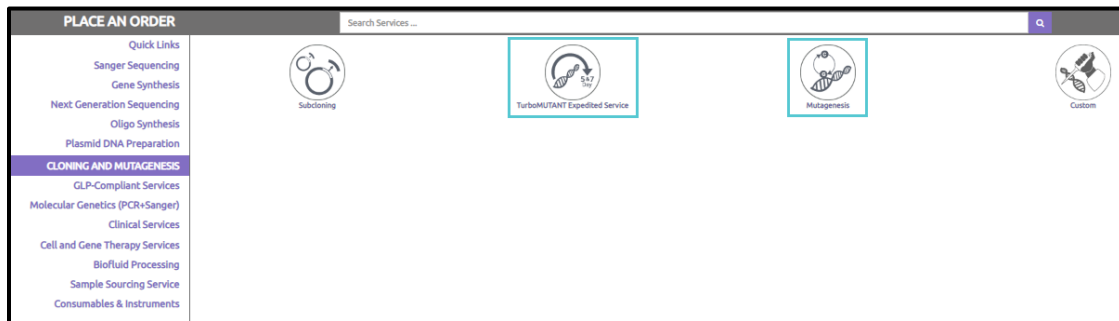


## MUTAGENESIS ORDERING GUIDE

### GETTING STARTED

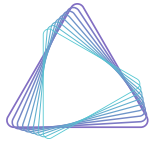
Log into your online Azenta account → Select “Cloning and Mutagenesis” tab on the left side → Select either the “Mutagenesis” or “TurboMUTANT” bubble.



### ORDER INFORMATION

The top of the form is composed of five optional fields (order name, order comments, promotion code, and coupon code) and two required fields (Total # of sequences and Service Priority). Additionally, there is an option to submit any documents with your inquiry.

Click “?” next to the fields for additional information or click “? Help” on the right-hand side for help options.



## Step 1: Sequence

STEP 1: SEQUENCE		Step 2: Mutagenesis requirements	Step 3: Codon Optimization	Step 4: DNA Preparation			
Mutant Name	Starting Template Name	Starting Template Size (total, in bp)	Starting Template Whole Plasmid Sequence	Antibiotic Selection	Other Antibiotic	My Vector is at Azenta	Tracking #
EXAMPLE	EXAMPLE	123	AGTGGGGGGA	Ampicillin	My Antibiotic	No	

This tab will include all information pertaining to the template plasmid.

**Mutant Name:** Please enter the name for your final construct.

**Starting Template Name:** Please enter the name of the starting template plasmid.

**Starting Template Size:** Please enter the size (in bp) for your template plasmid.

**Starting Template Whole Plasmid Sequence:** Please enter the full reference sequence of the starting template plasmid *before mutagenesis*.

**Antibiotic Selection:** Please enter the antibiotic resistance for template plasmid. If not listed within the dropdown menu, please select the option "Other" and list the antibiotic resistance within the field *Other Antibiotic*.

**My Vector is at Azenta:** Has this vector been used for, or generated in a previous Gene Synthesis, Cloning, or Mutagenesis order? To note that these vectors are at our facility, please select Yes and enter the original tracking number (30-XXXXXXXX) for this previous order within field Tracking #.

*Helpful note: we store any starting material provided, or final constructs generated at our facility for up to **two years** to be used for any future orders. For more information, please find our [Sample Storage Policy here](#).*

## Step 2: Mutagenesis Requirements

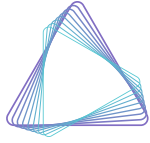
Step 1: Sequence	STEP 2: MUTAGENESIS REQUIREMENTS		Step 3: Codon Optimization	Step 4: DNA Preparation				
Mutant Name	Sequence Confirmation of Template Insert Prior to Mutagenesis	Sequence Type	Sequence of the Insert prior to mutagenesis	Will the sequence of the Insert post mutagenesis be provided?	Sequence of the Insert post mutagenesis	ChangeMutation	DeleteMutation	InsertMutation
EXAMPLE	No	DNA	Double click to select...	No	ATGC	AATATCCAAANAKKCC	ATCCGAGAG	ATGGCCCCCTC

This tab will include all information pertaining to the desired mutation requirements.

**Sequence Confirmation of Template Plasmid Prior to Mutagenesis:** Do you require sequence verification of your desired region of interest (containing the mutation location) prior to performing mutagenesis? Should the team observe any discrepancies compared to the provided reference sequence for your template plasmid you will be notified via email. For reference, this is an *optional* step. If not required, please select "no" from the dropdown menu.

**Sequence Type:** Will the desired mutation be noted in nucleic acid or amino acid format?

**Sequence of Insert Prior to Mutagenesis:** The region of interest containing the mutation location. Please note, this should reflect the original sequence prior to mutagenesis. We will perform sequence verification of this region post mutagenesis.



**Will the Sequence of the Insert Post Mutagenesis be provided?:** *Optional field.* Should you have the final nucleotide sequence for the region of interest post mutagenesis, please feel free to select "Yes".

**Sequence of the Insert Post Mutagenesis:** *Optional field.* If "Yes" was selected for the previous field, please enter the final nucleotide sequence in this field.

### How to enter your desired mutation:

Please note, the mutation location is in reference to the insert sequence entered. Not in reference to the full template plasmid sequence.

**For substitution mutations,** please enter in the format [original sequence][starting position][mutant sequence].

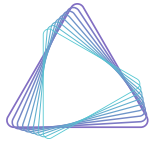
- Example: R23A. The mutant construct will contain one amino acid change, "R" replaced by "A" at amino acid position 23bp in the insert sequence.
- Example: AAA1CCC; AAAA20CCC. The mutant construct will contain two changes, "AAA" replaced by "CCC" starting at position 1 and "AAAA" replaced by "CCC" starting at position 20bp in the insert sequence.

**For deletion mutations,** please enter in the format [starting base/amino acid and position] – [ending base/amino acid and position]

- Example: R23-R23. The mutant construct will contain one amino acid deletion, "R" deleted at amino acid position 23bp in the insert sequence.
- Example: A2-C5 C1-A20. The mutant construct will contain two deletions, from "A" at position 2 to "C" at position 5, and from "C" at position 1 to "A" at position 20bp in the insert sequence..

**For insertion mutations,** please enter in the format [sequence to be inserted][starting position].

- Example: R23. The mutant construct will contain one amino acid insertion, "R" added after amino acid position 23bp in the insert sequence.
- Example: ATCG2 CCCC10. The mutant construct will contain two insertions, "ATCG" added after position 2 and "CCCC" added after position 10bp in the insert sequence.



## Step 3: Codon Optimization

Step 1: Sequence	Step 2: Mutagenesis requirements	STEP 3: CODON OPTIMIZATION	Step 4: DNA Preparation
Mutant Name *	Codon Optimization	Expression Host *	Other Expression Host
EXAMPLE	Yes	Homo Sapien	My Expression
1			Restriction Enzyme to Avoid AatII Double click to select...

This tab will include all information pertaining to Codon Optimization if “amino acid” was selected as the sequence type.

**Expression host:** Desired species for expression. This way, we can ensure the resulting mutation is optimized for this species.

**Restriction Enzymes to Avoid:** Are there any restriction sites you would like not included as a result of the mutation? Enter these sites here.

## Step 4: DNA Preparation

Step 1: Sequence	Step 2: Mutagenesis requirements	Step 3: Codon Optimization	STEP 4: DNA PREPARATION
Mutant Name *	Scale *	Endotoxin Free (applicable for large scale preps only) *	
EXAMPLE	Maxi	Yes	
1	Mini Scale, free of charge	No	

Would you like large-scale DNA preparation as an add-on? This information will be noted here.

For our anticipated yields, kindly see [here](#).

## Next Steps?

After you submit your inquiry, a member of our Project Management team will review the details of your project. Typically, we expect to provide a non-obligation quotation to your account within one business day. Should we require any additional information, you will be contacted promptly via email.

## Any Questions?

Please feel free to contact a member of our Project Management team by emailing us at [GS@Azenta.com](mailto:GS@Azenta.com), or giving us a call at 1- 908-222-0711 ext. 3 (United States) or +49-341 520 122-41 (Europe/UK).