TWIST TIPS

MEETING MINIMUM LENGTH REQUIREMENTS FOR GENES IN CUSTOM VECTORS

Twist Bioscience uses a proprietary DNA synthesis platform to generate high-quality, NGS-verified, custom gene sequences of 300–5,000 bp in length. Our convenient online ordering platform facilitates the design and ordering of clonal genes. It also offers the option of delivery in a selection of Twist Cloning and Expression Vectors or a custom vector. When you submit a sequence for synthesis, the scoring algorithm checks the sequence to determine whether it can be synthesized, and one of the first things it checks is whether the sequence meets the minimum length requirement of 300 bp.

WHAT IF THE SEQUENCE IS <300 BP?

When synthesizing sequences that are <300 bp, **you can use one of the following two options** to bring the insert size up to 300 bp:

OPTION 1

If possible, add non-coding DNA to either end of the insert

When using the first option, check that your sequence is compatible with non-coding DNA, and that the filler sequence does not interfere with your vector or gene's functionality. For example, if your vector contains gene expression machinery upstream of your defined insertion point, it may not be suitable to add filler sequence at the 5' end of your gene. Additionally, when adding filler sequence to the end of a protein coding gene, ensure your gene has defined at least one stop codon, two if you prefer, to avoid readthrough.

OPTION 2

Transfer a portion of your custom vector sequence to the insert and move the insertion point in the custom vector

- Note that the transfer of vector sequence to the insert sequence creates a new custom vector sequence, which requires a separate onboarding.

Onboarding increases the time to delivery and adds a one-time fee to your order, but the guidelines provided here will help you get the most out of your custom vector and maximize the chances for successful synthesis. Any changes to your strategy after onboarding has begun will require onboarding to be performed again, incurring additional fees.

TRANSFERRING SEQUENCE FROM THE VECTOR

To increase the insert size by incorporating a portion of the vector sequence, add the vector sequence to the insert in any configuration. For example, if you have a 100 bp insert, add 200 bp of the sequence from your custom vector by either adding 75 bp to the 5' end and 125 bp to the 3' end or by adding 100 bp to each end.
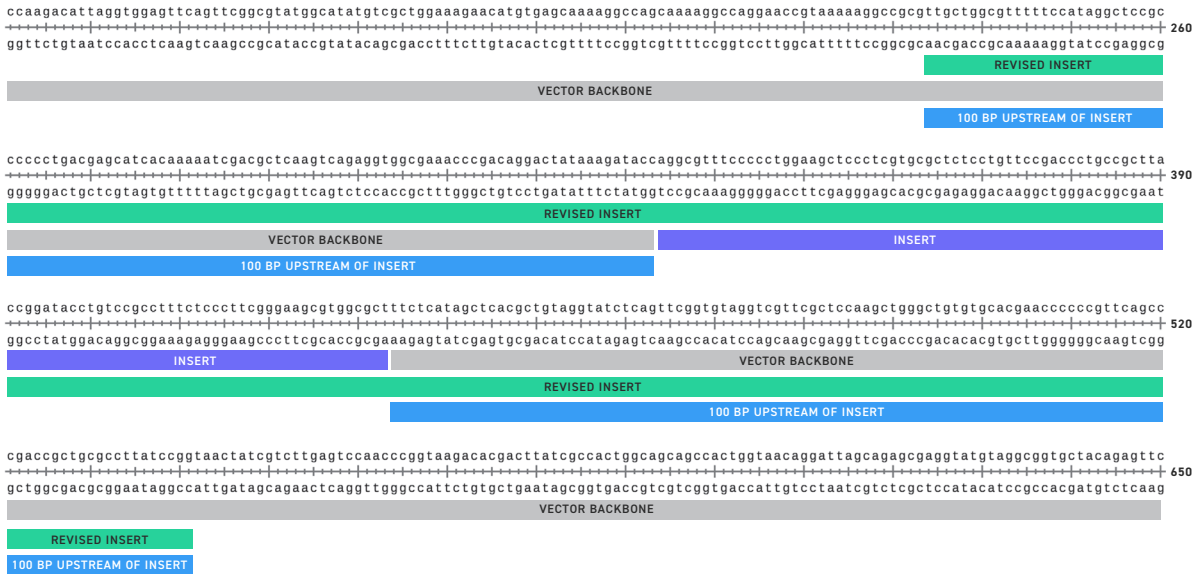

Adding vector sequence to increase insert size to 300 bp. The 100 bp insert in this example (purple) is located at the desired insertion point within a custom vector. To increase the insert size, 100 bp sequences from the vector (blue) are added to the insert sequence to generate a new 300 bp insert (green) and a revised sequence for the custom vector.

Avoid using vector sequences that contain features such as origins of replication, restriction enzyme sites, promoters, terminators, tags, and antibiotic resistance cassettes when adding to the insert. These features can cause synthesis failures because they often contain high GC regions, repeats, and homopolymers that can be difficult to synthesize.

CREATING A NEW CUSTOM VECTOR INSERTION SITE

The sequences you add to your insert to achieve the minimum size requirement are removed from the vector sequence, creating a new custom vector backbone sequence. Include the sequences in the **Insert** and **Vector + Insert** sequences in the Custom Vector Onboarding Form, which can be found by clicking **Custom Vector Onboarding** in our online ordering platform.

For more details, watch the Custom Vector Submission Tutorial or contact the Twist Support Team at customersupport@twistbioscience.com.
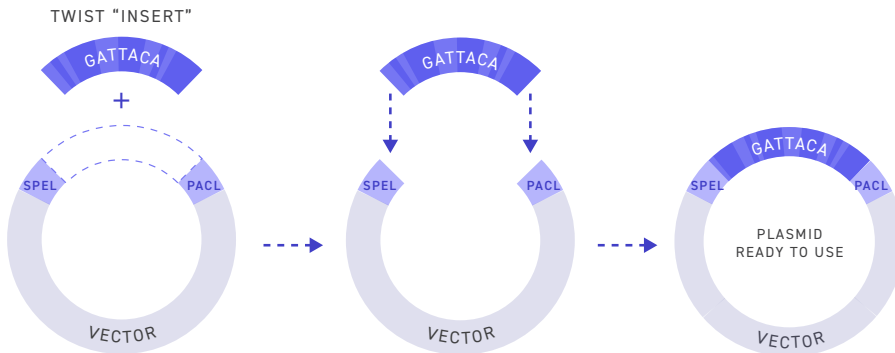
To minimize the need for additional onboarding, add the same upstream and downstream sequence to each new insert when using that same custom vector. If you need to modify your strategy for that vector after the onboarding process is initiated, the new strategy will require additional onboarding.

Twist synthesizes the gene sequence as submitted. Use the "export table" function to check your sequences before finalizing your order:

Once the csv file is downloaded, your insert sequence is listed in column H and the vector + insert sequence is listed in column I:

NAME	INSERTION POINT NAME	VECTOR NAME	INSERT LENGTH	VECTOR LENGTH	CONSTRUCT LENGTH	INSERT SEQUENCE	CONSTRUCT SEQUENCE	SCORE
Test_Gene	BamHI_XhoI	pET-24(+)	1447	5198	6645	TTGGGTACCGGG...	TTGGGTACCGGGCC...	STANDARD

For more help in optimizing the design of your gene sequences, please contact the Twist Support Team at customersupport@twistbioscience.com or through our online ordering platform.

These Products are subject to certain use restrictions as set forth in Twist's Supply Terms and Conditions
www.twistbioscience.com/supply-terms-and-conditions

CONTACT US sales@twistbioscience.com or visit twistbioscience.com