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.

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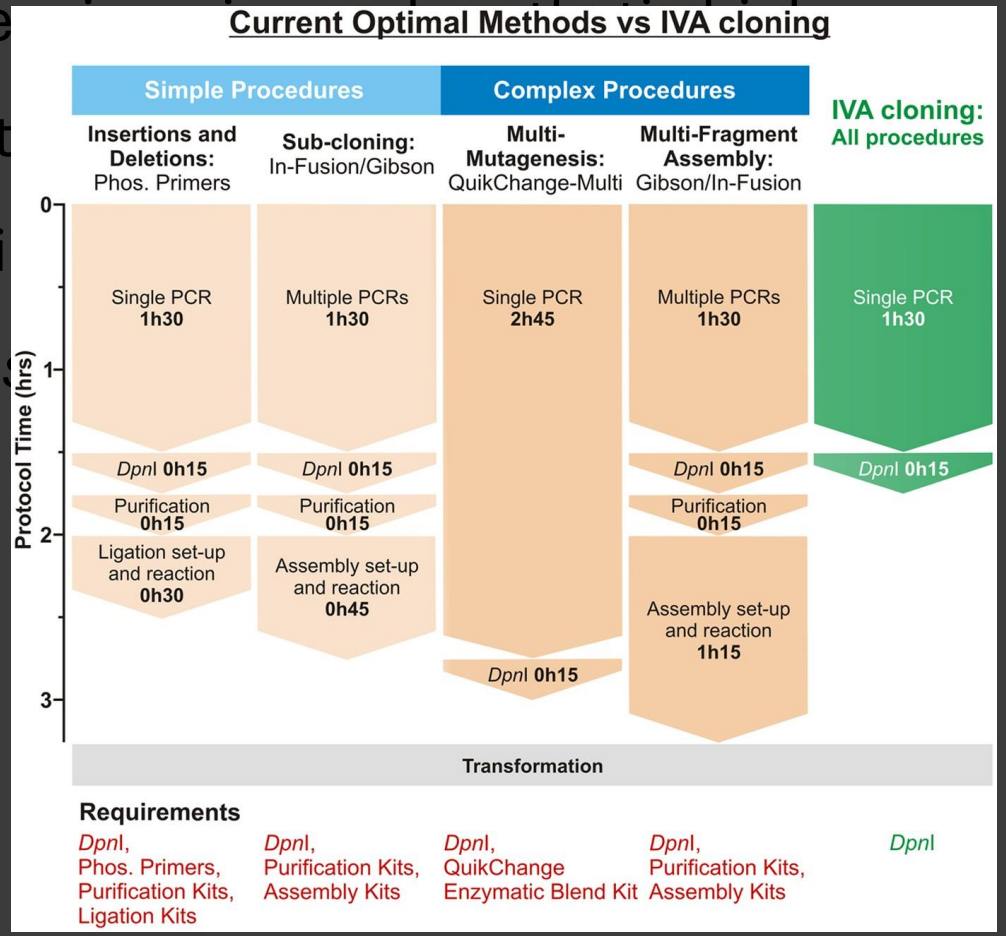
***In vivo* Assembly (IVA) takes advantage of host cell machinery to modify and fuse DNA fragments without *in vitro* construction.**



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

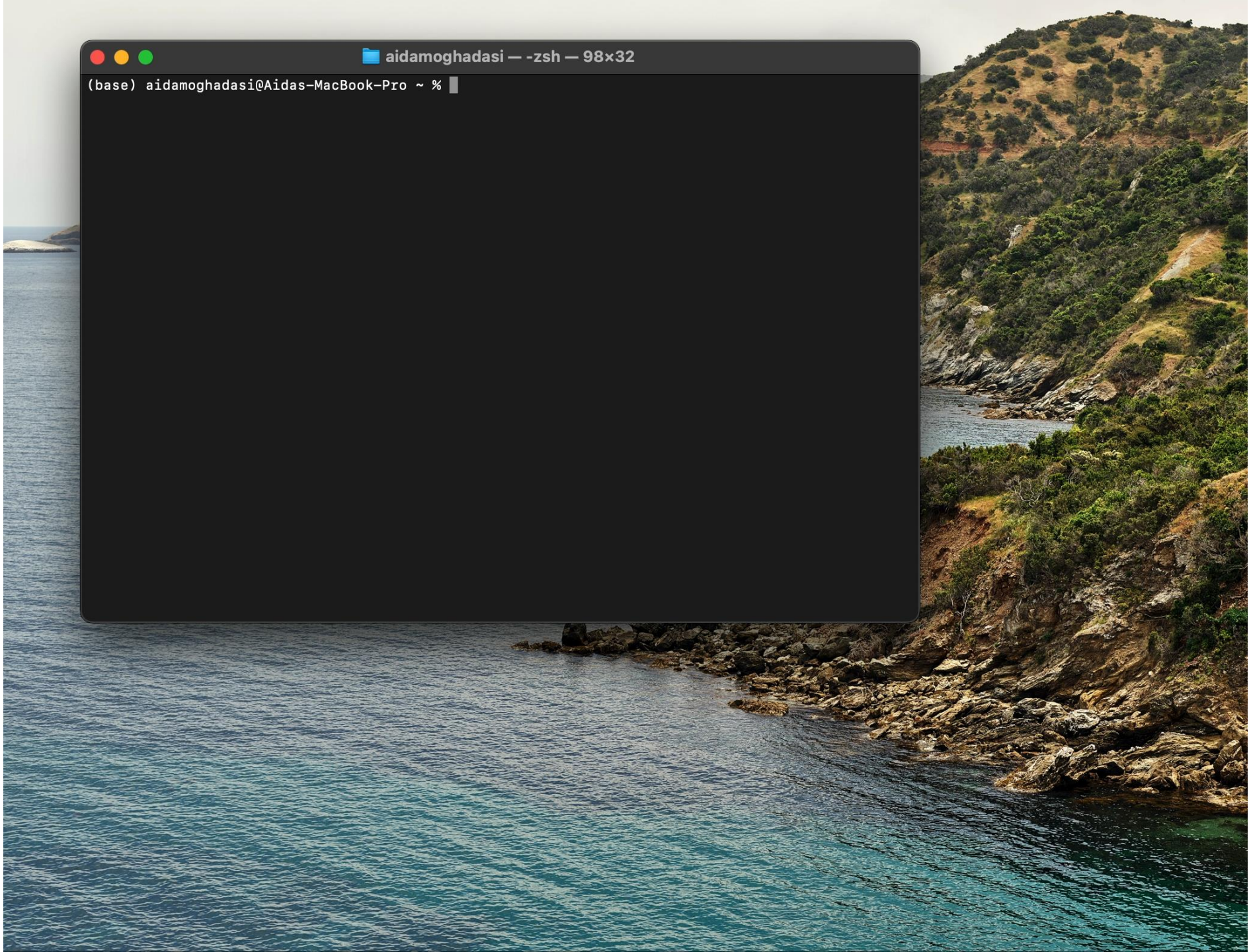
Template DNA sequence and additional sequences (inserts/fragments)

Outputs from DesignPR

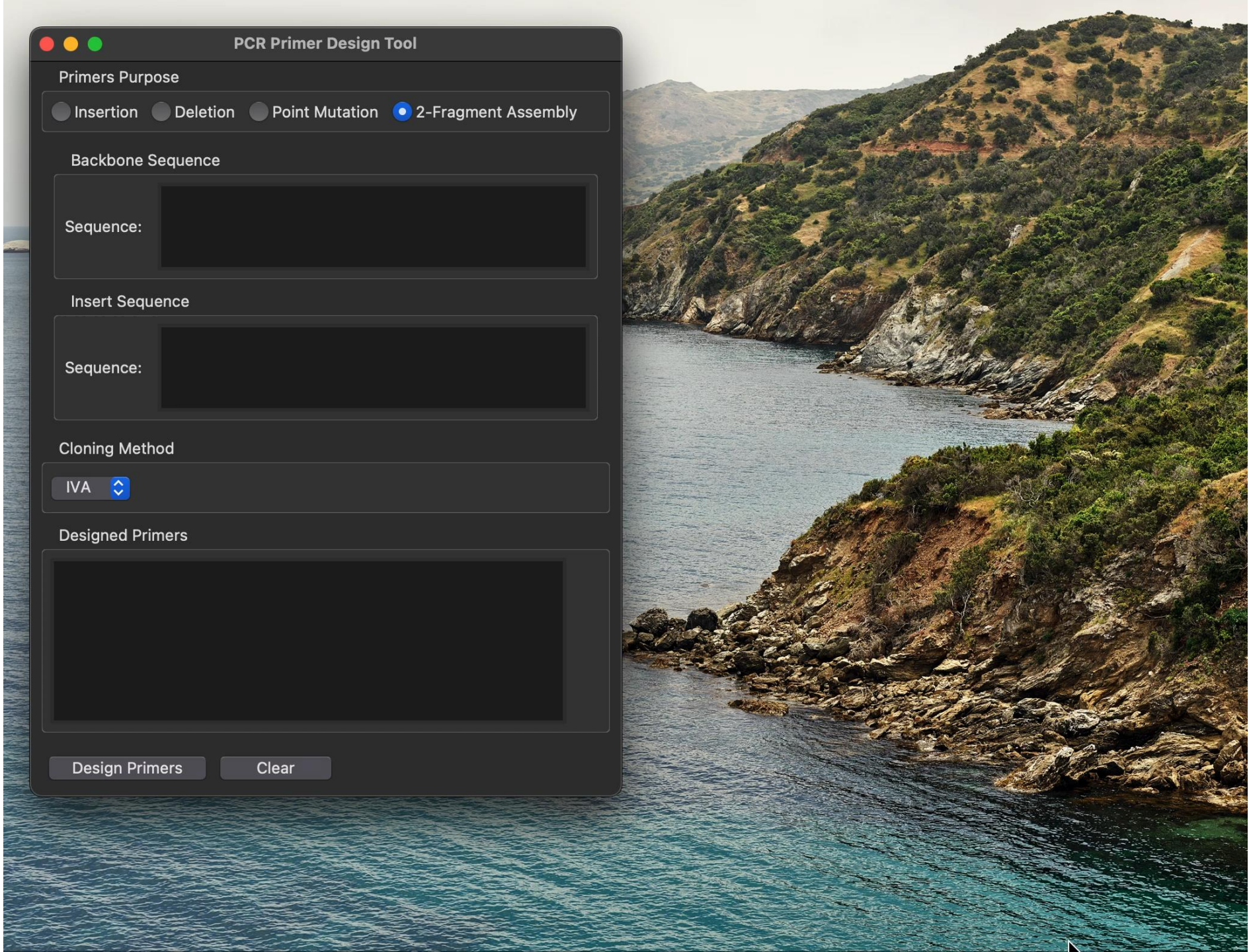
Primer designs sequences (forward and reverse)

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

Demo


aidamoghadasi — -zsh — 98×32
(base) aidamoghadasi@Aidas-MacBook-Pro ~ %

Demo


PCR Primer Design Tool
Primers Purpose
Insertion
Deletion
Point Mutation
2-Fragment Assembly
Backbone Sequence
Sequence:
Insert Sequence
Sequence:
Cloning Method
IVA
Designed Primers
Design Primers
Clear

GitHub Repo

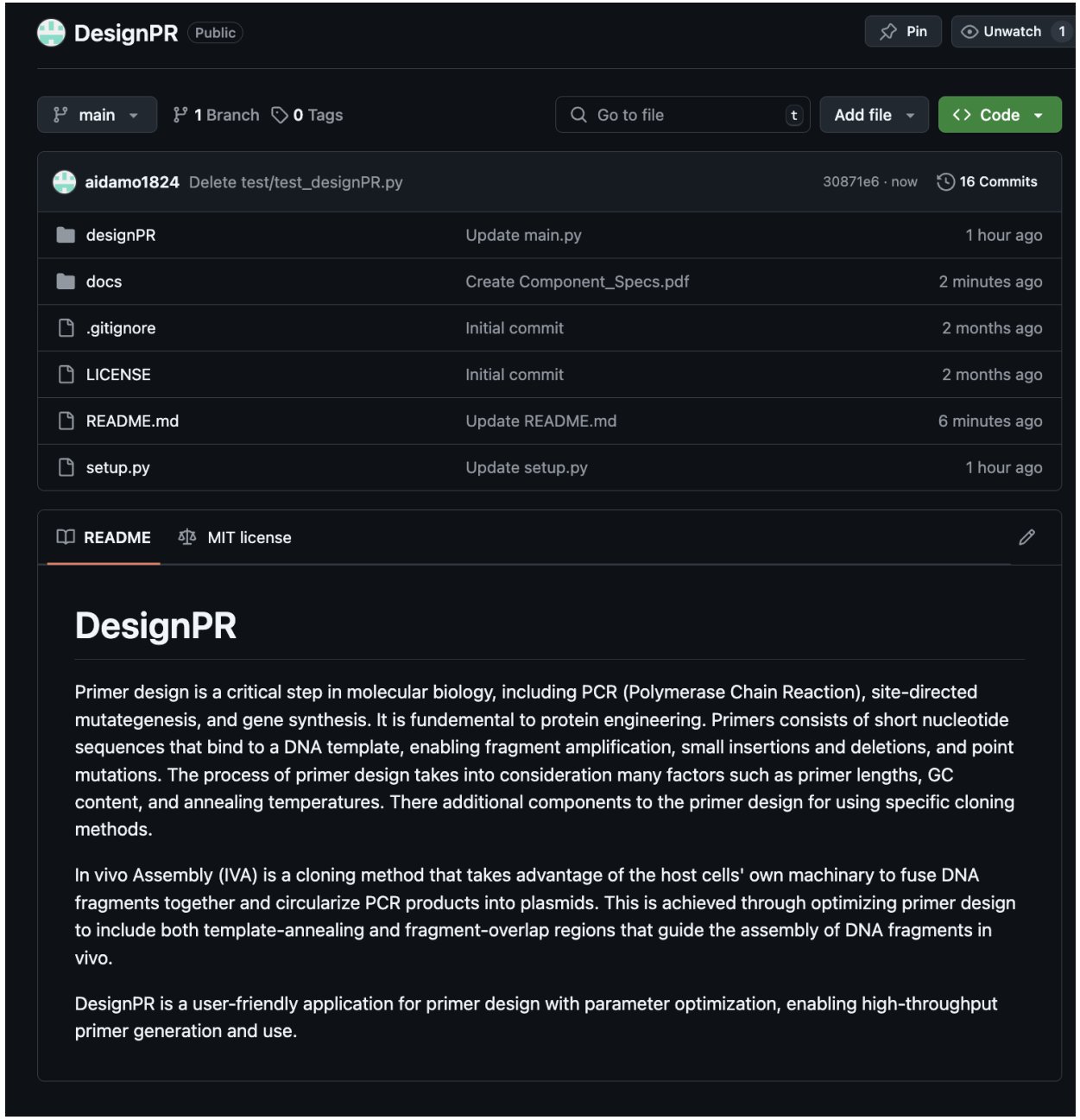
setup.py

designPR

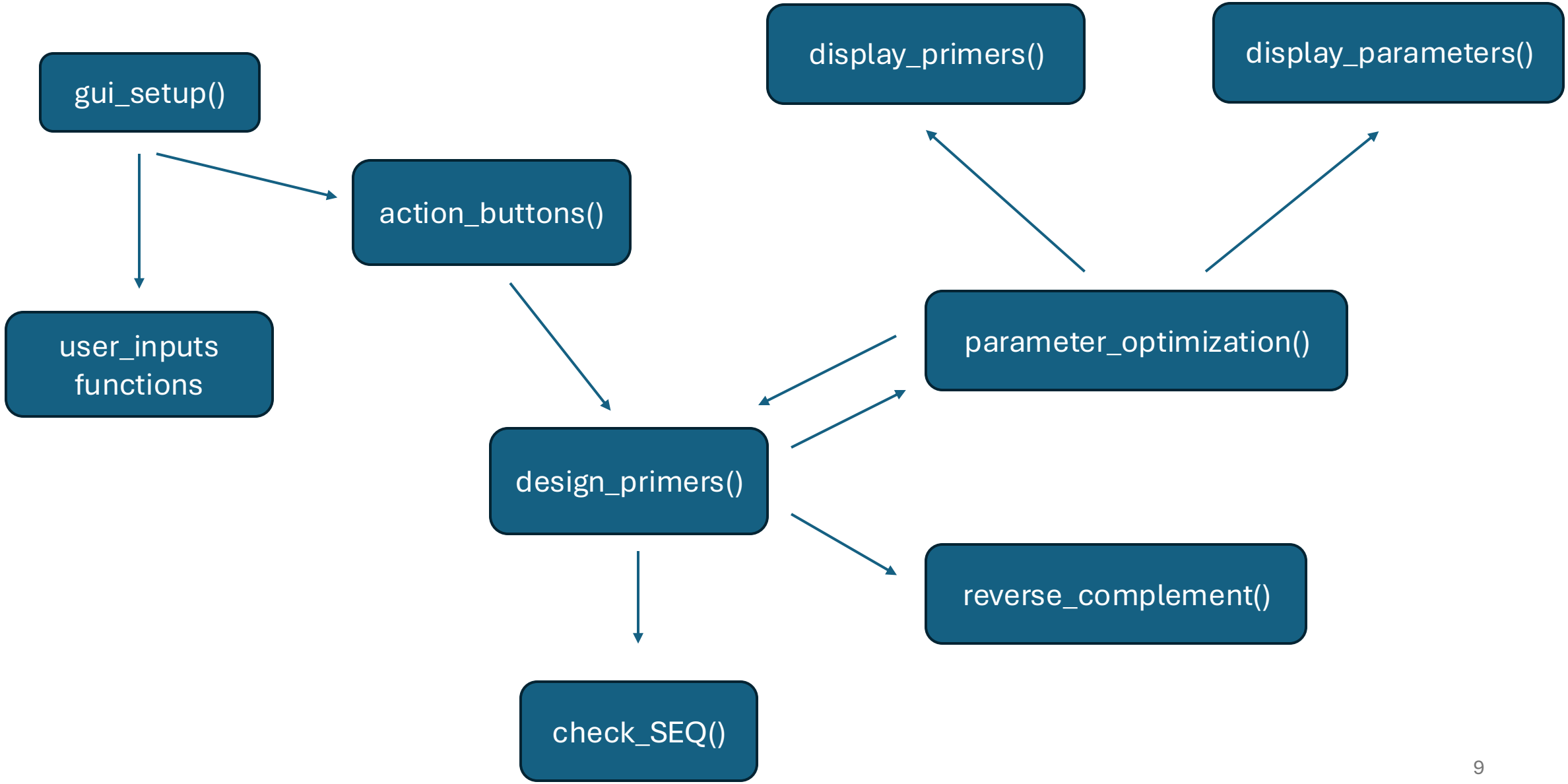
main.py
test.py

docs

Functional_Specifications.pdf
Component_Specifications.pdf
Presentations.pdf
DesignPR_userGuide.pdf



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