



GeneArt® Gene Synthesis
Online Portal Tutorial

Introduction

This tutorial is intended to help new users with placing an order for gene synthesis services, including the steps needed for gene optimization prior to placing an order. Video tutorials are also available at www.lifetechnologies.com/genesynthesis

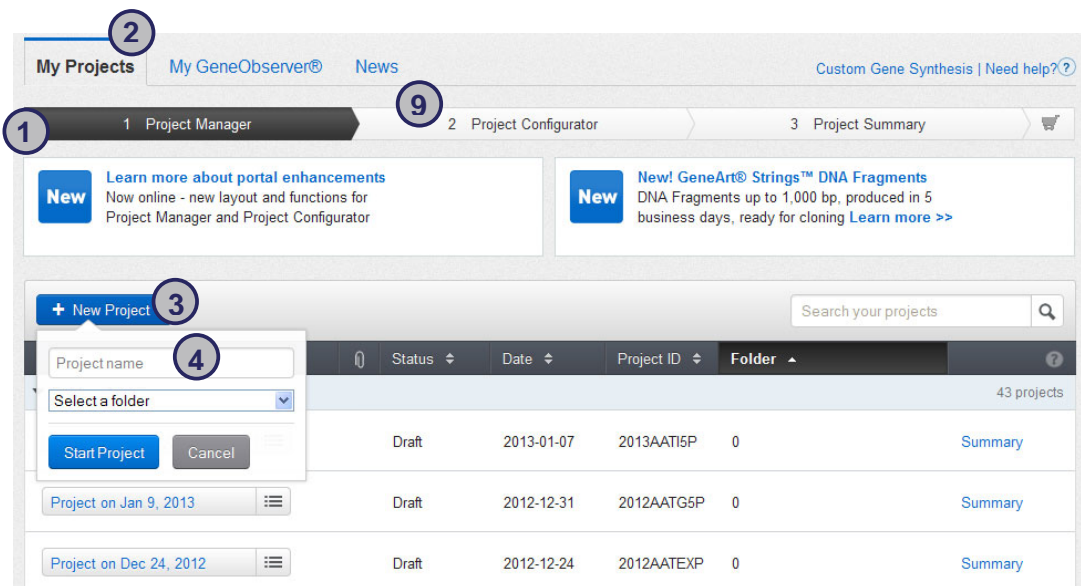
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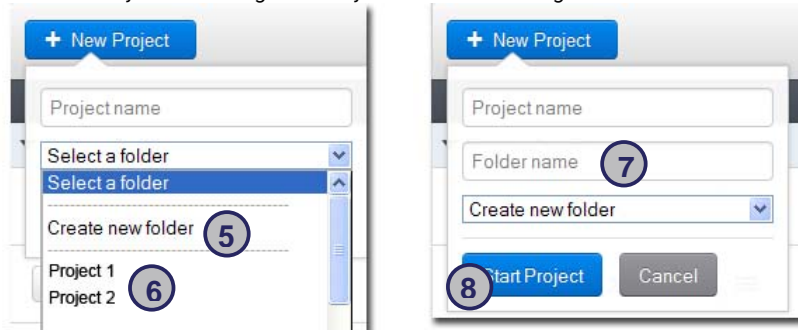
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1. Setting Up a Project

1.1. How to Start an Online Request

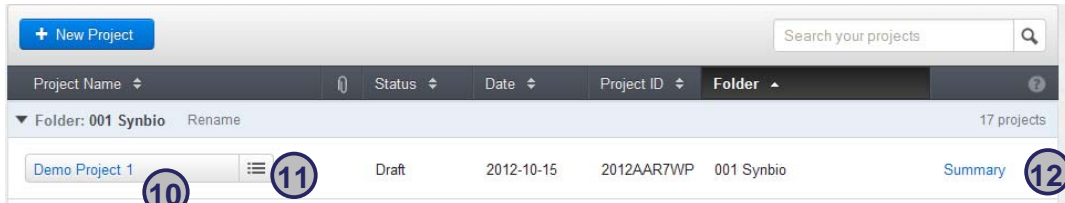


- After login, your starting screen will be the “Project Manager” (1) on the “My Projects” tab (2). The Project Manager will allow you to create new projects and organize existing ones.
- For any kind of GeneArt® online service, first click the button “+ New Project” (3), name your project (4), and then click “Select a folder”.
- On the subsequent dropdown menu you can either choose “Create new folder” (5) or select from your existing ones if you are a returning user (6).

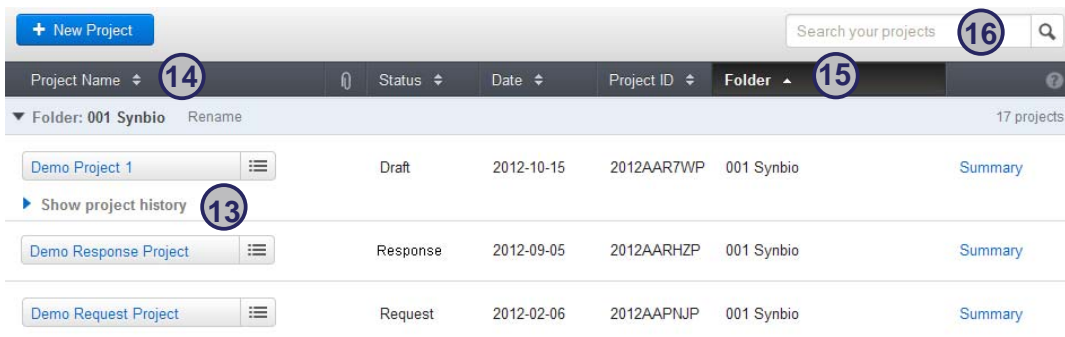


- If you are creating a new folder, the next step is to enter a “Folder name” (7).
- Then click the “Start Project” button (8). You will be directed to the “Project Configurator” (9), where you can design the service parts of your new project and enter your requirements.
- You can go back to the “Project Manager” step by clicking on the “My Projects” tab at any time (2).

1.2 Project Manager Features to Organize Projects



- Click this button (10) to edit or view your project in the Project Configurator.
- Hover over (11) to display a menu where you can rename, move, or delete your project. You can also add attachments.
- Click (12) to immediately move to the project summary, e.g., for ordering.

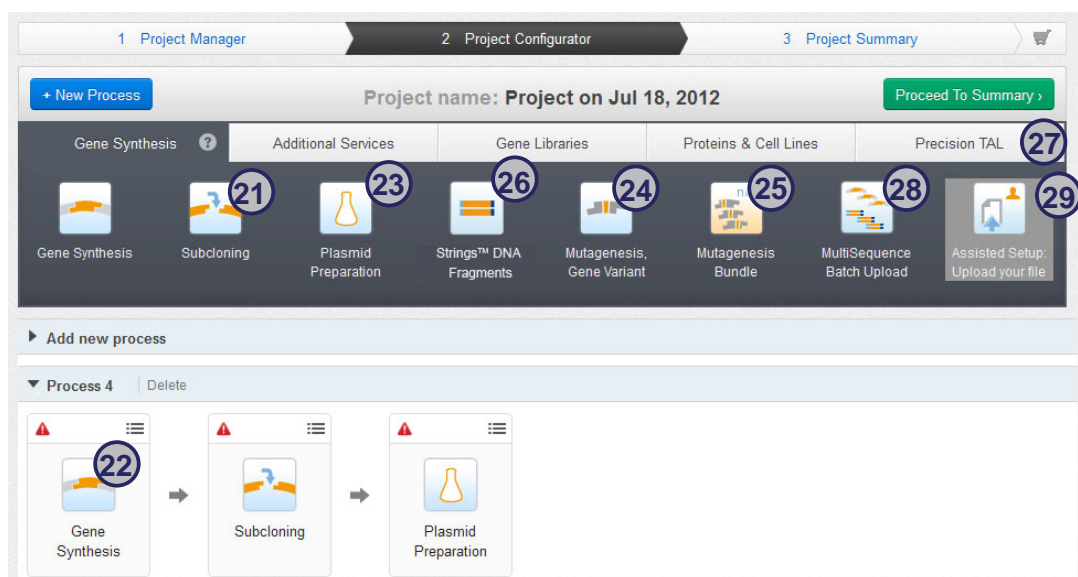


- Some projects will require review by our specialists, and different project versions will be created during this review. To see a chronological history of the status points in the project, click "Show project history"(13).
- Click Project Name, Status, Date, Project ID, or Folder in the header bar (14) to sort your projects by that group. You can toggle the sort order by clicking the same group header again.
- The current active sort group is indicated by darker highlighting (15).
- To search, type a project ID, project name, folder name, or gene name (case-insensitive, no asterisks) here (16).

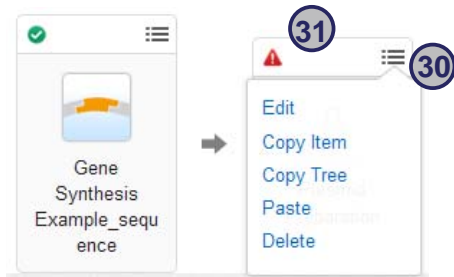
1.2. How to Use the Project Configurator for Project Setup, e.g., Gene Synthesis

The screenshot displays the 'Project Configurator' interface. At the top, there are three tabs: '1 Project Manager', '2 Project Configurator' (which is active), and '3 Project Summary'. Below the tabs, a header bar shows '+ New Process' on the left, 'Project name: Project on Jul 18, 2012' in the center, and 'Proceed To Summary >' on the right. A horizontal menu below the header lists five service groups: 'Gene Synthesis' (with a question mark icon), 'Additional Services', 'Gene Libraries', 'Proteins & Cell Lines', and 'Precision TAL'. Underneath this menu is a grid of eight service icons: 'Gene Synthesis', 'Subcloning', 'Plasmid Preparation', 'Strings™ DNA Fragments', 'Mutagenesis, Gene Variant', 'Mutagenesis Bundle', 'MultiSequence Batch Upload', and 'Assisted Setup: Upload your file'. A blue callout box with a bell icon contains the text: 'Start by creating your first process, as described below. Add another process as required. This can help save shipping fees. All of the processes together make up the project. Please note that each project requires a new, separate cart..'. Below this is a section titled 'Process 1' with a dropdown arrow. On the left of this section is a dashed box containing a 'Gene Synthesis' icon and the number '20', with the text 'Drag a service icon here.' below it. To the right of this box is the heading 'How to get started' followed by instructions: 'Start by dragging a product or service icon from the menu above to the box at left. Click the icon to enter required information. You can drag & drop dependent products or services onto what you already created (e.g. Subcloning onto Gene Synthesis). Once your set-up is complete, proceed to summary. □Add to cart□ then allows price verification before continuing to checkout and ordering. Some projects require specialist review before they can be ordered. For these, please proceed with □Send for Review□ on the project summary page.'

- The Project Configurator displays all available services as drag & drop icons (19), organized in tabs of service groups, e.g., Gene Synthesis (17). To receive more information about each service, and how to use it, click the question mark (18).
- To start a gene synthesis project, drag the Gene Synthesis icon (19) onto the drop area (20).



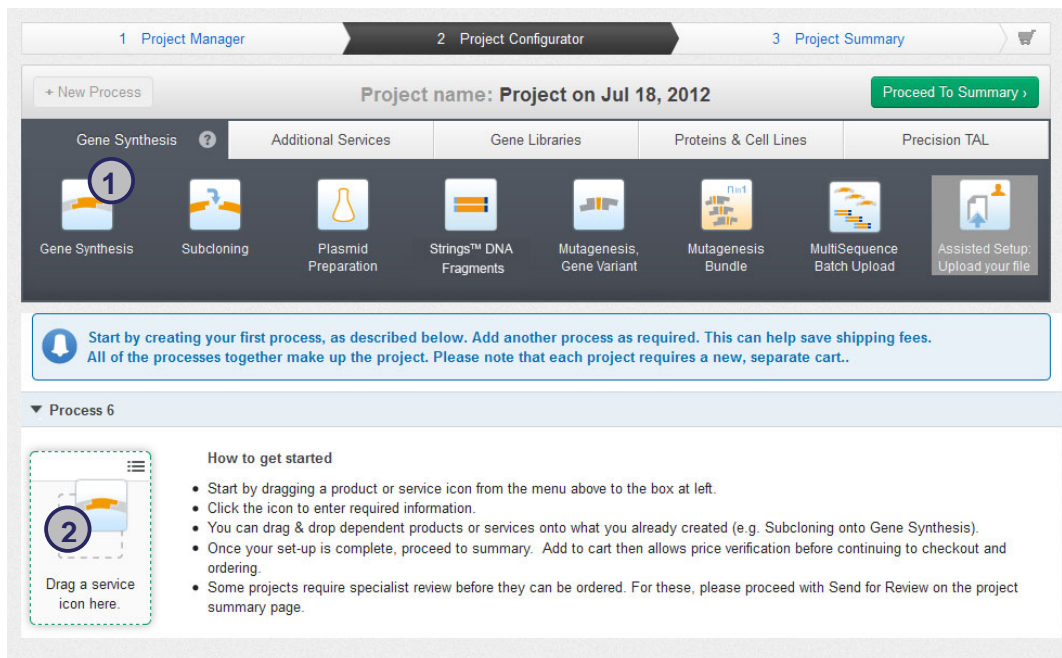
- To order additional services for your project, such as subcloning (21), drag and drop the icon for the service you need onto the Gene Synthesis icon that you created in the Process area (22).
- If you require larger amounts of your clone DNA, you can add the Plasmid Preparation service (23). If you want us to only produce your plasmid DNA, start a Process with just the Plasmid Preparation icon.
- Add any additional services the same way. Allowed services will be highlighted by a green frame when mousing over an icon in the upper panel.
- If you require site-directed mutagenesis or gene variations, use the Mutagenesis icon (24). For larger numbers of constructs, use the Mutagenesis Bundle icon (25).
- If you need ready-to-clone gene fragments (a fast and affordable alternative to full gene synthesis), use the Strings™ DNA Fragments icon (26).
- For precise targeting and editing of a genome, you can request clones for custom DNA-binding proteins with various effectors, using the Precision TAL icon on the Precision TAL tab (27).
- We also offer the option to enter several sequences at once using the MultiSequence Batch Upload icon (28).
- For assisted setup with the help of our specialists or for projects that need a higher level of customization, please use the Assisted Setup icon (29), which is a file upload function (supported file formats: doc, docx, xls, xlsx, ppt, pptx, pdf, zip, rar, gb, fas, jpg, jpeg, bmp, png, gif, tif, tiff, and txt). As much as possible, we try to process projects via the automated ordering on the online portal. For Assisted Setup, additional charges may apply.



- Each icon comes with a menu of functions (30) to help you enter and edit your information (“Edit”) or organize parts of your projects. “Copy Item” allows you to copy a service that already has been created and defined (e.g., subcloning) and apply it to another item (e.g., a gene) by “pasting” it into the target menu.
- All icons are accompanied by a status indicator in the top left corner, for a status overview.
 - ✓ A green check mark indicates that a setup is complete, i.e., has all information required to technically process the request. Please ensure that you have added any individual information and requirements nevertheless. You can then “Proceed To Summary”.
 - ⚠ A red exclamation mark (31) lets you know that more information about the requested service is needed. Please click the icon to open the service form and complete it.
The red status might also appear if you have copied a service. To address this, you simply open the form, then save and close it again without adding any information. In addition, if some service information is changed the dependent services might need to be validated again. Please open the form, adjust relevant information if necessary, and then save and close it. If no changes are required, just save and close it.
 - ⓘ A yellow status indicator lets you know that, in its current form, your project is not suitable for immediate ordering.
Subcloning: Please review cloning information for errors.
Mutagenesis: Does not fulfill variant criteria; you may need the Gene Synthesis service.
In either case, you can “Proceed To Summary” to submit for specialist review.
- Our “Gene Synthesis and Online Optimization” tutorial in chapter 2 covers the details of completing the Gene Synthesis service request form, including how to complete online sequence optimization for maximum expression in your host.
- Other tutorial chapters cover ordering of our Subcloning service (chapter 3) and Mutagenesis with Single and Multiple Gene Variants (chapters 4 and 5).

2. Gene Synthesis and Online Optimization

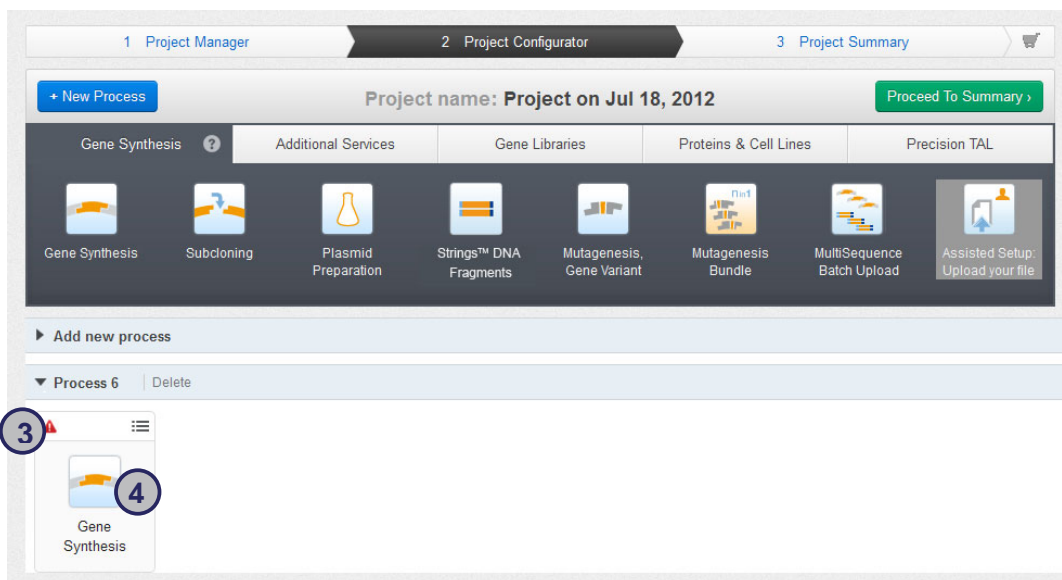
2.1. Gene Synthesis Project Setup



The screenshot shows the 'Project Configurator' interface for 'Project on Jul 18, 2012'. The top navigation bar includes '1 Project Manager', '2 Project Configurator', and '3 Project Summary'. A '+ New Process' button is on the left, and a 'Proceed To Summary >' button is on the right. The main menu contains several service icons: Gene Synthesis (circled 1), Subcloning, Plasmid Preparation, Strings™ DNA Fragments, Mutagenesis, Gene Variant, Mutagenesis Bundle, MultiSequence Batch Upload, and Assisted Setup: Upload your file. Below the menu is a blue notification box with a bell icon and text: 'Start by creating your first process, as described below. Add another process as required. This can help save shipping fees. All of the processes together make up the project. Please note that each project requires a new, separate cart..'. Underneath is a section for 'Process 6' with a 'How to get started' heading and a list of instructions. A circled '2' points to a dashed box labeled 'Drag a service icon here.'

- Start by dragging a product or service icon from the menu above to the box at left.
- Click the icon to enter required information.
- You can drag & drop dependent products or services onto what you already created (e.g. Subcloning onto Gene Synthesis).
- Once your set-up is complete, proceed to summary. Add to cart then allows price verification before continuing to checkout and ordering.
- Some projects require specialist review before they can be ordered. For these, please proceed with Send for Review on the project summary page.

- To begin, drag and drop the Gene Synthesis icon (1) onto the drop area (2).



The screenshot shows the 'Project Configurator' interface after the 'Gene Synthesis' icon has been dragged into the drop area. The 'Add new process' section is expanded, showing 'Process 6' with a 'Delete' button. The 'Gene Synthesis' icon is now in the drop area, circled with a '4'. A red exclamation mark is next to the icon, circled with a '3'.

- The red exclamation mark (3) indicates that more information is needed.
- Click the Gene Synthesis icon to open the form (4).

2.2. Entering a Sequence That Does Not Need Any Editing or Optimization

The screenshot shows the '1. Enter Sequence' tab of a Gene Synthesis form. The form is divided into several sections:

- Sequence name *** (5): A text input field.
- Sequence type *** (6): Radio buttons for 'DNA' (selected) and 'Protein'.
- Enter sequence *** (7): A large text area for pasting the sequence.
- Biosafety classification *** (8): Radio buttons for 'Level 1' and 'Level 2'.
- General information** (9): Checkboxes for 'Sequence encodes antibiotic resistance', 'Sequence contains ori', and 'Sequence encodes toxic protein'.
- Add-on options (small additional fees apply)**: A dropdown for 'Vector resistance choice' (set to 'No preference') and a checkbox for 'TSE-free production'.
- Special requirements request**: A checkbox with a help icon.
- Add service**: Checkboxes for 'Mutagenesis', 'Subcloning', 'Plasmid Preparation', 'Stockagar', 'Glycerol Stock', and 'Extended Documentation'.

At the top, there are tabs for '1. Enter Sequence', '2. Edit Sequence (optional)', '3. Optimize Sequence (optional)', 'Sequence Summary' (10), and 'Save & close' (11).

- The Gene Synthesis form has three main tabs: the Enter Sequence tab, the Edit Sequence tab, and the Optimize Sequence tab.
- The Enter Sequence tab is for entering the sequence and providing all basic information.
- Enter your sequence name (5), choose whether to enter your sequence as DNA or protein (6), and paste your sequence into the tool (7).
- Select the biosafety level for your project (8).
- The lower part of the form (9) allows you to add general information about your sequence and select additional service options.
- Please note that speed upgrades can be chosen later in the process on the project summary page, before you add your project to the cart.
- The Enter Sequence tab is the only tab you need to complete if your sequence is already defined and ready to order.
- At any time you can review your sequence in the Sequence Summary tab (10). Here you can download the sequence in different formats, as well as a printable PDF version of the sequence summary; see chapter 2.4.
- If no further edits or optimization are required, you can proceed to order by clicking the “Save & close” button (11).
- For checkout and ordering, please read the “Adding a Project to Cart and Placing Your Order” tutorial in chapter 7 to see how that process works.

2.3. Editing and Optimizing a Sequence

2.3.1. Defining the ORF in Preparation for Optimization

The screenshot displays a software interface with four tabs: "1. Enter Sequence", "2. Edit Sequence (optional)", "3. Optimize Sequence (optional)", and "Sequence Summary". A "Save & close" button is located in the top right corner. The "2. Edit Sequence" tab is active, showing a sequence editor with a text area containing DNA sequence data. The sequence is displayed in a grid format with line numbers on the left (1, 51, 101, 151, 201, 251, 301, 351, 401) and amino acid translations above the sequence. A search bar at the top right of the sequence area shows "Cursor Position: 0" and "Sequence Length: 594bp".

On the left side, there is a sidebar with a "Detected ORFs" section. It shows a list of ORFs, with one selected: "7 to 588". Below this, there are fields for "Start codon" and "Stop codon", with a "Show more" link. A "Feature Map" section at the bottom left shows "Protected sites (0)", "ORF (1)", "Protected areas (0)", and "Motifs to avoid (0)". The ORF (1) is highlighted with a red 'x' icon.

Numbered callouts (12-16) are placed on the interface to highlight key features:

- 12: Points to the "Detected ORFs" section.
- 13: Points to the "Define ORFs" section.
- 14: Points to the "Show more" link.
- 15: Points to the "Start codon" and "Stop codon" fields.
- 16: Points to the "Feature Map" section.

- The Edit Sequence tab is required to edit and prepare your sequence for optimization.
- Start here to choose (12) or define (13) the ORF you need for optimization. If your ORF is not displayed, click the "Show more" link (14).
- You can also add start and stop codons to your ORF (15).
- All your annotations (ORF, cloning sites, etc.) are shown in the Feature Map (16).

2.3.2. Selecting 5' and 3' Cloning Sites

1. Enter Sequence | **2. Edit Sequence (optional)** | 3. Optimize Sequence (optional) | Sequence Summary | Save & close

ORF

5' Cloning Sites **17**

Detected Sites Add New Sites / UTR

Selected sites will remain unchanged and will not be created within the ORF during optimization.

18 1-6 BamHI [GGATCC]
 5-10 NcoI [CCATGG]
[+ Show more](#)

Example_sequence Cursor Position: 0 Sequence Length: 594bp

1. GGATCCATGGGGGTGCACGAATGTCCTGCCTGGCTGTGGCTTCTCCTGTC
 51. CCTGCTGTGCTCCCTCTGGGCCTCCCAGTCTGGGGCGCCCCACCACGCC
 101. TCATCTGTGACAGCCGAGTCTGGAGAGGTACCTCTTGGAGGCCAAGGAG
 151. GCCGAGAATATCACGACGGGCTGTGCTGAACACTGCAGCTTGAATGAGAA
 201. TATCACTGTCCCAGACACCAAAGTTAATTTCTATGCCTGGAAGAGGATGG
 251. AGGTCGGGCAGCAGGCCGTAGAAGTCTGGCAGGGCCTGGCCCTGCTGTGC
 301. GAAGCTGTCTGCGGGGCCAGGCCCTGTTGGTCAACTCTTCCCAGCCGTG
 351. GGAGCCCTGCAGCTGCATGTGGATAAAGCCGTCAGTGGCCTTCGCAGCC
 401. TCACCACTCTGCTTCGGGCTCTGGGAGCCCAGAAGGAAGCCATCTCCCT
 451. CCAGATGGGCCCTCAGCTGCTCCACTCCGAACAATCACTGCTGACACTTT
 501. CCGCAAACCTCTCCGAGTCTACTCCAATTTCTCCGGGAAAGCTGAAGC
 551. TGTACACAGGGGAGGCCTGCAGGACAGGGGACAGATGAAAGCTT

3' Cloning Sites **17**

Add/Delete/Exchange

Feature Map (0)

- To annotate cloning sites (17), just select from the detected ones (18) or select from a list (19) or define new ones yourself (20).
- Selected sites will remain unchanged during optimization, and the motif will not get generated in the optimized sequence.

1. Enter Sequence | **2. Edit Sequence (optional)** | 3. Optimize Sequence (optional) | Sequence Summary | Save & close

ORF

5' Cloning Sites

Detected Sites Add New Sites / UTR

Choose enzyme from dropdown menu and enter position below. Or, click on the positions in the sequence at right.

- **19**

5' of nucleotide: 1 **20** Add

Enter custom sequence

5' of nucleotide: 1 Add

Example_sequence Cursor Position: 0 Sequence Length: 594bp

1. GGATCCATGGGGGTGCACGAATGTCCTGCCTGGCTGTGGCTTCTCCTGTC
 51. CCTGCTGTGCTCCCTCTGGGCCTCCCAGTCTGGGGCGCCCCACCACGCC
 101. TCATCTGTGACAGCCGAGTCTGGAGAGGTACCTCTTGGAGGCCAAGGAG
 151. GCCGAGAATATCACGACGGGCTGTGCTGAACACTGCAGCTTGAATGAGAA
 201. TATCACTGTCCCAGACACCAAAGTTAATTTCTATGCCTGGAAGAGGATGG
 251. AGGTCGGGCAGCAGGCCGTAGAAGTCTGGCAGGGCCTGGCCCTGCTGTGC
 301. GAAGCTGTCTGCGGGGCCAGGCCCTGTTGGTCAACTCTTCCCAGCCGTG
 351. GGAGCCCTGCAGCTGCATGTGGATAAAGCCGTCAGTGGCCTTCGCAGCC
 401. TCACCACTCTGCTTCGGGCTCTGGGAGCCCAGAAGGAAGCCATCTCCCT
 451. CCAGATGGGCCCTCAGCTGCTCCACTCCGAACAATCACTGCTGACACTTT
 501. CCGCAAACCTCTCCGAGTCTACTCCAATTTCTCCGGGAAAGCTGAAGC
 551. TGTACACAGGGGAGGCCTGCAGGACAGGGGACAGATGAAAGCTT

3' Cloning Sites

Add/Delete/Exchange

Feature Map (0)

2.3.3. Add, Delete, or Exchange Sequence Parts

The screenshot shows a software interface for editing a DNA sequence. The top navigation bar includes tabs for '1. Enter Sequence', '2. Edit Sequence (optional)', '3. Optimize Sequence (optional)', and 'Sequence Summary', along with a 'Save & close' button. The main window is divided into a left sidebar and a central sequence editor.

The sidebar on the left contains a tree view with 'ORF' selected. Under 'ORF', there are sub-items for '5' Cloning Sites', '3' Cloning Sites', and 'Add/Delete/Exchange' (which is highlighted with a blue circle and the number 21). Below these are buttons for 'Add', 'Delete', and 'Exchange'. A text input field labeled 'Enter custom sequence' is present, along with a '5' of nucleotide:' label and an 'Add' button. A note at the bottom of the sidebar reads: 'Enter positions manually or click on the positions in the sequence at right.'

The central sequence editor displays a DNA sequence of 594bp. The sequence is shown in a grid format with line numbers on the left (1, 51, 101, 151, 201, 251, 301, 351, 401). The first six nucleotides, 'GGATCC', are highlighted in yellow. Above the sequence, the text 'Example_sequence' and 'Cursor Position: 0' are visible. A search bar is located at the top right of the sequence editor.

Below the sequence editor is a 'Feature Map (3)' section. It contains four columns: 'Protected sites (1)', 'ORF (1)', 'Protected areas (0)', and 'Motifs to avoid (1)'. Under 'Protected sites (1)', there is an entry 'BamHI [GGATCC] 1-6' with a red 'X' icon. Under 'ORF (1)', there is an entry '7-588' with a red 'X' icon. Under 'Motifs to avoid (1)', there is an entry 'BamHI [GGATCC]'.

- On the Add/Delete/Exchange tab (21) you have separate options to add, delete, or exchange sequences. The exchange function works at the codon or amino acid level and therefore requires an ORF to be defined.

2.3.4. Optimizing a Sequence

- For optimization with our proprietary GeneOptimizer® algorithm, go to the Optimize Sequence tab (22).
- Define which expression host the sequence should be optimized for (23). Please note that depending on the expression organism, a feature will be available for adding the appropriate Kozak sequence (24).
- If you would like to receive a specialist optimization or review instead of optimizing online, you can select that option as well (25).
- Please note that there are optional functions that allow you to protect sites and motifs or exclude motifs from getting created during optimization (26).
- Ensure all sequence editing and specification is complete. Then click the “Optimize” button (27) to begin online optimization.
- The duration of optimization will depend on the sequence length and complexity. Typically, a standard sequence of approximately 1 kb will take about 30 seconds to be processed.

2.4. Sequence Summary

1. Enter Sequence
2. Edit Sequence (optional)
3. Optimize Sequence (optional)
28
31

Sequence name: Example_sequence

Sequence type: DNA

Biosafety level: Level 1

Vector resistancy: No Preference 29

TSE free: No

Sequence name / optimized for
Example_sequence/ Bos taurus

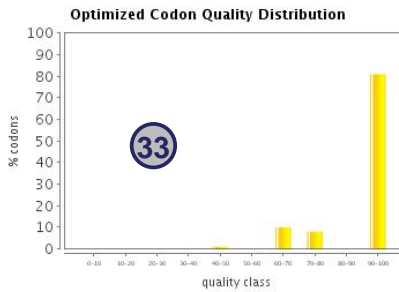
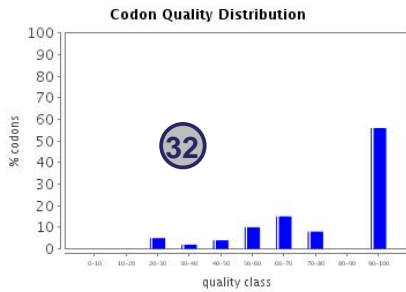
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Summary PDF
FASTA format
.GB format

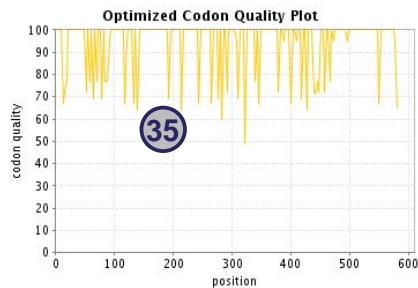
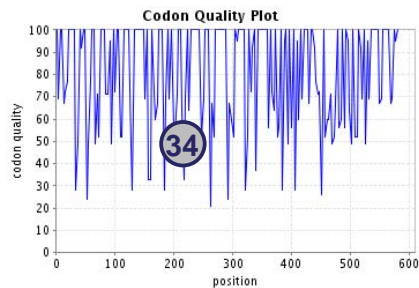
ORF	Protected sites	Protected areas	Motifs to avoid
7-588 [ATG...TAA]	1-6 BamHI [GGATCC]		

1.	M G V H E C P A W L W L L L S L L S L P L
70.	G G A I C C A T G G G C G T G C A C G A A T G T C C T G C C T G G C T G T G G C T G C T G A G C C T G C T G T C T C T G C C T C T G
139.	G L P V L G A P P R L I C D S R V L E R Y L L
208.	G G A C T G C C T G T G C T G G G A G C C C C T C C T A G A C T G A T C T G C G A C A G C A G G G T G C T G G A A A G A T A C C T G C T G
277.	E A K E A E N I T T G C A E H C S L N E N I T
346.	G A A G C C A A A G A G G C C G A G A A C A T C A C C A C C G G C T G C G C C G A G C A C T G C A G C C T G A A C G A G A A T A T C A C C
415.	V P D T K V N F Y A W K R M E V G Q Q A V E V
484.	G T G C C C G A C A C C A A A G T G A A C T T C T A C G C C T G G A A G A G G A T G G A A G T G G G C C A G C A G G C C G T G G A A G T G
553.	W Q G L A L L S E A V L R G Q A L L V N S S Q
	T G G C A G G G A C T G G C T C T G C T G T C T G A G G C C G T G C T G A G A G G A C A G G C C C T G C T C G T G A A C A G C A G C C A G
	P W E P L Q L H V D K A V S G L R S L T T L L
	C C T T G G G A A C C C T G C A G C T G C A C G T G G A C A A G G C C G T G T C T G G C C T G A G A A G C C T G A C C A C A C T G C T G
	R A L G A Q K E A I S P P D A A S A A P L R T
	A G A G C C C T G G G G G C C C A G A A A G A G G C C A T C T C C A C C T G A T G C C G C C T C T G C C G C C C C T C T G A G A A C C
	I T A D T F R K L F R V Y S N F L R G K L K L
	A T C A C G C C G A C A C C T T C A G A A A G C T G T T C A G G G T G T A C A G C A A C T T C C T G A G G G G C A A G C T G A A G C T G
	Y T G E A C R T G D R * A A G C T T
	T A C A C A G G C G A G G C C T G C A G A A C C G G C G A T C G C T A A A A G C T T

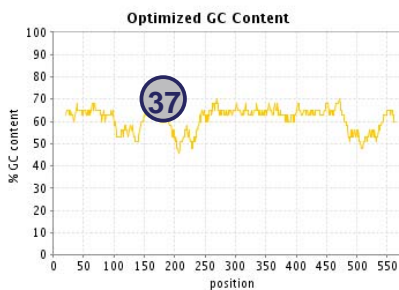
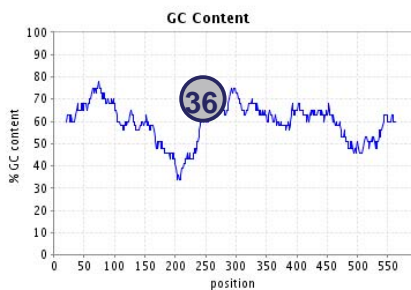
- The Sequence Summary tab (28) displays an overview of the actual sequence and all edits made (29). The sequence as well as the summary can be downloaded (30).



The histograms show the percentage of sequence codons which fall into a certain quality class. The quality value of the most frequently used codon for a given amino acid in the desired expression system is set to 100, the remaining codons are scaled accordingly (see also Sharp, P.M., Li, W.H., Nucleic Acids Res. 15 (3), 1987).



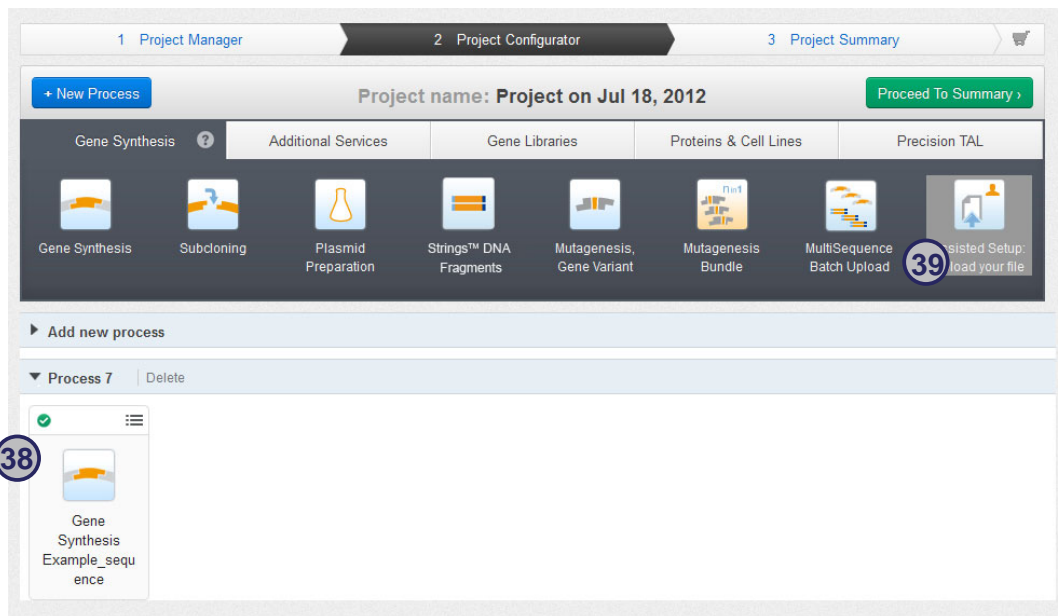
The plots show the quality of the used codon at the indicated codon position.



The plots show the GC content in a 40 bp window centered at the indicated nucleotide position.

- Graphical data display the quality of your sequence before and after optimization.
- The top two histograms (32) and (33) compare the quality distribution of codons in the sequence before and after optimization, in terms of the percentage of codons that fall into different quality classes. In the optimized sequence, the majority of codons used have quality values greater than 80.
- The second set of plots shows codon quality at each position in both non-optimized (34) and optimized sequences (35). Again in this view, it's clear that the number of codons with quality scores below 80 decreases dramatically after optimization.
- The final set of plots (36) and (37) shows GC content in the non-optimized and the optimized sequence. The optimization algorithm adjusts GC content for increased mRNA stability, when appropriate for the expression host.
- Please note, you can return to the previous tabs to make modifications at any time. Remember to click the "Optimize" button again each time when you want an optimized sequence.
- Save & close the form (31).

2.5. Information Complete

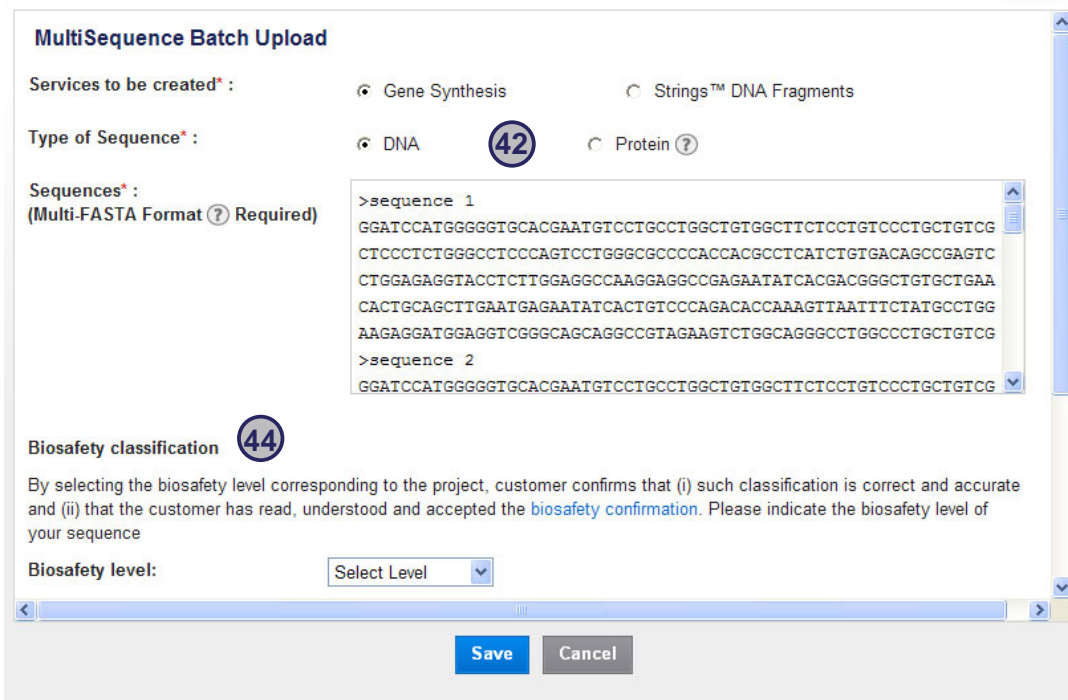


- The green status indicator should now be shown (38). If you are ready to place your order, click “Proceed To Summary” (39).
- The next step is to add the project to your cart and proceed to checkout. Please read the “Adding a Project to Cart and Placing Your Order” tutorial in chapter 7 to see how that process works.

2.6. MultiSequence Batch Upload

The screenshot displays the 'Project Configurator' interface for a project named 'Project on Jul 18, 2012'. The interface is divided into three main sections: '1 Project Manager', '2 Project Configurator', and '3 Project Summary'. A '+ New Process' button is located in the top left. The project name and a 'Proceed To Summary >' button are in the top right. Below this, a horizontal menu lists several services: Gene Synthesis, Subcloning, Plasmid Preparation, Strings™ DNA Fragments, Mutagenesis, Gene Variant, Mutagenesis Bundle, MultiSequence Batch Upload (marked with a blue circle '40'), and Assisted Setup: Upload your file. A blue callout box with a bell icon contains the text: 'Start by creating your first process, as described below. Add another process as required. This can help save shipping fees. All of the processes together make up the project. Please note that each project requires a new, separate cart..'. Below the menu, a section titled 'Process 8' is expanded, showing a 'How to get started' guide. This guide includes a dashed box labeled '41' with the text 'Drag a service icon here.' and a list of instructions: 'Start by dragging a product or service icon from the menu above to the box at left.', 'Click the icon to enter required information.', 'You can drag & drop dependent products or services onto what you already created (e.g. Subcloning onto Gene Synthesis).', 'Once your set-up is complete, proceed to summary. Add to cart then allows price verification before continuing to checkout and ordering.', and 'Some projects require specialist review before they can be ordered. For these, please proceed with Send for Review on the project summary page.'

- If you intend to order multiple sequences at the same time, our MultiSequence Batch Upload (40) can make your ordering process faster.
- To get started, drag and drop the MultiSequence Batch Upload icon (40) onto the drop area (41).



- The first step is to choose whether to enter DNA or protein sequences [42]. In either case, you'll need FASTA- or Multi-FASTA-formatted sequences ready for pasting into the sequence field, as in the example shown [43]. In this example we've entered three DNA sequences.
- Next, read the biosafety confirmation and select the biosafety requirements for your project [44].



CLOSE X

Biosafety classification

By selecting the biosafety level corresponding to the project, customer confirms that (i) such classification is correct and accurate and (ii) that the customer has read, understood and accepted the [biosafety confirmation](#). Please indicate the biosafety level of your sequence

Biosafety level:

Add service 45

- Subcloning
- Plasmid Preparation
- Stockagar
- Glycerol Stock
- Extended Documentation

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- If you need additional services that will be applied to all of your sequences, you can choose them here (45).
- When you're finished, click "Save" (46).



- For each sequence, a separate gene synthesis process is set up [(47), (48), (49)]. The red exclamation marks (50) indicate that more information is needed. You'll need to complete each Gene Synthesis process form.
- Click the first Gene Synthesis icon (51) to open the form.

1. Enter Sequence
2. Edit Sequence (optional)
3. Optimize Sequence (optional)
Sequence Summary
?
Save & close

Sequence name *

Enter sequence *

```

1   ATGGGGTGC ACGAATGTC TGGGCTG TGGCTTCTCC TGCCCTGCT GTCGCTCCCT
61  CTGGCCTCC CAGTCTGGG ACCCA CGCCTCATCT GTGACAGCCG AGTCTGGAG
121 AGGTACCTCT TGGAGGCCAA GAGGCGGAG AATATCACGA CGGGCTGTGC TGAACACTGC
181 AGCTTGAATG AGAATATCAC TGTCCCGAG ACCAARGTTA ATTTCTATGC CTGGAAGAGG

```

Sequence type *

DNA Protein

Biosafety classification *

By selecting the biosafety level corresponding to the project, customer confirms that (i) such classification is correct and accurate and (ii) that the customer has read, understood and accepted the [biosafety confirmation](#). Please indicate the biosafety level of your sequence

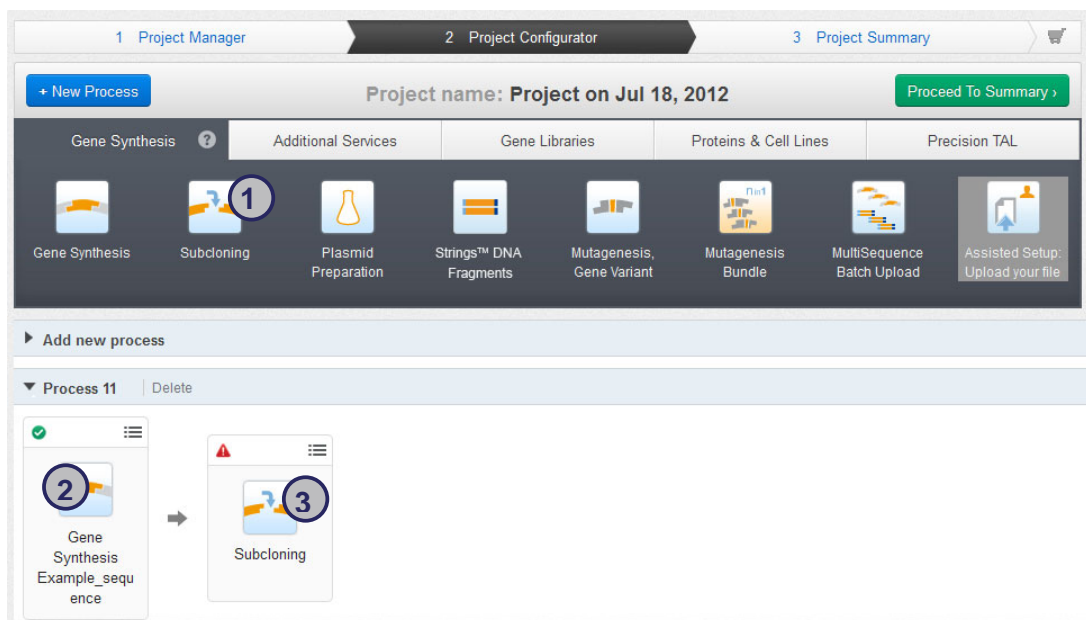
Level 1 Level 2

- The sequence (52) and an arbitrary name (53) are already filled in. You'll need to complete the rest of the form. To learn more about completing the Gene Synthesis form, please read the "Gene Synthesis and Online Optimization" tutorial in chapter 2.

- When you have completed the Gene Synthesis form, the green status indicator should be shown (54).
- Continue this process to complete the form for each gene synthesis construct in your project.
- Click the “Proceed To Summary” button (55). Then you can add the project to your cart and proceed to checkout. Please read the “Adding a Project to Cart and Placing Your Order” tutorial in chapter 7 to see how that process works.

3. Subcloning Into Vector of Your Choice

3.1. Subcloning Project Setup



- After setting up the gene synthesis project, drag and drop the Subcloning icon (1) onto the Gene Synthesis icon in the Process area (2). Please note: For subcloning, 5' and 3' cloning sites have to be specified in the Gene Synthesis form.
- Next, open the Subcloning form by clicking the Subcloning icon (3).

3.2. Enter Subcloning Requirements Using a Life Technologies Vector

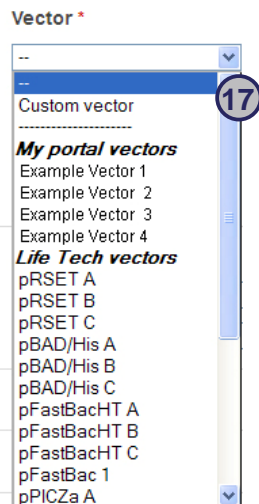
- Enter a name for the final plasmid (4).
- Then choose from the list of Life Technologies vectors (5).
- Cloning sites will be predefined, based on the cloning site information provided in the Gene Synthesis form (6).
- Next, the portal will automatically clone your predefined gene into the selected vector (7).
- If required, please select the alternative cloning strategy (8).

3.3. Enter Subcloning Requirements Using Your Own Vector

The screenshot shows the 'Enter Subcloning Requirements' form with the following fields and callouts:

- Final construct name ***: Example plasmid
- Resistance marker ***: Ampicillin (11)
- TSE-free production**:
- Vector source ***: I will provide (12)
- Vector name ***: Example vector (13)
- Vector ***: Custom vector (9)
- Copy number of vector ***: High copy (10)
- Vector sequence ***: ATCTCTGGGTGGCCCTTCTGCCCTCTCTCTGGTGGCCAGCGCGGA... (14)
- Upload vector sequence**: (15)
- Start cloning**: (16)
- Vector cloning sites**: Site 1: BamHI [GGATCC], Site 2: HindIII [AAGCTT]
- Insert cloning sites**: 5' Site: BamHI [GGATCC] (Pos. 1-6), 3' Site: HindIII [AAGCTT] (Pos. 595-600)

- If you require subcloning into a custom vector, choose the option “Custom vector” (9) from the list.
- Select the copy number of the destination vector (10) and the resistance marker (11).
- Indicate whether the vector is stocked at GeneArt® or is a new vector that will need to be sent to us (“I will provide”) (12).
- Provide us the vector name (13) and paste your vector sequence into the vector sequence field (14). Alternatively, upload your sequence (15).
- Then click “Start cloning” (16).
- As of October 2012, any vector ordered via the above-mentioned route will be added to your “My portal vectors” list (17) so that it is readily available for your next order.



Enter Subcloning Requirements Vector Summary Save & Close

Vector cloning sites

Site 1 * BamHI [GGATCC]

Site 2 * HindIII [AAGCTT]

Insert cloning sites

5' Site * BamHI [GGATCC] (Pos. 1-6)

3' Site * HindIII [AAGCTT] (Pos. 589-594)

Select your intended cloning strategy:

- The portal will automatically clone your predefined gene into the selected vector (18).
- If required, please select the alternative cloning strategy (19).

3.4. Vector Summary

The screenshot displays the 'Vector Summary' tab of a software interface. At the top, there are two tabs: 'Enter Subcloning Requirements' and 'Vector Summary', with the latter being active. A 'Save & Close' button is located in the top right corner, marked with a circled '23'. Below the tabs, the interface is divided into two main sections. The upper section contains a table of cloning requirements:

Final construct name	Example_plasmid	Resistance marker:	Ampicillin
Vector name:	Example_vector	Copy number of vector:	High copy
Vector source:	I will provide		
Vector cloning sites:	Site 1 /BamHI Site 2 /HindIII		
Insert cloning sites:	5' Site /BamHI 3' Site /HindIII		

The 'Vector cloning sites' field is marked with a circled '20'. Below this table is a circular plasmid map for 'Example_plasmid' (1225 bp). The map shows a blue arc representing the 'Example_sequence (594 bp)'. Two restriction sites are indicated: 'HindIII(2)' at the top and 'BamHI(590)' at the bottom. The map is marked with a circled '21'. To the right of the map, there is a 'Final plasmid sequence' label and a 'Download.GB' button, which is marked with a circled '22'. The interface includes a vertical scrollbar on the right and a horizontal scrollbar at the bottom.

- The Vector Summary tab includes your specifications (20), the final map (21), and the final vector information in GenBank® database format together with a download function (22).
- Click “Save & Close” (23).
- Proceed to the summary to add your gene synthesis and subcloning to the cart. Please read the “Adding a Project to Cart and Placing Your Order” tutorial in chapter 7 to see how that process works.

4. Strings™ DNA Fragments

4.1. How to Set Up a DNA Fragment Order

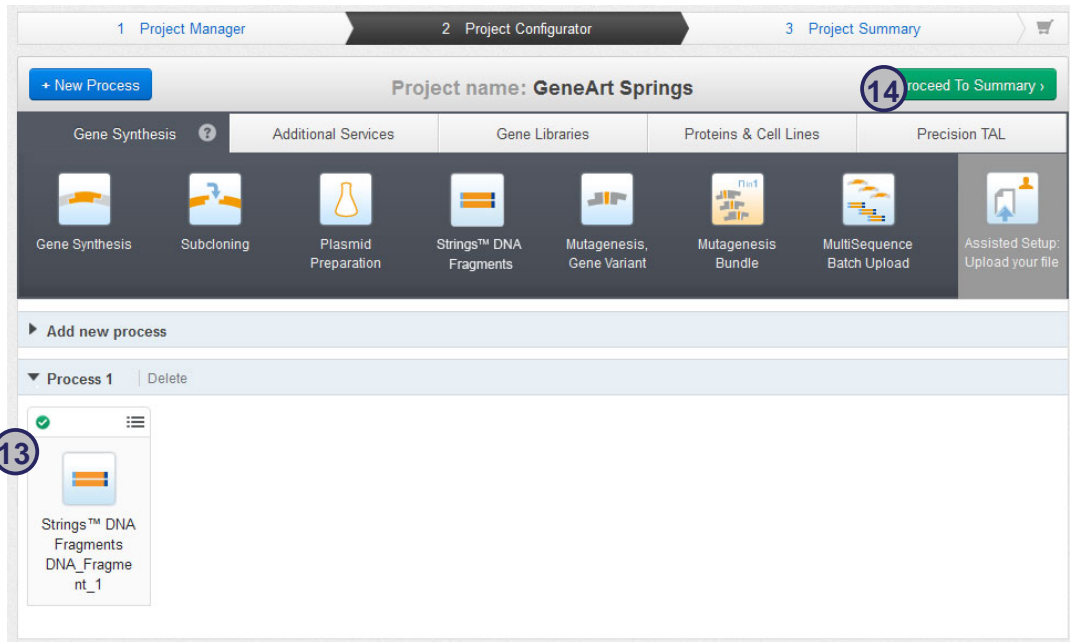
The screenshot shows the 'Project Configurator' interface for 'Project on Jul 18, 2012'. The top navigation bar includes '1 Project Manager', '2 Project Configurator', and '3 Project Summary'. Below the navigation bar, there is a '+ New Process' button and a 'Proceed To Summary >' button. The main area is divided into five tabs: 'Gene Synthesis', 'Additional Services', 'Gene Libraries', 'Proteins & Cell Lines', and 'Precision TAL'. Under the 'Gene Libraries' tab, several service icons are displayed: 'Gene Synthesis', 'Subcloning', 'Plasmid Preparation', 'Strings™ DNA Fragments' (circled with a '1'), 'Mutagenesis, Gene Variant', 'Mutagenesis Bundle', 'MultiSequence Batch Upload', and 'Assisted Setup: Upload your file'. Below the icons, a blue box contains instructions: 'Start by creating your first process, as described below. Add another process as required. This can help save shipping fees. All of the processes together make up the project. Please note that each project requires a new, separate cart..'. Under the 'Process 1' section, there is a 'How to get started' heading and a list of instructions. A dashed box labeled '2' is positioned over the 'Strings™ DNA Fragments' icon, with the text 'Drag a service icon here.'

- To set up a DNA Fragment order, drag the Strings™ DNA Fragments icon (1) onto the drop area (2).

The screenshot shows the 'Project Configurator' interface for 'Project on Jul 18, 2012'. The top navigation bar includes '1 Project Manager', '2 Project Configurator', and '3 Project Summary'. Below the navigation bar, there is a '+ New Process' button and a 'Proceed To Summary >' button. The main area is divided into five tabs: 'Gene Synthesis', 'Additional Services', 'Gene Libraries', 'Proteins & Cell Lines', and 'Precision TAL'. Under the 'Gene Libraries' tab, several service icons are displayed: 'Gene Synthesis', 'Subcloning', 'Plasmid Preparation', 'Strings™ DNA Fragments', 'Mutagenesis, Gene Variant', 'Mutagenesis Bundle', 'MultiSequence Batch Upload', and 'Assisted Setup: Upload your file'. Below the icons, a blue box contains instructions: 'Start by creating your first process, as described below. Add another process as required. This can help save shipping fees. All of the processes together make up the project. Please note that each project requires a new, separate cart..'. Under the 'Process 1' section, there is a 'How to get started' heading and a list of instructions. A dashed box labeled '3' is positioned over the 'Strings™ DNA Fragments' icon, with the text 'Drag a service icon here.' and a red exclamation mark. A dashed box labeled '4' is positioned over the 'Strings™ DNA Fragments' icon, with the text 'Drag a service icon here.'

- The red exclamation mark (3) indicates that more information is needed.
- Click the Strings™ DNA Fragments icon (4) to open the form.

- The Strings™ DNA Fragments form has three main tabs: the Enter Sequence tab (5), the Edit Sequence tab (6), and the Optimize Sequence tab (7).
- The Enter Sequence tab allows you to enter the sequence and provide all basic information.
- Enter your sequence name (8), choose whether to enter a DNA or protein sequence (9), and paste your sequence into the tool (10). If you enter a protein as an amino acid sequence, it is important to optimize it for your desired expression host.
- Editing and optimizing your gene works in the same way as for full gene synthesis. For details, see chapter 2.3, “Editing and Optimizing a Sequence”.
- The Sequence Summary tab (11) displays an overview of the actual sequence and all edits made. The sequence and the summary can be downloaded. For more details, see chapter 2.4, “Sequence Summary”.
- If you have entered all information, “Save and Close” the form (12).



- The green check mark status indicator should now appear (13). If you are ready to place your order, click “Proceed To Summary” (14).
- The next step is to add the project to your cart and proceed to checkout. Please read the “Adding a Project to Cart and Placing Your Order” tutorial in chapter 7 to see how that process works.

4.2. MultiSequence Batch Upload for Strings™ DNA Fragments

The screenshot shows the 'Project Configurator' interface for 'Project name: black label 2'. It features a navigation bar with '1 Project Manager', '2 Project Configurator', and '3 Project Summary'. Below the navigation bar, there are tabs for 'Gene Synthesis', 'Additional Services', 'Gene Libraries', 'Proteins & Cell Lines', and 'Precision TAL'. Under 'Additional Services', several icons are displayed, including 'Multi Sequence Batch Upload' which is circled with a blue '15'. Below this is a section titled 'Add new process' with a dashed box icon labeled '16' and the text 'Drag a service icon here.' To the right of this icon is a 'How to get started' section with a list of instructions:

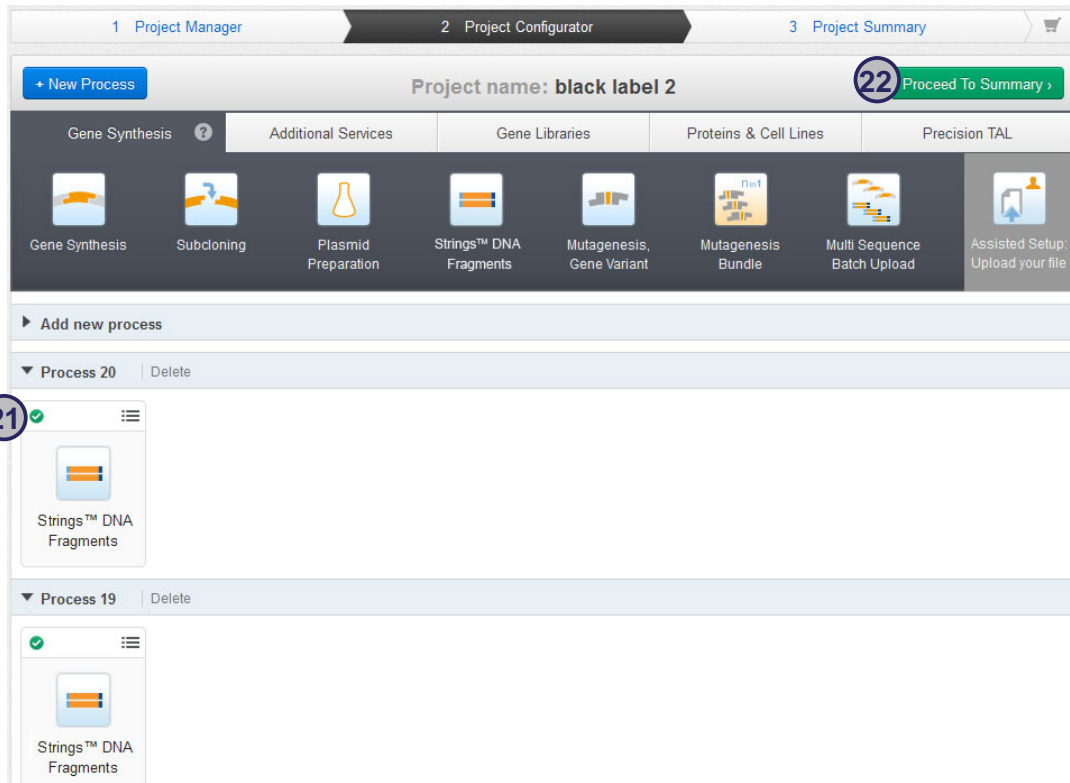
- Start by dragging a product or service icon from the menu above to the box at left.
- Click the icon to enter required information.
- You can drag & drop dependent products or services onto what you already created (e.g. Subcloning onto Gene Synthesis).
- Once your set-up is complete, proceed to summary. Add to cart then allows price verification before continuing to checkout and ordering.
- Some projects require specialist review before they can be ordered. For these, please proceed with Send for Review on the project summary page.

- If you intend to order multiple sequences at the same time, the MultiSequence Batch Upload (15) can make your ordering process faster.
- To get started, drag and drop the MultiSequence Batch Upload icon (15) onto the drop area (16).
- Select “Strings™ DNA Fragments” (17). The next step is to choose whether to enter DNA or protein sequences (18). If you enter a protein as an amino acid sequence, it is important to optimize it for your desired expression host. In either case, you’ll need FASTA-formatted sequences ready for pasting into the sequence field.
- In this example we’ve entered two DNA sequences (19).
- Then click “Save” (20).

The screenshot shows the 'MultiSequence Batch Upload' dialog box. At the top right is a 'CLOSE X' button. The dialog has the following sections:

- Services to be created* :** Radio buttons for 'Gene Synthesis' and 'Strings™ DNA Fragments' (17), with the latter selected.
- Type of Sequence* :** Radio buttons for 'DNA' (18) and 'Protein (?)', with 'DNA' selected.
- Sequences* :** A text area labeled '(Multi-FASTA Format (?) Required)' containing two DNA sequences (19):

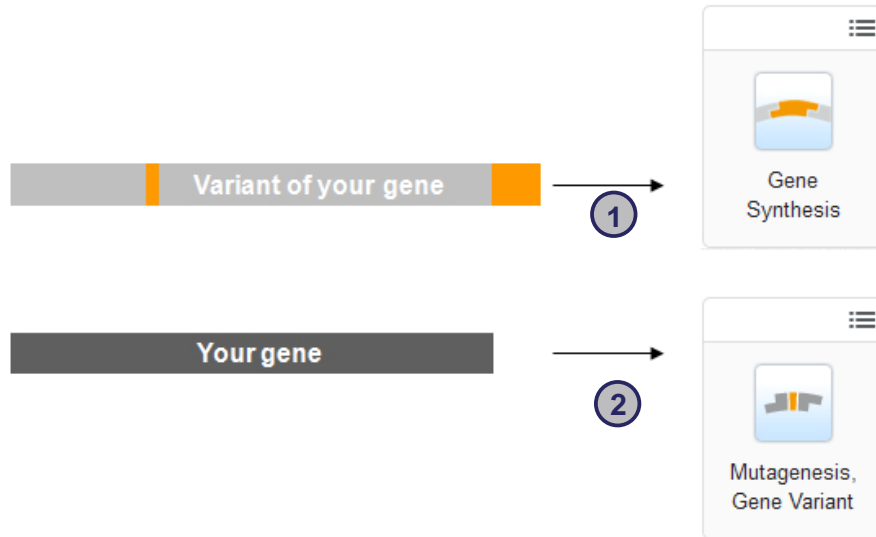

```
>sequence 1
ATGGGGGTGCACGAATGTCCTGCGCTGGCTGTGGCTTCTCCTGTCCCTGCTGTCGCTC
CCTCTGGGCCTCCCAGTCTCTGGCCACCACGCTCATCTGTGACAGCCGAGTC
CTGGAGAGGTACCTCTTGGAGCCAGGAGGCCGAGAATATCACGACGGGCTGTGCT
>sequence 2
ATGGGGGTGCACGAATGTCCTGCGCTGGCTGTGGCTTCTCCTGTCCCTGCTGTCGCTC
CCTCTGGGCCTCCCAGTCTGGGCCTGGCCACCACGCTCATCTGTGACAGCCGAGTC
CTGGAGAGGTACCTCTTGGAGCCAGGAGGCCGAGAATATCACGACTAGTTTAGCT
```
- Customer information**: A paragraph of text providing instructions on adding restriction enzymes and buffer nucleotides for subcloning, and adding 5' & 3' sequences for assembly.
- At the bottom are 'Save' (20) and 'Cancel' buttons.



- The green status indicators should be showing (21).
- Click the “Proceed To Summary” button (22). Then you can add the project to your cart and proceed to checkout. Please read the “Adding a Project to Cart and Placing Your Order” tutorial in chapter 7 to see how that process works.

5. Setup of Mutagenesis: Single Gene Variants

5.1. Which Sequence Qualifies as a Single Gene Variant?



- If you want to study a construct with mutations introduced by site-directed mutagenesis, or a variant of your gene, begin by determining which sequence is the longest version of your gene.
- You will need to order this sequence using the GeneArt® Gene Synthesis service (1), and set up the shorter version as a variant (2).

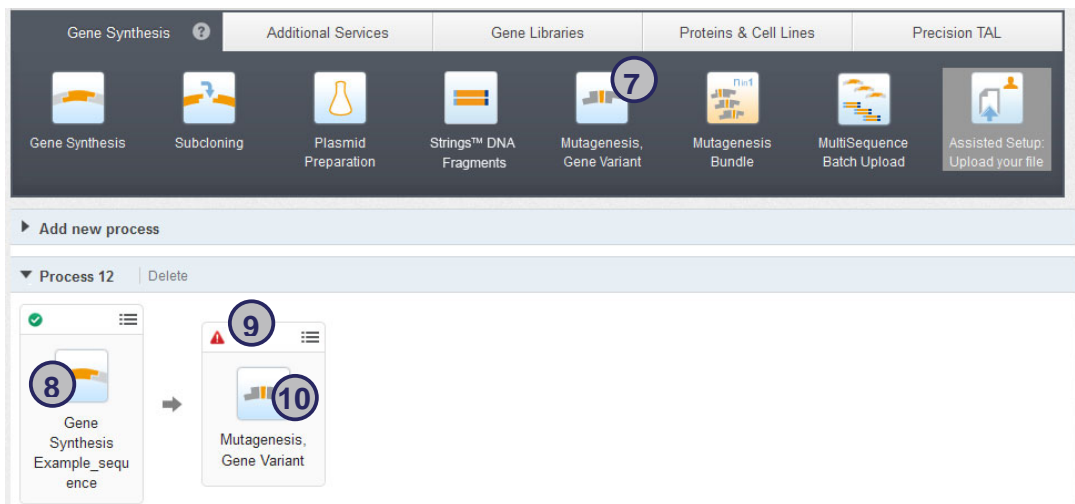
5.2. Single Gene Variant Project Setup

The screenshot shows the 'Project Configurator' interface for 'Project on Jul 18, 2012'. The top navigation bar includes '1 Project Manager', '2 Project Configurator', and '3 Project Summary'. A '+ New Process' button is on the left, and a 'Proceed To Summary' button is on the right. Below the navigation is a menu with categories: Gene Synthesis, Additional Services, Gene Libraries, Proteins & Cell Lines, and Precision TAL. Under 'Gene Synthesis', there are icons for Gene Synthesis (circled 3), Subcloning, Plasmid Preparation, Strings™ DNA Fragments, Mutagenesis, Gene Variant, Mutagenesis Bundle, MultiSequence Batch Upload, and Assisted Setup: Upload your file. A blue notification box states: 'Start by creating your first process, as described below. Add another process as required. This can help save shipping fees. All of the processes together make up the project. Please note that each project requires a new, separate cart.' Below this is the 'Process 1' section with a 'How to get started' guide. The guide includes a numbered step (4) with an icon and text: 'Drag a service icon here.' The text explains: 'Start by dragging a product or service icon from the menu above to the box at left. Click the icon to enter required information. You can drag & drop dependent products or services onto what you already created (e.g. Subcloning onto Gene Synthesis). Once your set-up is complete, proceed to summary. Add to cart then allows price verification before continuing to checkout and ordering. Some projects require specialist review before they can be ordered. For these, please proceed with Send for Review on the project summary page.'

- To set up the synthesis of the longest version of your gene, drag and drop the Gene Synthesis icon (3) onto the drop area (4).
- Next, work through the Gene Synthesis form by clicking the Gene Synthesis icon (5) and entering the requested data and information. To learn more about completing the Gene Synthesis form, please read chapter 2.

The screenshot shows the 'Project Configurator' interface for 'Project on Jul 18, 2012'. The top navigation bar includes '1 Project Manager', '2 Project Configurator', and '3 Project Summary'. A '+ New Process' button is on the left, and a 'Proceed To Summary' button is on the right. Below the navigation is a menu with categories: Gene Synthesis, Additional Services, Gene Libraries, Proteins & Cell Lines, and Precision TAL. Under 'Gene Synthesis', there are icons for Gene Synthesis, Subcloning, Plasmid Preparation, Strings™ DNA Fragments, Mutagenesis, Gene Variant, Mutagenesis Bundle, MultiSequence Batch Upload, and Assisted Setup: Upload your file. Below this is the 'Add new process' section. Under 'Process 11', there is a 'Gene Synthesis' item with a green status indicator (circled 6) and a 'Delete' button. The item is labeled 'Gene Synthesis Example_sequence' and has a circled 5 next to its icon.

- Note that if you choose to optimize your sequence using the GeneArt® GeneOptimizer® service, the variant sequence must be based on the optimized sequence.
- When you have completed the Gene Synthesis form, the green status indicator should be shown (6).
- Now let's see the process for ordering a single variant using the GeneArt® portal.



- To set up your variant, drag and drop the Mutagenesis icon (7) onto the Gene Synthesis icon in the Process area (8).
- The red exclamation mark lets you know that more information is needed (9).
- The next step is to open the Mutagenesis form by clicking the Mutagenesis icon (10).

5.4. Additional Information

CLOSE X

Please indicate the restriction enzymes present in your sequence intended for subcloning:

5' Restriction Site: **17**

3' Restriction Site: **17**

What GeneArt® Should Know:

TSE-free: **i**

Comment: Questions or comments will place your order on hold so that we can manually address your need.

Add service

18 Subcloning

Plasmid Preparation

Stockagar

Glycerol Stock

Extended Documentation

19

- If you plan to subclone your variant, enter the restriction sites you need in your sequence **(17)**. Note that the restriction sites must be present in the sequence you entered.
- If you need extra services, you can select them **(18)**.
- Click "Save" **(19)**.

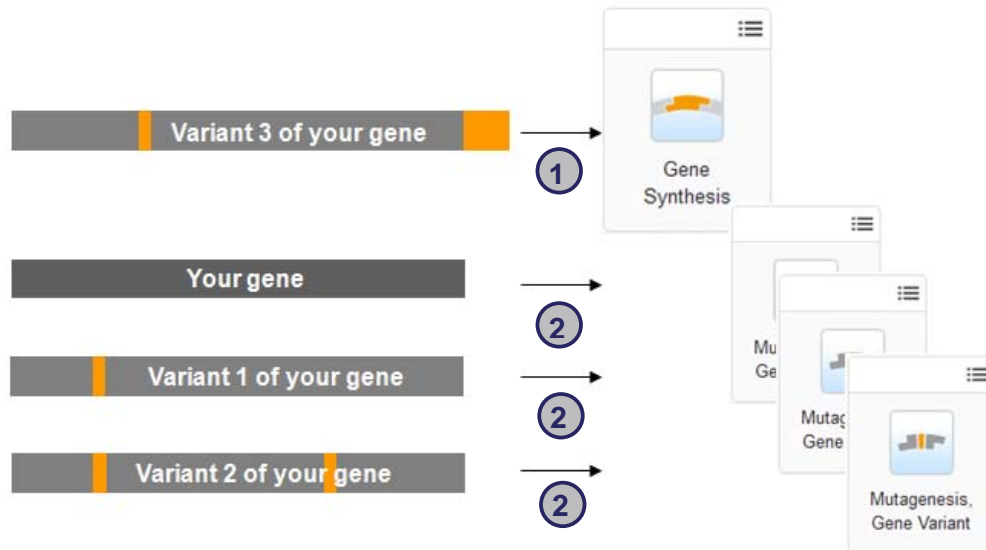
5.5. Information Complete

The screenshot displays a web interface for a laboratory workflow. At the top, a blue button labeled '+ New Process' is on the left, and a green button labeled '21 Proceed To Summary >' is on the right. The main header shows 'Project name: Project on Jul 18, 2012'. Below this is a navigation bar with five tabs: 'Gene Synthesis', 'Additional Services', 'Gene Libraries', 'Proteins & Cell Lines', and 'Precision TAL'. Underneath the tabs is a grid of service icons: 'Gene Synthesis', 'Subcloning', 'Plasmid Preparation', 'Strings™ DNA Fragments', 'Mutagenesis, Gene Variant', 'Mutagenesis Bundle', 'MultiSequence Batch Upload', and 'Assisted Setup: Upload your file'. A section titled 'Add new process' is visible, followed by a dropdown menu for 'Process 12' with a 'Delete' option. Below the dropdown, two process cards are shown. The first card, 'Gene Synthesis Example_sequence', has a green checkmark and a '20' in a blue circle. An arrow points to the second card, 'Mutagenesis, Gene Variant Mutagenesis_example', which also has a green checkmark and a '20' in a blue circle.

- The green status indicator should now be shown (20).
- Click the “Proceed To Summary” button (21). Then you can add the project to your cart and proceed to checkout. Please read the “Adding a Project to Cart and Placing Your Order” tutorial in chapter 7 to see how that process works.
- We have now used the Mutagenesis (Single Gene Variant) form to create a single variant.
- If you need several variants for your sequence, learn how to order them at the same time by reading the “Mutagenesis Bundles” tutorial in chapter 6.

6. Setup of Mutagenesis Bundles (Gene Variants)

5.1. Which Sequences Qualify as Mutagenesis Bundles?



- If you want to study mutations introduced by site-directed mutagenesis, or variants of your gene, begin by determining which sequence is the longest version of your gene.
- You will need to order this sequence using the GeneArt® Gene Synthesis service (1), and set up the shorter versions as variants (2).

6.2. Mutagenesis Bundle Project Setup

- To set up the synthesis of the longest version of your gene, drag and drop the Gene Synthesis icon (3) onto the drop area (4). Next, work through the Gene Synthesis form by clicking the Gene Synthesis icon (5) and entering the requested data and information. To learn more about completing the Gene Synthesis form, please read the “Gene Synthesis and Online Optimization” tutorial in chapter 2.
- Note that if you choose to optimize your sequence using the GeneArt® GeneOptimizer® service, all variant sequences must be based on the optimized sequence.

- When you have completed the Gene Synthesis form, the green status indicator should be shown (6).
- Now let's see the process for ordering multiple variants using the GeneArt® portal.
- If you want to order several mutants or variants, drag and drop the Mutagenesis Bundle icon (7) onto the Gene Synthesis icon in the Process area (5).

6.3. Entering Several Gene Variant Sequences

Variant Bundle CLOSE X

Please choose how you would like to create the variant sequences

8 with sequence from the parent service

Count(1-20)

9 with sequence given via multifasta

- Choose whether to create variants using the parent sequence service **(8)** or by uploading variant sequences using a multifasta form **(9)**.
- If you choose the parent service, the sequence used for the initial gene synthesis will be copied to the number of variant forms you specify. You can then introduce the variations you need into each Variant form.
- If you choose the multifasta form **(9)** you can paste all your variants into the system in one step.
- First we will set up two variants using the multifasta form. Choose the multifasta option **(9)**.

6.3.1. Setting Up a Mutagenesis Bundle With the Multifasta Option

Variant Information

DNA
 Protein(sequence will be back-translated and annotated as open reading frame)

Sequence* : (11)

TSE-free

Add service

Subcloning
 Plasmid Preparation
 Stockagar
 Glycerol Stock
 Extended Documentation

12

13

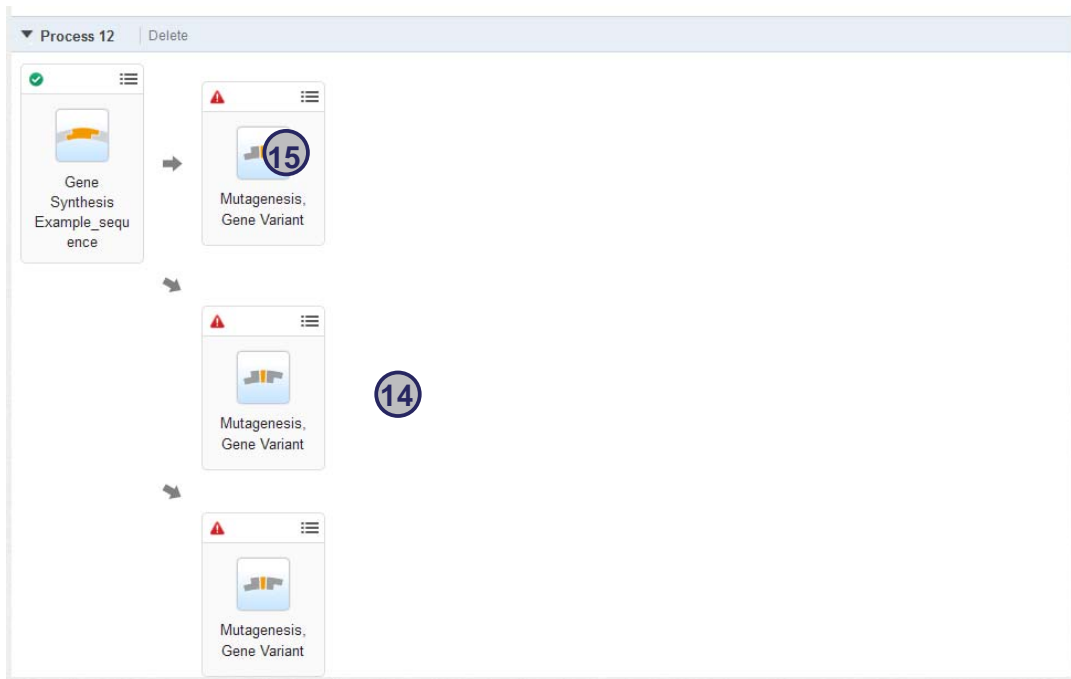
CLOSE X

- The form expands, and you can enter your prepared variants. Just copy and paste multifasta-formatted mutagenesis sequences (10) into the sequence field in the portal (11).

```

>Gene variant 1
GGATCCATGGGGGTGCACGAATGTCTGCCGTGGCTGTGGCTTCTCCGTGCCCTGTGCTGCCCTCTGGGCCCTCCAGTCTGGGGCCCCACCATTGCCATCTG
TGACAGCCGAGTCTGGAGAGGTACCTTTGGAGGCCAAGGAGGCCGAGAATATCACGACGGGCTGTGCTGAACACTGCAGCTTGAATGAGAATATCACTGTCCCAGA
CACC AAAGTTAAATTTCTATGCCCTGGAAGAGGATGGAGGTCGGGCAGCAGGCCGTAGAAGTCTGGCAGGGCTTGGCCCTGCTGTGGAAGCTGTCTGC GGGGCCAGGC
CCTGTTGGTCAACTCTTCCCAGCCGTGGGAGCCCTGCAGCTGTCATGTGGATAAAGCCGTCAGTGGCTTTCGCAGCTCACACACTGTGCTTGGGGCTTGGGAGCCCA
GAAAGGAGCCATCTCCCCTCCAGATGCGGCCCTCAGCTGCTCCACTCCGAACAATCACTGCTGACACTTTCCGCAAACTCTTCCGAGTCTACTCCAATTTCTCCGGGG
AAAGCTGAAGCTGTACACAGGGGAGGCCCTCAGGACAGGGGACAGATGAAAGCTT
>Gene variant 2
GGATCCATGGGGGTGCACGAATGTCTGCCGTGGCTGTGGCTTCTCCGTGCCCTGTGCTGCCCTCTGGGCCCTCCAGTCTGGGGCCCCACCATTGCCATCTG
TGACAGCCGAGTCTGGAGAGGTACCTTTGGAGGCCAAGGAGGCCGAGAATATCACGACGGGCTGTGCTGAACACTGCAGCTTGAATGAGAATATCACTGTCCCAGA
CACC AAAGTTAAATTTCTATGCCCTGGAAGAGGATGGAGGTCGGGCAGCAGGCCGTAGAAGTCTGGCAGGGCTTGGCCCTGCTGTGGAAGCTGTCTGC GGGGCCAGGC
CCTGTTGGTCAACTCTTCCCAGCCGTGGGAGCCCTGCAGCTGTCATGTGGATAAAGCCGTCAGTGGCTTTCGCAGCTCACACACTGTGCTTGGGGCTTGGGAGCCCA
GAAAGGAGCCATCTCCCCTCCAGATGCGGCCCTCAGCTGCTCCACTCCGAACAATCACTGCTGACACTTTCCGCAAACTCTTCCGAGTCTACTCCAATTTCTCCGGGG
AAAGCTGAAGCTGTACACAGGGGAGGCCCTCAGGACAGGGGACAGATGAAAGCTT
>Gene variant 3
GGATCCATGGGGGTGCACGAATGTACTGCCGTGGCTGTGGCTTCTCCGTGCCCTGTGCTGCCCTCTGGGCCCTCCAGTCTGGGGCCCCACCATTGCCATCTG
TGACAGCCGAGTCTGGAGAGGTACCTTTGGAGGCCAAGGAGGCCGAGAATATCACGACGGGCTGTGCTGAACACTGCAGCTTGAATGAGAATATCACTGTCCCAGA
CACC AAAGTTAAATTTCTATGCCCTGGAAGAGGATGGAGGTCGGGCAGCAGGCCGTAGAAGTCTGGCAGGGCTTGGCCCTGCTGTGGAAGCTGTCTGC GGGGCCAGGC
CCTGTTGGTCAACTCTTCCCAGCCGTGGGAGCCCTGCAGCTGTCATGTGGATAAAGCCGTCAGTGGCTTTCGCAGCTCACACACTGTGCTTGGGGCTTGGGAGCCCA
GAAAGGAGCCATCTCCCCTCCAGATGCGGCCCTCAGCTGCTCCACTCCGAACAATCACTGCTGACACTTTCCGCAAACTCTTCCGAGTCTACTCCAATTTCTCCGGGG
AAAGCTGAAGCTGTACACAGGGGAGGCCCTCAGGACAGGGGACAGATGAAAGCTT
  
```

- If you need extra services, you can select them under “Add service” (12).
- Click “Save” (13).





- The program has created three single variants (14).
- Open the first Mutagenesis form by clicking the icon (15).


- The name (16) and sequence field (17) are already filled in. You can change the name if you desire. Finish completing the form as described in the “Mutagenesis” tutorial in chapter 5.
- Click “Save” when you are finished (18).


+ New Process Project name: Project on Jul 18, 2012 20 Proceed To Summary >


Gene Synthesis ?	Additional Services	Gene Libraries	Proteins & Cell Lines	Precision TAL
------------------	---------------------	----------------	-----------------------	---------------



 Gene Synthesis



 Subcloning



 Plasmid Preparation


 Strings™ DNA Fragments


 Mutagenesis, Gene Variant

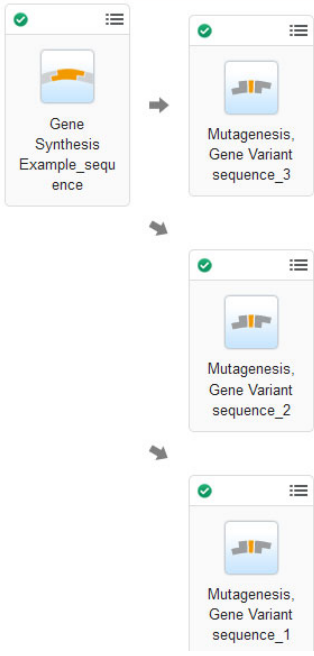

 Mutagenesis Bundle


 MultiSequence Batch Upload


 Assisted Setup: Upload your file

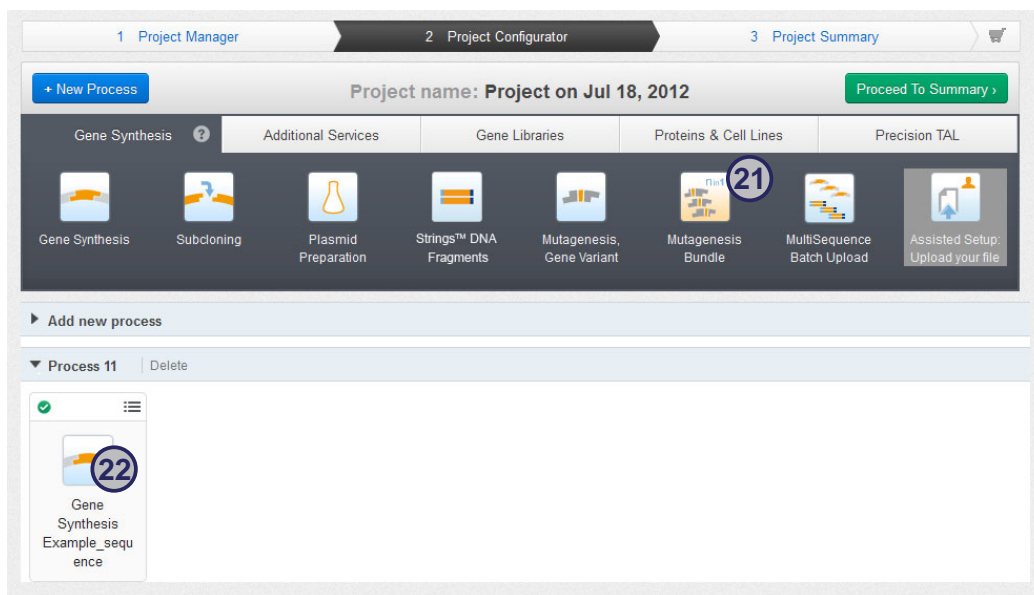
▶ Add new process

▼ Process 12 | Delete



- The green status indicator should be shown now”.
- Complete the remaining variants in the same way **(19)**.
- Once the form for every variant is completed, click “Proceed To Summary” **(20)** and send your project to GeneArt® scientists for review. Within 24 hours, the GeneArt® team will review your project and send you an email notification.
- When you receive a response from the GeneArt® team, you can add the project to your cart and proceed to checkout. Please read the “Adding a Project to Cart and Placing Your Order” tutorial in chapter 7 to see how that process works.

6.3.2. Setting Up a Mutagenesis Bundle With the Parent Service Option



- Now we'll set up two variants using the parent service rather than the multifasta method. Go back to the step where gene synthesis for the longest version of the gene has been set up. Drag and drop the Mutagenesis Bundle icon (21) onto the Gene Synthesis icon in the Process area (22).

CLOSE X

Variant Bundle

Please choose how you would like to create the variant sequences

with sequence from the parent service

Count(1-20)


with sequence given via multifasta


24


Save **Cancel**


- Choose the parent service option (23) and type in the desired number of variants—two in this example. Then click “Save” (24).


Gene Synthesis ? Additional Services Gene Libraries Proteins & Cell Lines Precision TAL



Gene Synthesis



Subcloning



Plasmid Preparation


Strings™ DNA Fragments


Mutagenesis, Gene Variant



Mutagenesis Bundle


MultiSequence Batch Upload



Assisted Setup: Upload your file

▶ Add new process


▼ Process 12 | Delete


Gene Synthesis
Example_sequence

→


Mutagenesis, Gene Variant

→


Mutagenesis, Gene Variant

25

- The program has created two single variants (25).
- Open the first form by clicking the icon (26).

CLOSE X

Single Variant

Your Variant Information

Sequence Name* : **27**

Sequence Type* : DNA[ACGT]
 Protein (Note: Please use an asterisk (*) to indicate translation stop.)

Sequence* :

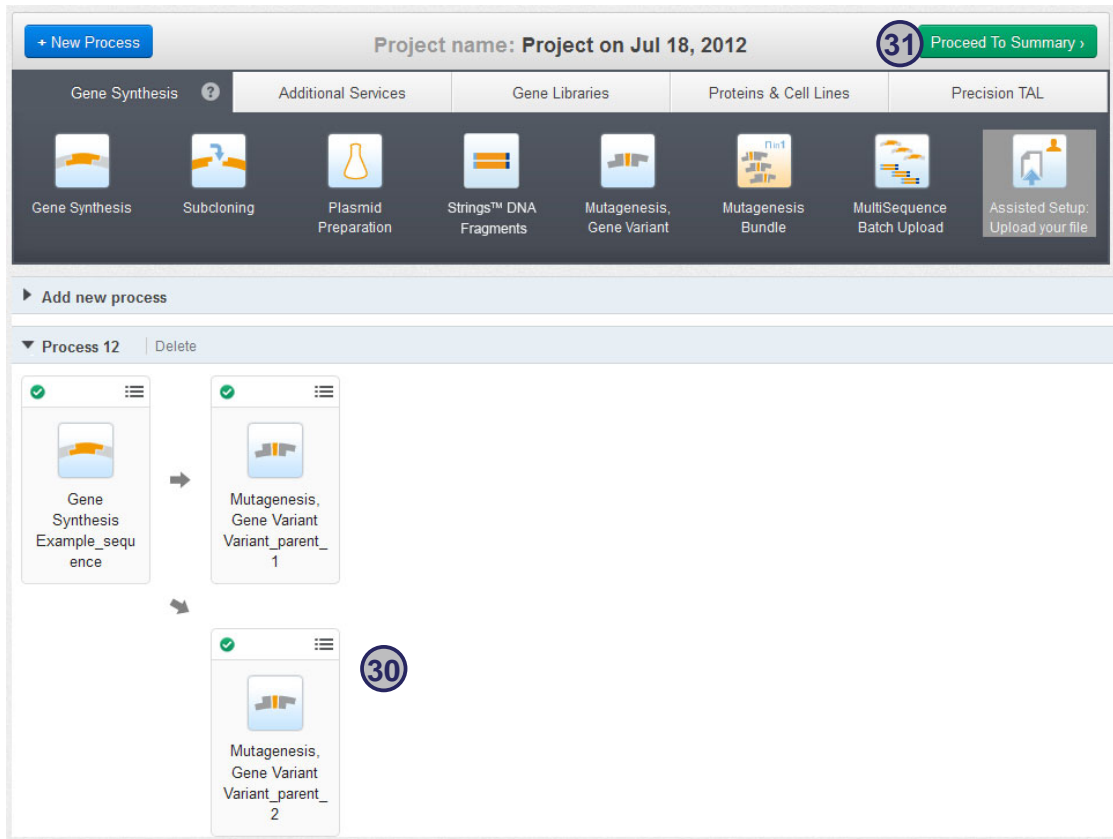
```
GGATCCATGGGGGTGCACGAATGTCTGCCTGGCTGTGGCTTCTCCTGTCCTGCTGCTGG
CTCCCTCTGGGCCTCCCAGTCCTGGGGCCGCCACCACGGCTCATCTGTGACAGCCGAGTC
CTGGAGAGGTACCTCTTGGAGGCCAAGGAGGCCGAGAATATCACGACGGGCTGTGCTGAA
CACTGCAGCTTGAATGAGAAT28TGTCCAGACACCAAAAGTTAAATTTCTATGCCTGG
AAGAGGATGGAGGTCGGGCAG28CCGTAGAAGTCTGGCAGGGCCTGGCCCTGCTGTCG
GAAGCTGTCTCTGCGGGGCCAGGCCCTGTTGGTCAACTCTTCCCAGCCGTGGGAGCCCTG
CAGCTGCATGTGGATAAAGCCGTCAGTGGCCTTCGCAGCCTCACCACTCTGCTTGGGGCT
CTGGGAGCCAGAAAGGAAAGCCATCTCCCTCCAGATGCGGCCTCAGCTGCTCCACTCCGA
```

Please indicate the restriction enzymes present in your sequence intended for subcloning:

5' Restriction Site:

3' Restriction Site: **29**

- The names of the parental sequence and the variant sequence are already filled in.
- You can change the name if you wish—in this example, we’ve chosen the name “Variant parent 1” **27**.
- Now type in your desired sequence modifications **28**.
- Finish completing the form as described in the “Mutagenesis” tutorial in chapter 5.
- Click “Save” **29**.



- The green status indicator should be shown now.
- Complete the remaining variants in the same way (30).
- Once the form for every variant is completed, click “Proceed To Summary” (31) and send your project to GeneArt® scientists for review. Within 24 hours, the GeneArt® team will review your project and send you an email notification.
- When you receive a response from the GeneArt® team, you can add the project to your cart and proceed to checkout. Please read the “Adding a Project to Cart and Placing Your Order” tutorial in chapter 7 to see how that process works.

7. Adding a Project to Cart and Placing Your Order

7.1. Proceed to Project Summary


The screenshot shows the GeneArt project summary interface. At the top, there is a header with a blue "+ New Process" button on the left, the project name "Project on Jul 18, 2012" in the center, and a green "1 Proceed To Summary >" button on the right. Below the header is a navigation bar with five tabs: "Gene Synthesis" (selected), "Additional Services", "Gene Libraries", "Proteins & Cell Lines", and "Precision TAL". Underneath the tabs is a row of service icons: Gene Synthesis, Subcloning, Plasmid Preparation, Strings™ DNA Fragments, Mutagenesis, Gene Variant, Mutagenesis Bundle, MultiSequence Batch Upload, and Assisted Setup: Upload your file. Below this is a section titled "Add new process" with a dropdown arrow. Underneath is a section for "Process 12" with a "Delete" link. The main area displays three process cards. The first card is "Gene Synthesis Example_sequence" with a green checkmark and a menu icon. An arrow points from this card to a second card, "Mutagenesis, Gene Variant Variant_parent_1", which also has a green checkmark and a menu icon. A second arrow points from the first card to a third card, "Mutagenesis, Gene Variant Variant_parent_2", which also has a green checkmark and a menu icon.










- Once you have completed defining your project, you can order GeneArt® services by clicking "Proceed To Summary" (1).

7.2. Project Summary

✔ Your project is finalized. For final pricing and delivery information please add your project to the cart. If you want to make changes to your project press the edit button in the cart. You will re-enter the project configurator.


Project Summary Back [add to cart](#) **5**

Project name: Project on Aug 10, 2012  [Download project report](#) **2**

Description	Name	Construct ID	Quantity	Download
Gene Synthesis Delivery upgrades (extra fee applies): <input type="checkbox"/> Express Service Reduces synthesis time by two business days. <input type="checkbox"/> SuperSPEED Service Reduces total synthesis time to five (for > 1.2 kb) or seven (for 1.2-1.8 kb) business days. 4	Example_sequence	12AAYGUP	595bp	 Download analysis report  Download your sequence in FASTA format 3  Download your sequence in .GB format
Mutagenesis	Gene_variant_2	12AAYGVP	595bp	 Download your sequence in FASTA format  Download your sequence in .GB format
Mutagenesis	Gene_variant_1	12AAYGWP	596bp	 Download your sequence in FASTA format  Download your sequence in .GB format
Subcloning	Example_plasmid	12AAYGXP	1	 Vector with insert  Download your sequence in .GB format

- The Project Summary page is displayed. Here you can download your overall project report **(2)**.
- Detailed files specific to different GeneArt® services are also available for download **(3)**.
- Choose Express or SuperSPEED Service if required **(4)**.
- If you are ready to check out, click the “add to cart” button **(5)** to proceed.


- Note: If your project requires review by GeneArt® scientists (yellow status indicator), the project summary will look different. Click “send for review” (6) to start the process. The average review time is one business day. Once completed, your sequence will be placed in your project folder and you can then proceed to order your gene as described above.








 **One or more services require review by GeneArt® scientist. Please send your project for review. Within 24 hours the GeneArt expert team will then perform the optimization and return the optimized sequence. You will get an email notification after your optimized sequence has been uploaded to your project**

Project Summary


Back
→ send for review
add to cart

6

Project name: Project on Aug 10, 2012  [Download project report](#)

Description	Name	Construct ID	Quantity	Download
Gene Synthesis Delivery upgrades (extra fee applies): <input type="checkbox"/> Express Service Reduces synthesis time by two business days. <input type="checkbox"/> SuperSPEED Service Reduces total synthesis time to five (for > 1.2 kb) or seven (for 1.2-1.8 kb) business days.	Example_sequence	12AAYGUP	595bp	 Download analysis report  Download your sequence in FASTA format  Download your sequence in .GB format
Mutagenesis	Gene_variant_2	12AAYGVP	284bp	
Mutagenesis	Gene_variant_1	12AAYGWP	596bp	 Download your sequence in FASTA format  Download your sequence in .GB format
Subcloning	Example_plasmid	12AAYGXP	1	 Vector with insert  Download your sequence in .GB format

Finished with your current sequence?

 **Send for Review** to have a scientist review your order and make recommendations.

Average Scientist Review Time: **24 hours**

7.3. Cart View

The screenshot shows the GeneArt shopping cart interface. At the top, there are three steps: 1 Shipping & Payment, 2 Review Order, and 3 Order Confirmation. A yellow warning box at the top left contains a note: "Note: At this time, the shopping cart cannot accommodate more than one GeneArt® project. To order an additional GeneArt® project, please add it to a second shopping cart." Below this is the "Cart" section with a "Rename cart" link and a circled "11" callout. There are buttons for "copy", "remove", and "add to favorites". A table lists items with columns for "Items", "Unit Size", "List Price", "Your Price", "Qty", and "Total". The first item is "Project on Aug 10, 2012—2012AARK6P" with a price of 838.08. Below it are three sub-items: "Subcloning -- Construct ID: 12AAYGRP" (290.00), "Variant -- Construct ID: 12AAYGSP" (179.00), and "Gene synthesis -- Construct ID: 12AAYGPP" (190.08). A "Variant -- Construct ID: 12AAYGQP" is also listed (179.00). At the bottom, it says "Estimated processing time for this project: 29 Days" with a circled "7" callout. On the right, the "Cart Summary" shows "Total Savings 0.00" and a "Begin Checkout" button with a circled "12" callout. A "Subtotal" of 838.08 is shown with a circled "10" callout. Other callouts include "8" and "9" on the "View | Edit | Remove" link, and "11" on the "Rename cart" link.

Items	Unit Size	List Price	Your Price	Qty	Total
Project on Aug 10, 2012—2012AARK6P View Edit Remove	1 Proj				838.08
Subcloning -- Construct ID: 12AAYGRP					
Catalog #: 854341DE GeneArt® Cloning into pcDNA™3.1 (+) • Final Construct Name:Subcloning_Example • Vector Name:pcDNA_3.1(+)	Ea.	290.00	290.00	1	290.00
Variant -- Construct ID: 12AAYGSP					
Catalog #: 830105DE GeneArt® Mutagenesis<1 kb • Sequence Name:Variant_parent_1	Ea.	179.00	179.00	1	179.00
Gene synthesis -- Construct ID: 12AAYGPP					
Catalog #: 817003DE GeneArt® Synthetic Gene<3 kb Online Order Rate • Gene Name:Example_sequence	Ea.	0.32	0.32	594	190.08
Variant -- Construct ID: 12AAYGQP					
Catalog #: 830105DE GeneArt® Mutagenesis<1 kb • Sequence Name:Variant_parent_2	Ea.	179.00	179.00	1	179.00

- You'll see detailed information about your order, including the estimated processing time (7). You can edit your project (8), or remove the project from your cart (9). If you do this, the project will remain as a "draft" in your project folder, and you can place it in the cart later. The price of your project is also displayed in the cart (10).
- The note at the top of the page is a reminder that you can have only one project in the cart at a time, but a project can include as many sequences as you wish (11).
- Check this page carefully, and if the information is correct, click "Begin Checkout" (12).

7.4. Shipment & Payment Information

1 Shipping & Payment 2 Review Order 3 Order Confirmation


Shipping & Payment Information

< Back Continue to Review >

Confirm your billing & shipping addresses

	Billing Address - Acct. # 68430609	Shipping Address - Acct. # 68430611
My Default Address	Example Billing address	Example Billing address
Additional Addresses	Attn ACCOUNTS PAYABLE	Example name
	Example street Example city Example country	Example street Example city Example country
		Edit address

Confirm your shipping method

Standard Shipping	Standard Shipping
	 Most in-stock items ship within 24 hours.

Order Summary

Total Savings	0.00
Subtotal	855.90
Handling Charge	66.00
(USD) TOTAL:	921.90

13

- Please provide all of the required shipping and billing information in the upper part of the page.
- A price update with handling charge is shown (13).

Customer Service 800 955 6288 [Quick Order](#) Hello, [Warren Coffeen](#) ▾

Select your payment method

Purchase Order Information

Purchase Order

Credit Card

My approver will pay

Enter your PO number
 14 Your PO number will be standardized in our system; all letters will be capitalized and special characters removed.

PO Reference # (optional)
 This will be included in the order documentation for your reference.

Contract (optional)
 This will be included in the order documentation for your reference.

Order Summary

Total Savings 0.00

Subtotal 855.90

Handling Charge 66.00

(USD) TOTAL: 921.90

Indicate your order preferences

American Recovery Reinvestment Act
 This order is being paid for with money from the American Recovery and Reinvestment Act.

GeneArt Terms & Conditions
 To meet the requirements a Biosafety and a Biosecurity check will be performed by Life Technologies Corporation or its affiliates. This might require further interaction with the customer, and Life Technologies Corporation and/or its affiliates expressly reserve the right to withdraw from this order. To proceed with this order please read and agree to the Gene Synthesis Terms and Conditions below which shall apply to any and all services (including but not limited to gene synthesis, cloning, plasmid preparation, generation of gene variants / sequential permutations, production of gene libraries and protein production) ordered via this web page.

* By checking this box the customer has read understood and accepted the [Gene Synthesis Terms and Conditions](#). 15

Approval Routing ▾

Email Preferences ▾

Special Instructions ▾

Enter cost center and invoice codes

Consolidated and Summary Invoice Customers: Please indicate your institution's invoice field sort values below (e.g., Cost Center, Grant, Project, Department, G/L, Business Unit, etc.)

Sort Value 1

Sort Value 2

< Back
Continue 16 Review >

- In the lower part of the page select a payment method.
- Please note: If you choose to pay with a purchase order and you don't have a PO number, please enter an internal reference number **(14)**
- UK and Italian purchasers: please confirm your VAT exemption, if applicable.
- Read the GeneArt® Terms and Conditions, and agree by ticking the box **(15)**.
- Then click "Continue to Review" **(16)**.

7.5. Review Order

1 Shipping & Payment
2 Review Order
3 Order Confirmation

< Back
Place My Order >

Send Bill to:

LINN BENTON COMMUNITY COLLEGE
68430609

Attn: Example
Example street
Example city
Example country

Ship Order to:

LINN BENTON COMMUNITY COLLEGE
68430611

Example name
Example street
Example city
Example country

Payment Information:

PO Number: 123456

Shipping Method: **Standard Shipping**

Edit Shipping & Payment

Items	Unit Size	List Price	Your Price	Qty	Total
Project on Aug 10, 2012 2012AARK6P	1 Proj				855.90
Subcloning -- Construct ID: 12AAYGRP					
Catalog #: 854341DE GeneArt® Cloning into pcDNA™3.1 (+)	Ea.	290.00	290.00	1	290.00
<ul style="list-style-type: none"> Final Construct Name:Subcloning_Example Vector Name:pcDNA_3.1(+) 					
Variant -- Construct ID: 12AAYGSP					
Catalog #: 830105DE GeneArt® Mutagenesis<1 kb	Ea.	179.00	179.00	1	179.00
<ul style="list-style-type: none"> Sequence Name:Variant_parent_1 					

Order Summary

Total Savings 0.00

Subtotal 855.90

Handling Charge 66.00

(USD) TOTAL: 921.90

- The Review Order page gives a summary of all your order information: shipping, billing, and payment information, your order details, and the total price of the order.
- Confirm the information, then click “Place My Order” (17).

7.6. Order Confirmation

Checkout
18

1 Shipping & Payment
2 Review Order
3 Order Confirmation

Order Confirmation

Logout
Continue Shopping

Thank you for your order!
 A confirmation email will be sent to the email address(es) that you specified. Track the status of your order through [My Account](#).
 Need product documentation? Find what you need by visiting [Technical Resources](#).

Order Number 22014733
Placed on 10 Aug 2012

Send Bill to:	Ship Order to:	Payment Information:
LINN BENTON COMMUNITY COLLEGE 68430609 Attn: Example ACCOUNTS PAYABLE 6500 PACIFIC BLVD SW ALBANY OR 97321 UNITED STATES	LINN BENTON COMMUNITY COLLEGE 68430611 Attn: Patty Petzel Biology WOH 206 6500 PACIFIC BLVD SW ALBANY OR 97321 3755 UNITED STATES	PO Number: 123456
Shipping Method: Standard Shipping		

Items	Unit Size	List Price	Your Price	Qty	Total
Project on Aug 10, 2012 2012AARK6P Process:1	1 Proj				855.90
Subcloning -- Construct ID: 12AAYGRP					
Catalog #: 854341DE GeneArt® Cloning into pcDNA™3.1 (+)	Ea.	290.00	290.00	1	290.00

- After placing your order, an “Order Confirmation” page will appear with all of the details. You can print this page for your records (18).
- You will also receive a confirmation email.

8. Contacts at GeneArt

For further information or if you have questions, please don't hesitate to contact us at:

Email: geneartsupport@lifetech.com

Europe:

Phone: +49 (0)941 942 76-100

Fax: +49 (0)941 942 76-780

America and Asia:

Phone: 800-955-6288, option 4/4/1

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