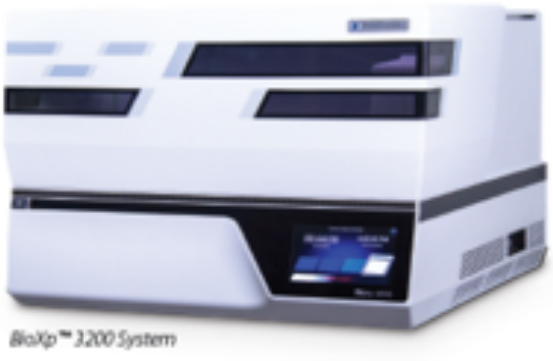
Yale School of Medicine | W.M. Keck Foundation

Oligo Synthesis Resource

New Service

Custom clones at your fingertips:
Custom synthesis and clone construction

BioXp™ 3200 SYSTEM



BioXp™ 3200 System

- **Double Stranded DNA**
- **Custom Clones**
- **Libraries**

Clone BioXp™ Tiles directly into your vector

BioXp™ 3200 System:

- **Delivers** workflow control in obtaining DNA or clones
- **Streamlines** your genomic processing
- **Offers** flexibility with multiple applications
- **Expands** your scientific capacity to accelerate discovery
- **Provides** faster turnaround than conventional methods

Interested? Please Contact:

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https://medicine.yale.edu/keck/oligo/
https://sgidna.com/bxp3200.html

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SYNTHETIC GENOMICS®

BioXp™ Custom Cloning Vector Preparation Guide

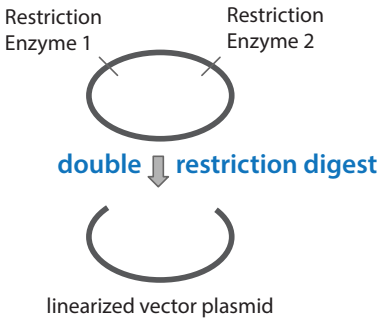
Follow this guide to prepare and analyze your vector for custom cloning before use on the BioXp™ 3200 System.

Select one of the following vector preparation options.

Self-preparation

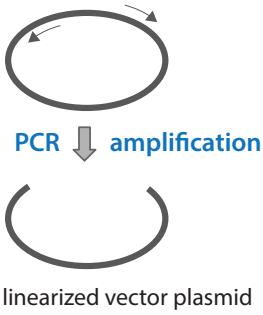
Option A: Vector preparation by double restriction digest

See pages 2–3, 5–12 for detailed instructions



Option B: Vector preparation by PCR amplification

See pages 2, 4–12 for detailed instructions



Custom vector preparation service

Or, SGI-DNA can prepare your vector for you.
Visit sgidna.com/bioxp or contact customerservice@sgidna.com for more information.

Contents

Before Starting: Determine Vector Concentration

Materials

- Cloning vector
- We recommend quantifying dsDNA with a Qubit® Fluorometer and Qubit® dsDNA BR Assay Kit or with a NanoDrop™ Spectrophotometer (Thermo Fisher Scientific)

Protocol

Follow the Qubit® dsDNA BR Assay Kit protocol provided with your kit using 1 µL of the cloning vector.

1. Record the data: Qubit® reading _____ ng/µL (concentration of vector).

2. Proceed to one of the following preparation protocols:

    - If you are preparing your vector by restriction digest, go to "Linearize Vector by Restriction Digest (Option A)" on page 3.

    - If you are preparing your vector by PCR amplification, go to "Linearize Vector by PCR Amplification (Option B)" on page 4.

Linearize Vector by Restriction Digest (Option A)

We recommend setting up a minimum of 16 parallel digestions to generate sufficient quantities of linearized vector. Scale-up may cause inefficient digestion.

Some reactions may require sequential digestion, depending on enzyme buffer compatibility.

Confirm that the double-digest does not create compatible cohesive ends.

Materials

- Vector, with known concentration
- Appropriate restriction enzymes

Protocol

1. Combine the following components in parallel reactions. **Add restriction enzymes last.**

Component	Volume
0.5–1.0 µg vector	χ µL
NEB Buffer, e.g. CutSmart®	2.5 µL
Restriction enzyme 1	1.0 µL
Restriction enzyme 2	1.0 µL
Distilled water	20.5–χ µL
Total volume	**25 µL**

2. Invert samples multiple times to mix the restriction enzymes well. Perform a quick spin to collect droplets.
3. Incubate reactions at 37°C for 2 hours.
4. Proceed to "Analyze and Prepare Linearized Vector for the BioXp™ System" on page 5.

Linearize Vector by PCR Amplification (Option B)

Before starting

Dilute vector to 2 ng/µL with sterile water. Each PCR amplification reaction requires 1 µL of vector template. Only dilute the amount of vector needed for the immediate PCR reaction.

Materials

- Diluted vector
- Primers
- High-fidelity DNA Polymerase
  (We recommend Phusion® PCR Master Mix, Thermo Fisher Scientific Cat. No. F531)

Protocol

1. Thaw all reagents on ice.
2. Vortex each reagent briefly. Perform a quick spin to collect all droplets.
3. Combine the following components in a PCR tube.

Component	Concentration	Volume (µL)
Phusion PCR Master Mix	—	50.0
Custom vector	2 ng/µL	1.0
Vector amplification 5' primer	100 µM	0.5
Vector amplification 3' primer	100 µM	0.5
Molecular biology grade water	—	48.0
Total Volume	—	**100**

4. Mix the reaction. Perform a quick spin to collect all droplets. Place the PCR tubes into a thermocycler and run the following conditions.

Step	Temperature	Duration	Number of cycles
Initial denaturation	98°C	1 minute	1 Cycle
Amplification	98°C	10 seconds	30 Cycles
	Primer T_m	30 seconds	
	72°C	30 seconds per kb	
Final extension	72°C	5 minutes	1 Cycle
Hold	10°C	—	1 Cycle

5. After the PCR amplification is complete, add 1 µL of DpnI enzyme to each PCR reaction.
6. Incubate at 37°C for 1 hour.
7. Incubate at 80°C for 20 minutes to inactivate DpnI.
8. Run 5 µL of each PCR sample on a 0.8% agarose E-gel to confirm the product size.
9. Analyze the gel. Only one single band should be evident.
   - If you observe a smear or unspecific bands, the PCR has failed.
   - If a fragment of the correct size is visible on the gel, proceed to "Analyze and Prepare Linearized Vector for the BioXp™ System" on page 5.

Analyze and Prepare Linearized Vector for the BioXp™ System

Gel Electrophoresis

Materials

- Linearized Vector
- 1% Agarose Gel, TAE Buffer and Gel Apparatus
- Loading Dye
- 1 kb Ladder

Protocol

1. Prepare a 1% agarose gel and place in a gel box with freshly prepared TAE buffer.
2. Prepare vector DNA for electrophoresis by combining the following reagents. Volumes listed in the following tables are dependent on your vector preparation method.

Restriction digestion reaction

Volume	Reagent
4 µL	6X Loading dye
25 µL	Linearized vector (digestion reaction)
29 µL	**Total Volume**

PCR amplification reaction

Volume	Reagent
20 µL	6X Loading dye
100 µL	Linearized vector (PCR reaction)
120 µL	**Total Volume**

3. Load the agarose gel according to the following table:

	Lane 1	Lane 3	Lane 3–12
Volume	5 µL	—	29 µL
Component	1 kb DNA Ladder (100 ng/µL)	Leave empty	Vector DNA with loading dye

4. Run gel at 90 V for approximately 45 minutes.
5. After electrophoresis, visualize the gel with transilluminator gel imager.

   NOTE: Confirm the presence of a band of the expected size before proceeding to gel extraction.

Gel Extraction

Materials

- Gel Extraction Kit
  (We recommend the MinElute® Gel Extraction Kit, Qiagen Cat. No. 28604)

Protocol

Follow the MinElute® Gel Extraction protocol provided with your kit, with the following exceptions or instructions:

1. Place the column into a clean colorless 1.5-mL microcentrifuge tube. Elute DNA by adding 15 µL of pre warmed EB Buffer (10 mM Tris-HCl, pH 8.5), let stand for 2–4 minutes, and centrifuge for 1 minute.

2. Consolidate all eluted DNA (linearized, purified vector) into one microcentrifuge tube.

Determine Linear Vector Concentration

The following steps are required.

Materials

- Linearized and purified cloning vector
- We recommend using the Qubit® Fluorometer and Qubit® dsDNA BR Assay Kit , or a NanoDrop™ Spectrophotometer (Thermo Fisher Scientific).

Protocol

Follow the Qubit® dsDNA BR Assay Kit protocol provided with your kit using 1 µL of the linearized, purified cloning vector.

Record the data: Qubit® reading _____ ng/µL (concentration of linear vector).

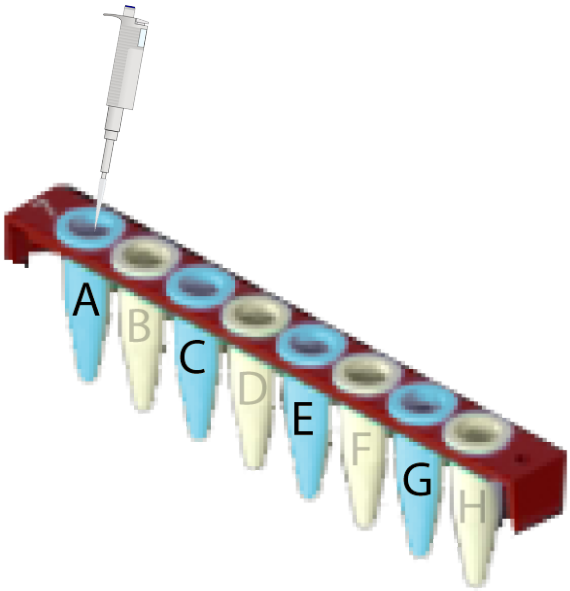
Guidelines to prepare the BioXp™ Custom Vector Strip

NOTE: Only prepare strips when you are ready to load a BioXp™ Custom Cloning job.

1. Confirm that your vector is at the appropriate concentration according to the size of the vector. Adjust the concentration, if necessary.

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

2. Add the prepared linear vector to wells **A, C, E** and **G** of a BioXp™ Vector strip according to the following instructions.

Number of BioXp™ Cloning Reactions	≤16	>16
Volume of prepared linear vector to add to strip wells	12 µL	18 µL
Add the prepared linear vector to **wells A, C, E and G** (highlighted with blue) of a BioXp™ Vector strip **Note:** Do not seal the strip		

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

Version 1.1 11.23.16