

DesignPR: A primer design tool optimized for *In vivo* Assembly

BIOEN 537
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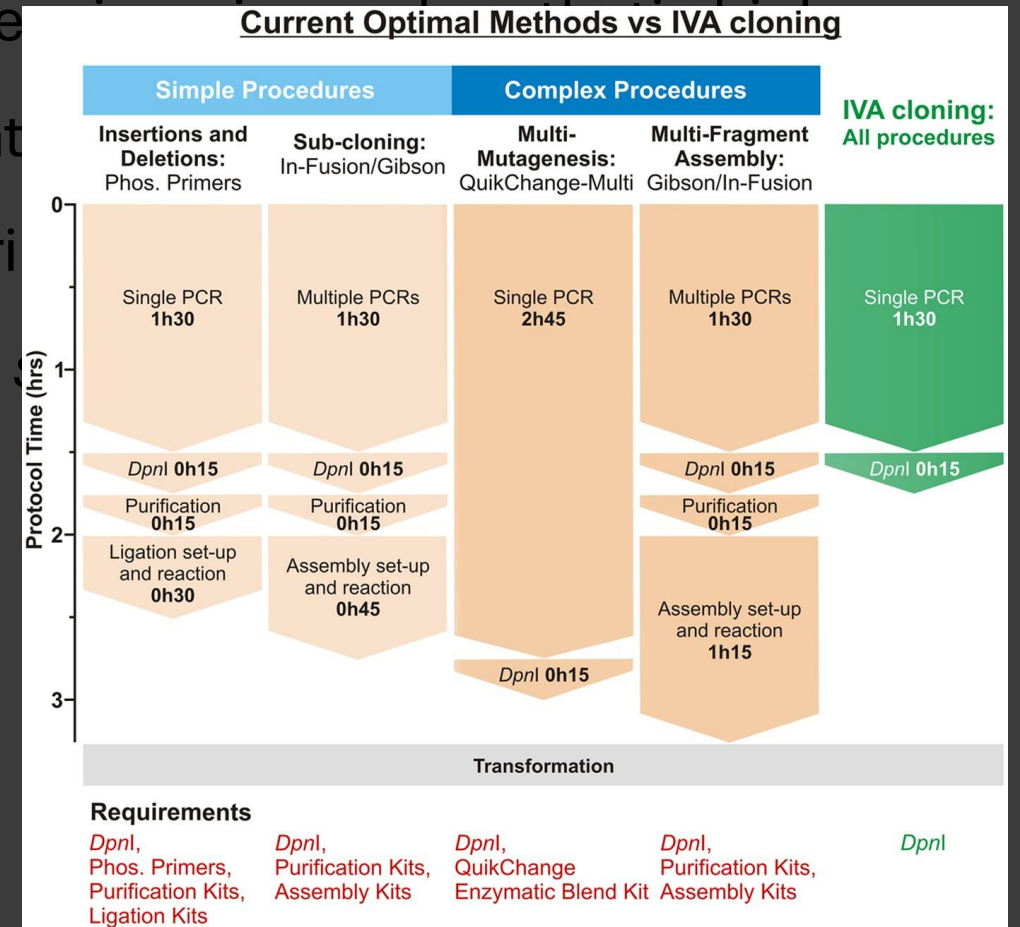


Background: *In vivo* Assembly

- Molecular cloning is fundamental to protein engineering and synthetic biology.
- Cloning involves PCR amplification/modification and directed assembly of DNA fragments, followed by propagation in bacteria cells.
- Traditionally, the amplified DNA fragments go through *in vitro* enzyme-based assembly methods like Gibson and In-Fusion.

Background: *In vivo* Assembly

- Molecular cloning is fundamental to protein engineering
 - Cloning involves PCR amplification/modification of DNA fragments, followed by propagation in bacteria
 - Traditionally, amplified DNA fragments are *in vitro* assembled using methods like Gibson and In-Fusion
- In vivo* Assembly (IVA) takes advantage of host cell machinery to modify and fuse DNA fragments without *in vitro* construction.**



Problem Statement

IVA relies heavily on appropriate primer design for positive-colony formation.

- The primer design requires **combination of many factors**
 - Homologous regions that anneal to the template DNA
 - Overlap regions that guide the assembly of DNA fragments *in vivo*
 - Modifications (insertions, deletions, and point mutations)
- The designed primers require further optimization for GC content, length and annealing temperatures.

DesignPR is a user-friendly application for primer design with parameter optimization, enabling high-throughput primer design.

Use Cases

Information from users

Primer Purpose Insertion, Deletion,
Point Mutation or 2-Fragment Assembly

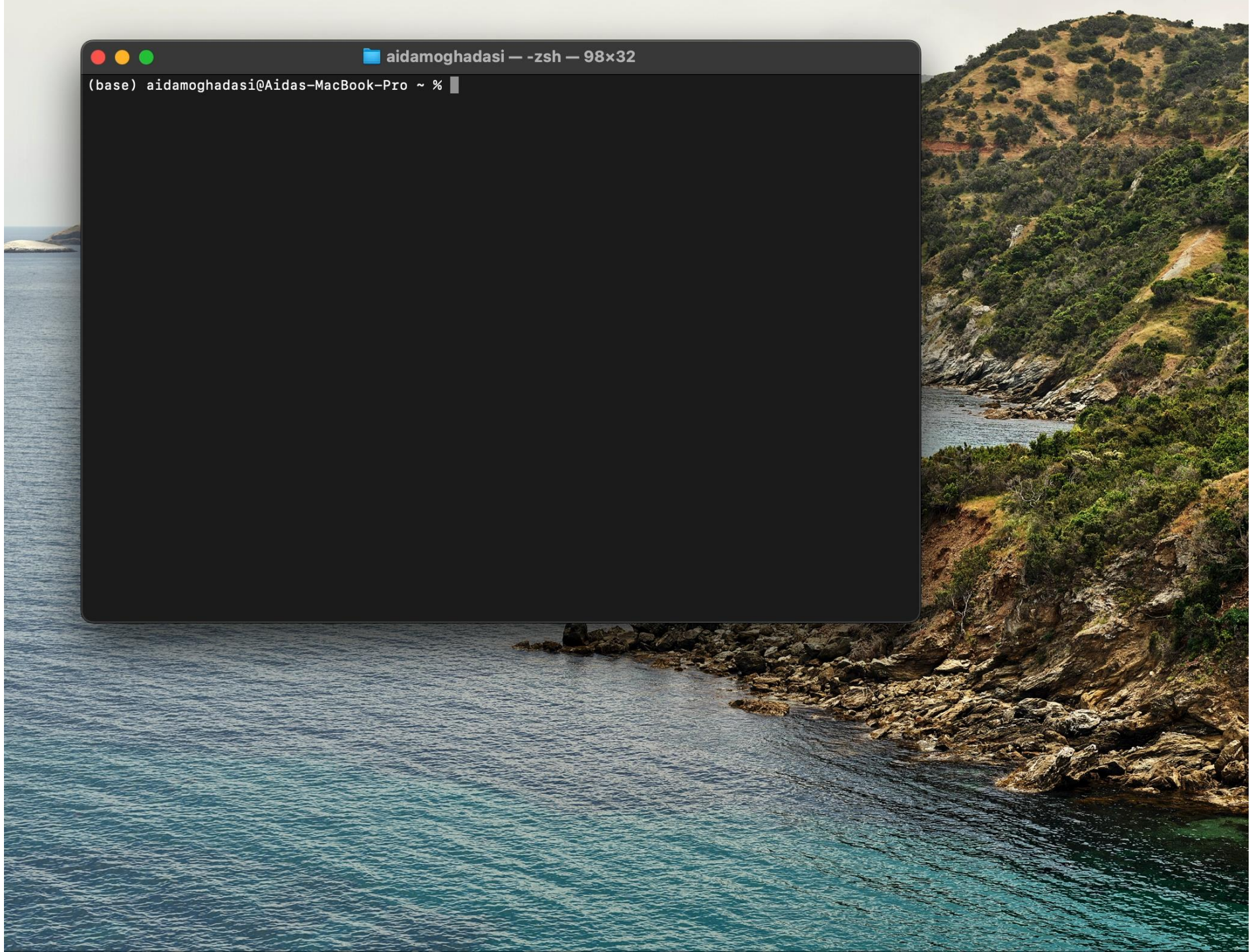
Template DNA sequence and additional
sequences (inserts/fragments)

Outputs from DesignPR

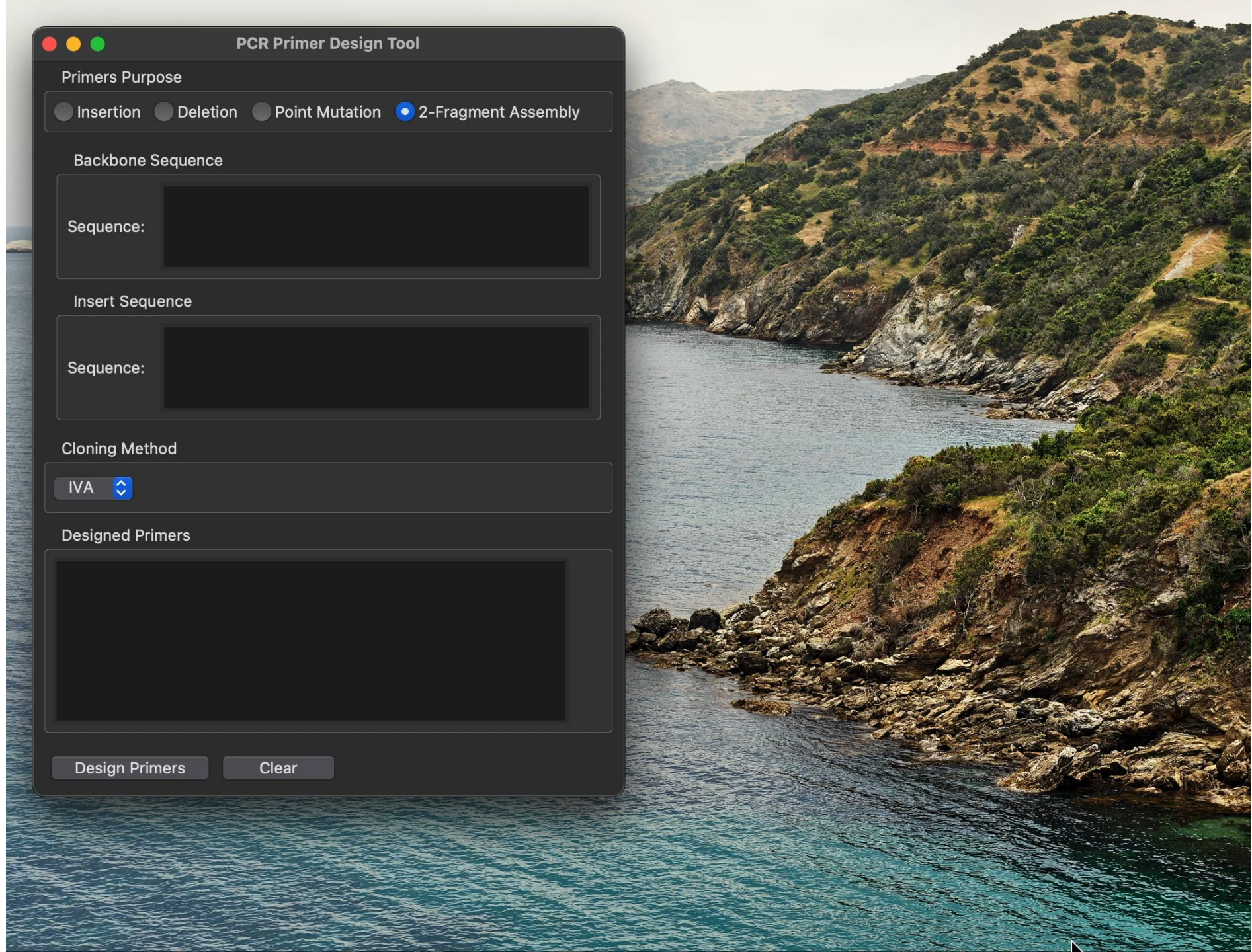
Primer designs sequences (forward and
reverse)

Optimized parameters: GC count,
length and annealing temperatures for
complete primers as well as for
homologous and overlap regions

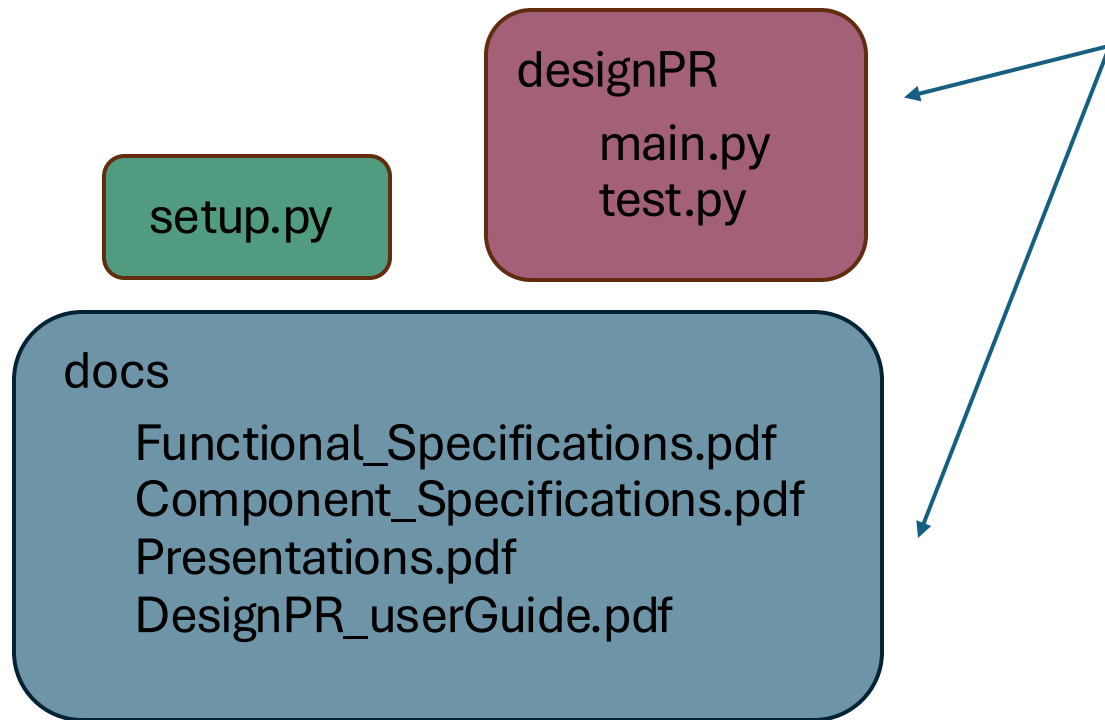
Demo



Demo



GitHub Repo



The screenshot shows the GitHub repository page for **DesignPR**, which is a public repository. The repository has 1 branch (main) and 0 tags. The commit history shows 16 commits, with the latest commit being a deletion of `test/test_designPR.py` by user `aidamo1824`.

File	Commit Message	Time Ago
designPR	Update main.py	1 hour ago
docs	Create Component_Specs.pdf	2 minutes ago
.gitignore	Initial commit	2 months ago
LICENSE	Initial commit	2 months ago
README.md	Update README.md	6 minutes ago
setup.py	Update setup.py	1 hour ago

The repository includes a **README** file and an **MIT license**. The README content is as follows:

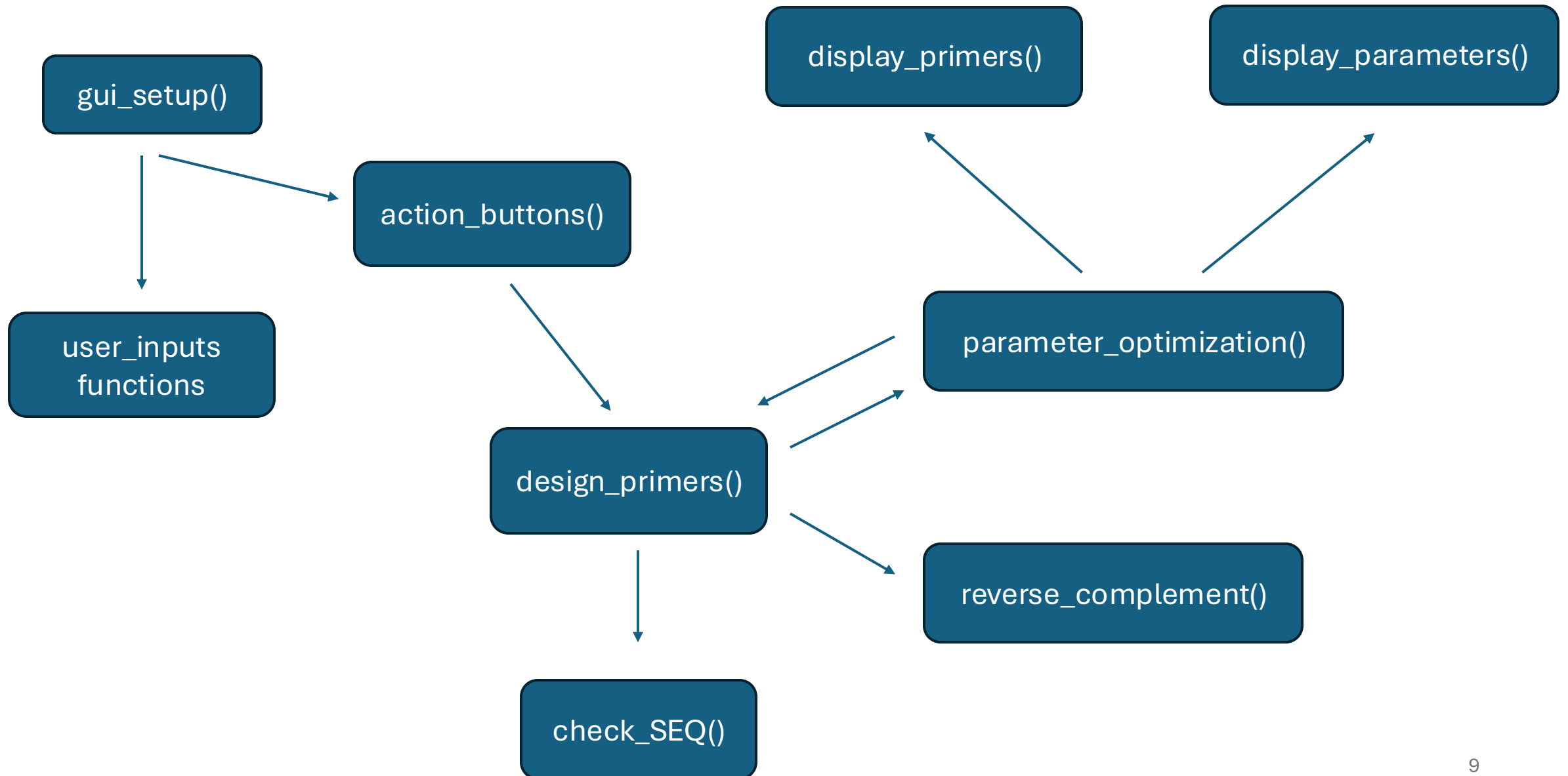
DesignPR

Primer design is a critical step in molecular biology, including PCR (Polymerase Chain Reaction), site-directed mutagenesis, and gene synthesis. It is fundamental to protein engineering. Primers consists of short nucleotide sequences that bind to a DNA template, enabling fragment amplification, small insertions and deletions, and point mutations. The process of primer design takes into consideration many factors such as primer lengths, GC content, and annealing temperatures. There additional components to the primer design for using specific cloning methods.

In vivo Assembly (IVA) is a cloning method that takes advantage of the host cells' own machinery to fuse DNA fragments together and circularize PCR products into plasmids. This is achieved through optimizing primer design to include both template-annealing and fragment-overlap regions that guide the assembly of DNA fragments in vivo.

DesignPR is a user-friendly application for primer design with parameter optimization, enabling high-throughput primer generation and use.

Project Structure



Lessons Learned

- Set up GUI such that it is compatible with pip install
- Update GUI live with action buttons
- Feedback loops to continuously optimize parameters

Future Directions

- Additional parameters to consider partial binding and off-target amplifications
- Add multi-fragment assembly
- Expand primer optimization for other cloning methods like KLD, Gibson, and Golden Gates Assembly