Background:

Primer design is critical to PCR, site-directed mutagenesis, and gene synthesis accuracy and efficiency. It is fundamental to protein engineering. Primers consist 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 are 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 python package that aims to provide a user-friendly interface and enable high throughput primer design generation and optimization.

User profile:

Research scientists with an understanding of PCR reactions, site-directed mutagenesis and molecular cloning techniques. No computational skills are necessary to use this GUI and package.

Use cases:

Objective A: Generate primer designs to insert a short nucleotide sequence in a DNA sequence

User-GUI interaction:

- Define primer purpose as “Insertion”
- Input a valid DNA sequence, **starting at the insertion site**
- Input a valid insert sequence (for “insertion” it must be under 15 nucleotides)
- Press “Design Primers”

Expected result: forward and reverse primer design outputs appear in result box of the GUI in addition to optimization parameters (GC content and annealing temperature)

Objective B: Generate primer designs to delete a short nucleotide sequence in a DNA sequence

User-GUI interaction:

- Define primer purpose as “Deletion”

- Input a valid DNA sequence, **starting at the deletion site**
- Input the sequence to be deleted, the delete sequence must be a part of DNA sequence above
- Press “Design Primers”

Expected result: forward and reverse primer design outputs appear in result box of the GUI in addition to optimization parameters (GC content and annealing temperature)

Objective C: Generate primer designs to assemble two DNA fragments

User-GUI interaction:

- Define primer purpose as “2-Fragment Assembly”
- Input a valid backbone sequence, **starting at the assembly site** (this is usually the longer sequence)
- Input a valid insert sequence, must be greater than 15 nucleotides
- Press “Design Primers”

Expected result: forward and reverse primer design outputs appear in result box of the GUI in addition to optimization parameters (GC content and annealing temperature). The designed primers should have two regions where they 1) anneal to the template DNA and 2) overlapping region between the two fragments