

BioXp™ cloning kit — Loading map and checklist

Each BioXp™ cloning kit includes *module A* (+4 °C), *module B* (-20 °C), and *Module C* (-20 °C). If order includes amplification, kit will also include *module D* (-20 °C).

Preparing the BioXp™ custom vector strip

1. If starting with circular vectors, linearize by restriction digesting 1 µg vector DNA with 30–50 Units enzyme (total reaction volume of 50 µL) at 37 °C for three hours.
2. Adjust the vector concentration and optional constant region concentration according to the table:

Vector size (kb)	Concentration (ng/µL)	Constant region concentration (ng/µL)
3–5	15–20	20–45
5–7	20–25	25–30
7–9	25–30	20–25
9–12	30–35	15–25

For multi-fragment assemblies only: An optional constant region may be added directly onto the custom vector strip. Recommended constant region sequence length is ~1 kb. Contact us at help@codexdna.com for longer constant region sequences and overlap design recommendations.

Constant region concentrations are recommended based on vector length, but it is recommended that for sequences < 1 kb, a 5-fold molar excess to the vector is used. Use the following formula for calculations: to include a constant region, combine equal volumes of vector and constant region to prepare linear vector.

$$\frac{\text{pmol}}{\mu\text{L}} = 1.55 \times \frac{\text{ng}/\mu\text{L}}{\text{bp}}$$

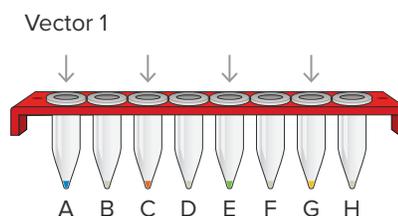
To include a constant region, combine equal volumes of vector and constant region to prepare linear vector.

3. Determine the volume of prepared linear vector to add to wells A, C, E, and G of the red BioXp™ vector strip.

Module A kit size	Volume
8 reactions	9 µL per well
16 reactions	14 µL per well
24 reactions	17 µL per well
32 reactions	21 µL per well

Example: To prepare the vector strip for a 16-reaction cloning kit, prepare a single 10 kb vector at a concentration of 30–35 ng/µL. Add 14 µL of prepared vector to the appropriate strip wells (A, C, E, and G). Total amount of required vector = 1.68 µg.

4. If using multiple vectors in a single BioXp™ run, be sure to add the appropriate linearized vector to wells A, C, E, and G of the vector strip. Refer to your specific BioXp™ job summary documentation that ships with kit to confirm vector designations in the vector strip.



	1	2	3	4	5	6	7	8	9	10	11	12
A	1	5	9	13	17	21	25					
B	2	10	18	26								
C	3	11	19	27								
D	4	12	20	28								
E	5	13	21	29								
F	6	14	22	30								
G	7	15	23	31								
H	8	16	24	32								

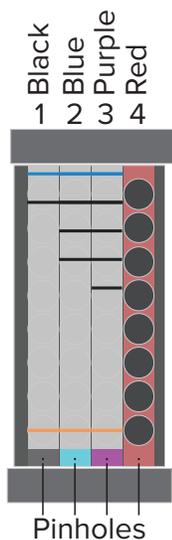
Custom cloning vector strip location	Corresponding BioXp™ plate location
● A	A1–4 B1–4
● C	C1–4 D1–4
● E	E1–4 F1–4
● G	G1–4 H1–4

Note: Do not seal the strip. Ensure that no air bubbles have been introduced and that the resuspended vector is at the bottom of the strip wells.

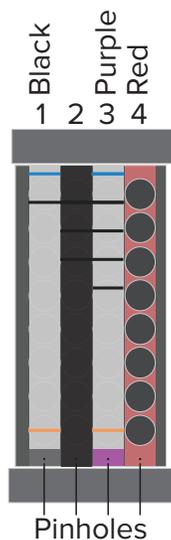
Loading the BioXp™ system

1. If the door is closed, select **Unlock Door** from the instrument LCD screen and open the door.
2. Thaw -20 °C components as directed below:
 - DNA assembly reagent plate (at 25 °C for one hour or on ice for at least three hours)
 - Gibson Assembly® cloning strip and (optional) RapidAMP™ strip (15 minutes on ice)
3. Load tips by aligning the tip tray notch with the **upper left corner** of each tip tray retainer.
 - Load 3 x 50 µL tips
 - Load 1 x 200 µL tips
4. Add a minimum of 12 mL freshly prepared 70% ethanol to the reusable **ethanol reservoir**.
 - Load **ethanol reservoir** in the right-most reservoir retainer position of the instrument deck.
Note: Do not discard the ethanol reservoir after the run; keep for future use.
5. Load plates stored at 4 °C.
 - Load the recovery plate onto the recovery chiller with the notch in the upper left corner.
 - Load the **Oligo Vault™ plate** so that the notch is positioned in the **upper left corner** of the thermocycler.
6. Vortex the thawed strips for ten seconds and then briefly spin the strips. Visually inspect the wells to ensure that they are completely thawed. **Load strips in the order listed and shown below, with the strip pinhole closest to the front of the instrument.**
 - Load the black **DNA purification strip** into position #1
 - Load the blue **RapidAMP™ strip** into position #2 or leave empty
 - Load the purple **Gibson Assembly® cloning strip** into position #3
 - Load the red **pUCGA or custom vector strip** into position #4

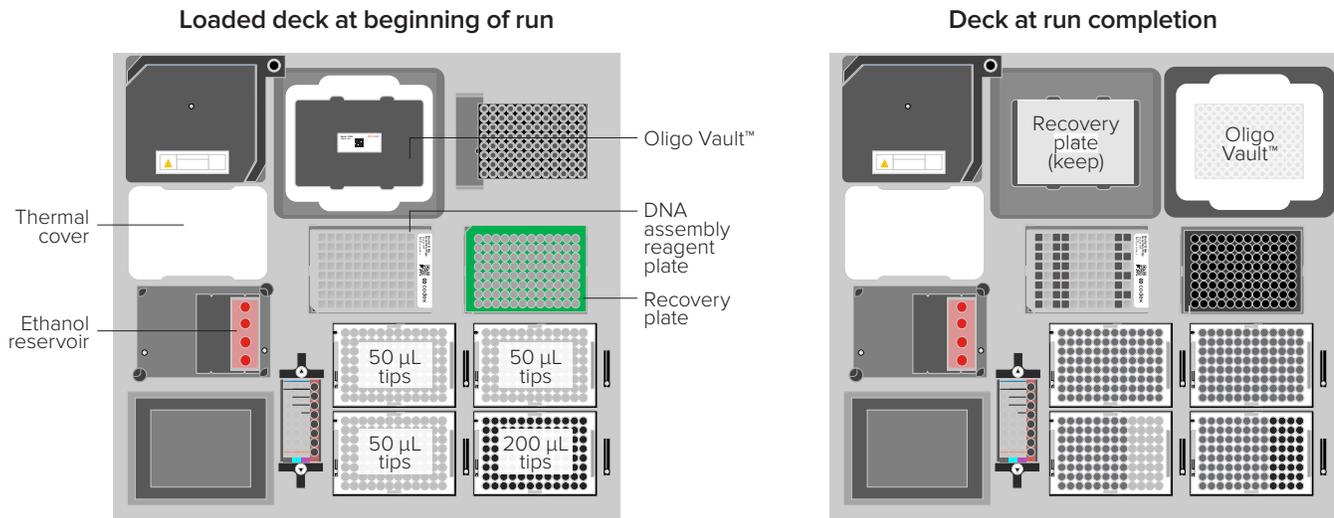
**Cloning and
RapidAMP™ amplification**



Cloning



7. **Secure strips** with the spring-loaded arms while holding strips securely in place.
8. Spin the thawed **DNA assembly reagent plate** for one minute at 500 rpm. Visually inspect the wells to ensure that they are completely thawed before loading the plate.
 - Load **DNA assembly reagent plate** onto reagent chiller, with notch in the **lower left corner** and barcode on the right.
 - Note: Be certain that the plate is properly seated within the chiller.*
9. Refer to the image below. Confirm that all components are securely seated. Close the door.
10. After the deck inspection, press **Start Now** or **Delay Start** (no more than two hours) to begin the run.



Final DNA product location

At the end of the run, save the following items:

- **Recovery plate** – All DNA products are now located in the recovery plate at the end of the run. Seal and store the recovery plate at 2 °C to 8 °C for up to one week or at –20 °C for up to one year.
- See plate map provided with oligo vault shipment for specific locations of each fragment and final product

Recommendations

Analyze fragments

We recommend evaluating the build success of sequences by running a gel containing the uncloned/unamplified BioXp™ gene fragments from the recovery plate before transforming clones.

Post-RapidAMP™ amplification restriction digest

Refer to the BioXp™ cloning with amplification - Quick reference manual for additional information.

Transformation

For chemically competent cells, dilution of cloning reactions is not required but we recommend plating a higher volume of the recovery mix (200 µL), especially for smaller (1 kb) sequences. We recommend using MAX Efficiency™ DH5α™ competent cells (Thermo Fisher cat. no. 18258012), NEB® 5- alpha competent *E. coli* (NEB cat. no. C2987). If other cells are used, be certain to use competent cells with a transformation efficiency $\geq 1 \times 10^9$ CFU/µg pUC19.

For electrocompetent cells, dilute cloning reactions with a 1:3 dilution ratio before transformation (e.g. for each well, transfer 10 µL cloning reaction to a clean tube or plate and add 30 µL molecular biology grade water). We recommend using TransforMax™ EPI300™ Electrocompetent *E. coli* (Lucigen cat. no. EC300110).

Recovery plate contents and location

Final product (cloned or cloned and amplified DNA)	Uncloned/unamplified fragments
Wells A1–H4	Wells A5–H8

- **Ethanol reservoir** —empty and dry for next use
- **Tip waste bin**

Identifying error-free clones

Cloning on the BioXp™ system has been optimized for high cloning efficiency, which does not necessarily correlate with high colony output post-transformation but results in a higher percentage of full-length clones. Screen clones by colony PCR or restriction enzyme digest to identify full-length clones prior to sequencing. We recommend sequencing 2 to 4 full-length clones for fragments < 1000 bp, 4 to 8 full-length clones for fragments 1001-1800 bp and 8 to 16 full-length clones for fragments 1801-3600 bp.

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 For technical assistance, contact help@codexdna.com.