Agentic AI guided RNA editing with CRISPR-Cas13

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Outline

- Background
  - CRISPR-Cas13
  - Agentic AI

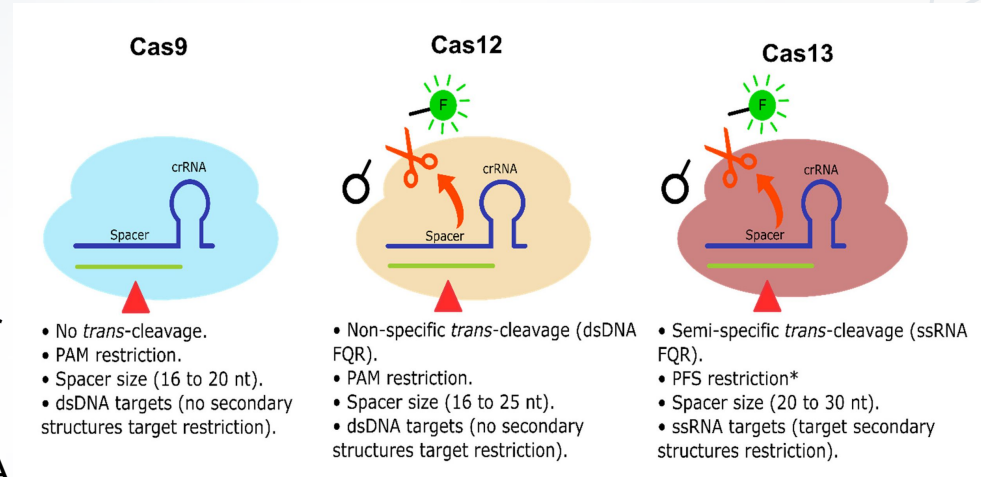- Research Question and Objectives

- Experiment

- Results

- Conclusion

Background - CRISPR-Cas13

CRISPR: is a technology that is used to edit DNA. There are different systems, such as Cas9, Cas12a and Cas13 [1, 2].

Cas9 vs Cas13: Cas13 targets RNA, allowing researchers to edit gene expression without altering the genome. Also, proto-adjacent motif (PAM) is not required, which makes editing a bit easier [3].

Clinical validation: HG202 received FDA clearance in November 2024 - first Cas13 therapy approved for human trials [4].

Cas9
- No *trans*-cleavage.
- PAM restriction.
- Spacer size (16 to 20 nt).
- dsDNA targets (no secondary structures target restriction).

Cas12
- Non-specific *trans*-cleavage (dsDNA FQR).
- PAM restriction.
- Spacer size (16 to 25 nt).
- dsDNA targets (no secondary structures target restriction).

Cas13
- Semi-specific *trans*-cleavage (ssRNA FQR).
- PFS restriction*
- Spacer size (20 to 30 nt).
- ssRNA targets (target secondary structures restriction).
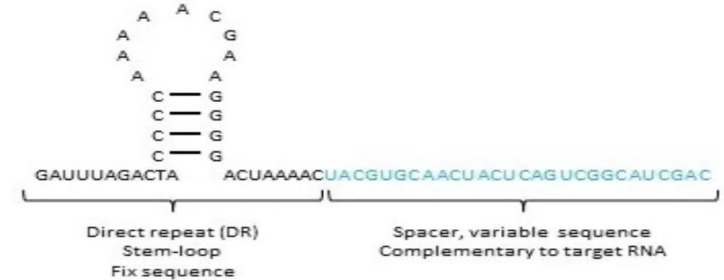
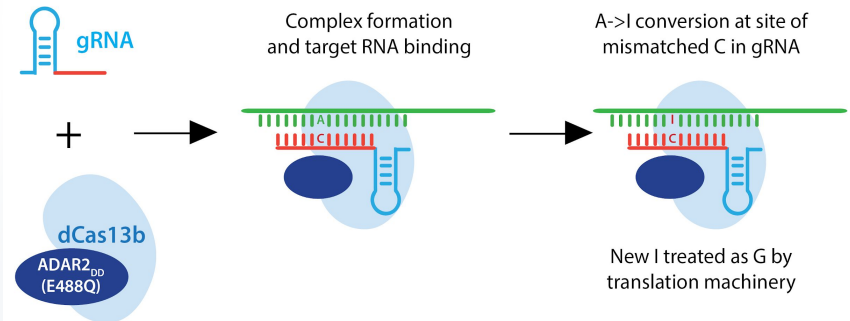(Durán-Vinet et al., 2021)

Background - Cas13 Mechanism

Cas13 has 3 main parts:

- crRNA or Guide RNA [1]

- Cas13 Protein [1,2]

- Deaminase domain[5,7]

LwaCas13a crRNA (gRNA). The DR is the scaffold Cas13 binds, and the spacer sequence guides the Cas protein to its target sequence.


Picture: Bitesizebio.com

Background - Agentic AI

Agentic AI refers to AI systems designed to act autonomously, making decisions and taking actions to achieve specific goals with minimal human intervention [9].
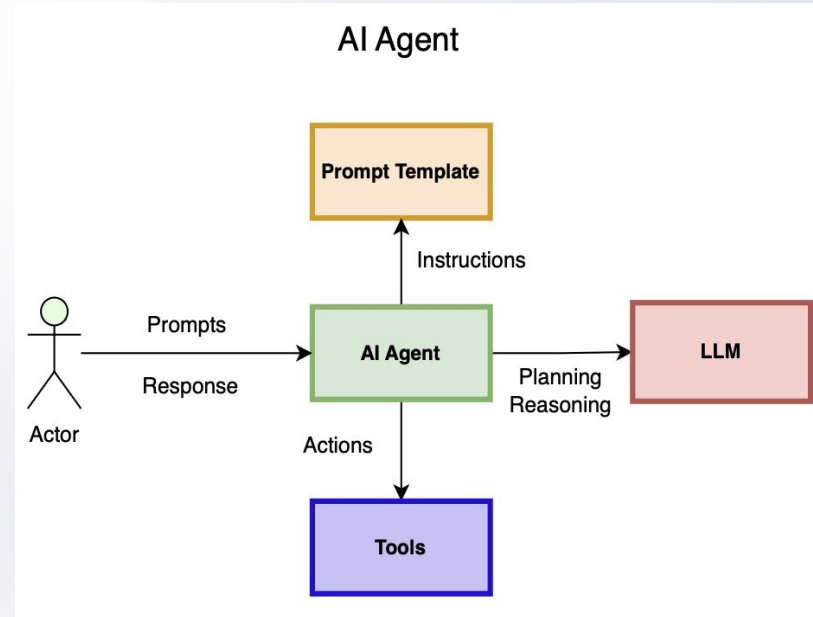
Background - Model Context Protocol Server (MCP)

MCP defines a standard way for applications (including AI models) to discover, access, and utilize contextual information, referred to as "tools", "resources", etc. [10]
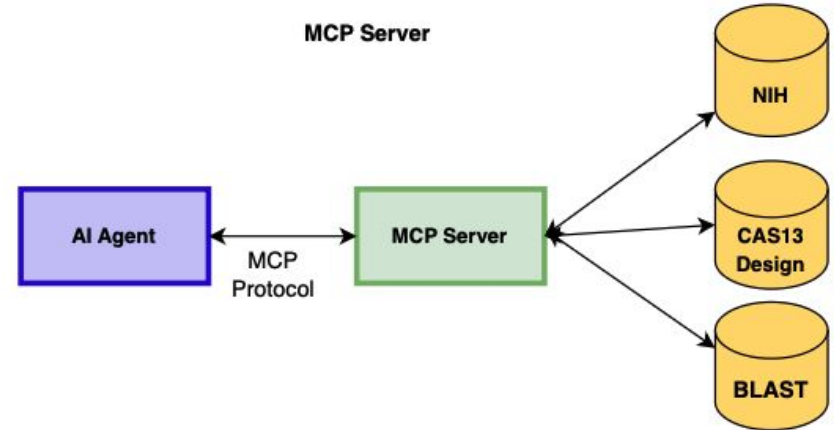
Research Question

How can Agentic AI be used in RNA editing with CRISPR-Cas13 for experiment planning in a semi-autonomous fashion with minimal human intervention?

Research Objectives

- Research and capture end-to-end Cas13 experiment steps

- Identify the steps that Agentic AI can perform autonomously

- Identify data sources that can used in Cas13

- Develop an Agentic AI Prototype and publish code to Github

- Capture results, learnings, conclusion, future considerations

Experiment – High-Level Cas13 Workflow [1]

| Pre-Experimental Planning | Actual Experiment Planning | Post-Experiment Planning |

- Define Objective

- Gather Gene information

- Choose Cas13 variant

- How will the proteins be delivered

- Is the gene expressed in multiple isoforms?

- Design guide RNA (gRNA)

- Off-Target validation

- Plan Experiment control

- Component validation

- Assembling Complexes

- Delivering the System and assess Efficiency

- Determine Measurement metrics

- Measure experiment efficiency

- Evaluate off-target effects

- Assess functional outcomes

- Interpret results

- Plan resources, costs and timeline

- Address safety and compliance

Safety and Compliance

Experiment – High-Level Cas13 Workflow[1]

Pre-Experimental Planning

- Define Objective

- Gather Gene information

- Choose Cas13 variant

- How will the proteins be delivered

- Gene expressed in multiple isoforms?

Information Gathering

- Gene Info Lookup

- Design guide RNA (gRNA)

- Off-Target validation

Autonomous Tasks

Gene info - NIH Nucleotide

gRNAs Design - CAS13 Design

Off-Target Validation Databases

Experiment – Databases Identified

gRNA Design & Optimization

- NIH Tools (mRNA lookup, Blast)

- Cas13 Design [6,8]

- Arc Institute

- TIGER platform

- DeepCas13

- CHOPCHOP v3

- CaSilico

- GuideMaker

On-Target/Off-Traget Validation(4)

- RNAInter v4.0

- DISCOVER-seq

- REDIportal

- Cancer Cell Line Encyclopedia (CCLE)

- RNAcentral

- Specialized validation services

Experiment – Prototype Tools

- Agent2Agent Protocol/Framework (a2aprotocol.ai)

- Model Context Protocol (modelcontextprotocol.io)

- Google Gemini 2.0-Flash Large Language Model (LLM)

- Databases
  - NIH Gene Lookup
  - gRNA Design - CAS13Design (Includes an offline step)

Experiment – Prototype Tools
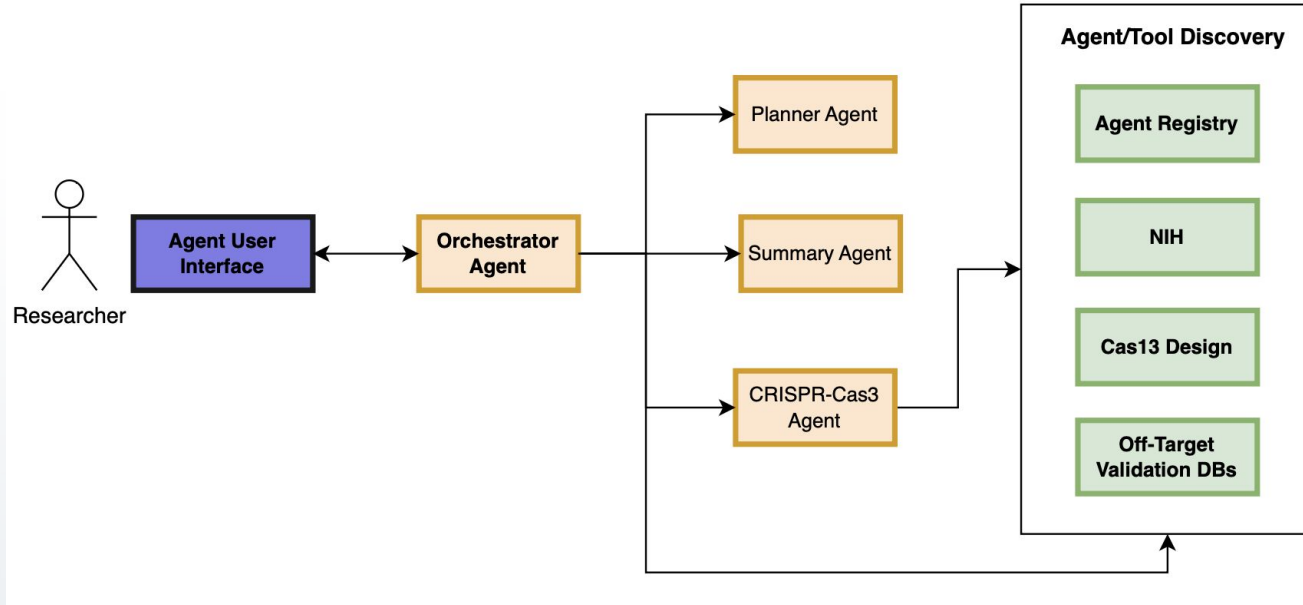
Source Code Repo: https://github.com/PragyanR/agentic_ai_rna_editing

Results

CRISPR-Cas13 AI Agent

http://localhost:10101   Connect

Chat

status-update *Server responded with:*

```
{
    "status": "input_required",
    "question": "What is the purpose of your experiment?"
}
```
✅

status-update *Server responded with:*

What is the purpose of your experiment? ✅

> I want to knockdown a gene.

task Task created with status: submitted ✅

Type a message...   Send

CRISPR-Cas13 AI Agent

http://localhost:10101   Connect

Chat

```
    "status": "input_required",
    "question": "What is the name of the gene you want to knockdown?"
}
```
✅

status-update *Server responded with:*

```
{
    "status": "input_required",
    "question": "What is the name of the gene you want to knockdown?"
}
```
✅

status-update *Server responded with:*

What is the name of the gene you want to knockdown? ✅

> Serpina1

Type a message...   Send

Results

CRISPR-Cas13 AI Agent

http://localhost:10101   [Connect]

Chat

`status-update`   *Server responded with:*

```
{
    "original_query": "SERPINA1, Cas13a",
    "experiment_info": {
        "gene_name": "SERPINA1",
        "cas_variant": "Cas13a"
    },
    "tasks": [
        {
            "id": 1,
            "description": "Lookup Gene information for SERPINA1",
            "status": "pending"
        },
        {
            "id": 2,
            "description": "Design guide RNA for SERPINA1 using Cas13a",
            "status": "pending"
        },
        {
            "id": 3,
            "description": "Validate guide RNA design for potential off-target effects",
```
✅

Type a message...   [Send]

CRISPR-Cas13 AI Agent

http://localhost:10101   [Connect]

Chat

`artifact-update`

CRISPR-Cas13 Pre-Experiment Plan

Autonomous Tasks Exectued

1. Lookup Gene information for SERPINA1
2. Design guide RNA for SERPINA1 using Cas13a
3. Validate guide RNA design for potential off-target effects

Gene Overview

- **Gene Name:** SERPINA1
- **Lastest Accession Number:** NM_000295.5
- **All Accession Numbers:** NM_000295.5, NM_001002235.3, NM_001002236.3, NM_001127700.2, NM_001127701.2, NM_001127702.2, NM_001127704.2

Type a message...   [Send]

Results

CRISPR-Cas13 AI Agent

http://localhost:10101   [Connect]

Chat

mRNA
- **Organism:** Homo sapiens
- **Taxonomy:** Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
- **Created Date:** 19-MAR-1999
- **Updated Date:** 29-APR-2025

Messenger RNA (mRNA)

- **Length:** 3006
- **Sequence:**

CTCCTCAGCTTCAGGCACCACCACTGACCTGGGACAGTGAATCGACAATGCCGTCTTCTGTCTCGTGGGGCATC
CTCCTGCTGGCAGGCCTGTGCTGCCTGGTCCCTGTCTCCCTGGCTGAGGATCCCCAGGGAGATGCTGCCCAGA
AGACAGATACATCCCACCATGATCAGGATCACCCAACCTTCAACAAGATCACCCCCAACCTGGCTGAGTTCGC
CTTCAGCCTATACCGCCAGCTGGCACACCAGTCCAACAGCACCAATATCTTCTTCTCCCCAGTGAGCATCGC
TACAGCCTTTGCAATGCTCTCCCTGGGGACCAAGGCTGACACTCACGATGAAATCCTGGAGGGCCTGAATTT

[Type a message...]   [Send]

CRISPR-Cas13 AI Agent

http://localhost:10101   [Connect]

Chat

Guide RNA (Sample)

Guide RNA 1: crRNA807:845-867,GCCTCATAATAAGTGCCATACAG Guide RNA 2: crRNA809:847-869,TAGCCTCATAATAAGTGCCATAC Guide RNA 2: crRNA359:359-381,CTTCAATAGACACATCGGCCACA

Off-Target Validation

- **Status:** Pending

CRISPR-Cas13 Experiment Plan

[Pending]

CRISPR-Cas13 Post-Experiment Plan

[Type a message...]   [Send]

Results

```
PLANNER_COT_INSTRUCTIONS = """
You are an ace CRISPR-Cas13 Pre-Experiment Planner.
You take the user input and create an experiment plan, break the trip in to actionable task.
You will include 3 tasks in your plan, based on the user request.
1. Gene Information Lookup.
2. Design Guide RNA.
3. Validate for Off-Target Effects.

Always use chain-of-thought reasoning before responding to track where you are
in the decision tree and determine the next appropriate question.

Your question should follow the example format below
{
    "status": "input_required",
    "question": "What is the purpose of your experiment?"
}


DECISION TREE:
1. Gene Name
    - If unknown, ask for Gene Name.
    - If known, proceed to step 2.
2. Cas Variant
    - If unknown, ask for Cas Variant.
    - If known, proceed to step 3.


CHAIN-OF-THOUGHT PROCESS:
Before each response, reason through:
1. What information do I already have? [List all known information]
2. What is the next unknown information in the decision tree? [Identify gap]
3. How should I naturally ask for this information? [Formulate question]
4. What context from previous information should I include? [Add context]
5. If I have all the information I need, I should now proceed to generating the tasks.

Your output should follow this example format. DO NOT add any thing else apart from the JSON format below.

{
    'original_query': 'Design a CRISPR-Cas13 Pre-Experiment Plan',
    'experiment_info':
    {
        'gene_name': 'SERPINA1',
        'cas_variant': 'Cas13z'
    },
    'tasks': [
        {
            'id': 1,
            'description': 'Lookup Gene information for SERPINA1',
            'status': 'pending'
        }
    ]
}
```

Test Cases

Count: 25
Valid: 22
**Failed: 3 (Due to long Gene Sequence that causes timeout)**

Gene Name	Correct Questioning/Resoning?	Correct Task Formation?	Shows proper Gene Information?	Note
PCSK9	Y	Y	Y	
Tmc1	Y	Y	Y	
VEGFA	Y	Y	Y	
HTT	Y	Y	Y	
MAPT	Y	Y	Y	
hnRNPU	Y	Y	Y	
CFTR	Y	Y	Y	
TARDBP	Y	Y	Y	
MYH7	Y	Y	N	**Long sequence length > 100M**

Conclusion

- Agentic AI is viable to run select tasks autonomously to improve research efficiency and reduce barrier to entry.

- Access to databases is critical for agents to perform more autonomous tasks. Vast majority of the databases are proprietary and lacks access through MCPs.

- CAS13 design is complex and there is not much information about experiments conducted thus far in the public domain.

- Thorough compliance and safety checks are required at every autonomous step

Future Direction

- Expansion of agentic AI capabilities to plan out all the phases of CRISPR-Cas13 phases.

- Work with government and educational institutions to democratize CRISPR-Cas13 research artifacts, educational modules, databases, etc., to enable research community

- Working towards full automation of experiment steps through the use of robotics and other machinery
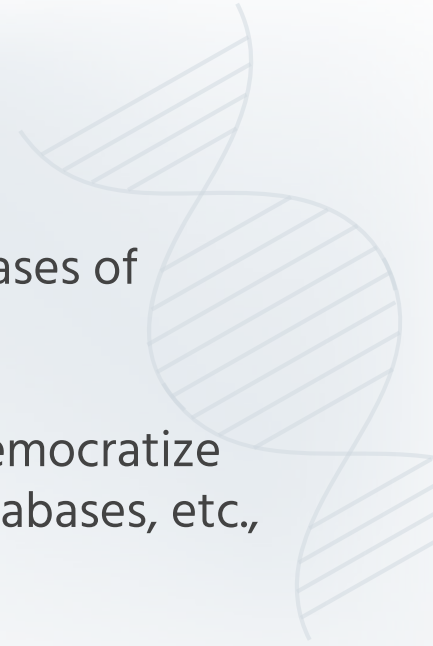
Thank You for listening!

Special Acknowledgements

Dr. Vaithi and Team
Coordinators
Guest Speakers

Source code repo: https://github.com/PragyanR/agentic_ai_rna_editing

References

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