BioXp™ Multi Vector Custom Cloning

What is BioXp™ Custom Cloning?

DNA fragments built on the BioXp™ system are directly cloned into a customer supplied vector using Gibson Assembly® methodology. This application **eliminates subcloning,** saving time and effort.

How do I prepare for my BioXp™ Custom Cloning submission?

DNA fragments and vectors need to be designed with homologous overlaps to enable Gibson Assembly® cloning on the BioXp™ system. Vector linearization prep protocols are on the BioXp™ Custom Cloning web page [pdf]. Contact customerservice@sgidna.com if you need help with the design.

Tips for BioXp™ Custom Cloning

- Organize your FASTA file according to vector location before submission into portal
- Sequences are filled vertically on the BioXp™ plate (A1-A8, B1-B8, etc)
- Vectors loaded in the 8-well strip must align with insert well location
- Load correct volume and concentration of vector based on total number of reactions

Step 1: Organize your FASTA file and submit into the BioXp Portal

Sequences must be submitted in the correct order in the FASTA file, according to vector location.

The map below outlines which sequence aligns with the vectors loaded into the 8-well strip:

- First sequence (S1) in the FASTA file is built in A1 and is assembled into vector in the A location.
- The second sequence in the FASTA file (S2) is in location B1 and is combined with the vector in the A location. Rows C and D utilize the vector in location C, etc.
- If you do not have enough sequences to fill a column in the plate, include a **spacer sequence** in your FASTA file to align your inserts to clone with the correct vector. The spacer sequence is a 30-mer that will allow us to build the sequences so they correspond with the custom vector.

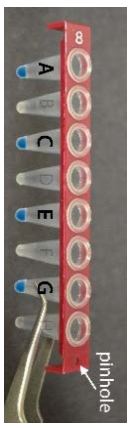
Spacer sequence example:

```
>Spacer1
  GGAAGTTTGTCTAGATCTCAGGCGTGGATG
```

Final Plate Map
(Sequence 1-32)

	1	2	3	4	5
A	S1 A	S9 A	S17 A	S25 A	
B	S2 A	S10 A	S18 A	S26 A	
C	S3 C	S11 C	S19 C	S27 C	
D	S4 C	S12 C	S20 C	S28 C	
E	S5 E	S13 E	S21 E	S29 E	
F	S6 E	S14 E	S22 E	S30 E	
G	S7 G	S15 G	S23 G	S31 G	
H	S8 G	S16 G	S24 G	S32 G	

Vector Strip



Sequences in rows A and B are assembled into vector position A
Sequences in rows C and D are assembled into vector position C
Sequences in rows E and F are assembled into vector position E
Sequences in rows G and H are assembled into vector position G

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Step 2: Load BioXp components according to protocol

Step 3: Determine the proper quantity of vector to load

Load enough vector in the appropriate wells of the strip to accommodate the number of reactions in the plate, including spacer sequences.

Number of BioXp™ Cloning Reactions	1-16	17-32
Volume of prepared linear vector to add to strip wells	12 µL	18 µL

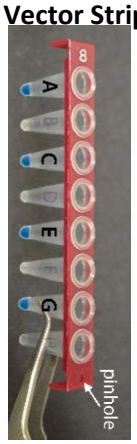
Step 4: Load Vectors in Correct Orientation

Add the appropriate linearized vector to wells A, C, E, and G of the BioXp™ Vector strip. Do not seal the strip.

Final Plate Map
(Sequence 1-32)

	1	2	3	4	5
A	S1 A	S9 A	S17 A	S25 A	
B	S2 A	S10 A	S18 A	S26 A	
C	S3 C	S11 C	S19 C	S27 C	
D	S4 C	S12 C	S20 C	S28 C	
E	S5 E	S13 E	S21 E	S29 E	
F	S6 E	S14 E	S22 E	S30 E	
G	S7 G	S15 G	S23 G	S31 G	
H	S8 G	S16 G	S24 G	S32 G	

Vector Strip



Sequences in rows A and B are assembled into vector position A
Sequences in rows C and D are assembled into vector position C
Sequences in rows E and F are assembled into vector position E
Sequences in rows G and H are assembled into vector position G

Please contact customerservice@sgidna.com if you have any questions or need additional support.