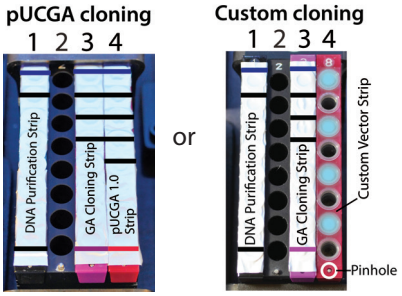
BioXp™ Loading Map and Checklist– Cloning

Each BioXp™ Cloning kit includes Module A (+4°C), Module B (–20°C), and Module C (–20°C).

1. Load reagents for a new run according to the following instructions:
   - Use appropriately sized reagents for your job; reagent sizes are denoted by stickers on boxes, e.g. S or L
   - 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** (30 minutes at room temperature or 1 hour on ice)
   - **GA Cloning Strip** and **Vector Strip** (30 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

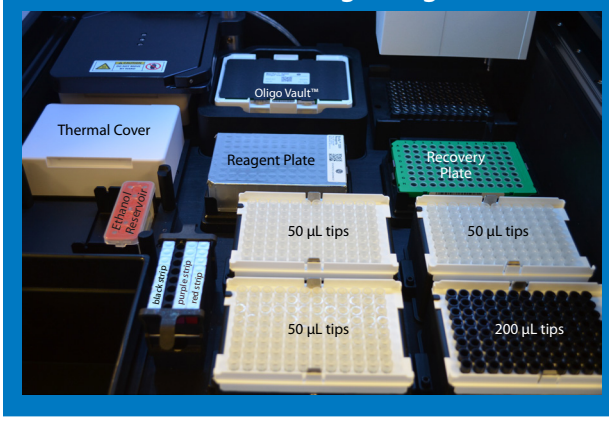
     Note: Do not discard the **Ethanol Reservoir** after the run; keep for future use
5. Load plates stored at 4°C:
   - ☐ Load the **Recovery Plate** onto the Recovery Chiller with the notch in the **upper left corner**
   - ☐ Load the **Oligo Vault™ Plate** into the Thermocycler with the notch in the **upper left corner**
6. Briefly spin the strips. **Load in the order listed and shown below**, with the **strip pinhole closest to you**
   - ☐ Load the black **DNA Purification Strip** into position #1
   - ☐ Leave position #2 empty
   - ☐ Load the purple **GA Cloning Strip** into position #3
   - ☐ Load the red **pUCGA 1.0** or **Custom Vector Strip** into position #4



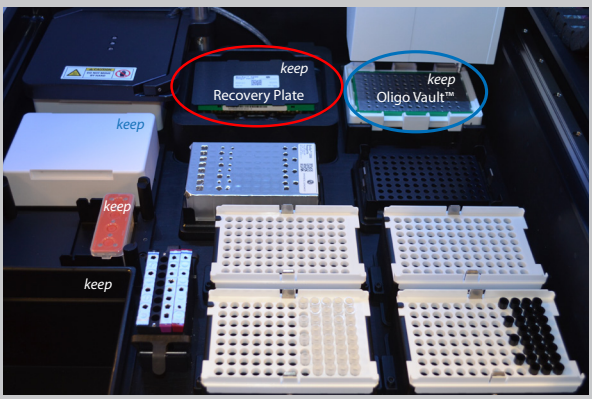
7. Secure strips with spring-loaded arms while holding the strips in place.
8. Spin the thawed **DNA Assembly Reagent Plate** for 1 minute at 500 rpm.
   - ☐ Load **DNA Assembly Reagent Plate** onto Reagent Chiller, notch in the **lower left corner**

     Note: Be certain that the plate is properly seated within the chiller.
9. Refer to the photo in the lower left panel below. Confirm that components are securely seated. Close the door.
10. After the deck inspection ends, press **Start Now** or **Delay Start** to begin the run.

Loaded deck at beginning of run



Deck at run completion



Guidelines to prepare the BioXp™ custom vector strip

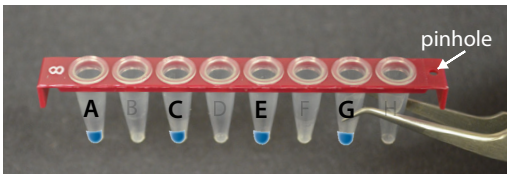
1. Adjust the vector concentration according to the table:

Vector Size (kb)	Concentration (ng/µL)
3–5	15–20
5–7	20–25
7–9	25–30
9–12	30–35

2. Determine the volume of prepared linear vector you will add to wells **A, C, E,** and **G** of a BioXp™ Vector strip.

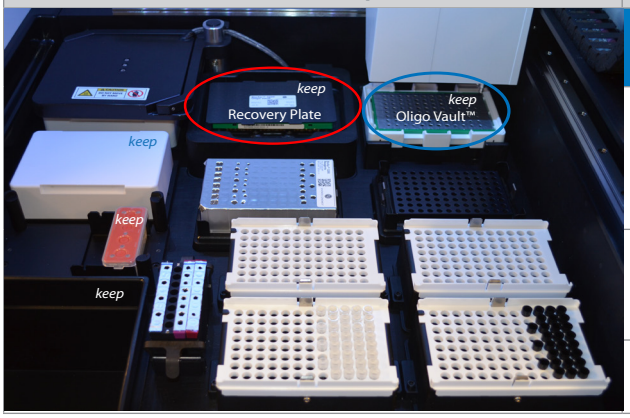
Number of BioXp™ Cloning Reactions	Volume
≤16 Reactions	12 µL
>16 Reactions	18 µL

3. Add 12 or 18 µL of prepared linear vector to **wells A, C, E and G** of a BioXp™ Vector strip. Do not seal the strip. Load the prepared custom strip on the deck with the pinhole closest to the front of the instrument..



EXAMPLE: To prepare the vector strip for 8 cloning reactions, prepare a 10 kb vector at a concentration of 30–35 ng/µL. Add 12 µL of prepared vector to the four strip wells (A, C, E and G).
Total amount of required vector = 1.44 to 1.68 µg.

Final DNA product location

Deck at run completion	Items to keep		
	**DNA product**	**Located within**	**Storage instructions**
	Cloning Reaction (wells A1–H4)	**Recovery Plate**	Seal and store at 2°C to 8°C for up to one week or at –20°C for up to a year.
	Uncloned **Tiles** (wells A5–H8)	**Oligo Vault™ Plate** (foil covered)	Seal and store at 2°C to 8°C for up to one week.
	Ethanol Reservoir (empty and rinse for next use)		

Tips

After the BioXp™ run is complete, cloning reactions are ready for transformation. We recommend diluting cloning reactions 1:2 with molecular biology grade water before transformation.

Analyze Tiles– We recommend evaluating the success of the assembly reaction by running a gel containing the uncloned BioXp™ Tiles from the Oligo Vault™ Plate before transforming clones

Transformation– We recommend using *E. cloni* 10G Chemically Competent Cells (Lucigen Cat. No. 60107, free samples available at **Lucigen.com**) or TransforMax™ EPI300™ Electrocompetent *E. coli* (Lucigen Cat. No. EC300110). If other cells are used, be certain to use competent cells with a transformation efficiency ≥1 × 10^9 CFU/µg pUC19.

Additional product information is available at **www.sgidna.com/bioxp**

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