate the DNA fragment and cut the vector.
 - Transformation: Introduce the recombinant DNA into bacteria.
 - Selection and Screening: Identify successful clones.
 - Extraction: Harvest the recombinant DNA.
 - Analysis: Confirm the success of the cloning.
 - More complex cloning experiments might require repeating these steps.

Step-by-Step Process:

Manipulation of DNA: Start with a plasmid vector and the DNA fragment of interest.

The DNA is cut using restriction enzymes, allowing the fragment to be inserted into the vector.

Transformation: Introduce the recombinant plasmid into a bacterial host.

Selection and Screening: Only bacteria with the recombinant plasmid will survive on selective media (like antibiotics).

Extraction: Once successful bacteria are identified, extract the recombinant DNA for further use.

Analysis: Confirm that the recombinant DNA is correct and ready for experiments.

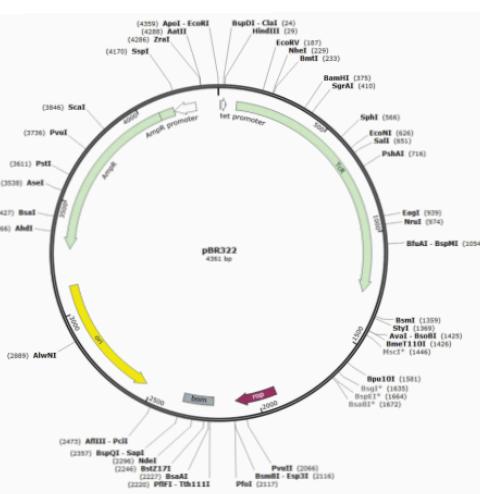


Figure 2. Map of *E. coli* plasmid pBR322.

This streamlined process can be done

using specialized software like SnapGene, which helps with visualizing cloning strategies and ensuring successful DNA manipulation.

Detailed Steps in the Cloning Cycle

Step 1: Manipulation of DNA

- First, the DNA fragment (the insert) is cut from its source using **restriction enzymes**, which act as molecular scissors. The plasmid (vector) is also cut with the same restriction enzyme.

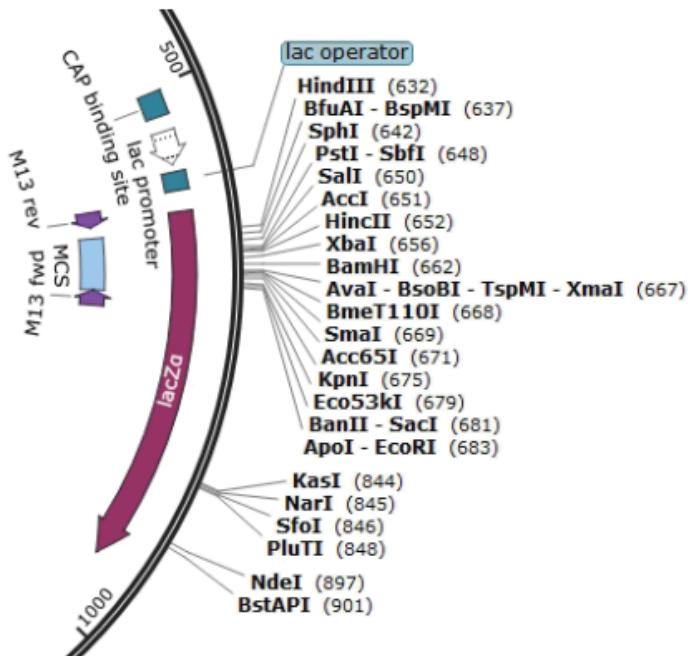


Figure 4. Enzyme restriction sites in pUC19.

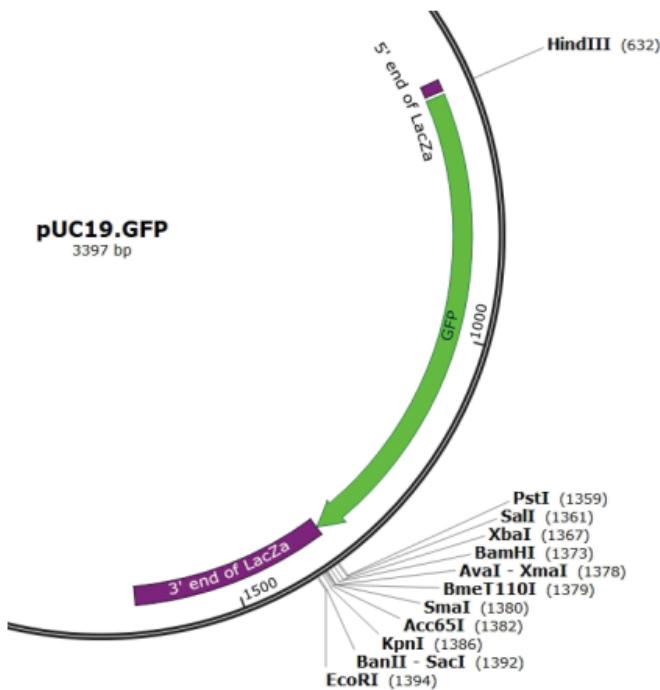


Figure 14. Cloning of the GFP sequence into the MCS disrupts LacZα.

- The insert and the plasmid are then **ligated** together using **DNA ligase**, which "glues" them into a recombinant plasmid. This recombinant plasmid can now carry the DNA

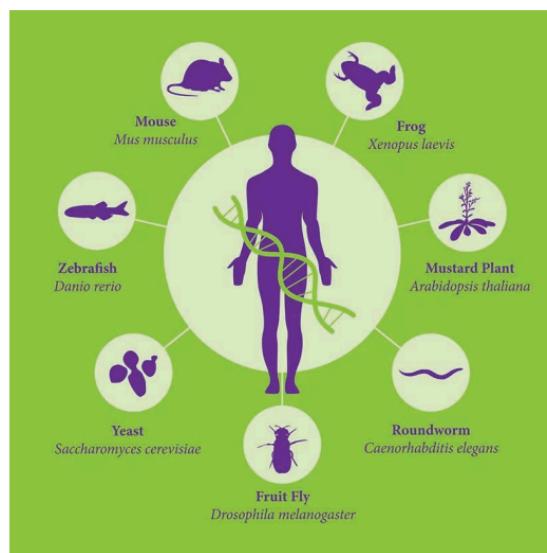


Figure 5. Organisms commonly used in biological research.

Step 2: Transformation

- The recombinant DNA is introduced into bacterial cells via **transformation**. This can be done using **chemically competent cells** (treated with calcium chloride) or **electroporation** (shocking the cells with an electric field).
- Only a small fraction of the bacterial cells will successfully take up the recombinant DNA.

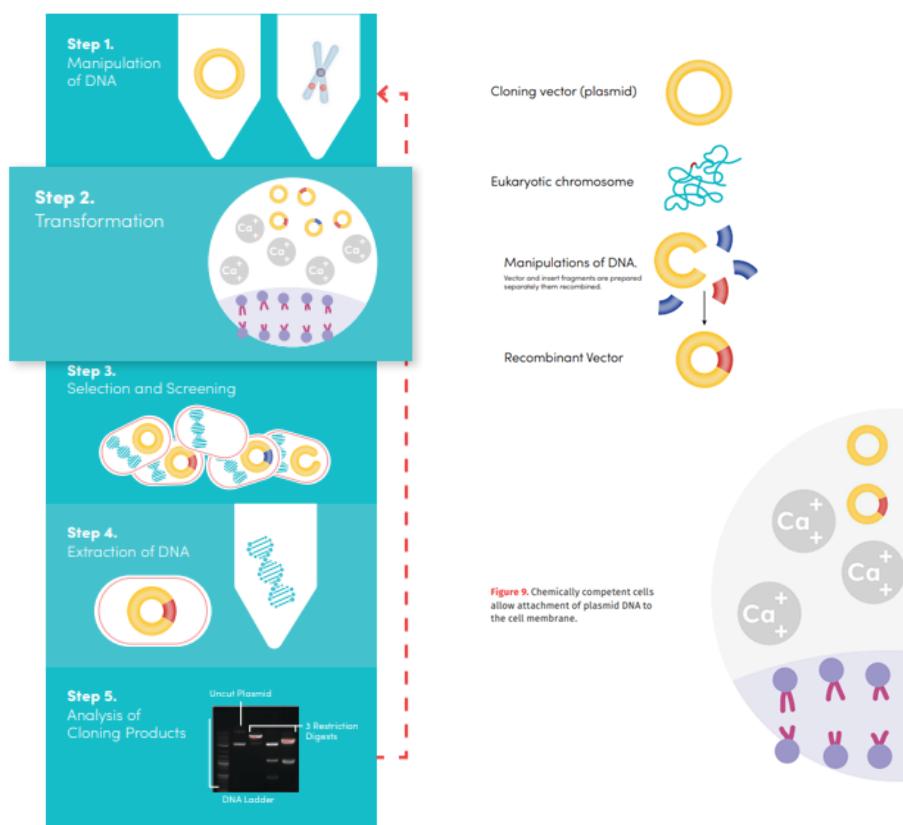


Figure 6. An overview of the plasmid cloning cycle.

Figure 9. Chemically competent cells allow attachment of plasmid DNA to the cell membrane.

Step 3: Selection and Screening

- **Selection:** After transformation, the bacteria are grown on agar plates containing an antibiotic. Only bacteria with the plasmid (which contains an antibiotic resistance gene) will survive.
- **Screening:** Bacteria are then screened to find those that have the correct DNA insert. This is often done using a **blue-white screen**, where the bacteria turn blue or white depending on whether the plasmid contains the DNA insert. For example, if the DNA insert disrupts the LacZ gene in the plasmid, the bacteria will appear white, indicating the presence of the insert.

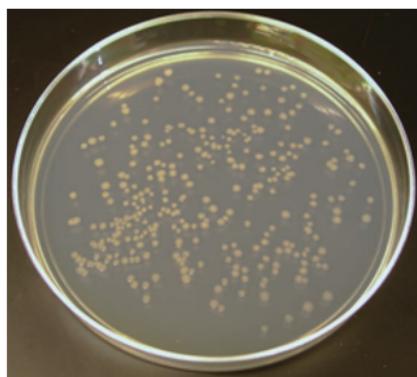
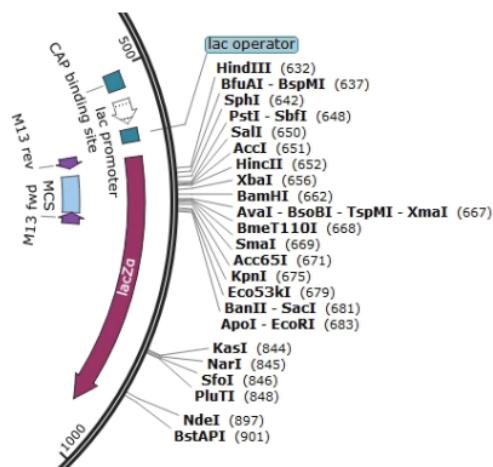


Figure 10. Bacterial colonies that are antibiotic resistant grow in an agar plate.



Step 4: Extraction

- Once the correct bacterial clones are identified, the plasmid DNA is isolated from the bacteria using a method called **miniprep**. This allows for a small amount of plasmid DNA to be purified for further analysis.

Step 5: Analysis and Verification

- To confirm that the clone contains the correct insert, the plasmid DNA is analyzed using techniques such as **sequencing**, **restriction digestion**, or **diagnostic PCR**. These methods help verify that the insert is the correct size and sequence.
- If the cloning procedure was successful, the recombinant DNA can be used in further experiments.

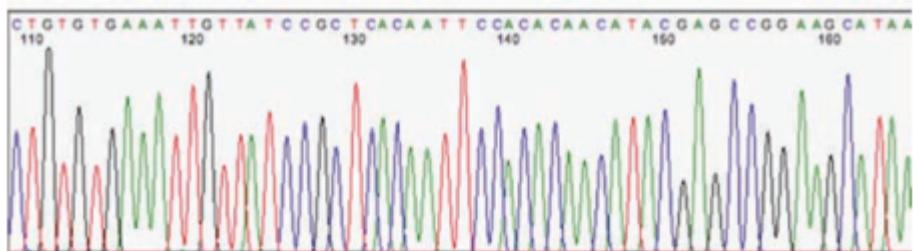


Figure 15. Sample sequence that is given once a clone is submitted for sequencing.

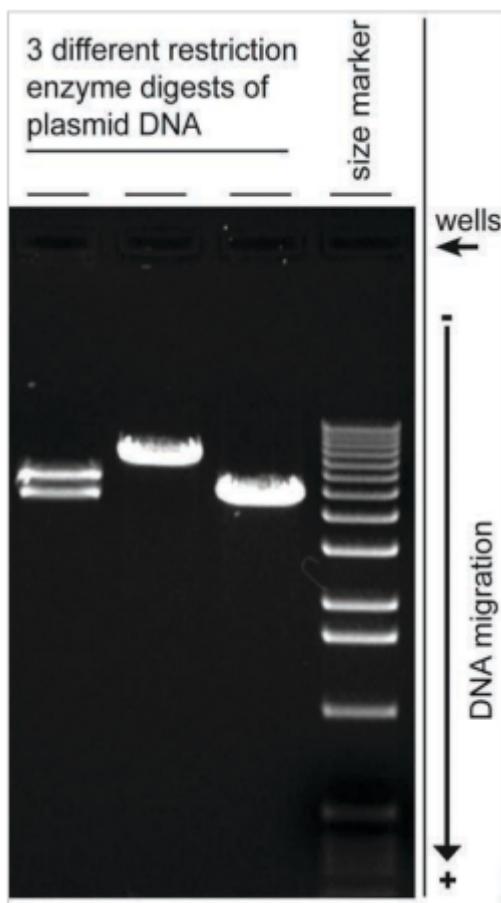


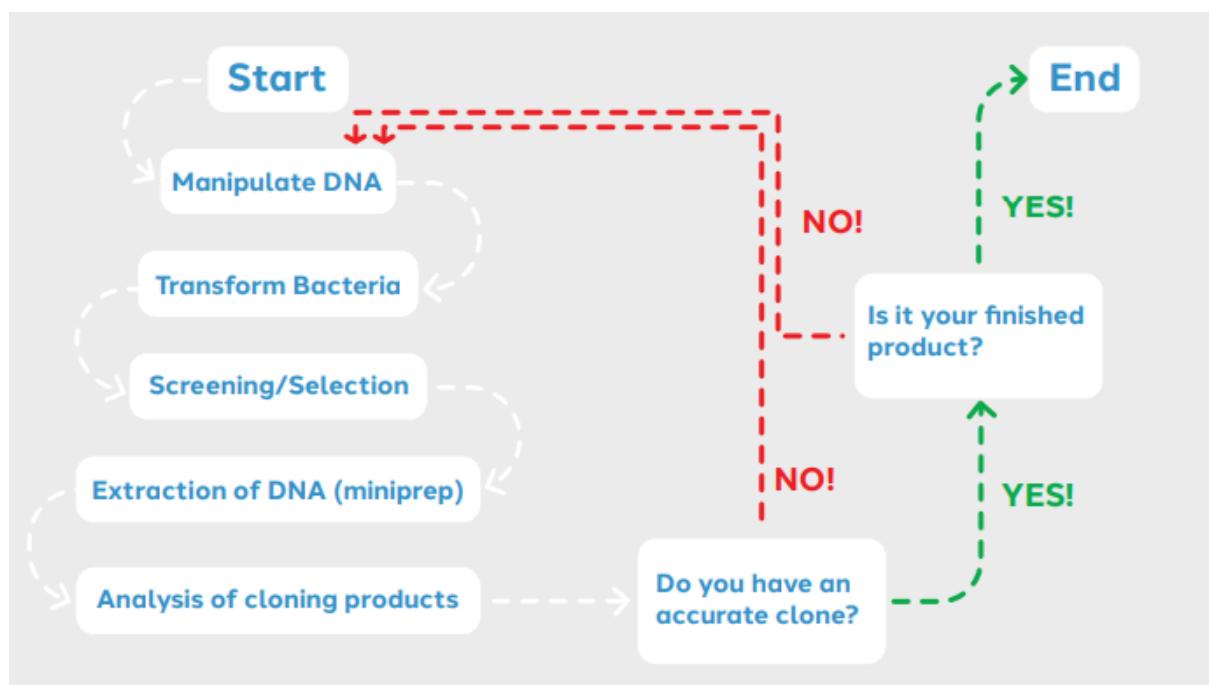
Figure 16. Restriction digests of plasmid DNA.

Tools for the Cloning Process

SnapGene is a popular tool that helps researchers visualize, document, and annotate their cloning work. It can help:

- **Document sequences:** Create and import DNA sequences.
- **Annotate sequences:** Mark features like restriction sites and primers.
- **Verify clones:** Compare your clone's sequence to a reference sequence using alignment tools.
- **Visualize cloning strategies:** Ensure that your plasmid contains all the necessary elements, like antibiotic resistance genes and the insert, before starting your experiment.

The **Plasmid Cloning Cycle** involves multiple stages, each critical to successfully cloning DNA into a plasmid. With the right tools like **SnapGene**, researchers can streamline the cloning process and increase the accuracy of their experiments, ensuring reliable and reproducible results.



Cloning Strategy and Using SnapGene for Molecular Constructs

Visualizing and Planning Cloning in SnapGene

SnapGene provides a digital platform for planning and visualizing your cloning strategy before performing the hands-on procedures in the lab. Using SnapGene's tools, you can simulate cloning experiments, create molecular constructs, and visualize key elements like restriction sites, multiple cloning sites (MCS), and antibiotic resistance genes. Here's how SnapGene aids in the cloning process:

1. **Actions Menu:** This menu allows you to simulate cloning techniques such as restriction and insertion cloning, Gateway cloning, Gibson and HiFi Assembly, and InFusion cloning.

You can visualize the process from beginning to end, ensuring that your construct is designed properly before moving to the lab bench.

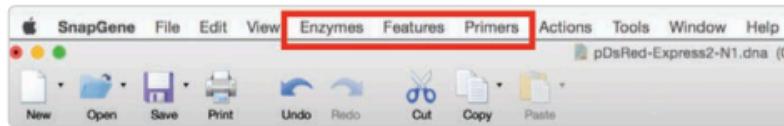


Figure 18. Annotating in SnapGene.

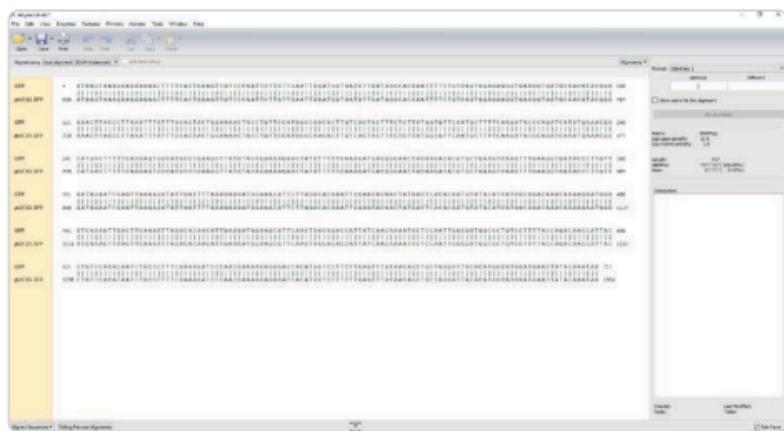


Figure 19. Aligning sequences in SnapGene for cloning verification.

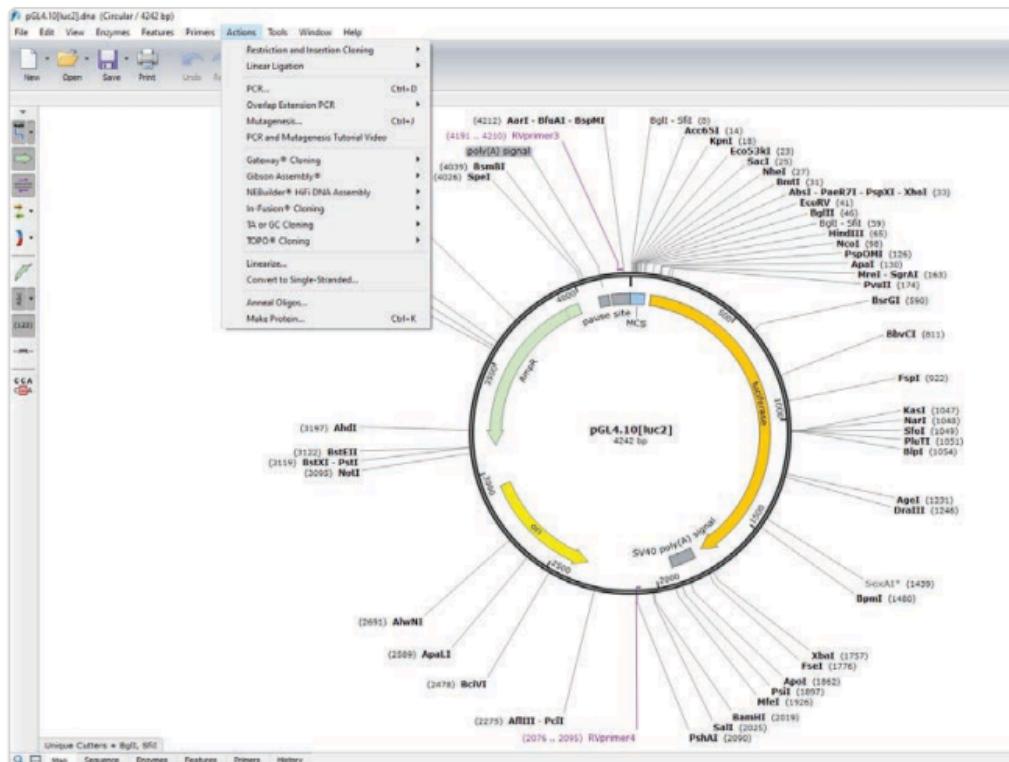


Figure 20. Action menu in SnapGene.

3. Tools for Verification:

- SnapGene has a **Simulate Agarose Gel** function, which helps predict your results for PCR reactions or restriction digests, allowing you to check whether the cloning procedure was successful.
- You can also use SnapGene's **NCBI searches, sequence assembly, and sequence alignment** tools to verify that your clone is correct by comparing it to reference sequences.

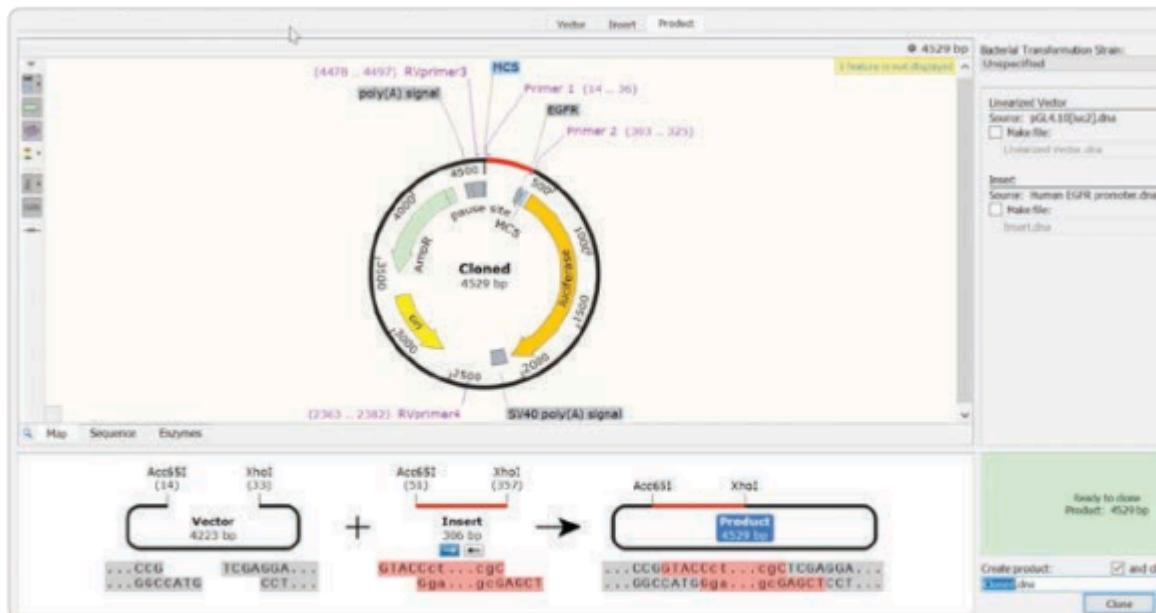


Figure 25. Simulation and visualization of restriction enzyme cloning in SnapGene.

Documentation and Annotation:

- SnapGene's **File Menu** helps you create and document DNA sequences.
- The **Enzyme, Features, and Primers Menus** let you annotate specific regions of your sequences, which is essential for creating complete maps of your constructs.

By visualizing the cloning process in silico, SnapGene helps you troubleshoot potential issues and optimize your cloning strategy before spending time and resources on bench work.

Tips for Cloning Success

Molecular cloning can be tricky, but by following these tips, you can avoid common pitfalls and improve your success rate:

Tip 1: Respect Murphy's Law

Cloning can be full of surprises, so be prepared for things to go wrong. By following the guidelines and understanding the protocol, you can mitigate potential issues.

Tip 2: Understand the Protocols

It's crucial to understand the purpose of each step in your cloning protocol. Familiarizing yourself with the reasons behind each action will help you perform the procedure more effectively and identify which steps require extra care.

Tip 3: Prepare Abundant, High-Quality Nucleic Acids

The quality of your starting material is vital. Ensure your DNA is free of contaminants, as this can affect cloning efficiency. Pay attention to the DNA purification process to yield clean and concentrated DNA.

Tip 4: Accurately Quantify Your Nucleic Acids

Accurate quantification is essential, especially when working with small amounts of DNA. Use a UV spectrophotometer, such as NanoDrop, to ensure your DNA concentration is correct and that the sample is of good quality (with a 260/280 ratio between 1.80 and 2.00).

Tip 5: Store and Handle DNA Properly

Protect your DNA from damage, especially UV damage, by storing it at -20°C and using longer wavelength UV (UVA) when purifying DNA from gels.

Tip 6: Understand Molar Ratios

Many cloning protocols specify the molar ratios of insert to vector (typically ranging from 2:1 to 5:1). Understanding these ratios helps ensure efficient ligation. Molar ratios are based on the size and molecular weight of the fragments being cloned.

Tip 7: Master Pipetting

Accurate pipetting is crucial for cloning success. Ensure that your pipetting technique is precise to minimize errors and maintain consistency in your experiments.

Tip 8: Double-Check Your Work

Molecular cloning often involves multiple steps and reagents. Always double-check your calculations, reagents, and volumes to avoid costly mistakes.

Tip 9: Save Reaction Intermediates

Cloning protocols can go awry, so it's a good idea to save leftover reaction intermediates. If something goes wrong in one step (e.g., transformation), you can go back and retry using your saved materials.

Conclusion: Efficiency and Troubleshooting with SnapGene

SnapGene is an invaluable tool that helps streamline the cloning process, allowing you to design, visualize, and verify your constructs in silico before performing the experiment in the lab. By following the tips outlined in this chapter and using SnapGene's powerful tools for annotation, simulation, and verification, you can increase the likelihood of a successful cloning experiment. Additionally, being prepared for challenges and knowing how to troubleshoot can make all the difference in achieving your cloning goals.

Key Tips and Cloning Techniques in SnapGene

Cloning Tips for Success

In addition to the practical steps involved in molecular cloning, here are some key tips to further improve your cloning success:

Tip 10: Safely Store and Document Finished Products

Proper storage is essential to prevent degradation of your reagents and plasmid constructs. Ensure that you follow the recommended storage conditions to maintain the integrity of your samples. Also, accurate documentation of your work will help you troubleshoot problems in the future and avoid confusion during the cloning process.

Tip 11: Don't Rely on Kits Without Understanding the Steps

While kits can be extremely useful, it's essential to understand what each step in the kit is accomplishing. If something goes wrong, this understanding will help you troubleshoot more effectively and determine the cause of failure. Avoid blindly following protocols; being informed about the process gives you the ability to fix issues and refine your approach.

Tip 12: Simplify Your Cloning Protocol

Complex cloning protocols often introduce more chances for error. If possible, try to minimize the steps involved in your cloning project. Fewer steps reduce the risk of mistakes, such as mislabeling or loss of DNA, and improve your overall cloning efficiency.

SnapGene's Key Cloning Techniques

SnapGene provides several advanced tools that simplify and streamline common molecular cloning techniques. Whether you're working with traditional methods or newer, more efficient approaches, SnapGene helps visualize and simulate each process, making it easier to avoid errors before you even begin the physical experiment.

Restriction Enzyme Cloning

Restriction enzyme cloning is one of the most common methods for preparing inserts and vectors for cloning. SnapGene makes this process highly efficient by allowing you to simulate the cutting and ligation of both plasmids and inserts. The software can help you see the final product, highlighting your insert in red and the plasmid in black (Figure 25). By visualizing the entire cloning process, SnapGene allows you to confirm that everything is aligned properly before you even pick up a pipette.

<https://www.snapgene.com/series/simulate-restriction-cloning>

Gateway Cloning

Gateway cloning is a recombination-based cloning method that does not require restriction enzymes. Instead, this technique uses a two-step recombination process involving **attL** and **attB** sites. The first step (BP reaction) creates an entry clone, while the second (LR reaction) generates an expression clone. SnapGene supports visualizing both reactions, helping you manage and track the process from start to finish (Figures 26 and 27). Gateway cloning is particularly useful when you need to move large DNA fragments or need precise control over insert orientation.

Gibson Assembly

Gibson Assembly is another restriction enzyme-free method that allows multiple DNA fragments to be joined into a vector in a single reaction. The technique works by exploiting overlapping sequences between fragments, which are joined by a specific enzyme. SnapGene helps by automatically designing the primers for Gibson Assembly and allowing you to visualize the assembly process (Figure 28). This tool can handle complex assemblies where multiple fragments are involved, saving you significant time in the lab.

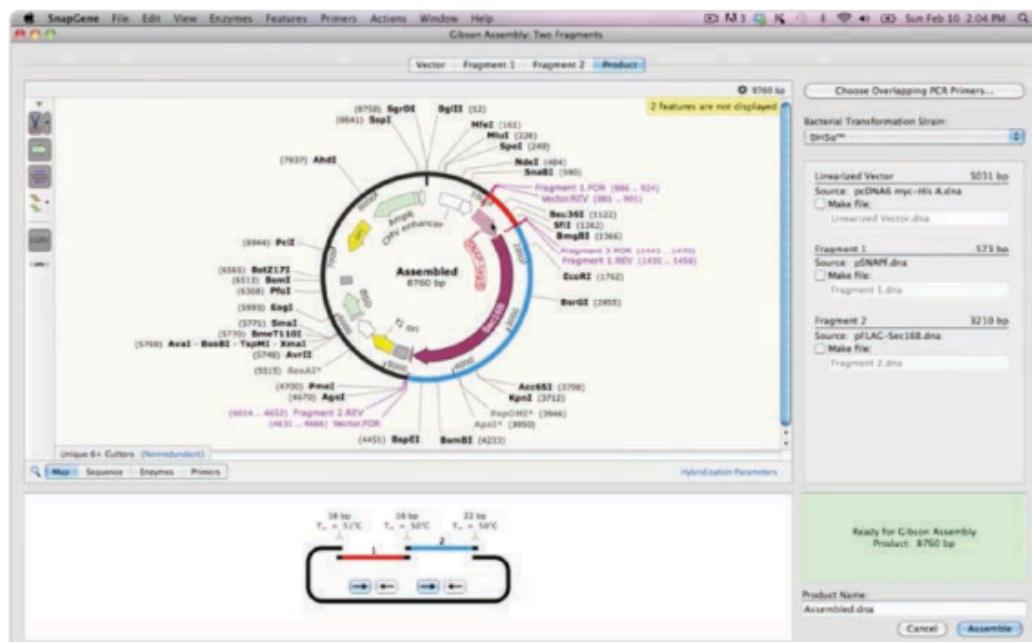


Figure 28. Product in SnapGene's simulation of Gibson Assembly performed with a vector and two fragments. Primers are indicated in purple. The fragments are shown in blue and red.

In-Fusion Cloning

In-Fusion cloning is an efficient method that joins inserts with linearized vectors through the annealing of complementary ends. Unlike Gibson Assembly, In-Fusion does not require ligation but instead relies on the use of specialized enzymes to create the desired recombinant DNA. SnapGene simulates this process by allowing you to visualize the fragments and the vector, ensuring everything is correctly aligned before performing the cloning reaction (Figures 29 and 30). This technique is known for being quick and highly accurate, especially when cloning multiple fragments at once.

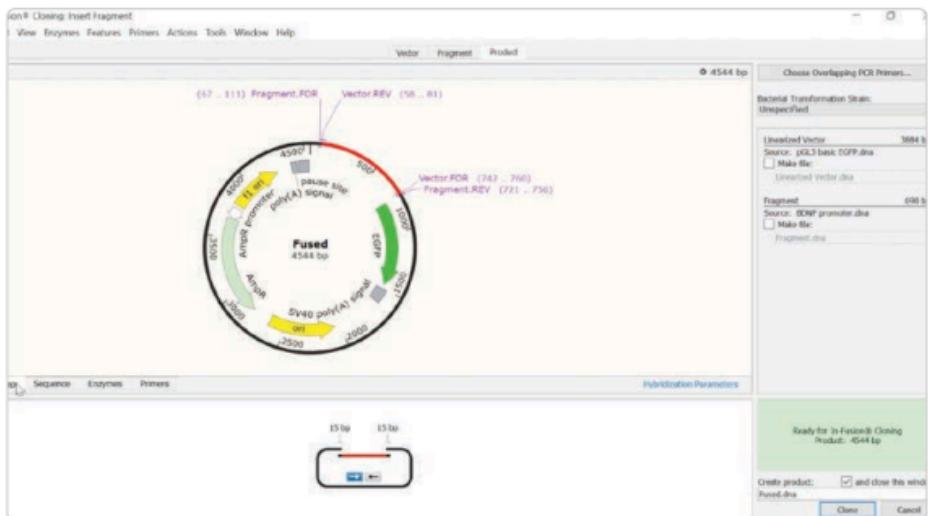


Figure 30. The product of In-Fusion Cloning simulation in SnapGene. The fragment is in red and the plasmid is in black. Primers are indicated in purple.

TA Cloning

TA cloning is a relatively simple method that exploits the addition of "A" overhangs to the 3' ends of PCR products, which can then be inserted into vectors with complementary "T" overhangs. SnapGene makes it easy to simulate TA cloning, showing you how the insert and vector align with their overhangs and how they will ligate together (Figures 31 and 32). This method is commonly used for cloning PCR products and is particularly effective for smaller fragments.

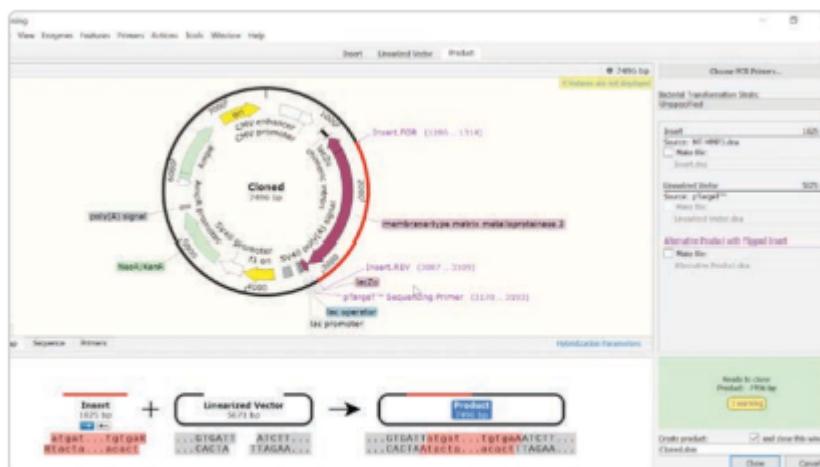


Figure 32. Snapshot of simulation of TA cloning in SnapGene. The resulting product is a plasmid with the indicated insert (in red).

<https://www.snapgene.com/resources>



MCQs on SnapGene and Molecular Biology Tools

Which company currently owns SnapGene?

- A. Thermo Fisher Scientific
- B. Agilent Technologies
- C. Dotmatics (Insightful Science)
- D. Qiagen

Which of these views in SnapGene allows visualization of enzyme cut sites and annotations?

- A. Sequence View
- B. Map View
- C. Gel Simulation View
- D. Both A and B

Which tool in SnapGene simulates the insertion of a PCR product into a plasmid?

- A. Restriction Cloning
- B. Insert Fragment
- C. Assembly Wizard
- D. Ligation Reaction

Which of the following can be used to verify your cloning design in SnapGene?

- A. Sequence Alignment
- B. Gel Simulation
- C. Feature Annotation
- D. All of the above

Which file format is native to SnapGene for saving annotated DNA constructs?

- A. .gb
- B. .dna
- C. .fa
- D. .txt

Which of the following is NOT a cloning technique directly supported in SnapGene?

- A. Gibson Assembly
- B. CRISPR-Cas9 Genome Editing
- C. Golden Gate Assembly
- D. In-Fusion Cloning

The SnapGene 7.0 update introduced all of the following EXCEPT:

- A. Tabbed interface
- B. Primer auto-annotation
- C. Cloud-based lab notebook
- D. Advanced file search

Benchling:

2. **Benchling** is a cloud-based platform where you can visualize, annotate, and edit DNA sequences, including those from GenBank files.

- **How to Open:** Upload your GenBank file to your Benchling workspace → The platform will display the sequence, annotations, and provide editing tools.

Benchling for R&D: is a cloud-based platform that helps life science organizations manage, standardize, and accelerate research. It replaces paper notebooks and scattered spreadsheets with a single digital workspace that integrates scientific data, processes, and collaboration.



A Lasting Informatics Backbone for Growing Biologics Companies

The complexity of biologics R&D makes informatics a strategic necessity. But it doesn't make sense to invest many months implementing a system that is hard-coded and won't be able to meet your needs a year from now.

The only platform natively designed for biologics R&D

Large molecule R&D involves complex processes, numerous hand-offs, and samples with extensive interlinkages. Benchling is natively built to model the complexity of these processes and samples and provide full experimental context across handoffs. It's a lasting informatics infrastructure that drives the development of your organization's scientific platform.

A screenshot of the Benchling software interface. On the left, a sidebar shows a tree view of studies: BIOT 0919 (Status: In progress) containing SCRE001 (Status: Complete), SEQU001 (Status: Complete), CREA001 (Status: Completed and reviewed), CREA001 (Status: Not started), EXP0001 (Status: Complete), and SCRS001 (Status: In progress). Below this is a 'Screen for ScFv binding' section with an input sample table for '96WPRO12' and a grid for 'ELISA Results Structured Data' showing various sample positions A1 through A5.

Core Capabilities

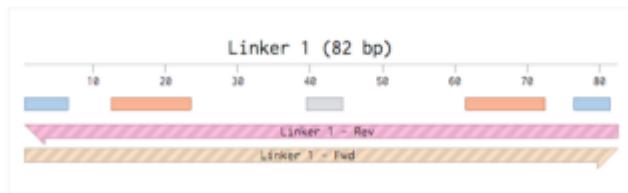
- **Electronic Lab Notebook (ELN):** A digital notebook for documenting experiments, capturing results and images, linking metadata, and ensuring compliance with audit trails and e-signatures.
- **Molecular Biology Tools:** Supports DNA and protein sequence design, alignment, primer design, cloning methods (e.g., Golden Gate, Gibson), CRISPR guide design, and antibody annotation.

- Registry & Inventory Management: Centralized systems to register, track, and manage biological entities (plasmids, cell lines, antibodies) and reagents with location tracking, barcoding, and label generation.
- Workflows: A visual interface to plan, automate, and monitor multi-step R&D processes across teams.
- Benchling Connect (Lab Automation): Integrates directly with lab instruments to automate data capture and reduce manual errors.
- Insights (Analytics): Built-in dashboards and reports for analyzing experimental data, KPIs, and resource use—enabling data-driven decisions without coding.
- Benchling AI: AI tools assist with notebook entry drafting, data structuring, and predictive modeling (e.g., protein design).
- Compliance & Security: Features like version control, audit trails, and e-signatures support GxP/GMP and IP protection requirements.
- Collaboration: Cloud-based, real-time editing and sharing across distributed teams, ensuring everyone works from a single, consistent dataset.

Linker Preparation

All linkers should be ordered from IDT as oligonucleotides. For Linkers 1 and 3, PAGE purification is required. Phosphorylation is using T4 PNK (NEB) as per manufacturer's protocol where specified. Annealing is carried out in 1X CutSmart buffer in a PCR machine that is set to ramp from 98°C to 20°C over 60 minutes. All completed adapters should be drop-dialyzed against ddH₂O for 20 minutes before using a dialysis disc.

1. Linker 1 (82nt)



An example of a weighted decision matrix. Note how there are columns for multiple vendors, which makes it easy to compare across vendors in the same sheet.

A	B	C	D	E	F	G	H	I	J	K	L	M	N
Weighted Decision Matrix			Vendor Scoring										
			Category Weight	Topic Weight	Vendor 1	Vendor 2	Vendor 3						
6	Core Functionality		30										
7	1.1	Strong biology support including molecular biology functionality to support sequence design		30									
8	1.2	Support for free form input to design experiments		10									
9	1.2	Ability to integrate with Reg and inventory systems		10									
10	1.3	Ability to store and manage assay dev data		10									
11	1.4	Robust, secure collaboration tools incl. notebook sharing [incl. audit trails], Configurable witnessing workflows including reminders and alerts.		10									
12	1.5			10									
13	1.6			10									
14	1.7			10									
15					0	0	0						
16					0								
17													
18	Vendor Assessment		15										
19	2.1	Proven record of successful ELN deployments		20									
20	2.2	Reference checks - vendor provided and TRV		20									
21	2.3	Technology stack assessment		10									
22	2.4	Implementation and long term support strategy (same team?)		15									
23	2.5	Domain expertise required for successful implementation.		15									
24	2.6	"gut-feeling" score		20		0	0	0					
25													

Typical Applications

- Drug Discovery & Development: Streamlines experimental design, cloning, and data analysis for antibody, gene, and cell therapy R&D.
- Sample & Data Management: Tracks thousands of biological samples through experiments and storage.
- Workflow Optimization: Automates complex lab processes and inter-team requests.
- Data Centralization: Creates a unified, searchable repository improving reproducibility and accessibility.
- Regulatory & IP Readiness: Ensures data integrity (ALCOA+), compliance, and traceability for patents or filings.
- Scalability: Serves startups and large pharma alike across biotech, agritech, and bioprocess development.

<https://www.benchling.com/>

Learn how Benchling is rebuilding biotech for the AI era. [Read the blog](#)

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Set the new speed of R&D

Benchling, your hub for [Scientific analytics](#)

Request a demo

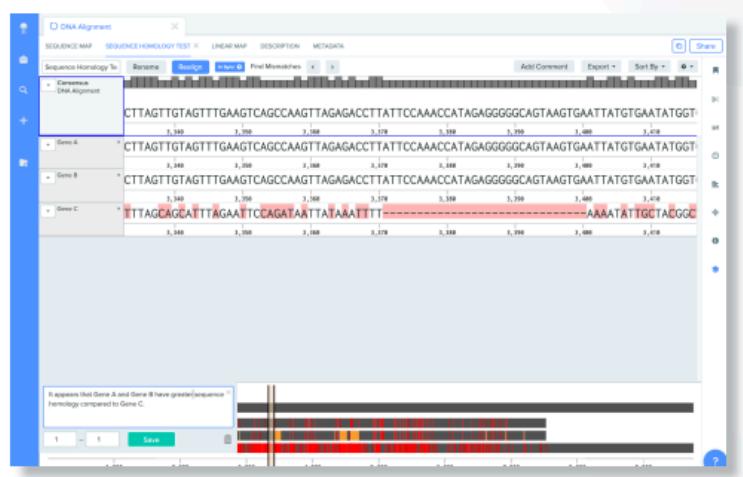
Welcome to Benchling! How can we help your research?

Tools and Analysis within Benchling

- Secondary structure prediction for nucleotides (homo- and mono-dimers)
- Automatic primer design based on the Primer3 wizard
- Drag-and-click / manual primer design
- Thermodynamic parameters and nucleotide properties (GC content, Length, Melting temperature)

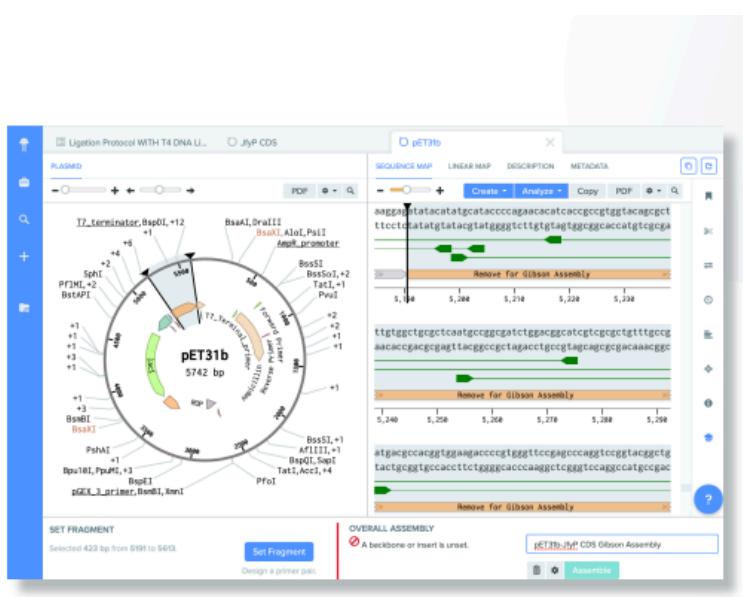
Tools and Analysis within Benchling

- ▶ Sequence imports from external databases or various file formats
- ▶ DNA and protein sequence alignments
- ▶ NCBI BLAST searches for DNA sequences
- ▶ Sequence annotation



Tools and Analysis within Benchling

- ▶ Sequence visualization and annotation for manual cloning
- ▶ Restriction cloning assembly wizard with primer design to incorporate restriction sites
- ▶ Gibson cloning assembly wizard with primer design to add sequence complementarity
- ▶ Golden Gate cloning assembly wizard with primer design for including BsaI sites



The image shows the Benchling landing page. It features a 'Get started for free' section with fields for 'Email' and a 'Continue' button, or a 'Sign up with Google' button. Below this is a link 'Already have an account? Sign In'. To the right, there are two boxes: 'Notebook' (described as for multi-day experiments) and 'Molecular Biology' (described as for sequencing, alignments, plasmid design, primers, and CRISPR guides). Both boxes include a 'Show me an entry' or 'Open a sequence' button.

This screenshot shows the 'pBR322_EGFR' project in the Benchling Molecular Biology Suite. The left sidebar lists projects like 'Example Project', 'Popular Addgene Plasmids', 'Protocol Repository', and 'Example Entry'. The main area displays a 'SEQUENCE MAP' with a DNA sequence and restriction enzyme sites (AatII, ZraI, NotI, DraIII). Below it is a circular 'LINEAR MAP' of the plasmid, labeled 'pBR322_EGFR' with a size of 5968 bp. The map shows various restriction sites and regions: 'EGFR promoter' (blue), 'EGFR sig_peptide' (yellow), 'EGFR_CDS' (green), 'EGFR_RBS' (red), and 'EGFR_3_prime_UTR' (pink). A legend at the bottom indicates positions 1 through 10. On the left, a sidebar titled 'Explore Molecular Biology' offers options like 'Open a sequence', 'Split your workspace', 'Add annotations', 'Run a digest', 'View an alignment', 'Import a new sequence', and 'Earn 1GB of extra space!'. A progress bar at the top of this sidebar shows 17% completion.

Bacterial Gene Editing Using Benchling —

Benchling provides an integrated digital platform for designing, executing, and tracking bacterial gene editing workflows. It connects *in silico* design (sequence editing and plasmid construction) with *wet lab* documentation, data capture, and analysis.

Typical Workflow

1. Design the Gene Editing Strategy (Molecular Biology Suite)

- **Import Sequences:** Upload the bacterial genome or target region from databases (e.g., NCBI).
- **Design gRNAs:** Use CRISPR tools to identify and score optimal guide RNAs, checking for off-target effects.

- **Create Donor Templates:** Design homologous recombination (HR) or HDR templates with desired edits and flanking homology arms.
- **Plasmid Construction:** Virtually assemble gRNA, Cas9, and donor DNA into expression vectors using cloning wizards (Gibson or Golden Gate).

2. Plan and Document Experiments (ELN & Workflows)

- **Protocol Documentation:** Record detailed experimental steps, link to sequences, and include reagent data.
- **Workflow Setup:** Use Benchling Workflows to assign tasks, manage experiment stages (e.g., cloning, transformation, screening), and track progress.

3. Perform Experiments and Capture Data (Notebook & Inventory)

- **Sample Tracking:** Manage plasmids, bacterial strains, and reagents using barcoded inventory entries.
- **Data Capture:** Record experimental results, gel images, sequencing data, and notes directly in the ELN.

4. Analyze and Verify Edits (Molecular Biology Suite & Insights)

- **Sequence Verification:** Import sequencing data to align against the reference and confirm edits.
- **Data Analysis:** Use Insights dashboards to evaluate editing efficiency and compare results across experiments.

Key Benchling Features Used

- **Molecular Biology Suite:** gRNA design, sequence alignment, cloning simulation, HDR/HR template design.
- **Electronic Lab Notebook (ELN):** Centralized experiment documentation with audit trails and e-signatures.
- **Registry & Inventory:** Tracking engineered strains, plasmids, and reagents for full lineage visibility.
- **Workflows:** Coordination of multi-step editing projects across R&D teams.
- **Insights:** Data analytics and visualization to assess experiment performance.

- **Compliance:** Built-in version control and traceability for IP and regulatory requirements.
-

Benefits

- Unified platform connecting design, lab work, and analysis.
Streamlined project tracking and reproducibility.
Reduced manual errors and improved collaboration.
Audit-ready documentation supporting regulatory and IP needs.
-

MiniProject: Design Oligos for detecting the sickle cell anemia mutation in the HBB gene.

1. Oligos (Oligonucleotides):

- **Definition:** "Oligo" is short for **oligonucleotide**, which refers to a short sequence of nucleotides (DNA or RNA). Oligos can vary in length but typically range from 10 to 100 nucleotides.
Purpose: Oligonucleotides can serve a variety of purposes:
 - **Probes** for detecting specific sequences (e.g., in ASO hybridization).
Primers for amplifying DNA or RNA (e.g., in PCR).
Custom sequences for research or therapeutic purposes (like in gene editing, RNA interference, etc.).
Antisense or sense oligos for gene silencing or expression regulation.
- **Length:** Oligos can be shorter or longer than primers, depending on their application (typically 10-100 nucleotides).
Types: They can be **DNA or RNA**, depending on the experiment.

2. Primers:

- **Definition:** Primers are **a type of oligonucleotide** that are specifically designed to serve as a starting point for DNA synthesis during processes like PCR, sequencing, or reverse transcription.
Purpose: The primary function of primers is to provide a free 3'-OH group to which nucleotides can be added during DNA synthesis (in PCR or sequencing). They are essential for initiating the replication or amplification of DNA.
Length: Primers are typically 18-30 nucleotides long, though this can vary slightly.
- **Types:**
 - **Forward Primer:** Binds to the 3' end of the target strand and helps amplify the complementary (opposite) strand.
Reverse Primer: Binds to the 5' end of the target strand and amplifies the

other strand.

Oligos can be used for a broader range of purposes, including **probe hybridization, gene silencing, gene expression regulation, or PCR primers**.

Primers are specifically used to **initiate DNA amplification** during PCR or sequencing.

- **Length:**

- Oligos can be **longer** than primers (e.g., 100 bp oligos for probe applications).
Primers are typically **shorter** (usually 18-30 nucleotides).

- **Context:**

- **Primers** are always a type of oligo used in PCR, but not all oligos are primers. Oligos can also be used as **probes** in hybridization assays or in other applications where they aren't used to start DNA replication.

1. Understanding the Mutation

Sickle cell anemia is caused by a **point mutation** in the HBB gene that leads to the substitution of glutamic acid (E) with valine (V) at position 6 of the beta-globin protein. Specifically, the mutation changes the DNA sequence from:

- **Normal sequence:** GAG (glutamic acid)
- **Mutant sequence:** GTG (valine)

The nucleotide change is an **A to T transversion** in the second position of the sixth codon of the HBB gene.

- **Normal DNA Sequence (around codon 6):**

- ...GAG T... (glutamic acid encoded by GAG)

- **Mutant DNA Sequence (sickle cell):**

- ...GTG T... (valine encoded by GTG)

2. Designing Allele-Specific Oligonucleotide (ASO) Probes for Hybridization

To detect this mutation via **ASO hybridization**, we need to design probes that can differentiate between the normal and mutant sequences. Here's the process:

a. Normal-Specific Probe

- This probe will be complementary to the normal HBB sequence and will hybridize only with the wild-type DNA.
- Example of a **19-mer** probe sequence for the wild-type sequence:
 - **Normal Probe Sequence:** ...GAG TCG...
 - This probe will bind specifically to the wild-type (normal) sequence.

b. Mutant-Specific Probe

- This probe will be complementary to the mutant sequence and will hybridize only with sickle cell DNA.
- Example of a **19-mer** probe sequence for the mutant (sickle cell) sequence:
 - **Mutant Probe Sequence:** ...GTG TCG...
 - This probe will bind specifically to the sickle cell sequence.

3. Designing Allele-Specific PCR (AS-PCR) Primers

In **Allele-Specific PCR**, we design primers that amplify the target region depending on whether the individual carries the wild-type or the mutant allele. Here's how to design the primers:

a. Common Reverse Primer

- The reverse primer will bind to a downstream region common to both the normal and mutant sequences. Its sequence will not be affected by the mutation at codon 6.
- Example (reverse primer):
5' - TTT GAA GAG CTC TGG GAC C - 3' (This sequence is complementary to the region downstream of codon 6.)

b. Normal-Specific Forward Primer

- This primer will be designed such that the 3' end perfectly matches the normal sequence (the **A** at codon 6).
- Example (forward primer for wild-type):
5' - CTC GAG GAG GAG GAG GAG A - 3' (This primer ends with an A to match the wild-type GAG codon.)

c. Sickle-Specific Forward Primer

- This primer will be designed to end with a **T** at the mutation site, matching the sickle cell mutation (GTG codon).
- Example (forward primer for mutant):
5' - CTC GAG GAG GAG GAG T - 3' (This primer ends with a T to match the mutant GTG codon.)

4. PCR Reaction Design

- In **separate PCR reactions**, we can amplify the normal or mutant alleles:
 - **Normal Allele PCR:** Amplifies only when the normal-specific forward primer is used in combination with the common reverse primer.
 - Mutant Allele PCR:** Amplifies only when the sickle-specific forward primer is used in combination with the common reverse primer.
 - Heterozygous (Carrier) PCR:** Amplifies both normal and mutant alleles, showing two distinct PCR products.

PCR Conditions:

- Use **appropriate annealing temperatures** to ensure that the primers bind only to their specific target sequences.
Ensure that the **melting temperatures (Tm)** of the primers are similar to guarantee efficient amplification.

5. Primer Design Criteria

- **Length:** Primers are typically between 18 and 24 nucleotides.
GC Content: Aim for a GC content of 40-60% for stable binding.
Melting Temperature (Tm): Primers should have a Tm between 55-65°C.
Avoid Secondary Structures: Check for potential primer-dimer formation and secondary structures using bioinformatics tools (e.g., Primer3).

6. Practical Application: PCR Amplification & Detection

1. **Sample Preparation:** DNA is extracted from the individual's blood sample.
2. **PCR Reaction:** Perform separate PCR reactions using the **normal-specific** and **sickle-specific** primers.
3. **Gel Electrophoresis:** Analyze the PCR products by gel electrophoresis.

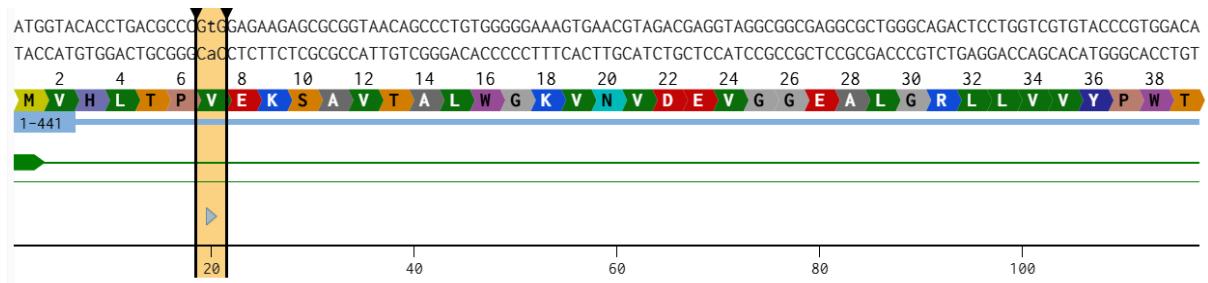
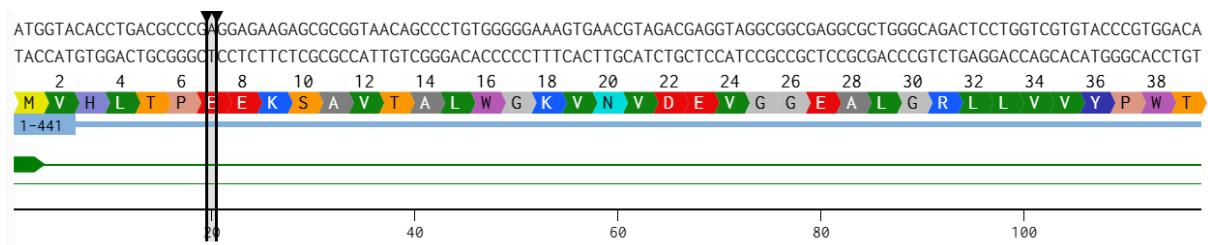
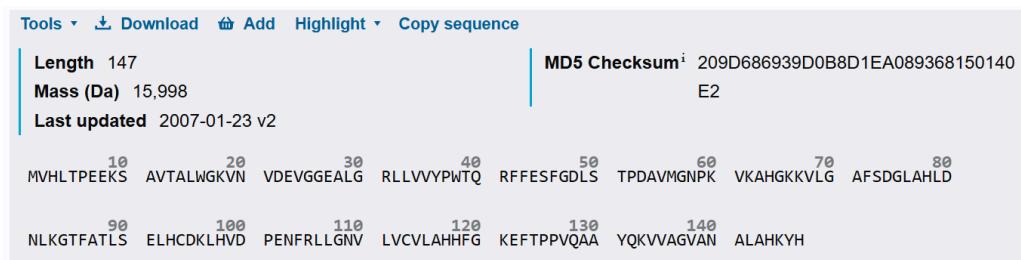
- **Wild-type individuals** will show a single band (amplified by the normal-specific primer).
- **Sickle cell homozygous** individuals will show a single band (amplified by the sickle-specific primer).
- **Heterozygous carriers** will show two bands (one from each primer pair).

Alternatively, fluorescent or probe-based detection systems can be used to identify the presence of the PCR product for each allele.

7. Bioinformatics Tools

For optimal primer design and probe creation, consider using tools like:

- **Primer3** (for designing primers)
- OligoCalc** (for calculating melting temperature and primer specificity)
- Benchling** (for sequence visualization and analysis)



1. Length of the Oligo:

- **Primers:** Typically **18-30 nucleotides** long.

- **Probes:** Can range from **18 to 50 nucleotides**, depending on the application, but **19-25 nucleotides** is a common range for good specificity.
 - Short oligos may not provide enough specificity.
 - Longer oligos tend to form more stable hybridization with the target, but they can also have increased risk of secondary structures.

Why it matters: The length determines the specificity of binding and the melting temperature (Tm) of the oligo.

2. Melting Temperature (Tm):

- **Tm** is the temperature at which half of the oligo is bound to its complementary strand. For optimal hybridization and amplification, you want primers to have similar Tm values.

For primers:

- Tm should be between **55°C and 65°C**.
- For **probe-based assays** (e.g., ASO hybridization), aim for a Tm around **60-70°C**.

To calculate Tm, you can use the **Nearest-Neighbor Method** or use online tools (e.g., **OligoCalc** or **Primer3**).

Why it matters: A well-matched Tm ensures that primers or probes bind efficiently and with specificity at the appropriate temperature during PCR or hybridization.

3. GC Content:

- Ideal GC content: **40-60%**.
 - **High GC content** (greater than 60%) can make the oligo too stable and difficult to denature during PCR or hybridization.
 - **Low GC content** (less than 40%) can result in weak hybridization and less specificity.

Why it matters: GC pairs form **3 hydrogen bonds** (compared to 2 for AT pairs), so a higher GC content generally means stronger binding. However, if too high, it can lead to secondary structures like hairpins.

4. Avoid Secondary Structures:

- **Hairpins:** Self-complementary regions within an oligo that cause it to bind to itself, forming a loop.
- **Dimerization:** The formation of dimers between two oligos (primer-primer dimers) or between the probe and primer.

Why it matters: Secondary structures reduce the efficiency of primer binding and can prevent successful amplification or hybridization. Ensure primers and probes do not form self-dimers or hairpins.

- Use tools like **Primer3** or **OligoAnalyzer** to check for secondary structures.
-

5. Specificity:

- **Specificity** is crucial to avoid binding to unintended targets. To achieve this:
 - **For primers:** Ensure that the 3' end of the primer is complementary to the target sequence to prevent non-specific binding.
 - **For probes:** Make sure the probe is complementary only to the target sequence (not homologous sequences).

Why it matters: Non-specific binding can lead to false results or weak amplification, especially when using PCR primers or in hybridization assays.

- **Tool: BLAST or primer BLAST** can help you check whether the sequence matches the intended target only.
-

6. 3' End Considerations (for Primers):

- The **3' end** of the primer should be as specific as possible to ensure efficient and accurate extension during DNA synthesis (e.g., in PCR).
 - Avoid having **G or C** at the 3' end (unless it's part of the target), as these bases can sometimes cause unwanted binding or extension in certain conditions.

- **G/C clamp:** A **G or C** at the 3' end of the primer is often used strategically to enhance the specificity and stability of the primer during PCR.

Why it matters: The primer's 3' end is where DNA synthesis starts, so it needs to bind perfectly to the complementary strand to ensure the amplification is accurate.

7. Avoid Repetitive Sequences:

- Avoid **repetitive sequences** (such as "AAAA" or "GGGG"), as they may result in ambiguous binding or unstable hybridization.
- **Homopolymer runs** (like "GGGG" or "TTTT") can lead to poor PCR efficiency.

Why it matters: Repetitive sequences can cause slippage during DNA synthesis, leading to errors and reduced amplification efficiency.

8. Optimal Primer Pairing:

- When designing **two primers (forward and reverse)** for PCR:
 - **Similar Tm values:** Forward and reverse primers should have Tm within **2-5°C** of each other for efficient amplification.
 - **Complementary sequences:** Ensure that the primers bind to the target region without forming secondary structures (e.g., primer dimers or hairpins).

Why it matters: If primers have a large Tm difference, the amplification process may not work efficiently, as one primer may bind too weakly, or the other may melt prematurely.

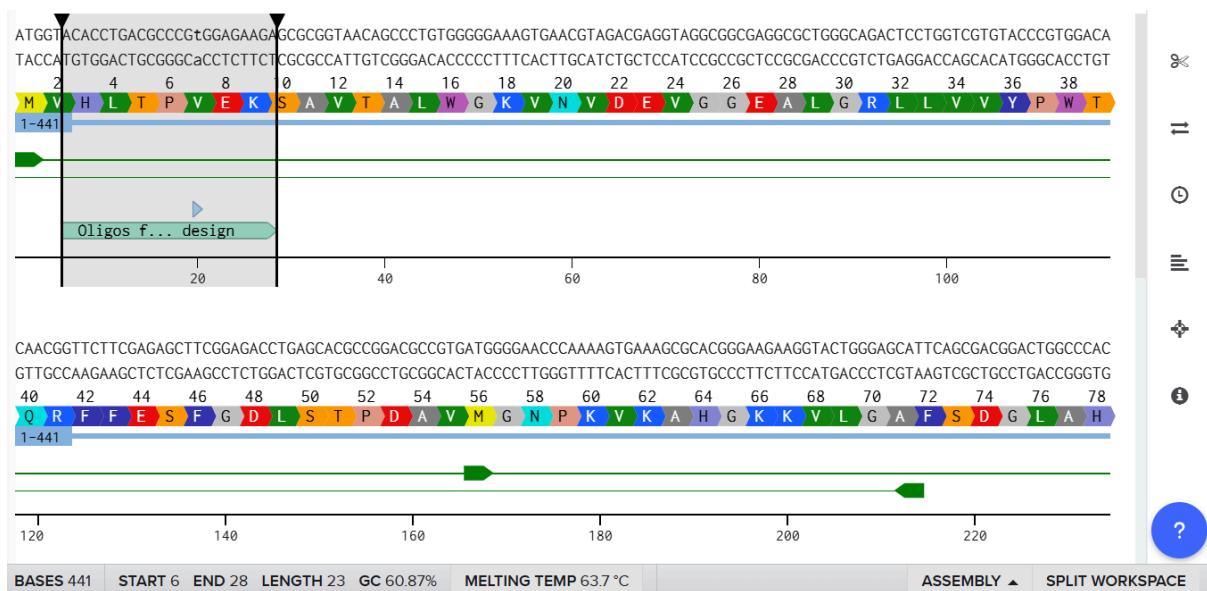
9. Designing Probes for Hybridization:

- **Probe Length:** Typically, **19-25 nucleotides**.
- The probe should have a high **GC content** (about 50-60%) to ensure stable binding, but avoid long homopolymer runs or excessive GC content (>70%).
- **5' End Fluorescent Labeling** (for detection in fluorescence-based assays like FRET or TaqMan).

Why it matters: The probe needs to hybridize to its target efficiently under stringent conditions, so it must be the right length and have appropriate GC content.

10. Other Considerations:

- **Avoid binding to homologous sequences** (for specificity): For example, in a clinical assay, if you are detecting a mutation in the HBB gene (like sickle cell mutation), the oligos should be specific to that exact mutation site.
- **Probe Design for Allele-Specific Hybridization:** For allele-specific probes (such as in ASO hybridization), the probe should be designed to match perfectly with either the normal or mutant sequence at the mutation site (in the case of sickle cell: **GAG → GTG**).



<https://benchling.com/s/seq-FeM9Uquhfiz0ps9sEl2R?m=slm-AuFp53stkqi6S43NcxWf>

GFP mRNA

SEQUENCE MAP

Linear Map | Plasmid | Design Primer | Share | ...

Create v Analyze v

Primer Pair | Jump to Primer | Set from Selection |

Design

Strand: Forward | Reverse

Bases: 5' attaGAATTCTATGAG
TAAAGGAGAAGAACT
TTTCACTGGAG

3'

Location: 56 | 712

Overhang: 10 | 10

Cut Site: HindIII | AAGCTT

Use the dropdown above to look up restriction sites.

Explore Molecular Biology

BASES 922 START 712 END 742 LENGTH 31 GC 32.26% MELTING TEMP 56.8 °C ASSEMBLY ▾ SPLIT WORKSPACE

Projects / Example Project

pUC19

Pick Assembly Strategy

Digest and Ligate (selected)

Gibson

Golden Gate

Start | Cancel

Virtual Digest | Plasmid | ...

ASSEMBLY ▾ SPLIT WORKSPACE

The screenshot shows the BioEdit software interface with the following details:

- Left Sidebar:** Projects / Example Project, Search bar, Type dropdown, Filters button.
- Middle Panel:** Sequence Map of GFP mRNA [26-742] (620-670 bp) relative to pUC19. The map shows restriction sites: EcoRI, BpuI, BamHI, Eco55KI, KpnI, SacI, BmrI, and SmaI. A "source" region is indicated between 635 and 665 bp. A preview window shows the sequence TAA agcttgg ATTTCGA acc cacttaaG1.
- Right Panel:** GFP mRNA [26-742] (723 bp) relative to pUC19 (2.6 kb). The map shows restriction sites: HindIII, EcoRI, PvuII, PvuI, DrdI, NdeI, BstAPI, L4440, L4440+3, PciI, +1, AfI, III, and MspI. A circular diagram highlights the 1060 bp MCS region. A yellow arrow points to the "Reverse Orientation" button.