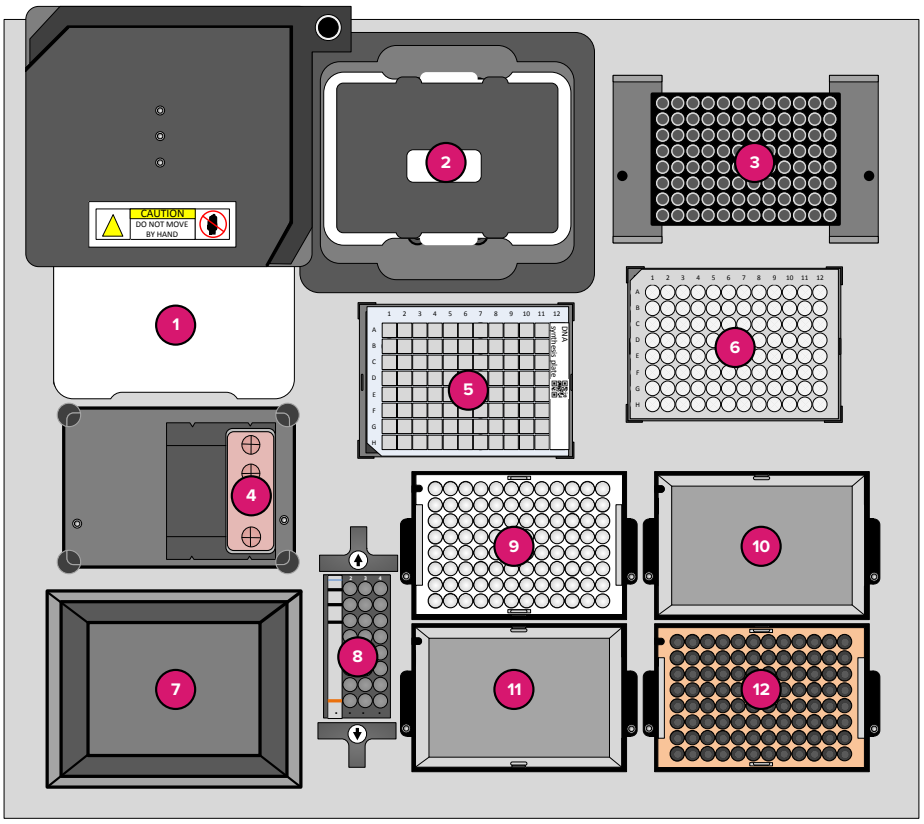
BioXp® 3250 Select DNA cloning kit — Loading map and checklist

Each BioXp Select DNA cloning kit (Golden Gate or Gibson Assembly) includes the necessary modules to prepare template DNA, vector, and cloning reagents.



1. Chiller cover storage
2. Reaction plate
3. Purification station
4. Ethanol reservoir
5. Reagent block
6. DNA input plate
7. Waste bin
8. Strip holder
9. Tip tray #1
10. Tip tray #2
11. Tip tray #3
12. Tip tray #4

Figure 1. BioXp 3250 system layout without labware.

DNA cloning for Gibson Assembly Kit

# of reactions	Kit SKU	Reagent block (Mod J)	Gibson strip (Mod C)	Positive control	50 µL tip trays	200 µl tip trays
8	BX-SEL-CLNGA-08	BX-J1	BX-C08-BSPC2	BSPC-2	1	1
24	BX-SEL-CLNGA-24		BX-C24-BSPC2			

Table 1. Consumables matrix for the BioXp Select DNA Cloning for Gibson Assembly kit

DNA cloning for Golden Gate Assembly, BsaI Kit

# of reactions	# of fragments	Kit SKU	Reagent block (Mod J)	Golden Gate strip (Mod L)	Positive control	50 µL tip trays	200 µl tip trays
8	≤ 4	BX-SEL-CLNGG4A-08	BX-J1	BX-L1-BsaI	BSPC-4	1	1
	≥ 5	BX-SEL-CLNGG10A-08					
24	≤ 4	BX-SEL-CLNGG4A-24		BX-L2-BsaI			
	≥ 5	BX-SEL-CLNGG10A-24					

Table 2. Consumables matrix for the BioXp Select DNA cloning for Golden Gate Assembly kits, using BsaI restriction enzyme.

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DNA cloning for Golden Gate Assembly, BsmBI Kit

# of reactions	# of fragments	Kit SKU	Reagent block (Mod J)	Golden Gate strip (Mod L)	Positive control	50 uL tip trays	200 µl tip trays
8	≤ 4	BX-SEL-CLNGG4B-08	BX-J1	BX-L1-BsmBI	BSPC-4	1	1
	≥ 5	BX-SEL-CLNGG10B-08					
24	≤ 4	BX-SEL-CLNGG4B-24		BX-L2-BsmBI			
	≥ 5	BX-SEL-CLNGG10B-24					

Table 3. Consumables matrix for the BioXp Select DNA cloning for Golden Gate Assembly kits, using BsmBI restriction enzyme.

Prepare DNA input plate on the bench

1. Prepare the empty DNA input plate by loading your DNA fragment and vector mixture.

   ☐ The following is recommended per well:

		Golden Gate kits	Gibson kits
Total volume (fragment+vector)		12µl	7µl
Vector amount		50-75 ng	20-50 ng
Molar ratio (fragment: vector	<1kb fragment	3:1	3:1
	≥1kb fragment	2:1	1:1
	pDNA input	1:1	N/A

Table 4. Input DNA template loading volume and ratios by kit type.

Note: For additional specific details about molar ratios, including accounting for multiple fragments, and other concentrations of input DNA templates, please refer to the BioXp Select DNA cloning user guide found on the Telesis Bio resources page.

   ☐ Load input DNA in the appropriate wells depending on the number of reactions in your kit (Figures 2 and 3). Mix by pipetting gently 3-4 times.

2. Spin the filled DNA input plate for one (1) minute at 1,000g to remove any bubbles and collect all the liquid at the bottom of the wells before use.

3. Maintain the DNA input plate on ice until the start of the run.

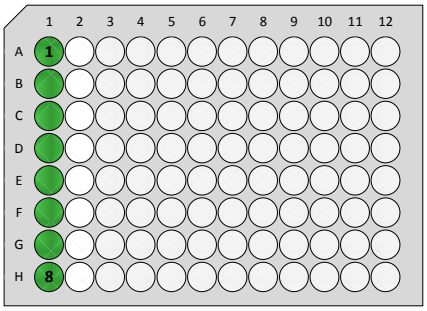

Figure 2. Input DNA fragment and vector loading plate map for 8 reaction kits. (Golden Gate and Gibson Assembly share the same well) positions).

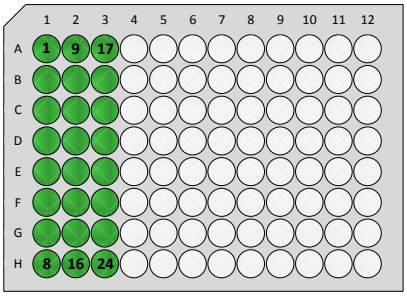

Figure 3. Input DNA fragment and vector loading plate map for 24 reaction kits (Golden Gate and Gibson Assembly share the same well) positions.)

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Prepare and load the BioXp system deck

Loading plates and reagents into the BioXp 3250 system

1. Thaw the **cloning strip** from either Module L or Module C (stored at -80°C) on ice for up to 60 minutes. Alternatively, strips can be thawed at room temperature for 10 minutes, then placed on ice for up to an hour until use.
2. Briefly spin the **cloning strip** for 10-15 seconds in a benchtop mini centrifuge to remove bubbles and collect the liquid at the bottom of the wells.
   - ☐ Load the **cloning** strip in column one (Figure 4) of the strip holder (Position 8 in Figure 1).
   - ☐ The other 3 strip holder locations remain empty.
3. Load the filled **DNA input plate** (Position 6 in Figure 1). Take care so that liquid is not dislodged from the bottom of the plate wells while loading the DNA input plate on the BioXp 3250 system. If liquid is dislodged, spin the plate again and re-load.
4. Load fresh (unopened) **tip racks** on the deck by aligning the tip tray notch with the upper left corner of each tip tray retainer.
   - ☐ Load 1 x 50 µL tip rack (Position 9 in Figure 1)
   - ☐ Load 1 x 200 µL tip rack (Position 12 in Figure 1)
5. Load the empty reusable ethanol reservoir in the right-side of the holder and ensure the lid is snapped closed (Position 4 in Figure 1)
   - ☐ Although empty, the ethanol reservoir must be present for proper system initialization.

   Note: Do not discard the **ethanol reservoir** after the run; keep for future use.
6. Load the empty **Reaction plate** (Position 2 in Figure 1). Ensure that the white plate carrier and the black metal plate cover are both present. Ensure the plate cover is oriented so the job type and QR code are facing you.
7. Load the **Reagent block** (Position 5 in Figure 1). Ensure the label is on the right-hand side of the plate and that the notch in the plate plastic is in the lower left corner. Make sure the plate is secured flush with the plate holder.
8. Refer to Figure 5 showing a loaded deck at the beginning of a run. Confirm that all components are securely seated. Close the hood, and if prompted click next.
9. After the deck inspection finishes, press **Start** to begin the run.

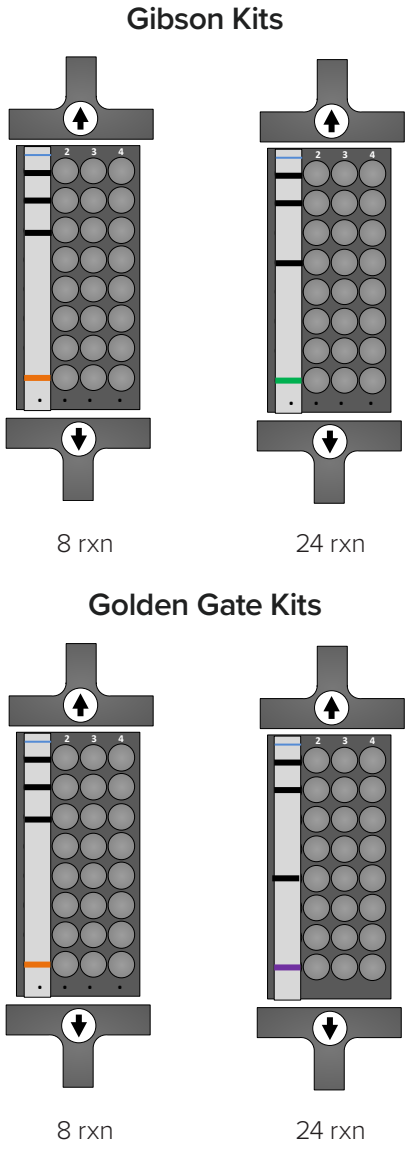

Figure 4. Correct strip layouts for Select DNA cloning Gibson and Golden Gate kits, including barcoding stripes. **Note:** Both Gibson cloning strips ship with a purple frame strip. Golden Gate cloning strips have the same stripe pattern regardless of restriction enzyme, but BsaI ships with a black frame strip and BsmBI a white frame strip.

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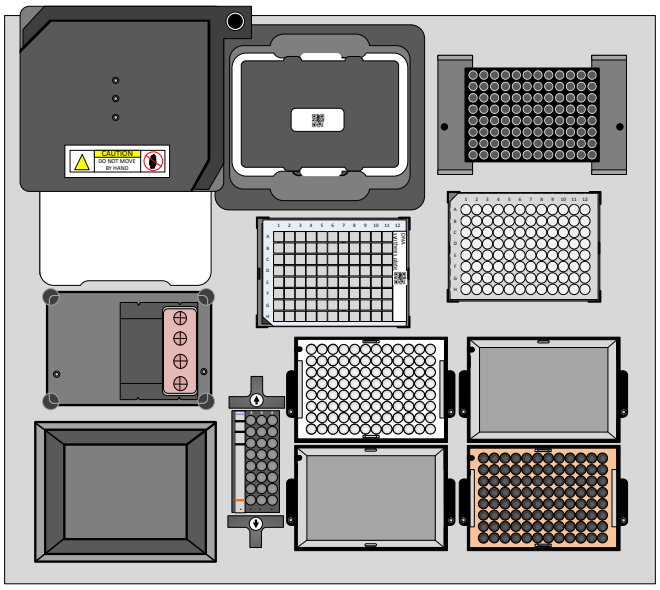

Figure 5. Loaded deck at the beginning of run (Example 8-reaction cloning job)

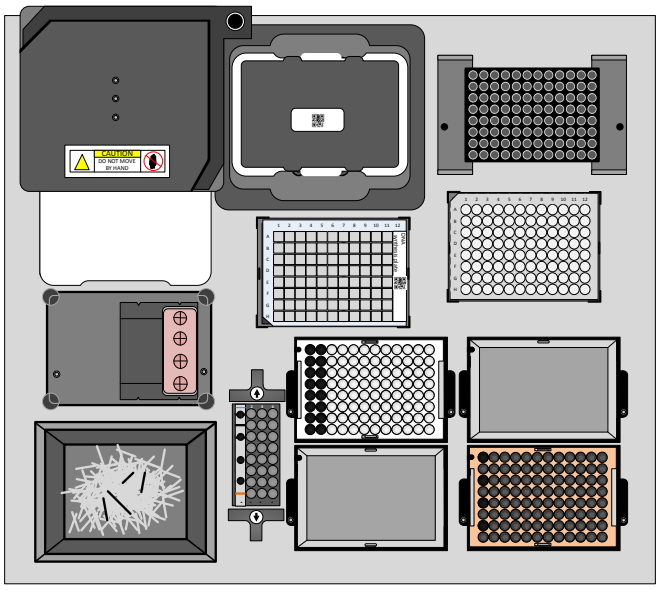

Figure 6. Deck upon run completion (Example 8-reaction cloning job)

Finishing the run and collecting reaction products

Figure 6 shows deck configuration upon completion of successful run. At the end of the run, save the following items:

- ☐ Save the **Reaction plate** (Position 2 in Figure 1). This plate contains the final cloned DNA products, see Figures 7 and 8 below. Seal the plate before removing it from the white carrier to avoid dislodging the sample from the wells. Store the sealed **Reaction plate** at –20 °C for up to six months.
- ☐ Save the **Ethanol reservoir** for future runs.

All other labware can be discarded. Saving and re-racking of pipette tips is not recommended.

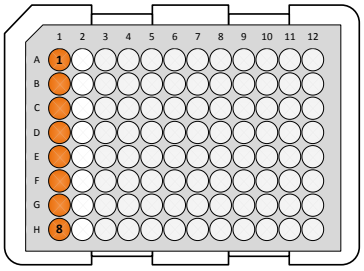

Figure 7. Reaction plate map after completed run for 8 reaction kit (Golden Gate and Gibson Assembly share the same well positions).

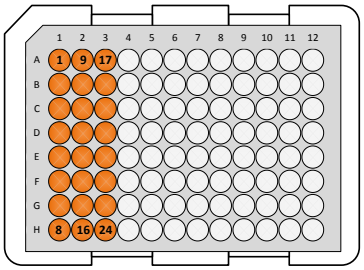

Figure 8. Reaction plate map after completed run for 24 reaction kit (Golden Gate and Gibson Assembly share the same well positions).

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For technical assistance, contact help@telesisbio.com

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