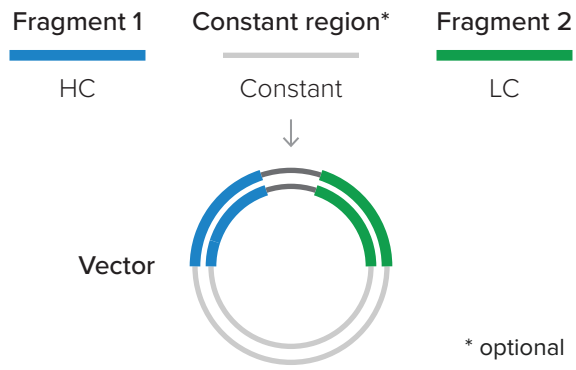
BioXp™ multi-fragment cloning — Quick reference manual

BioXp™ multi-fragment cloning allows you to synthesize two *de novo* sequences (up to 32 total) and assemble them into your custom vector (up to 12 kb) in a single run using the Gibson Assembly® method. This application eliminates sub-cloning steps, saving you time and effort. You may also include an optional constant region. Once you determine the DNA fragments and vector you'd like to clone, please use the following guidelines to design your sequences with correct overlap design and arrage your sequences in final FASTA file format.

Design and organize your sequences

Step 1: Determine which design best fits your workflow

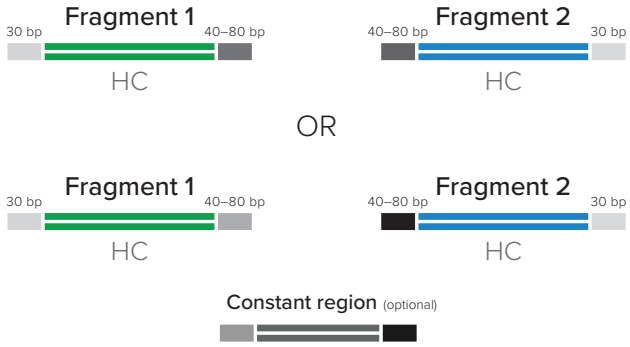
- If your sequences are between 600 bp and 3.6 kb, they are eligible for multi-fragment cloning.
- Multi-fragment cloning can assemble and combine two fragments between 300 bp to 1.8 kb each, cloning them both into a custom vector. Alternatively, multi-fragment cloning allows for the addition of a constant region sequence along with two fragments into a custom vector.



Note: Fragment 1 and fragment 2 are your sequence pair to be synthesized by the BioXp™ system and cloned into your vector.

Step 2: Add homologous overlaps to your sequences

- Add 30 bp homologous overlap of your vector to sequences 1 and 2 and at least 40–80 bp overlap between them. If a constant region is to be added, be sure to design fragment 1 with 40 bp overlap on the3' end and design fragment 2 with 40 bp overlap to the 5' end.



Design BioXp™ fragments with homologous overlaps to enable Gibson Assembly® cloning on the BioXp™ system. Vector linearization prep protocols are available at codexdna.com. Contact help@codexdna.com if you need additional assistance with the design.

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Step 3: Arrange your sequence pairs in the FASTA file format

- All sequences need to be arranged in pairs to be cloned together. Each sequence pair will need to be in order on the FASTA file with fragment 1 coming before fragment 2.
- See sequence pair arrangement to the right for reference. Note: Use the complementary excel worksheet available on the portal for additional guidance.
- Multi-fragment cloning allows you to clone sequence pairs using up to 4 different vectors with an optional constant region sequence per run, providing 4 full clones per vector or 16 total full reactions per BioXp™ run.
- DNA is built sequentially by columns on a BioXp™ recovery plate. Vectors are incorporated into the plate by rows. Sequences must be submitted in the correct order in the FASTA file, according to vector location. In the ordering portal, enter the sequences in FASTA format in the order they will be built on the plate:

Clone 1	Fragment 1 1of2/vector 1 [well A5]
	Fragment 2 2of2/vector 1 [well B5]
Clone 2	Fragment 3 1of2/vector 2 [well C5]
	Fragment 4 2of2/vector 2 [well D5]
Clone 3	Fragment 5 1of2/vector 3 [well E5]
	Fragment 6 2of2/vector 3 [well F5]
Clone 4	Fragment 7 1of2/vector 4 [well G5]
	Fragment 8 2of2/vector 4 [well H5]

- If you do not plan to build sequences in all wells of a column, add spacer sequences corresponding to the empty wells of that column.
  >Spacer1
  GGAAGTTTGTCTAGATCTCAGGCGTGGATG

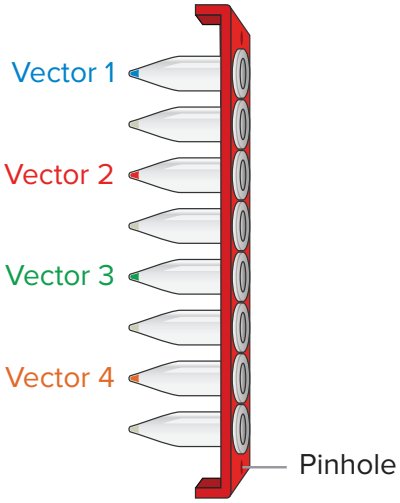
Vector	Sequence	Sequence name	Output
1	1 fragment 1	construct_01_1of2	Clone 1
	2 fragment 2	construct_01_2of2	
2	3 fragment 3	construct_02_1of2	Clone 2
	4 fragment 4	construct_02_2of2	
3	5 fragment 5	construct_03_1of2	Clone 3
	6 fragment 6	construct_03_2of2	
4	7 fragment 7	construct_04_1of2	Clone 4
	8 fragment 8	construct_04_2of2	
5	9 fragment 9	construct_05_1of2	Clone 5
	10 fragment 10	construct_05_2of2	
6	11 fragment 11	construct_06_1of2	Clone 6
	12 fragment 12	construct_06_2of2	
7	13 fragment 13	construct_07_1of2	Clone 7
	14 fragment 14	construct_07_2of2	
8	15 fragment 15	construct_08_1of2	Clone 8
	16 fragment 16	construct_08_2of2	
5	17 fragment 17	construct_09_1of2	Clone 9
	18 fragment 18	construct_09_2of2	
6	19 fragment 19	construct_10_1of2	Clone 10
	20 fragment 20	construct_10_2of2	
7	21 fragment 21	construct_11_1of2	Clone 11
	22 fragment 22	construct_11_2of2	
8	23 fragment 23	construct_12_1of2	Clone 12
	24 fragment 24	construct_12_2of2	
5	25 fragment 25	construct_13_1of2	Clone 13
	26 fragment 26	construct_13_2of2	
6	27 fragment 27	construct_14_1of2	Clone 14
	28 fragment 28	construct_14_2of2	
7	29 fragment 29	construct_15_1of2	Clone 15
	30 fragment 30	construct_15_2of2	
8	31 fragment 31	construct_16_1of2	Clone 16
	32 fragment 32	construct_16_2of2	

Step 4: Select multi-fragment cloning option on the portal and submit your FASTA file

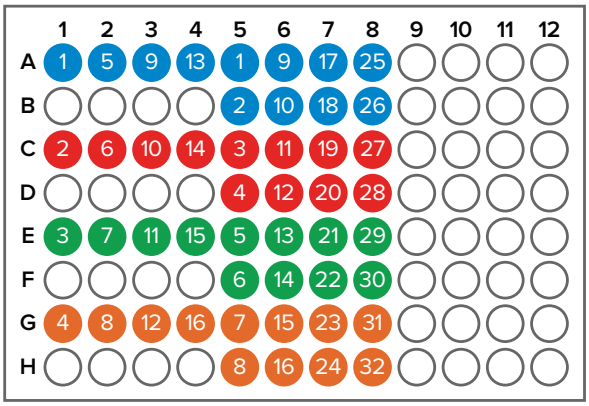
BioXp™ cloning kit

The BioXp™ cloning kit will come with an empty vector strip. Refer to the included *BioXp™ cloning kit — Loading map and checklist* for specific instruction on how to load your linearized vector and constant region sequence (optional). The loading map and checklist will also provide guidance on loading volumes and concentrations based on vector size. Once your BioXp™ run completes, the final products will be located in the BioXp™ recovery plate.

Vector +/- constant region



BioXp™ recovery plate



Custom cloning vector strip well location	Location of clones in recovery plate
A	A1–4
C	C1–4
E	E1–4
G	G1–4

For technical assistance, contact help@codexdna.com.