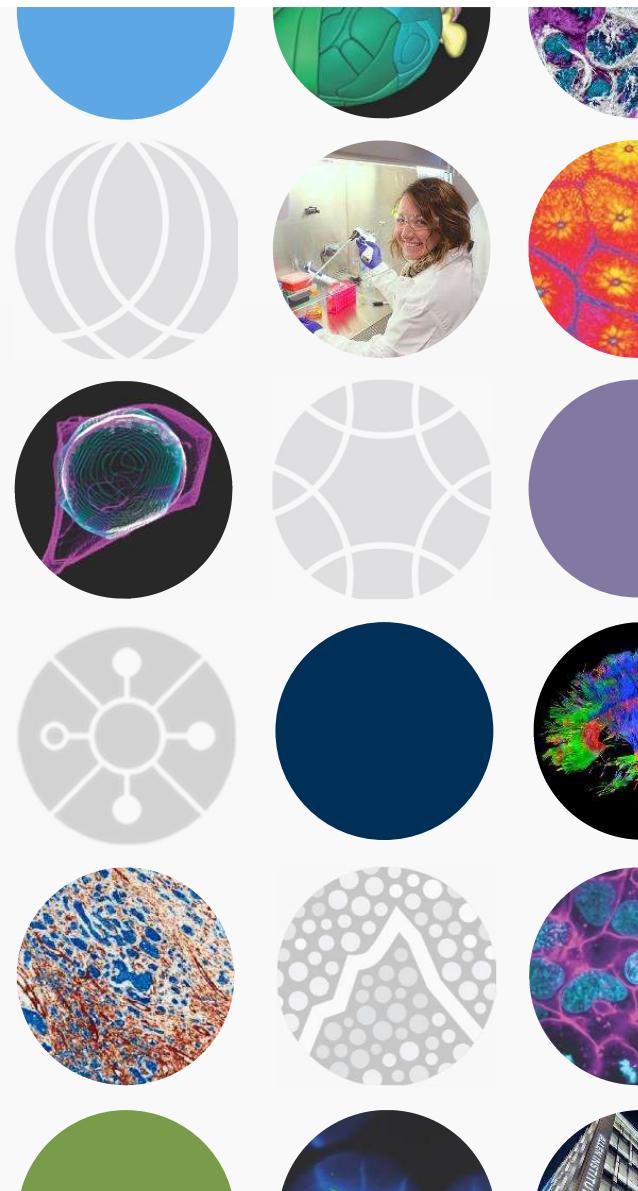




# Basic Plasmid Designer Prototype

Adapting CRISPR-GPT



# CRISPR-GPT

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- AI-assisted framework for **designing and evaluating CRISPR guide RNAs**
- Integrates **large language models (LLMs)** with established CRISPR design principles
- Translates **user intent and experimental constraints** into actionable design outputs
- **Not a generalist platform like Biomni**

# CRISPR-GPT Adaptation

A small prototype for plasmid design within the CRISPR-GPT framework.

# CRISPR-GPT Adaptation

- Added a fifth design option to support expression plasmid design.
- Improved user prompting to better handle plasmid design chat-based inputs.
- Updated state logic to include simplified, step-by-step workflows for plasmid design.
- Implemented relevant classes and functions to support the design goals.
- Tested a basic use case and successfully at two levels of difficulty, obtained a passing and failing design.

# Meta-Mode Test 1

pcDNA3.1(+) (Pulled from defined plasmid library) and eGFP  
(provide raw sequence)

# Enter Meta-Mode



AI Assistant for gene editing

Chatbot

Welcome to CRISPR-GPT. I can help with the following tasks. Please select one to continue.

1. Meta-Mode (Step-by-Step Guidance on Pre-defined Meta-Task)
2. Auto-Mode (Customized Guidance on Free-style User Request)



1

# Design an Expression Plasmid



AI Assistant for gene editing

Chatbot

{'Thoughts': 'The user has selected Meta-Mode for step-by-step guidance on a pre-defined meta-task. Presumably, this task involves structured guidance on a topic related to CRISPR technology.', 'Choice': '1'}

Please select the general gene editing scenarios to continue.

1. Generating a Knockout Using CRISPR.
2. CRISPR Base Editing Without Double-Strand Breaks. (Not Supported in Lite version)
3. Generating Small Insertion/deletion/base editing through Prime Editing. (Not Supported in Lite version)
4. Activation or Repression of Target Genes Using CRISPR. (Not Supported in Lite version)
5. Designing an Expression Plasmid.

# Choose a backbone from the Library

The screenshot shows a dark-themed AI interface for CRISPR-GPT. At the top left is the logo 'CRISPR-GPT' with a DNA helix icon. Below it is the subtitle 'AI Assistant for gene editing'. A blue button labeled 'Chatbot' is visible. The main area contains a text box with the following content:

{'Thoughts': 'The user has chosen option 5, which involves designing an expression plasmid. This process entails designing a plasmid that can drive the expression of a gene of interest in a host organism. Essential elements typically include a promoter, the gene of interest, a terminator, and often a selectable marker. Additional considerations might involve the choice of vectors, compatibility with host organisms, and regulatory sequences to optimize expression levels.', 'Choice': '5'}

Now, let's start designing your expression plasmid construct. We will guide you through a step by step process as listed below:

1. Selecting an expression plasmid backbone.
2. Designing the gene insert.
3. Selecting output format for your construct.

Which expression plasmid backbone would you like to use?

1. pcDNA3.1(+)
2. pAG
3. I have my own plasmid backbone
4. I know the plasmid name/details

Please select 1, 2, 3, or 4.

At the bottom right of the interface, there is a small red-bordered box containing the number '1'.

# Provide the insert sequence

The screenshot shows a dark-themed chat interface for CRISPR-GPT. At the top left is the CRISPR-GPT logo with the text "AI Assistant for gene editing". Below it is a "Chatbot" button with a speech icon. The main area contains a message from the bot:

{'Thoughts': "The user has entered '1', which suggests they selected the first option. This corresponds to the pcDNA3.1(+) CMV promoter vector.", 'BackboneName': 'pcDNA3.1(+)', 'CustomDetails': 'N/A', 'Status': 'confirmed'} Final Result pcdna3.1(+)

We can help you design your gene insert for expression plasmids.

Do you already have the exact DNA sequence for the gene you want to express?

If YES: Please provide the sequence (in FASTA or raw format)

If NO: Please tell us the gene name or protein you want to express (e.g., "EGFP", "human TP53"), and we can look up and extract the sequence for you.

A large gray box at the bottom contains a long DNA sequence:

```
ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCATCCTGGTCGAGCTGGACGGCGACGTAAACGCCACAAGTTCAGCGTGTCCGGCGA  
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCTGAAGTTCATCTGCACCACCGCAAGCTGCCGTGCCCTGGCCCACCCCTCGTGACCACCCCTGA  
CCTACGGCGTGCAGTGCTTCAGCCGCTACCCGACCATGAAGCAGCACGACTTCTCAAGTCCGCCATGCCGAAGGCTACGTCAGGAGCGCACCA  
TCTTCTCAAGGACGACGGCAACTACAAGACCCCGCCGAGGTGAAGTTCGAGGGCGACACCCCTGGTAACCGCATCGAGCTGAAGGGCATCGACTTCA  
AGGAGGACGGCAACATCCTGGGCACAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATATCATGGCCACAAGCAGAAGAACGGCATCAAGGTGAA  
CTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTGCCGACCACTACCAGCAGAACACCCCCATGGCGACGGCCCCGTGCTGCTGCCG  
ACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGCCTGCTGGAGTCGTGACCGCCGCCGGATCA  
CTCTCGGCATGGACGAGCTGTACAAGTAA
```

# Override safety step

The screenshot shows the CRISPR-GPT AI Assistant for gene editing. At the top, there's a logo with a DNA helix icon and the text "CRISPR-GPT". Below it, a subtext says "AI Assistant for gene editing". A "Chatbot" button is visible. In the main area, a message box contains the following text:

Error occurred. Error Message: Warning: Your input contains a possibly an identifiable private human/patient sequence that should not be supplied to a public LLM model. Please consider removing the sequence. To ignore the warning and continue, add [IGNORE HIPAA RULE] anywhere in your input. Let's try again.

Below this message, a sequence of DNA bases is shown:

eGFP  
ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCTACCTGGTCAGCTGGACGGCACGTAAACGGCCACAAGTTACGGTGTCCGGC  
GAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCTGAAGTTCATCTGCACCACCGGAAGCTGCCGTGCCCTGGCCACCCCTCGTACCC  
TGACCTACGGCGTGCAGTGCCTCAGCGCTACCCGACCACATGAAGCAGCAGCAGACTTCTCAAGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGC  
ACCATCTTCTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTGAGGGCGACACCCCTGGTAACCGCATCGAGCTGAAGGGCATCGA  
CTTCAAGGAGGACGGCAACATCCTGGGCACAAGCTGGAGTACAACATAACAGCCACAACGTCTATATCATGCCGACAAGCAGAAGAACGGCATCAA  
GGTGAACCTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTGCCGACCACCTACAGCAGAACACCCCCATCGCGACGGCCCGTGTG  
CTGCCCGACAACCAACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGCCTGCTGGAGTTCGTACCGCCG  
CCGGGATCACTCTCGGCATGGACGGAGCTGTACAAGTAA

A red rectangular box highlights the text "[IGNORE HIPAA RULE]" at the bottom of the sequence.

# Confirm choices and proceed



AI Assistant for gene editing

Chatbot

Gene: eGFP

Sequence provided: ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAG (length: 717)

Great! We have identified your target gene. Here's a summary of your construct:

1. Target Gene: {gene\_name}
2. Plasmid Backbone: {plasmid\_backbone}

Would you like to proceed with this construct, or would you like to make any modifications?



proceed with this construct



# Select output (raw)

The screenshot shows a dark-themed chatbot interface for CRISPR-GPT. At the top, the logo 'CRISPR-GPT' is displayed next to a DNA double helix icon, with the subtitle 'AI Assistant for gene editing'. A 'Chatbot' button is visible on the left. The main conversation area contains the following text:

Great! We have identified your target gene. Here's a summary of your construct:

1. Target Gene: eGFP
2. Plasmid Backbone: pcDNA3.1(+)

Would you like to proceed with this construct, or would you like to make any modifications?

Excellent! Your expression construct design is complete. We are ready to provide you with the final sequence for ordering.

In what format would you like to receive your plasmid sequence for ordering?

1. GenBank format (.gb) - Complete annotation with features and metadata
2. FASTA format (.fasta) - Sequence header and nucleotide sequence
3. Raw sequence string - Nucleotide sequence only

Please select your preferred format (1, 2, or 3). You can use this sequence file to submit to your DNA synthesis vendor (e.g., GenScript, IDT, Synthego, etc.).

A small number '3' is located in the bottom right corner of the main text area.

# Observe Output!

 CRISPR-GPT

AI Assistant for gene editing

**Chatbot**

Your construct sequence is ready:

**CONSTRUCT SEQUENCE:**

```
GACGGATCGGGAGATCTCCGATCCCCTATGGTCACTCTCAGTACAATCTGCTGTGCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTTGG  
AGGTCGCTGAGTAGTGCAGAACATTAAAGCTACAACAAGGCAAGGCTGACCGACAATTGCGATGAAGAATCTGCTTAGGGTTAGGCCTTTCGCTG  
CTTCGCGATGAGTCAGGGCCAGATAACGCGTTGACATTGATTAGTACTAGTTAATAGTAATCAATTACGGGGTCAATTAGTCAGGCCATATGGAGTC  
CGGGCTACATAACTACGCTAAATTGGCCCGCTGACGCCAACGACCCCGCCATTGACGCTAATAATGACGTATGTTCCATAGTAAACGCCAATA  
GGGACTTTACATTGACGCTCAATTGGGAGATTACGGTAACTGGGCACTTGGCAGTACATCAAGTGTATCATGCGAAAGTACGCCCTTATTGACGTC  
AATGACGGTAATGGCCGCGCTGGATTAGCCAGTACATGACCTTATGGGACTTCTACTTGGCAGTACATCACTGTTAGTATGCTATGCTTACCTGGT  
GATGCGGTTTGGCAGTACATCAATTGGGTTGGTAGCGGTTGACTCACGGGATTTCAGTCTCCACCCATTGACGTCAATTGGAGTTTGGC  
ACCAAATCAACGGGACTTCCAAAATGCGTAACAACCTCGCCCCATTGACGCAAATGGCGGTAGGCCTGTACGGTGGGAGGTCTATAAGCAGAGCT  
CTCTGCTTAACTAGAGAACCCACTGCTTACTGCTTATCGAAATTAAATGACTCACTATAGGGAGACCCAAGCTGCTGGCTTAAACTAAAGCTGGTAC  
CGAGCTCATGGTGAGCAAGGGCAGGGAGCTGTTACCGGGGGGGTGGCCTACCTGGTCAGCTGAGCGGAGCTAAAGGGCAACAGGGCTACGGTAC  
CCGGCAGGGCAGGGGAGTGCACCTACGGCAAGCTGACCCTAAGTTACATGCAACCCGGCAAGCTGCCGTGCCCTGGCCACCCCTGTGACC  
ACCCGACTCACGGCTGAGTCTCAGCCGCTACCCGACACATGAAGCAGCACGACTTCTCAAGTCCGCCATGCCGAAGGCTACGTCCAGGAG  
CGCACCATCTCTCAAGGACGACGGCAACTACAAGACCCGGCCAGGTGAAGTTCAGGGCAGACCCCTGGTGAACCGCATGAGCTGAAGGGCATC  
GACTTCAAGGAGGACGGCAACATCTGGGCAAGCTGGAGTACAACAAACAGGCAACAGCTATATCATGGCCGACAAGCAGAAAGCAGCATCA  
AGGTGAACCTCAAGATCCGCCAACACATCGAGGAGCAGGAGCTGCACTGGCCGACCACTACCGAGAACACCCCGAGCGAGGCCCTGTGCTG  
CTGCCGACAACCCACTACCTGAGCAGCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCATCACATGGCTCTGCTGGAGTTCTGACCGCCGC  
○ Plasmid Backbone: pcDNA3.1(+)  
○ Total Size: 6148 bp  
○ Insertion Method: mcs (at position 928)  
○ Output Format: RAW_SEQUENCE
```

This sequence is ready for synthesis and expression testing.

Your expression construct is complete and ready for synthesis!

Would you like to:

1. Download/save your construct design
2. Modify any aspect of your design and reorder
3. Start a new plasmid design project

# Plasmid Validation

Using a gene editor and the Rubric

# Plasmid Validation

## Design Summary:

- Gene: eGFP
- Plasmid Backbone: pcDNA3.1(+)
- Total Size: 6148 bp
- Insertion Method: mcs (at position 928)
- Output Format: RAW\_SEQUENCE

This sequence is ready for synthesis and expression testing.

Your expression construct is complete and ready for synthesis!

Would you like to:

1. Download/save your construct design
2. Modify any aspect of your design and reorder
3. Start a new plasmid design project

Using preferred plasmid editor tool (like Benchling or Snapgene)

## 1. Upload the newly generated sequence

- Upload as a circular DNA sequence
- Auto Annotate.

## 2. Manually Recreate the Plasmid:

- Upload the backbone as a circular DNA Sequence from known sequence (either from the library or pulled manually from addgene or other repository).
- Add insert DNA sequence to the site indicated in the outputs
- Auto Annotate

3.

# Plasmid Validation

## Design Summary:

- Gene: eGFP
- Plasmid Backbone: pcDNA3.1(+)
- Total Size: 6148 bp
- Insertion Method: mcs (at position 928)
- Output Format: RAW\_SEQUENCE

This sequence is ready for synthesis and expression testing.

Your expression construct is complete and ready for synthesis!

Would you like to:

- Download/save your construct design
- Modify any aspect of your design and reorder
- Start a new plasmid design project

## 3. Start Comparison

- Copy the generated plasmid sequence and verify that the expected sequence can be found within it, confirming full insertion at the specified location (then verify the location itself is valid).
- Check the total plasmid size as a quick sanity check; it should match expectations.
- If the size does not match, begin by verifying that the backbone sequence is correct.
- If the backbone matches, search for the insert within the plasmid.
- If needed, check individual plasmid components.
- Consider the possibility that the insert was placed a few nucleotides away from the specified position.
- Continue through the rubric systematically.

# Plasmid Validation

## 4. Conclusion

- Plasmid was valid.
- The plasmid backbone and insert sequences were preserved.
- The location was off by 1nt from what the program said, but otherwise the gene was inserted in the proper location downstream of the Promoter in the MCS, in the correct orientation.

# Rubric for Test 1

A	B	C	D	E	F	G	H
Critical Failure Present?	NO						
Overall Result	PASS						
Percentage	96%						
Section	Category	Checkpoint	Severity	Points	Pass (Y/N)	Score Earned	Notes
Input Validation	Backbone	Did the user provide a backbone sequence?	Info	0	y	0	
Input Validation	Backbone	If not, did the user select a predefined plasmid or indicate the backbone sequence for the program to pull?	Critical	2	y	0	
Input Validation	Backbone	Correct backbone sequence retrieved (if selecting from library)? Leave blank if not applicable.	Critical	2	y	0	
Input Validation	Backbone	Correct backbone sequence retrieved (if the user provided the URL)? Leave blank if not applicable.	Critical	2		0	
Input Validation	Backbone	Correct backbone sequence retrieved (if the user provided the name)? Leave blank if not applicable.	Critical	2		0	
Input Validation	Backbone	Backbone length correct (for example, the program in the chat will pull the sequence length, is this length that is output correct?)	Minor	0.5	y	0	
Input Validation	Backbone	Backbone sequence is valid DNA	Critical	2	y	0	
Input Validation	Insert	Did the user provide an insert sequence?	Info	0		0	
Input Validation	Insert	If not, did the user provide a Gene ID? (Leave blank if not applicable)	Critical	2		0	
Input Validation	Insert	If a gene ID was provided, was the correct insert retrieved?	Critical	2		0	
Input Validation	Insert	Insert sequence valid is DNA	Critical	2	y	0	
Input Validation	Insert	Insert length correct (for example, the program in the chat will pull the sequence length, is this length that is output correct?)	Minor	0.5	v	0	

# Video

The screenshot shows a dark-themed chat interface for CRISPR-GPT. At the top, there's a logo with a DNA helix icon and the text "CRISPR-GPT" followed by "AI Assistant for gene editing". A message from the "Chatbot" says: "ts: 'The user has chosen option 5, which involves designing an expression plasmid. This process entails designing a plasmid that can drive the expression of a gene of interest in a host organism. Essential elements typically include a promoter, the gene of interest, a terminator, and often a selectable marker. Additional considerations might involve the choice of vectors, compatibility with host organisms, and regulatory sequences to optimize expression levels.', 'Choice': '5'}".

Below this, the bot continues: "Now, let's start designing your expression plasmid construct. We will guide you through a step by step process as listed below:

1. Selecting an expression plasmid backbone.
2. Designing the gene insert.
3. Selecting output format for your construct.

Which expression plasmid backbone would you like to use?

1. pcDNA3.1(+)
2. pAG
3. I have my own plasmid backbone
4. I know the plasmid name/details

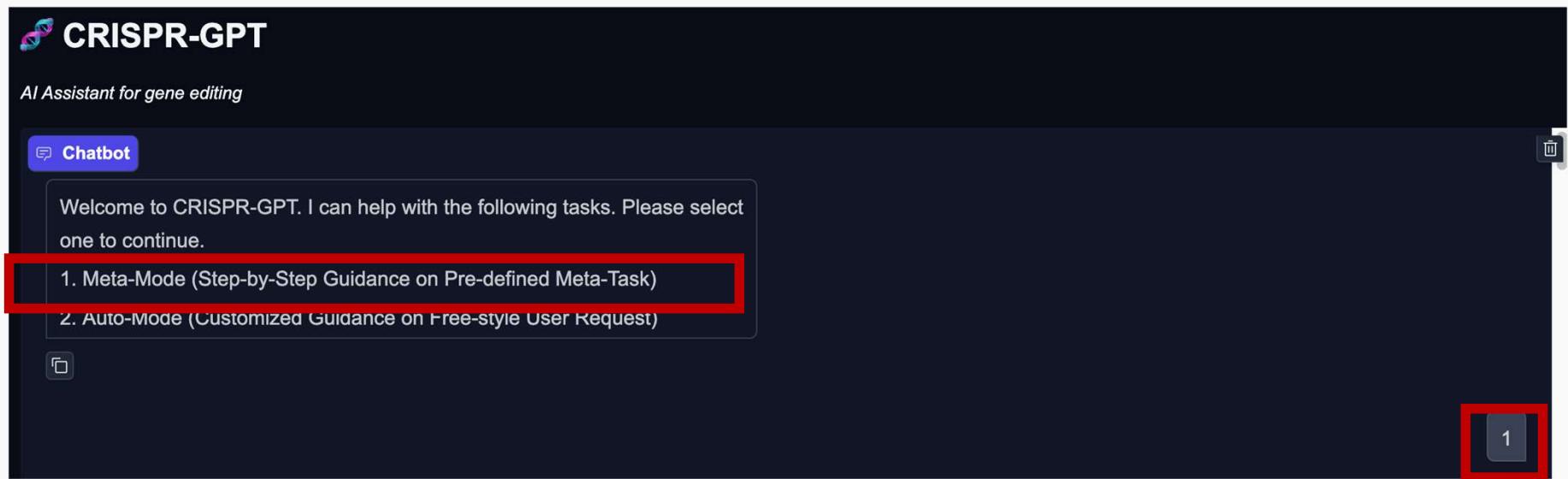
Please select 1, 2, 3, or 4.

At the bottom of the interface, there's a text input field with placeholder text "Ask about gene-editing..." and a blue "Send" button. To the right of the "Send" button is a small number "1" inside a box, likely indicating the count of messages sent.

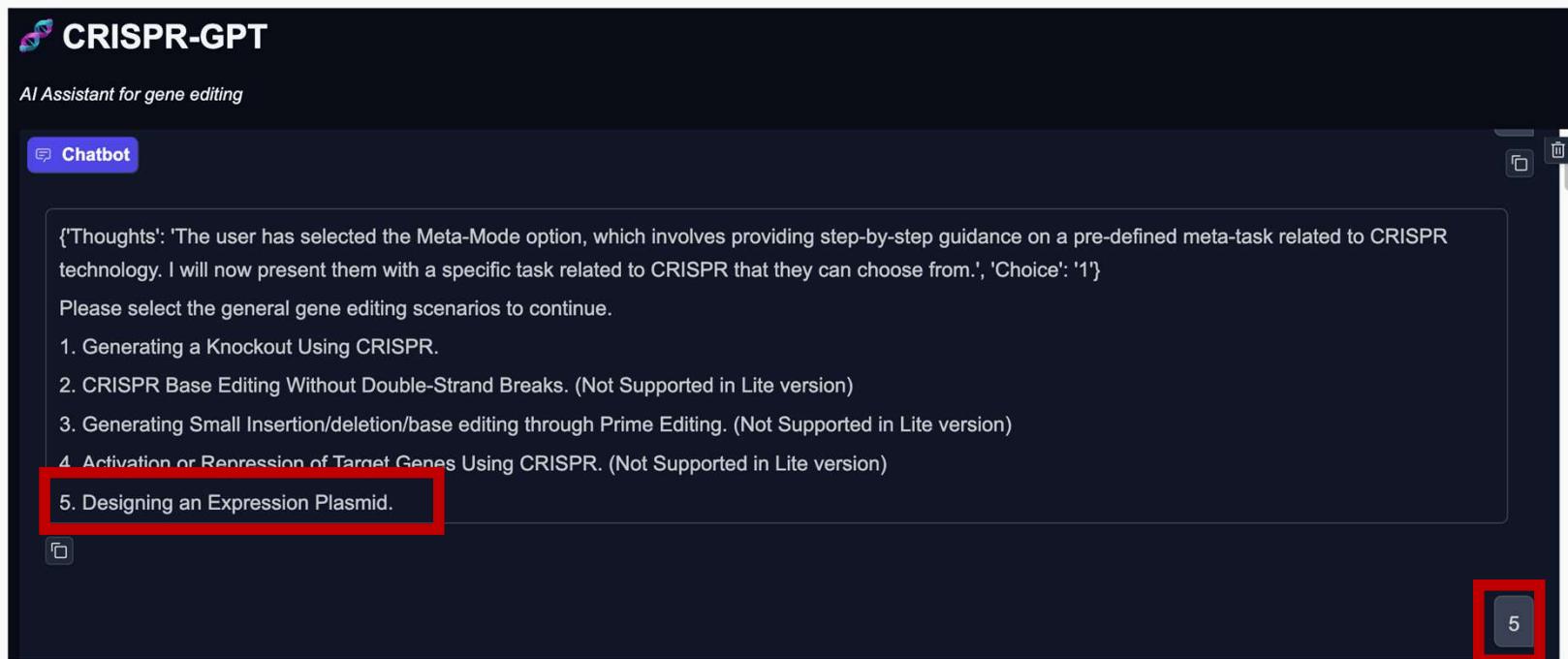
# Meta-Mode Test 2

pcDNA3.1(+) (Provide name and details) and eGFP(provide raw sequence)

# Enter Meta Mode



# Design an Expression Plasmid



# Choose to provide the plasmid name/details

The screenshot shows a dark-themed AI assistant interface. At the top, it says "CRISPR-GPT" with a small logo and "AI Assistant for gene editing". Below that is a "Chatbot" button. The main text area contains the following content:

{'Thoughts': 'The user has chosen option 5, which involves designing an expression plasmid. This task typically involves selecting a suitable vector, choosing the right promoter, introducing a gene of interest, and ensuring proper regulatory elements for successful gene expression in the desired organism.', 'Choice': '5'}

Now, let's start designing your expression plasmid construct. We will guide you through a step by step process as listed below:

1. Selecting an expression plasmid backbone.
2. Designing the gene insert.
3. Selecting output format for your construct.

Which expression plasmid backbone would you like to use?

1. pcDNA3.1(+)
2. pAG
3. I have my own plasmid backbone
- 4. I know the plasmid name/details**

Please select 1, 2, 3, or 4.

In the bottom right corner of the main window, there is a small red square containing the number "4".

# Provide name and some details

The screenshot shows a dark-themed interface for the CRISPR-GPT AI Assistant. At the top, it says "CRISPR-GPT" and "AI Assistant for gene editing". A "Chatbot" button is visible. The main text area contains the following conversation:

{'Thoughts': 'The user selected option 4, which indicates they have chosen their plasmid by name or reference. However, the user did not provide specific details about the plasmid name or reference.', 'BackboneName': "User's plasmid by name or reference", 'CustomDetails': 'Details not provided', 'Status': 'needs\_details'} Final Result user's plasmid by name or reference

You indicated you want to use a custom plasmid backbone.

Please provide one of the following:

Option A: Plasmid Sequence

- Paste the complete plasmid sequence in FASTA or GenBank format
- Example: >plasmid\_name or LOCUS plasmid\_name...

Option B: Plasmid Details

- Plasmid name (e.g., "pEGFP-N1", "pUC19")
- Key features: promoter type, selection marker(s), origin of replication, approximate size
- Example: "My plasmid has CMV promoter, Ampicillin resistance, pBR322 origin, ~6 kb"

Please provide as much detail as possible.

A red box highlights the "Option B: Plasmid Details" section. A callout box with a white background and black border contains the text: "Unfortunately, Option B did not work no matter how much detail (including the catalog number or the URL)". Another red box highlights the input field where the user typed "pcDNA3.1(+), CMV promoter, Kan and Amp r, 5.4kb".

# After error, provide sequence

We weren't able to extract a plasmid sequence from your input.

To use a custom backbone, please provide:

1. The plasmid name/identifier
2. The actual DNA sequence (in FASTA or raw ACGT format)

You can also try:

- Providing the sequence from a GenBank file
- Pasting the sequence from a plasmid repository
- Going back to select a standard backbone (pcDNA3.1(+) or pAG)

Please try again with the sequence included.

```
GACGGATGGGAGATCTCCGATCCCCATGGTCACTCTCAGTACAATCTGCTCTGATGCCGATAGTTAGCCAGTATCTGCTCCCTGCTGTGTGG  
AGGTCGCTGAGTAGTGCGCGAGCAAATTAAAGCTACAACAAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCCTTGCCTG  
CTTCGCGATGTACGGGCCAGATATAACGCGTTGACATTGATTATTGACTAGTTATTAAAGTAATCAATTACGGGGTCATTAGTCATAGCCCATAATGGAGTC  
CGCGTTACATAACTTACGGTAAATGGCCCGCTGGTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATA  
GGGACTTTCCATTGACGTCAATGGGTGGAGTATTACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACGTC  
AATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGACTTCCATTGGCAGTACATCACGTATTAGTCATCGCTTACCATGGT  
GATGCGGTTTGGCAGTACATCAATGGCGTGGATAGCGGTTGACTCACGGGGATTCCAAGTCTCCACCCATTGACGTCAATGGAGTTGTTGGC  
ACCAAAATCAACGGGACTTCCAAATGTCGTAACAACCTCCGCCCCATTGACGCAAATGGCGGTAGGCGTGTACGGTGGAGGTATATAAGCAGAGCT  
CTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAAACCGCTCACTATAGGGAGACCAAGCTGGCTAGCGTTAAACTTAAGCTTGGTAC
```

# Provide Insert Sequence

The screenshot shows the CRISPR-GPT AI Assistant for gene editing chatbot. The interface includes a logo, the text "CRISPR-GPT", and "AI Assistant for gene editing". A "Chatbot" button is visible. The main text area displays the following information:

Custom Backbone: pcDNA3.1(+)  
Sequence length: 5589 bp | Promoter: CMV (Cyomegalovirus) | Selection marker: Neomycin (G418 resistance) | Origin: SV40 origin, f1 origin  
We can help you design your gene insert for expression plasmids.

Do you already have the exact DNA sequence for the gene you want to express?  
If YES: Please provide the sequence (in FASTA or raw format)  
If NO: Please tell us the gene name or protein you want to express (e.g., "EGFP", "human TP53"), and we can look up and extract the sequence for you.

A large gray box contains a long DNA sequence:

```
ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCACGTAAACGCCACAAGTCAGCGTGTCCGGCGA  
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCCTGAAGTTCATCTGCACCACCGCAAGCTGCCGTGCCCTGCCCAACCGTACGTCCAGGAGCGCACCA  
CCTACGGCGTGCAGTGCTTCAGCCGCTACCCGACCACATGAAGCAGCACGACTTCAAGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGCACCA  
TCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCGAGGTGAAGTTCGAGGGCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCA  
AGGAGGACGGCAACATCCTGGGGACAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATATCATGGCCACAAGCAGAAGAACGGCATCAAGGTGAA  
CTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTGCCGACCACTACCAGCAGAACACCCCCATCGCGACGGCCCCGTGCTGCCCG  
ACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCAACGAGAAGCGCGATCACATGGTCTGGAGTCGTGACCGCCCGGGATCA  
CTCTGGCATGGACGAGCTGTACAAGTAA
```

# Proceed with design

The screenshot shows a dark-themed AI interface for CRISPR-GPT. At the top left is the CRISPR-GPT logo with a DNA helix icon. Below it is the text "AI Assistant for gene editing". A blue button labeled "Chatbot" is visible. The main area contains a conversation log:

Gene: EGFP  
Sequence provided: ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGTGGTGCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGC... (length: 717)  
Great! We have your target gene. Here's a summary of your construct:  
1. Target Gene: {gene\_name}  
2. Plasmid Backbone: {backbone\_name}

A red rectangular box highlights the message: "Would you like to proceed with this construct, or would you like to make any modifications?". To the right of this message are two input fields: one with a red border containing the letter "y" and another with a red border containing a small square icon.

# Choose output format

The screenshot shows the CRISPR-GPT AI Assistant interface. At the top, it says "CRISPR-GPT" and "AI Assistant for gene editing". Below that, there's a "Chatbot" button and a "yes" button with a checkmark. In the main area, the text says: "Excellent! Your expression construct design is complete. We are ready to provide you with the final sequence for ordering." It then asks, "In what format would you like to receive your plasmid sequence for ordering?" followed by a list of three options:

1. GenBank format (.gb) - Complete annotation with features and metadata
2. FASTA format (.fasta) - Sequence header and nucleotide sequence
3. Raw sequence string - Nucleotide sequence only

Option 3 is highlighted with a red box. Below the list, a note says: "Please select your preferred format (1, 2, or 3). You can use this sequence file to submit to your DNA synthesis vendor (e.g., GenScript, IDT, Synthego, etc.)." There is also a small "no" button with a square icon. In the bottom right corner of the main area, the number "3" is displayed inside a red box.

# Gather construct, and begin validation

The screenshot shows two views of the CRISPR-GPT interface. The left view shows a message from the Chatbot: "Your construct sequence is ready:" followed by the "CONSTRUCT SEQUENCE". The right view shows the full "Design Summary" which includes the construct sequence, a redacted section, and a "Design Summary" table.

**AI Assistant for gene editing**

Your construct sequence is ready:

CONSTRUCT SEQUENCE:

```
GACGGATCGGGAGATCCCGATCCCTATGGTGCAGTCAGTACAATCTGC  
AGGTGCGTAGTAGTGCGCGAGCAAATTAAAGCTACAACAGGAAGGCTT  
CTTCGGATGTACGGGCCAGATAACCGGTTGACATTGATTATTGACTAGTTT  
CGCGTTACATAACTTACGGTAATGGCCCGCCTGGCTGACCGGCCAACGACCC  
GGGACTTTCCATTGACGTCATGGGTGGAGTATTACGGTAAACTGCCACTT  
AATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGAC  
GATGCGGTTTGGCAGTACATCAATGGCGTGGATAGCGGTTGACTCACGGG  
ACCAAAATCAACGGGACTTCCAAATGCTGAACAACCTCCGCCCCATTGACGC  
CTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAAACGACTC
```

**AI Assistant for gene editing**

Chatbot CCCCATGGTGCAGAAGTAAGTTGGCCGAGTGTTCAGTCAGTCAGTAAGATGCTTCTGTGACTGGTGA  
CTGCATAATTCTCTACTGTCATGCCATCCGTAAGATGCTTCTGTGACTGGTGA  
CTGCAGTCAACCAAGTCATTCTGAGAATAGTGTATGCCGACCGAG  
TTGCTCTGGCCGGCGTCAACAGGGATAATACCGGCCACATAGCAGAAC  
GATCTACCGCTTGGAGATCCAGTCGATGTAACCCACTCGTCACCCACT  
GATCTACCGCTTGGAGATCCAGTCGATGTAACCCACTCGTCACCCACT  
AGGAAGGCAAATGCCAAAAAGGGAATAAGGGCACACGGAATGTTGA  
ACTCATACTCTCCCTTTCAATATTGAAGCATTATCAGGGTATT  
GTCTCATMGAACCACATCGCTCGTATGTTGTGGAATTGAGCGGATAACAA

Design Summary:

- Gene: eGFP
- Plasmid Backbone: pcDNA3.1(+)
- Total Size: 6109 bp
- Insertion Method: mcs (at position 928)
- Output Format: RAW\_SEQUENCE

This sequence is ready for synthesis and expression testing.  
Your expression construct is complete and ready for synthesis!

Would you like to:

- Download/save your construct design
- Modify any aspect of your design and reorder
- Start a new plasmid design project

# Rubric for Test 2

A	B	C	D	E	F	G	H
Critical Failure Present?	YES FAIL 78%						
Section	Category	Checkpoint	Severity	Points	Pass (Y/N)	Score Earned	Notes
Input Validation	Backbone	Did the user provide a backbone sequence? If not, did the user select a predefined plasmid or indicate the backbone sequence for the program to pull?	Info	0	n	0	
Input Validation	Backbone	Correct backbone sequence retrieved (if selecting from library)? Leave blank if not applicable.	Critical	2	y	0	
Input Validation	Backbone	Correct backbone sequence retrieved (if the user provided the URL)? Leave blank if not applicable.	Critical	2		0	
Input Validation	Backbone	Correct backbone sequence retrieved (if the user provided the name)? Leave blank if not applicable.	Critical	2	n	2	
Input Validation	Backbone	Backbone length correct (for example, the program in the chat will pull the sequence length, is this length that is output correct?)	Minor	0.5	n	0.5	After being able to just provide the Backbone name and retrieve the sequence, the sequence was manually provided. Size is off and the sequence contained the error at this point: "BackboneName": "pcDNA3.1(+)", "SequenceLength": "5589 bp", "Promoter": "CMV (Cytomegalovirus)", "SelectionMarker": "Neomycin (G418 resistance)", "Origin": "SV40 origin, f1 origin", "SequenceProvided": "yes",

# Graduated Testing Options

# Testing progression

- Meta Mode
  - Backbone selection
    - Select backbone sequence from library
    - Provide sequence directly
    - Provide backbone sequence URL
    - Provide backbone sequence name
    - Provide general direction (“expression in mammalian cells”)
  - Insert Selection
    - Provide sequence and name directly
    - Provide URL to sequence
    - Provide gene name
- Auto Mode (Same as above but in this mode)

# Plasmid Designs the tool should be able to make

- [Simple FP expression vectors](#)

13033	<a href="#">pcDNA3-YFP</a>	Mammalian expression vector for cloning your gene fused to YFP
13032	<a href="#">pcDNA3-mRFP</a>	Mammalian expression vector for cloning your gene fused to mRFP
13031	<a href="#">pcDNA3-EGFP</a>	Mammalian expression vector for cloning your gene fused to EGFP
13030	<a href="#">pcDNA3-CFP</a>	Mammalian expression vector for cloning your gene fused to CFP

- [Inducible GFP Expression](#): A more complex backbone
- [pCMV Cre](#)

# Additional Considerations

- Empty backbones may be hard to find. In the case an empty backbone cannot be found the designer would need to pull a version with an insert present, identify the insert and remove it before proceeding.

# Links

[Link to Rubric Document](#)

[Link to Rubric Excel](#)

[Adapted CRISPR-GPT Repo](#)

# THANK YOU

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[alleninstitute.org](http://alleninstitute.org)

