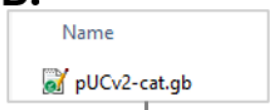
Demonstration of batch-mode cloning design

A.

```
# { ..................................... Example 1a: alanine scanning
GBparser.cloning_design_wrapper_1(
    base_filename='pUCv2-cat',              # template plasmid GB file
    feature_select='cat',                   # gene name
    mutation_codon_no=[42, 45],             # residues to be mutated
    replacement_codon=preferred_codon('A', species='ecoli'),  # mutate to Ala(A)
    plasmid_prefix='plasmid-',
    oligo_prefix='P',
    N_match_cutoff=2, YR_match_cutoff=4, GC_threshold=0,
    verbose=False)
GBparser.update_list('Oligo')
GBparser.update_list('Plasmid')
```

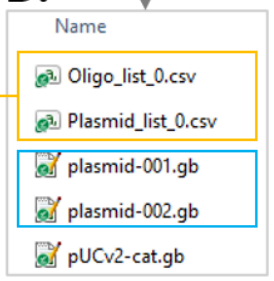
B.



C.

```
================================================================ Overview
---------------------------------------------------------------- plasmid-001
pUCv2-cat_T42A
    Primer pair Template    Product (bp)
    P02, P01    pUCv2-cat   3044
---------------------------------------------------------------- plasmid-002
pUCv2-cat_L45A
    Primer pair Template    Product (bp)
    P04, P03    pUCv2-cat   3044
```

D.



E.

Plasmid_list_0.csv

	A	B
1	Name	Definition
2	plasmid-001	pUCv2-cat_T42A
3	plasmid-002	pUCv2-cat_L45A

Oligo_list_0.csv

	A	B	C	D
1	Name	Length	Bases	Note
2	P01	27	aaggtctcaTATCCAGCTGAACGGTCT	designed on 30-JUL-2026 for construction of plasmid-001
3	P02	43	aaggtctcgGATATTGCAGCCTTTTTAAAGACCGTAAAGAAAA	designed on 30-JUL-2026 for construction of plasmid-001
4	P03	26	aaggtctcaAGGCCGTAATATCCAGC	designed on 30-JUL-2026 for construction of plasmid-002
5	P04	42	aaggtctcgGCCTTTGCAAAGACCGTAAAGAAAAATAAGCAC	designed on 30-JUL-2026 for construction of plasmid-002

Figure 1. Demonstration of alanine scanning.

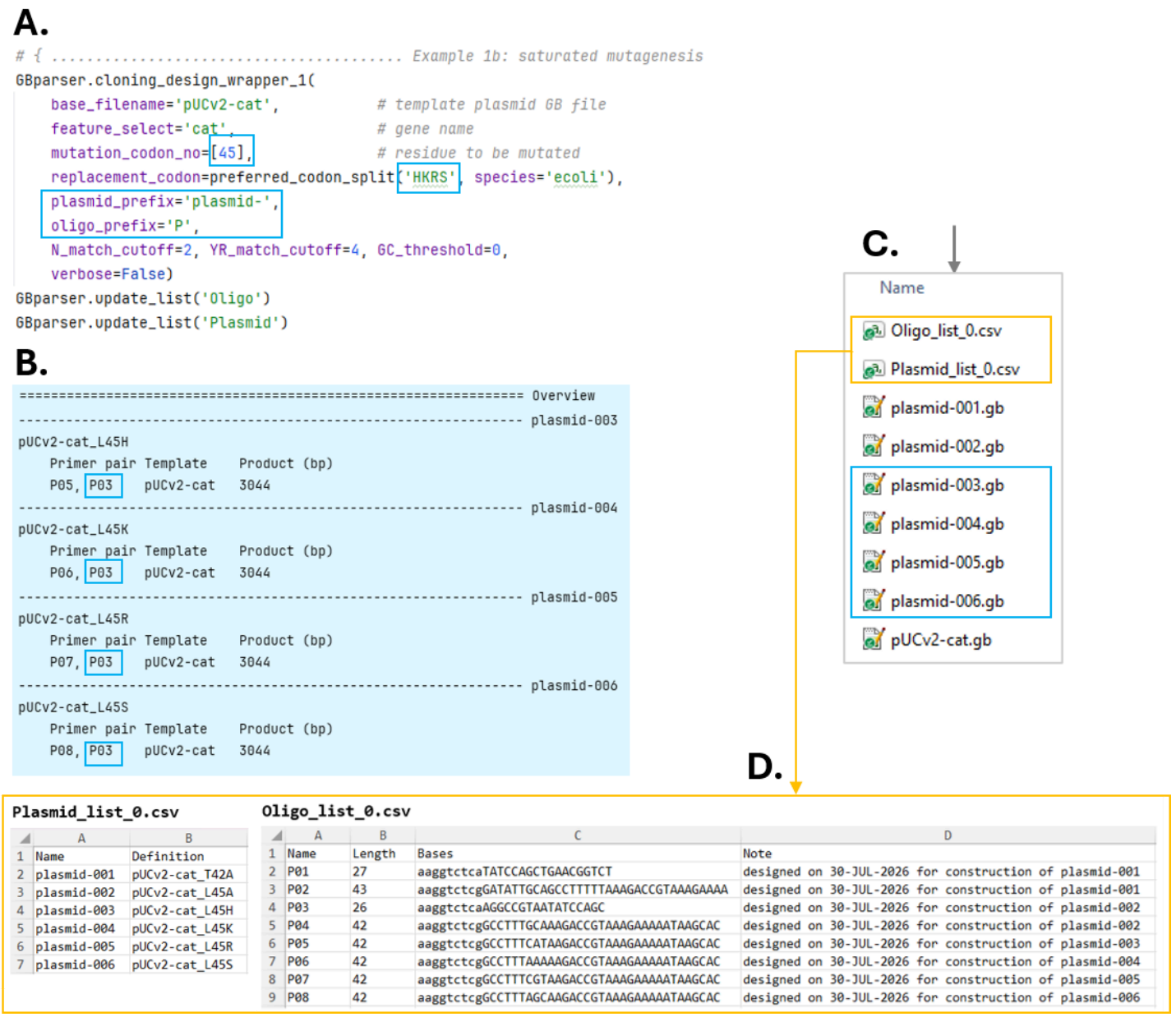
(A) Example code for independently mutating residues 42 and 45 (mutation_codon_no) of the *cat* gene (feature_select) in plasmid pUCv2-cat (base_filename) to alanine.

(B) Before execution, the working directory should contain the corresponding plasmid GenBank file *pUCv2-cat.gb* with the *cat* gene properly annotated.

(C) Standard output after execution, providing cloning instructions for each designed plasmid, for example, the blue-framed instruction indicates: "Create plasmid-001 by Golden Gate assembly of a 3044-bp PCR product amplified from pUCv2-cat using oligos P02 and P01."

(D) Working directory after execution. If *plasmid_list_0.csv* and *oligo_list_0.csv* files do not exist, they are created automatically. GenBank files (.gb) are generated for the designed plasmids.

(E) The designed plasmids and oligonucleotides (oligos) are stored in CSV format.



	A	B
1	Name	Definition
2	plasmid-001	pUCv2-cat_T42A
3	plasmid-002	pUCv2-cat_L45A
4	plasmid-003	pUCv2-cat_L45H
5	plasmid-004	pUCv2-cat_L45K
6	plasmid-005	pUCv2-cat_L45R
7	plasmid-006	pUCv2-cat_L45S

	A	B	C	D
1	Name	Length	Bases	Note
2	P01	27	aaggtctcaTATCCAGCTGAACGGTCT	designed on 30-JUL-2026 for construction of plasmid-001
3	P02	43	aaggtctcgGATATTGCAGCCTTTTTAAAGACCGTAAAGAAAA	designed on 30-JUL-2026 for construction of plasmid-001
4	P03	26	aaggtctcaAGGCCGTAATATCCAGC	designed on 30-JUL-2026 for construction of plasmid-002
5	P04	42	aaggtctcgGCCTTTGCAAAGACCGTAAAGAAAAATAAGCAC	designed on 30-JUL-2026 for construction of plasmid-002
6	P05	42	aaggtctcgGCCTTTCATAAGACCGTAAAGAAAAATAAGCAC	designed on 30-JUL-2026 for construction of plasmid-003
7	P06	42	aaggtctcgGCCTTTAAAAAGACCGTAAAGAAAAATAAGCAC	designed on 30-JUL-2026 for construction of plasmid-004
8	P07	42	aaggtctcgGCCTTTCGTAAGACCGTAAAGAAAAATAAGCAC	designed on 30-JUL-2026 for construction of plasmid-005
9	P08	42	aaggtctcgGCCTTTAGCAAGACCGTAAAGAAAAATAAGCAC	designed on 30-JUL-2026 for construction of plasmid-006

Figure 2. Subsequent saturated mutagenesis.

(A) Example code for independently mutating residue 45 to histidine, lysine, arginine, and serine (abbr. H, K, R, S respectively). By specifying plasmid_prefix and oligo_prefix, newly designed plasmids and oligos are indexed sequentially following the last existing plasmid or oligo with the same prefix.

(B) Standard output after execution. The algorithm automatically detects and reuses oligo P03, previously designed and stored in *oligo_list_0.csv*.

(C) Working directory after execution. If *plasmid_list_0.csv* and *oligo_list_0.csv* files already exist, they are updated. GenBank files (.gb) are generated for the designed plasmids.

(D) The newly designed plasmids and oligos are appended to the existing CSV lists.

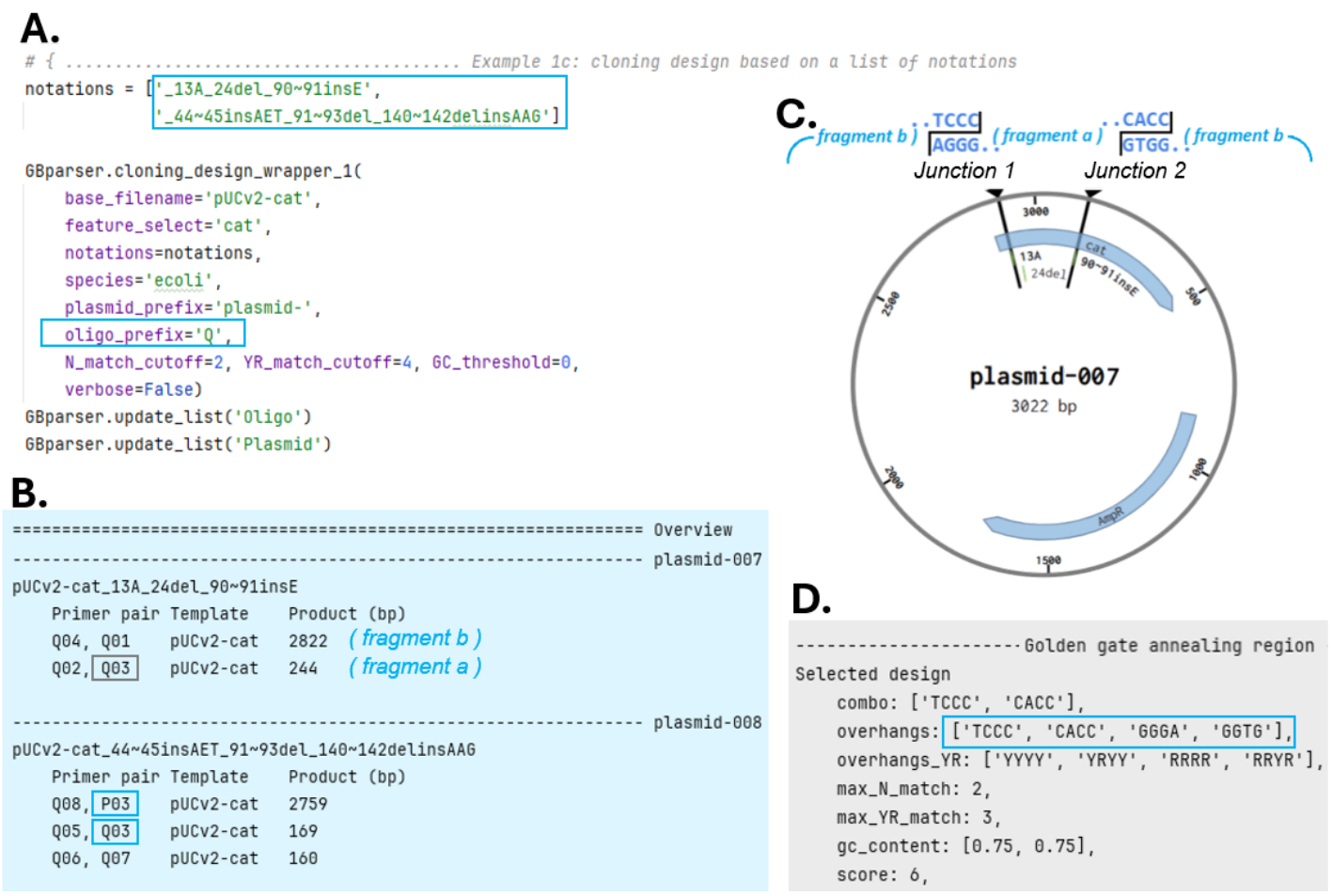


Figure 3.

Subsequent cloning design based on a list of mutation notations.

(A) Example code for designing two plasmids. The first encodes a mutated CAT protein with a glutamate inserted between residues 90 and 91 (*90~91insE*), deletion of residue 24 (*24del*), and substitution of residue 13 with alanine (*13A*). The second encodes a mutated CAT protein with residues 140–142 replaced by alanine–alanine–glycine (*140~142delinsAAG*), deletion of residues 91–93 (*91~93del*), and insertion of alanine–glutamate–threonine between residues 44 and 45 (*44~45insAET*). oligo_prefix is reset to *'Q'* to distinguish oligos designed in this run.

(B) Standard output after execution. The algorithm automatically detects and reuses oligos stored in *oligo_list_0.csv* (P03) as well as oligos generated in the same run (Q03)

(C) Plasmid-007 is assembled from two PCR fragments joined at junction 1 and junction 2. See Figure 4 for details.

(D) Assembly accuracy is determined by the orthogonality of the annealing ends (overhangs). The algorithm selects the optimal design based on multiple criteria, with overhang orthogonality (minimal pairwise identity) being a major factor. In this example, the maximum pairwise identity among the four overhangs is 2 (max_N_match). To account for ligase tolerance of noncanonical pyrimidine(Y)–purine(R) pairing, nucleotide sequences (A/T/C/G) are converted to Y/R representation and analyzed similarly (max_YR_match).

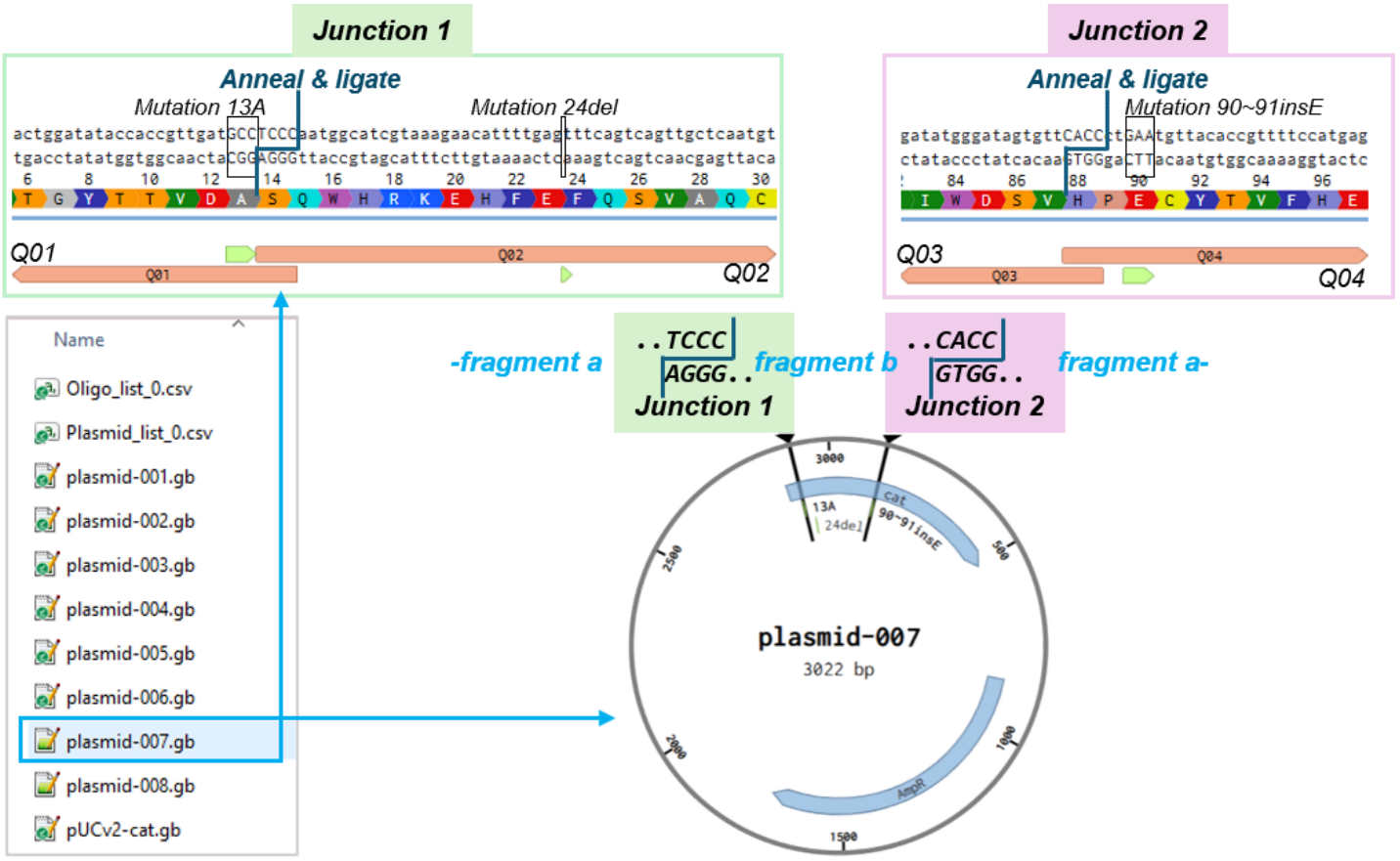


Figure 4. Assemble plasmid-007 from two PCR fragments.

GenBank files (.gb) can be visualized using various sequence visualization software (here, Benchling). Because mutations are introduced by PCR primers (oligos), they are placed at fragment junctions. The three mutation sites (13, 24, and 90–91) are grouped into two mutation regions (13–24 and 90–91), each assigned a fragment junction. Consequently, plasmid-007 is assembled from two PCR fragments.