CUSTOM VECTOR ONBOARDING GUIDE

BEFORE YOU BEGIN

Please ensure you have the following items and information before beginning:

1. A genbank (.gb) file with the complete vector sequence.

- **a.** The sequence must match the material that will be shipped to Twist.
- **b.** If you do not have your vector sequence in the .gb file format, we recommend using SnapGene, A Plasmid Editor, or the plasmid designer software of your choice to generate the required genbank (.gb) file.
- **c.** We also highly encourage you to annotate landmarks in the vector to help you identify the insertion site during the process. Please also make sure to identify the reading orientation of the vector.

2. The desired insertion site location identified.

- **a.** Insertion sites can be at a single base pair location or it can replace an entire section within the vector.

3. Information regarding your vector including antibiotic resistance, copy number, and construct stability.

- **a.** Please ensure the vector is either high copy or medium copy. We do not accept low copy plasmids at this time.
- **b.** Construct stability will guide your selection of optimal E. Coli strain. DH10B-like cells are available for most cloning applications and Stbl3-like cells are available for use with unstable constructs such as lentiviral expression vectors.

4. Please note we cannot accept any vector that has been grown with bacteriophage or that produces bacteriophage.

Note: While you are welcome to flank your insertion site with restriction sites, Twist uses a proprietary homology based cloning method that does not rely upon restriction sites. Therefore, the insertion site does not need to be flanked by restriction sites unless you require this for your downstream applications.

GENERAL PROCESS OVERVIEW

The Online Custom Vector Onboarding Process Consists of 5 General Steps:

1. Uploading your vector sequence

2. Answering questions regarding your vector

3. Selecting the insertion site(s)

4. Review and approval of your selection

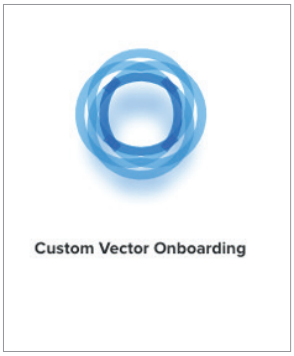
5. Completing your order

You will need to answer a series of questions regarding your vector to ensure that we have all the information needed to process your order correctly and efficiently. We will then ask you to identify the insertion site which can be created from either a single point or by replacing a portion of your vector. A downloadable construct with an example insert at your desired insertion site will be automatically generated which you will review before submitting your order.

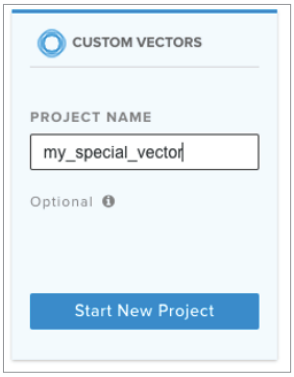
To Get Started:

1. Log into your ecommerce account (https://ecommerce.twistdna.com/app)

2. Locate the “Custom Vector Onboarding” app on the Home page.



3. Click on the “Custom Vector Onboarding” icon and enter a Project Name (optional).



4. After naming the project, click on the “Start New Project” button to proceed to the Custom Vector Onboarding page.

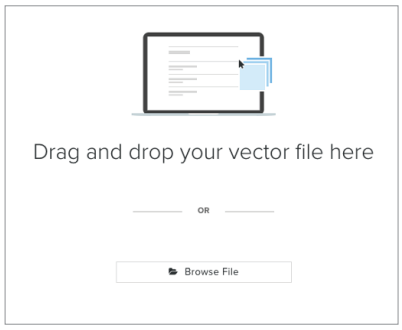
5. Before being able to continue with your draft, you will be presented with a pop-up description of the onboarding process. Once you are ready to continue, click the “Got it” button.

a. Note: This pop-up will only be displayed once. If you need to review the requirements for the onboarding process again, please click on the “Custom Vector Onboarding Guidelines” link below the “+ Onboard New Vector” button on the top right-hand corner.

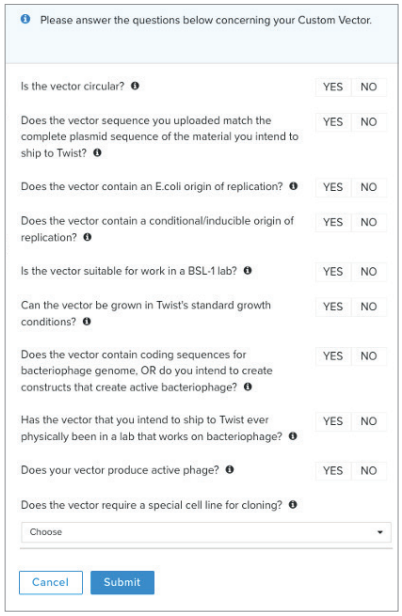
6. You will now be able to start Custom Vector Onboarding by clicking either one of the “+ Onboard New Vector” buttons.

Uploading a Vector and Completing the Vector Details

7. Clicking on the “+ Onboard New Vector” button will open a window where you can upload the genbank (.gb) file of your vector. The file can be uploaded by dragging and dropping the file into this window or alternatively, the file can be selected using the Browse File option. For an example genbank file, please click on “Sample” to download an example vector file to review.



8. Once the upload is complete, a new window with a series of questions will appear. An answer to each of these questions is required to continue.



a. Please answer the questions with a “YES” or “NO” to the best of your knowledge. If you do not know the answer or if it is an unaccepted answer, please reach out to Technical Support via email at customersupport@twistbioscience.com or by using the chat function available on our page.

9. Once the questionnaire is complete, click "Submit" and then click “Continue.”

10. On the next page you can view the annotated vector map and sequence. Please input/confirm the Vector Name, Antibiotic Resistance, Copy Number, and select the *E. coli* strain.

- **a.** Antibiotic resistance: Twist Custom Vector Onboarding is compatible with most common antibiotic resistance cassettes.If your desired antibiotic resistance is not available, please contact Technical Support.
- **b.** Copy Number: Please ensure your Custom Vector is either High Copy or Medium Copy.
- **c.** *E. coli* strain: Twist uses either “DH10B-like” or “Stbl3-like” competent cells.
  - **i.** DH10B-like Cells: suitable for most cloning applications
    - **1.** Genotype: F– mcrAΔ(mrr-hsdRMS-mcrBC) endA1 recA1Φ80dlacZΔM15ΔlacX74araD139Δ(ara,leu)7697galUgalK rpsL nupG λ– tonA (StrR)
  - **ii.** Stbl3-like Cells: suitable for cloning unstable constructs such as lentiviral expression vectors
    - **1.** recA13 supE44 ara-14 galK2 lacY1 proA2 rpsL20(StrR) xyl-5 λ– leu mtl-1 F– mcrB mrr hsdS20(rB–, mB–)

11. A Description box is supplied for your use. The text you add to the Description box will appear in the My Vectors page and provide you and your collaborators a description of your Custom Vector.

- **a.** Note that this Description box has a limit of 500 characters.

12. You have the option to upload a vector map that will be displayed to your collaborators, should you share the custom vector with other Twist users, but do not wish to share the vector sequence.

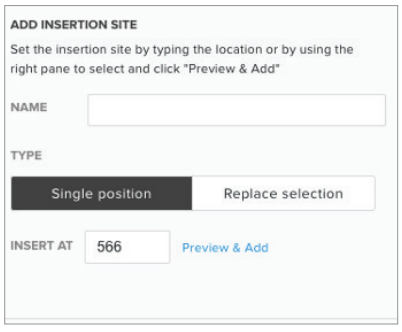
13. Once you have completed this section, click Continue to add insertion site(s).

Selecting an Insertion Site

14. To add an insertion site, start by creating a name for each insertion site request. We suggest a name that you will be able to easily identify in the future.

15. Next, select the type of insertion site by clicking “Single position” or “Replace selection”. Positioning your mouse cursor over each button will display a brief visual of each insertion type.

- **a.** Designing a Single Position Insertion Site
  - **i.** Select “Single position” to create an insertion site at a single position within the vector without removing any vector sequence.
  - **ii.** The easiest way to define a single position insertion site is to either enter a bp location in the available “INSERT AT” box or to go into the “Sequence” tab, navigate the sequence, and click between the two nucleotides where you wish you insert to appear.



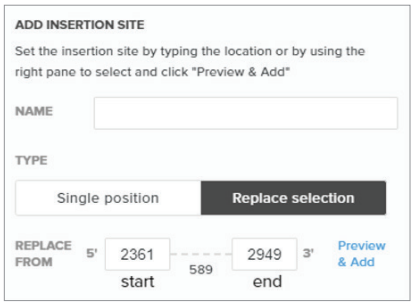
iii. Selecting an insertion site will result in a base pair number appearing in the box.

b. Designing a “Replace Selection” Insertion Site

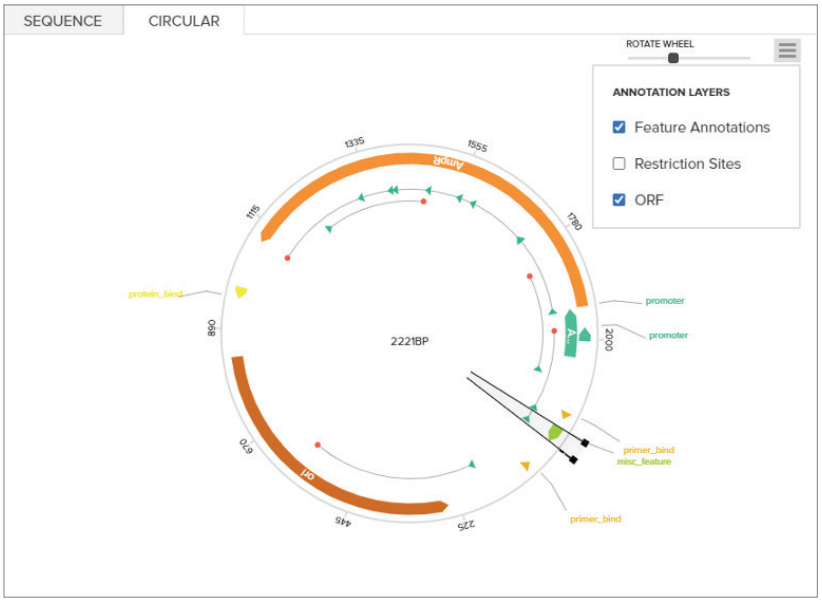
i. Select “Replace selection” to remove a section of vector sequence and replace it with an insertion site.

ii. Designing a “Replace Selection” Insertion Site will replace the portion of vector you select with an insertion site.

iii. To generate an Insertion Site, click on the “Replace Selection” button and input the starting and ending location of the portion of vector you wish to replace with an insertion site.



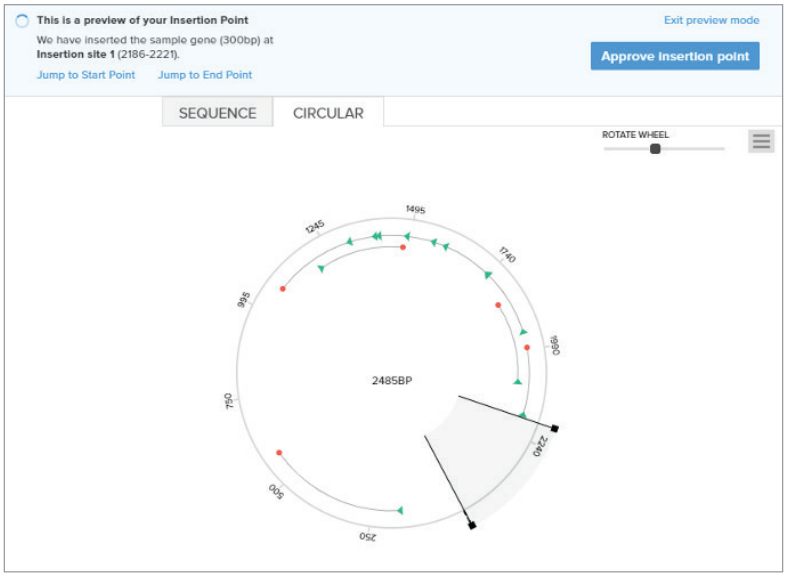
1. Alternatively, you can highlight the portion of sequence you want to replace with an insertion site in the sequence tab. Highlighting sequence will automatically trigger the “Replace Selection” insertion site option.



iv. If you annotated the sequence with the portion of sequence to be replaced, click on the annotation to select that specific sequence.

16. After making your selection, click on the “Preview and Add” link next to the number box, to review the insertion site, either in map form or sequence form.

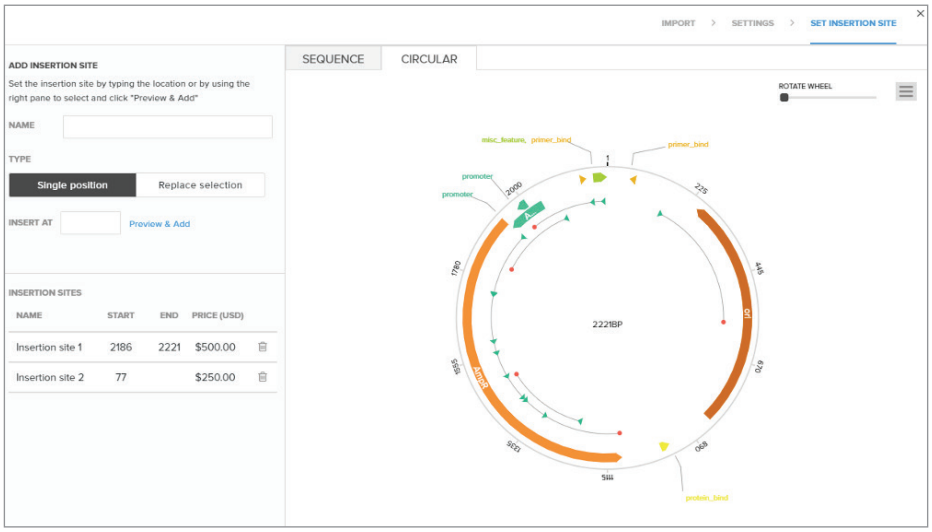
a. Note that at this point, an example 300 bp sequence will be inserted to illustrate what a potential insert would look like at the specified location. This sequence will be highlighted in the preview, with the start and end location numbers identified. This is solely for illustration; we will not synthesize this sequence or clone this sequence into your final custom vector.



i. Example 300 bp sequence: ATGCAGTTTTATTCTCTCGCCAGCACTGTAATAGGCACTAAAAGAGTGATGATAATCATGA GTGCTGAGCTAAGACGGCGTCGGTACATAGCGGTCTTACGGTCAGTCGTAATTCCTCACGAGTCCCGTCCAGTTGAGCG TATCACTCCCAATGTACAAGCAACCCGAGAAGGCTGTGCTTGGAGTCAATCGGATGTAGGATGGTCTCCAGACACGGGG CCACCACTCTTCACGCGTAAAGCAAGAACGTCGAGCAGTCATGAAAGTCTTAGTACCGGACGTGCCGTTTTACTGCGAATA

17. Once you review the insertion site, select “Approve insertion point”. The insertion site details will appear as a row under the “INSERTION SITES” on the left-side of the panel.

a. Note: Multiple insertion sites can be added to each Custom Vector. However, only one insert can be cloned into a vector at a time.



18. At this point, you can either:

a. Click “Continue”, to continue to checkout

b. Click “Back”, to review the vector details once more.

c. Add additional insertion sites.

d. Onboard additional vectors by clicking “+ Onboard New Vector” in the upper right.

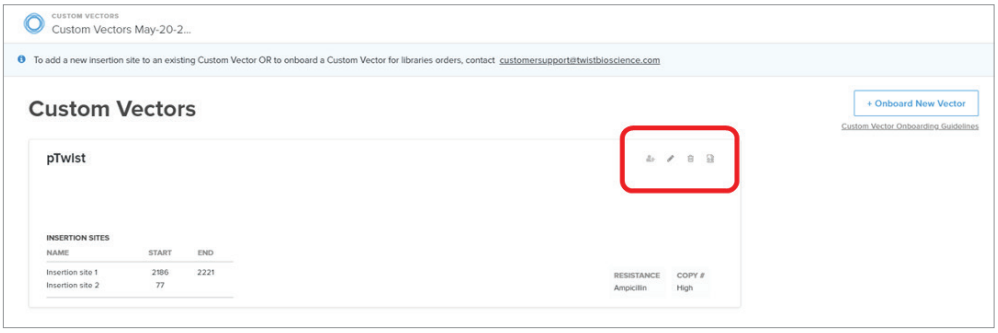
19. If you are ready to continue and check out, please go to step 20.

Generating a Quote

20. Once you have added all the insertion sites you need, you can close the pop-up window by clicking on the “Close” button.

21. In the next window, your custom vector draft will appear.

22. On the top right of the custom vector box, you will see 4 buttons: “*share*”, “*edit*”, “*download*” and “*delete*”. Currently the share functionality is unavailable, but the edit button will allow you to make additional modifications to the draft, the “*download*” button will allow you to download the genbank file with the insertion site(s) annotated, and the delete button would delete the draft. **We highly recommend you download the genbank file to review offline and confirm that the insertion design looks correct.**



23. When you have confirmed that the insertion is correct, you can request a quote by clicking “Get Quote” on the pricing panel.

24. In the “Quote Generation” page, you will need to provide your shipping address. However, this is for internal purposes as you will not be shipped any material at this point.

25. You can now generate a Quote that you can keep for your records or as a document for your procurement department. At this point, your draft is saved, and you can come back later to complete your order.

Checking Out

26. Once you are ready to check out, please complete the billing address and payment options.

27. Once complete, click Check Out to complete your order.

28. We will now email you instructions on how to ship your vector, and once the physical material is received, your onboarding start date and expected completion date.