BioXp™ cloning with amplification — Quick reference manual

Use this guide to evaluate the success of assembly and amplification reactions.

Prior to ordering sequences:

Design sequences to include a unique restriction site at a desired location; site will usually be outside of the genes of interest.

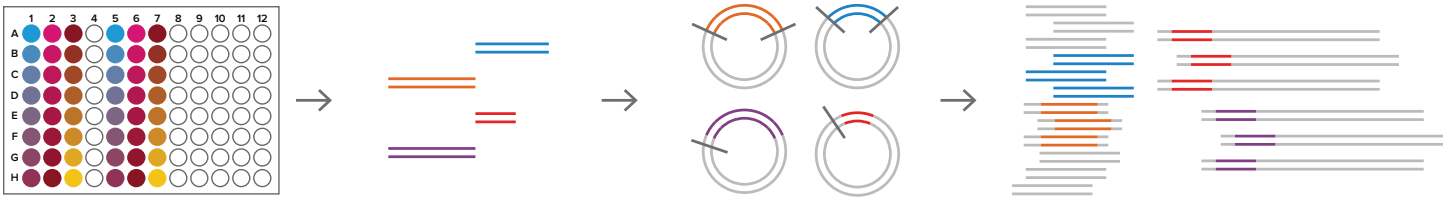
Following a BioXp™ cloning with amplification run:

1. Analyze BioXp™ fragments
2. Linearize cloned and amplified constructs via restriction digestion
3. (Optional) DNA cleanup
4. (Optional) quantification of amplified DNA prior to downstream applications

Pre-run design recommendations

It is common to linearize with a single- or double-restriction enzyme cut in a non-essential portion of their backbone. We recommend selecting suitable cut-sites for restriction digestion prior to gene synthesis and ensuring that there are no unwanted recognition sequences in the gene to be synthesized. It is necessary to include at least one unique restriction site in the initial construct design for concatemer linearization.

Cloned constructs with restriction sites and expected final linear products



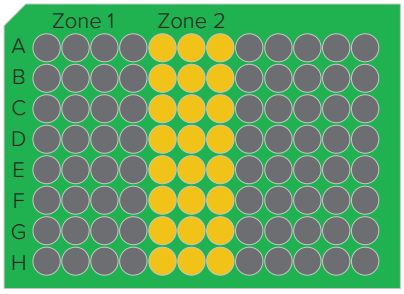
Up to 24 different fragments can be built simultaneously and will be available at the end of a BioXp™ run in wells A5–A7. Cloned and amplified product is in wells A1–H3.

During the BioXp™ run, all 24 fragments are cloned into up to 4 different vectors and then amplified using Gibson Assembly® RapidAMP™ technology. After the run, restriction digestion will linearize the amplfied product into the desired final fragment, ready for downstream applications.

Post-run analysis

Analyze BioXp™ fragments

Perform gel electrophoresis of ~5 µL of the uncloned BioXp™ fragments from zone 2 (positions A5–H7) of the recovery plate. The quality of the BioXp™ fragments is a key indicator of overall assembly and cloning with amplification efficiencies.



Location of BioXp™ fragments

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Expected results of BioXp™ fragments – Gel electrophoresis

These gel images show examples of completed runs with the assembled fragment of interest clearly distinguishable. In the gel photo on the left, notice the absence of off-target assemblies and/or intermediate by-products, which is indicative of optimal BioXp™ assembly. The gel image on the right shows the fragment of interest, as well as a by-product.

- If prominent secondary bands are present, it is possible that the Gibson Assembly® RapidAMP™ reaction contains populations of both species.
- If fragments are poorly built, continue with downstream analysis of the product. The robustness of the Gibson Assembly® RapidAMP™ technology allows for the amplification of small amounts of circularized DNA. Restriction analysis should be used to confirm the fidelity of the final product.

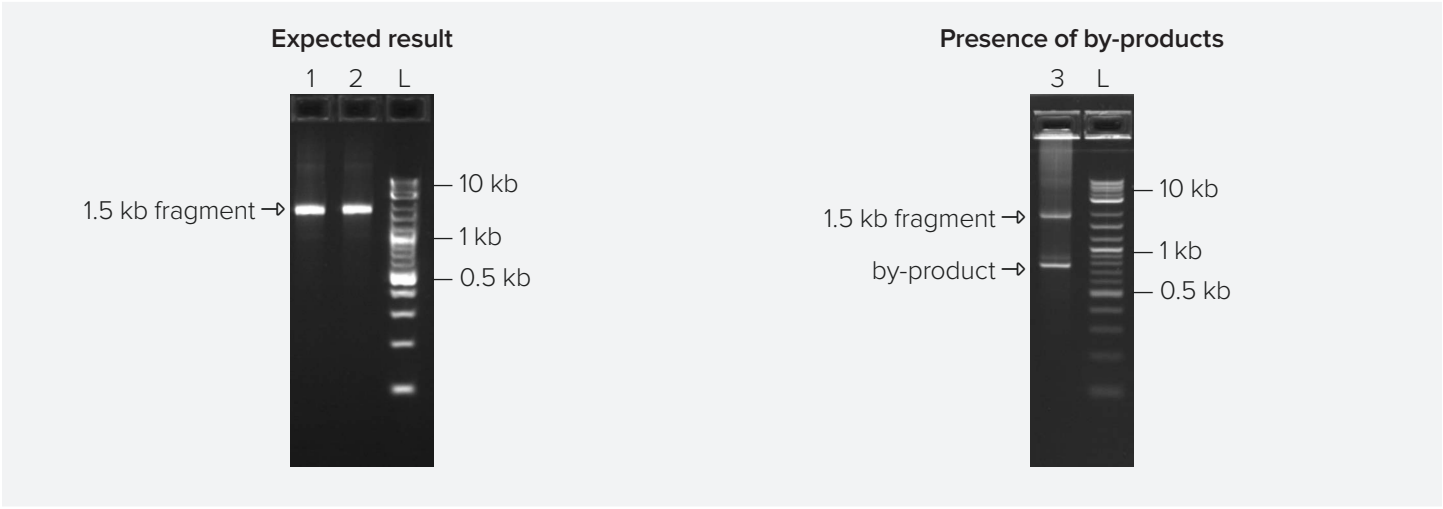
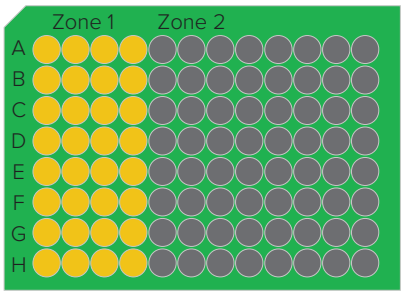


Figure 2. Representative electrophoresis results. Lanes 1, 2, and 3 contain 2 µL of assembled product from 3 different reactions. Lane L is a DNA Ladder.

Location of cloned and amplified product

Final cloned and amplified products are located in zone 1 (positions A1–H3) of the recovery plate



Final product location

Linearization of Gibson Assembly® RapidAMP™ amplified product

We recommend performing a restriction digestion after completion of the Gibson Assembly® RapidAMP™ reaction. The final amplified product is concatenated and highly branched. For best results, reduce to linear monomers by restriction digestion.

The amplified solution may be viscous. For optimal digestion, dilute the product 1:1 with restriction enzyme mix (see below). However, the product can be kept at a higher concentration by adding a more concentrated restriction enzyme mix, increasing the digestion incubation time, and vortexing occasionally during the incubation to ensure efficient digestion. Use your preferred restriction digestion protocol* or follow the generalized directions below.

Note: For complete DNA digestion, incubation times may need to be increased due to the high yield of amplified DNA. Heat inactivate the restriction enzyme if DNA is used in cell-based assays or IVT.

Linearization via restriction digestion

1. Add equal volume (~50 µL) of 1X restriction digest buffer (final buffer concentration will be 0.5X, accounting for presence of buffers in Gibson Assembly® RapidAMP™ reaction) and 2 µL restriction enzyme the Gibson Assembly® RapidAMP™ product. More restriction enzyme volume can be added, but total enzyme volume should remain < 10% (no more than 1 µL enzyme per 10 µL digestion).

Note: A typical restriction enzyme concentration from NEB is 20,000 units/ml. If using restriction enzyme with less units/ml, adjust the enzyme volume according to the enzyme units.

2. Digest at 37 °C for at least two hours. The digestion reaction can be scaled down or up as needed and can go overnight if the enzyme is high fidelity.

3. Heat inactivate the enzyme before going directly into transfection or another cell-based assay.

Optional transformation and sequencing

If clone banking or analyzing error rates, 2 µL of the digestion reaction can be transformed into one of our recommended cell lines, such as MAX Efficiency™ DH5α chemically competent cells, as per the recommended protocols from the manufacturer.

Determine linearized DNA yield (three options)

- **Qubit:** Vortex digestion reaction and then mix 5 µL with 5 µL water. Use 2 µL for BR dsDNA Qubit quantitation. The final concentration of the restriction digested RapidAMP™ product equals the Qubit concentration * dilution factor (2).
- **Densitometry:** Run gel with appropriate volume of digestion reaction against a reference of known quantity.
- **Nanodrop:** Clean up digestion reaction via bead-based or column purification prior to measuring to improve accuracy.

Optional further DNA processing

Purify digested reaction: For transient transfection, either digested and heat-inactivated DNA (straight from step 3 in linearization procedure) or DNA that has been further purified to remove reaction components will be effective. However, for sensitive cell-based applications as well as in vitro transcription, DNA cleanup has been shown to increase performance. We recommend bead-based purification, such as AMPure, to minimize loss of DNA during purification. Column or gel purification is also acceptable although more DNA will likely be lost.

Circularize monomers: Monomers may be circularized after restriction digestion using ligase. Optimization may be required.

For technical assistance, contact help@codexdna.com.