

BioXp™ Gibson Assembly® RapidAMP™ Design Guidelines and Downstream Recommendations

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

Prior to ordering sequences:

1. Design sequences to include a unique restriction site at a desired location. Most customers will want this site to be outside of genes of interest

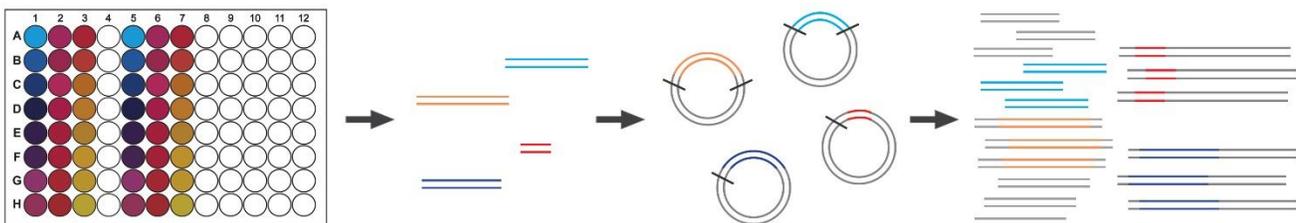
Following a BioXp™ RapidAMP™ Cloning run:

2. Analyze BioXp™ Tile Fragments
3. Linearize Cloned and Amplified Constructs via Restriction Digestion
4. Optional DNA Clean-up
5. Optional Quantification of Amplified DNA Prior to Downstream Applications

Pre-Run Design Recommendations

Most customers will want 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 ensure 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



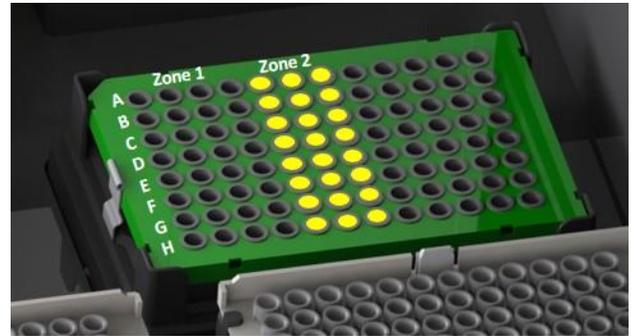
Up to 24 different fragments can be built simultaneously and will be available at the end of a BioXp run in wells A5 - H7. Cloned and amplified product is in wells A1 - H3.

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

Post-Run Analysis

Analyze BioXp™ Tiles

Perform gel electrophoresis of ~5 µL of the uncloned BioXp™ Tiles from Zone 2 (positions A5–H7) of the **Recovery Plate**. The quality of the BioXp™ Tiles is a key indicator of overall assembly and cloning/RapidAMP™ efficiencies.



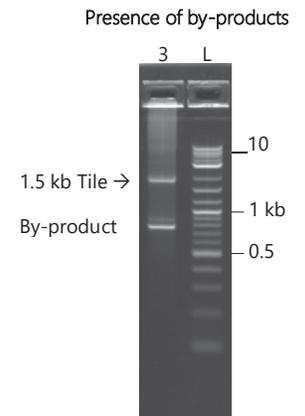
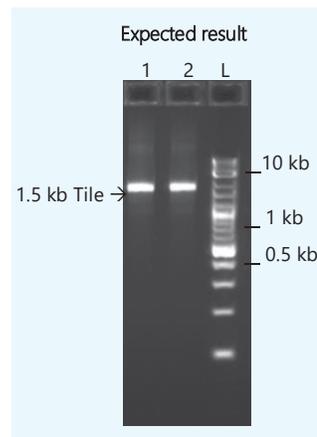
Location of BioXp™ Tiles

Expected Results of BioXp™ Tiles– 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.

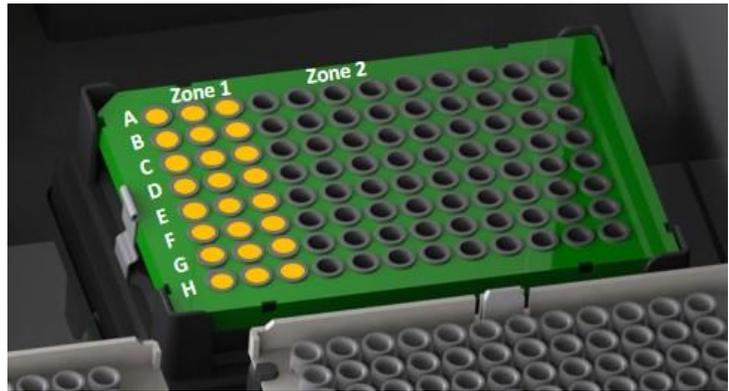
NOTE: If prominent secondary bands are present, it is possible that the RapidAMP™ reaction contains populations of both species.

NOTE: **If tiles are poorly built, continue with downstream analysis of the product.** The robustness of the 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.



Location of BioXp™ RapidAMP™ Cloned and Amplified Product

Final cloned and amplified products are located in Zone 1 (positions A1–H3) of the Recovery Plate.



Location of RapidAMP™ Product

Linearization of RapidAMP™ Amplified Product

We recommend performing a restriction digestion after completion of the 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 μ l) of 1X restriction digest buffer (final buffer concentration will be 0.5X, accounting for presence of buffers in RapidAMP™ reaction) and 2 μ l restriction enzyme RapidAMP™ product. More restriction enzyme volume can be added, but total enzyme volume should remain $\leq 10\%$ (no more than 1 μ l enzyme per 10 μ l 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 2 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 μ L of the digestion reaction can be transformed into one of our recommended cell lines, such as MAX Efficiency™ DH5 α Chemically Competent cells, as per the recommended protocols from the manufacturer.

Determine Linearized DNA Yield (3 options)

- **Qubit:** Vortex digestion reaction and then mix 5 μ l with 5 μ l water. Use 2 μ l 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 clean-up has been shown to increased 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 maybe be required.

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