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. Refer to *BioXp™ clones — User guide* for further vector linearization guidance.

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)
2–5	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@condexdna.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.

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

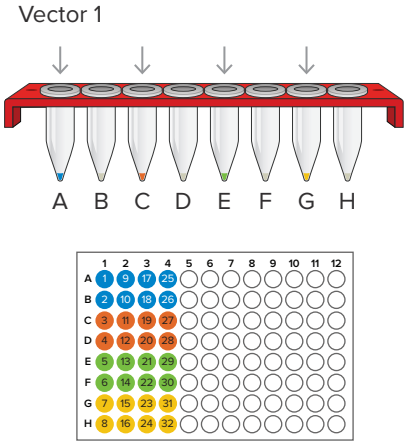
To include a constant region, combine equal volumes of vector and constant region to prepare linear vector.

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.



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.

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CODEX DNA

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)

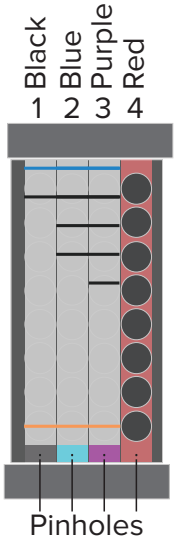
3. Load tips by aligning the tip tray notch with the **upper left corner** of each tip tray retainer.
   - ☐ Load 3 x 50 µL tips
   - ☐ Load 1 x 200 µL tips

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.

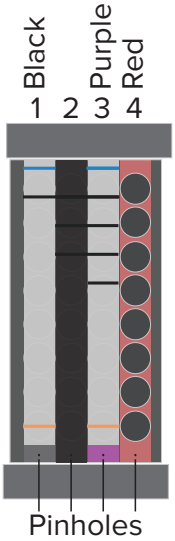
5. Load plates stored at 4 °C.
   - ☐ Load the recovery plate onto the recovery chiller with the notch in the upper left corner.
   - ☐ Load the **Oligo Vault™ plate** so that the notch is positioned in the **upper left corner** of the thermocycler.

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 **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.

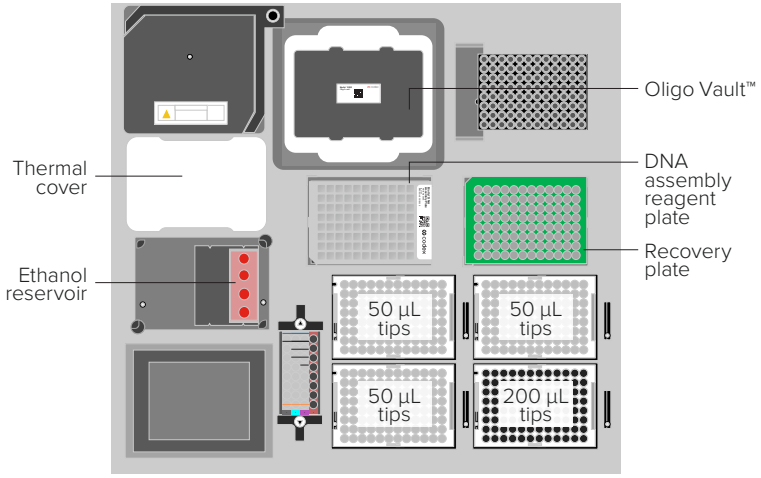
8. Spin the thawed **DNA assembly reagent plate** for one minute at 500 rpm. Visually inspect the wells to ensure that they are completely thawed before loading the plate.

   ☐ 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.

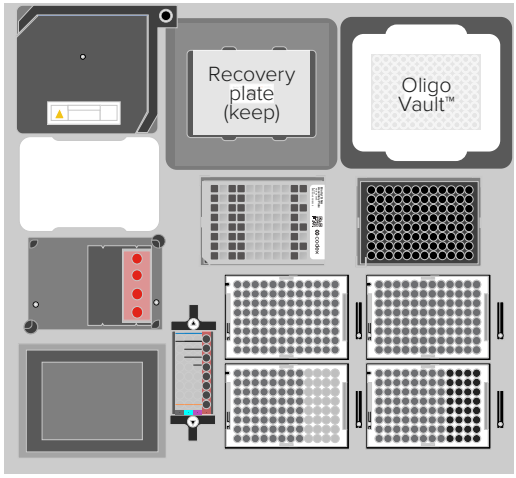
9. Refer to the image below. Confirm that all components are securely seated. Close the door.

10. After the deck inspection, press ***Start Now*** or ***Delay Start*** (no more than two hours) to begin the run.

Loaded deck at beginning of run



Deck at run completion



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.

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**

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 *BioXp™ clones — User guide* 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). If other cells are used, be certain to use competent cells with a transformation efficiency ≥ 1×10^8 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).

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.

Trademarks

- MAX Efficiency™ is a trademark of Thermo Fisher Scientific.
- Gibson Assembly® is a registered trademark of Codex DNA, Inc. Gibson Assembly® US patent nos. 7,776,532 and 8,435,736 and 8,968,999
- BioXp™, Oligo Vault™, and RapidAMP™ are trademarks of Codex DNA, Inc.

For technical assistance, contact help@codexdna.com.

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