

## Welcome to IDT's 454 FusionPrimers Guide!

This Quick Start Guide is designed to cover the basic steps for entering Gene Sequence information for 454 FusionPrimers. It covers guidelines for setting Global and Individual Target options for Primer Designs, reviewing Gene Results and includes instructions for placing an Order or saving information to place an Order at a later time.

### Getting Started

- 1 Access IDT's website at [www.idtdna.com](http://www.idtdna.com).
- 2 From IDT's main page, there are two methods to access IDT SciTools:
  - a On the **IDT main page**, on the **Menu**, click **SciTools**.
  - OR -
  - b On the **IDT main page**, click the **IDT SciTools link**.



### Accessing the 454 FusionPrimers Page

- 1 On the **IDT SciTools** page, click **454 FusionPrimers**.



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# Entering Gene Sequence Information

You can enter Gene Sequence information by typing Gene Sequence Accession Numbers directly into the box provided or by uploading an Accession Number file. Gene Sequence(s) in FASTA format can also be entered directly in the box provided or uploaded from a sequence file containing the gene sequence information in FASTA format.

## General Rules for Entering Gene Sequence Accession Numbers and Gene Sequences in FASTA Format

When entering information for Gene Sequence Accession Numbers or Gene Sequence(s) in FASTA format, if you type the information directly into the respective boxes, you can specify the Exon and Intron areas in your Gene Sequence. For more information about Exon and Intron areas, refer to the following section in this Guide: "Option One - Setting Global Target Selections".

**Note** If you type or enter your own sequence information, IDT's 454 FusionPrimer does not designate the CDS regions.

- 1 Exon areas are specified by using uppercase letters in your gene sequence information.
- 2 Intron areas are specified by using lower case numbers in the gene sequence information.

**Gene Entry | Login**

**Enter gene sequence accession numbers of interest (comma separated, maximum 10)**

NM\_001234,NM\_001235,NM\_001236,NM\_001111,NM\_000111

Upload Accession File

- OR -

**Enter gene sequence or sequence of interest (multiple sequences in FASTA format, positive orientation, maximum 10)**

```
>FASTA FORMAT 1
CAAGTATTTTCAGCCCCAGCCGGCCACACAGCTCGGATCTCCTC
CTGTGGATCCCCCAGCTCTGCGATGATGGCAGAAGAGCACACA
GATCTCGAGGCCAGATCGTCAAGGATATCCACTGCAAGGAGAT
TGACCTGGTGAACCGAGACCCCAAGAACATTAACGAGGACATAG
TCAAGgtaggctctgcaggcctgctcgggcgggagaggtgtcaggtttgcgag
acgtgggcgcttggcaggggagggtatctgctgcagacacagtttccctgaaaaga
```

Upload Sequence File

Clear Entry      Gene Selection

## Option 1 - Directly Entering Gene Sequence Accession Numbers

To enter information for your Gene Entry directly into the box provided:

- 1 On the **Gene Entry** page, in the **Enter Gene Sequence Accession Numbers of Interest** box, type or paste your **Gene Sequence Accession** numbers.
- 2 Click **Gene Selection**.

**Gene Entry | Login**

**Enter gene sequence accession numbers of interest (comma separated, maximum 10)**

NM\_001234,NM\_001235,NM\_001236,NM\_001111,NM\_000111

Upload Accession File

- OR -

**Enter gene sequence or sequence of interest (multiple sequences in FASTA format, positive orientation, maximum 10)**

Upload Sequence File

Clear Entry      Gene Selection

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## Option 2 - Uploading a Gene Sequence Accession Number File

**Note** If you choose to upload your own Accession Number file, the file **must** be a **text** file to upload correctly to the Gene Entry site.

To upload Gene Sequence information from a text file:

- 1 On the **454 FusionPrimers** page, under **Gene Entry**, click **Upload Accession File**.
- 2 In the **Enter the path for the text file of accession numbers** box, click **Browse** and locate the file you want.

The file path you select appears in the box.

- 3 If you are ready to proceed immediately with your selection, click **Gene Selection**.
- 4 To review your selected accession list before proceeding, click **View Accession List** and then on the **Gene Entry** page, review your accession numbers.
- 5 When you are ready to proceed, click **Gene Selection** to process your criteria.
- 6 To **cancel** your accession numbers, click **Clear Entry**.

Results for the Gene Sequence information you entered appear on the Target Locations page.

### Gene Entry | Login

Enter gene sequence accession numbers of interest (comma separated, maximum 10)

Upload Accession File

### Gene Entry | Login

Enter the path for the text file of FASTA formatted sequences

Fusion Primer Quick Start Guide\Source Files\Test FASTA

View Sequence List

Gene Selection

## Option 3 - Directly Entering Gene Sequence Information in FASTA Format

You can enter Gene sequence information in FASTA format by typing your sequence(s) directly into the box provided. A gene sequence in FASTA format consists of a single line description followed by lines of sequence data. The description line is distinguished from the sequence data by a "greater than" symbol (>). The word following the ">" symbol is the identifier name of the sequence. You do not use a space between the ">" symbol and the first letter of the identifier name. Immediately following the identifier name, hit Return (or Enter) and then on the next line, type your sequence information.

To enter Gene Sequence information in FASTA format directly into the box provided:

- 1 On the **Gene Entry** page, in the **Enter Gene Sequence or sequence of interest** box, type or paste your **Gene Sequence in FASTA format**.
- 2 Click **Gene Selection**.

### Gene Entry | Login

Enter gene sequence accession numbers of interest (comma separated, maximum 10)

Upload Accession File

- OR -

Enter gene sequence or sequence of interest  
(multiple sequences in FASTA format, positive orientation, maximum 10)

```
CAAGTATTTTCAGCCCCAGCCGGCCACACAGCTCGGATCTCCTC
CTGTGGATCCCCCAGCTCTGCGATGATGGCAGAAGAGCACACA
GATCTCGAGGCCAGATCGTCAAGGATATCCACTGCAAGGAGAT
TGACCTGGTGAACCGAGACCCCAAGAACATTAACGAGGACATAG
TCAAAGgtaggctctgcaggcctgcctcgcgggcggagagtgtcaggtttgcgag
acgtggcgcttgccagggagggtatctgctgcagacacagtttccctgaaaaaga
gacttggtgctctgttctctgtatcaccccccaaccccccccccaaaaaagg
```

Upload Sequence File

Clear Entry

Gene Selection

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## Option 4 - Uploading a Gene Sequence File in FASTA Format

You can upload a sequence information file in FASTA format by following the steps shown below.

- 1 On the **454 FusionPrimers Gene Entry** page, click **Upload Sequence File**.
- 2 In the **Enter the path for the text file of FASTA formatted sequence** box, click **Browse** and locate the file you want.  
The file path you select appears in the box.
- 3 To review the FASTA sequence file you uploaded before proceeding, click **View Sequence List**.

Enter the path for the text file of FASTA formatted sequences

Quick Start Guide\Source Files\FASTA Format Upload.txt

The FASTA sequence information you uploaded appears in the Gene Sequence box on the **Gene Entry** page.

Enter gene sequence or sequence of interest  
(multiple sequences in FASTA format, positive orientation, maximum 10)

```
>FASTA FORMAT 1
CAAGTATTTTCAGCCCCAGCCGGCCACACAGCTCGGATCTCCTC
CTGTGGATCCCCCAGCTCTGCGATGATGGCAGAAGAGCACACA
GATCTCGAGGCCAGATCGTCAAGGATATCCACTGCAAGGAGAT
TGACCTGGTGAACCGAGACCCCAAGAACATTAACGAGGACATAG
TCAAGgtaggctctgcaggcctgctcggcgggcgagagtgatcaggttgag
acgtggcgcttgccaggggagggtatctgctgcagacacagttccctgaaaaga
```

- 4 When you have reviewed your sequence information and are ready to proceed, click **Gene Selection** to process your criteria.  
Results for the Gene Sequence FASTA information you entered appear on the Target Locations page.
- 5 To **cancel** the Gene Sequence FASTA information you uploaded, click **Clear Entry**.

## Option 5 - Entering a Combination of Gene Accession Numbers and Gene Sequences in FASTA Format

You can enter a combination of Gene Accession Numbers and Gene Sequence Numbers (in Fasta format).

- 1 On the **454 FusionPrimers Gene Entry** page, in the **Enter Gene Sequence Accession Numbers of Interest** box, type, paste or upload your **Gene Sequence Accession Numbers**.
- 2 In the **Enter Gene Sequence or sequence of interest** box, type, paste or upload your **Gene Sequence FASTA format numbers**.
- 3 Click **Gene Selection**.  
Results for the Gene Sequence Accession and FASTA numbers appear on the Target Locations page.

Gene Entry | Login

Enter gene sequence accession numbers of interest (comma separated, maximum 10)

NM\_001234,NM\_001235,NM\_001236,NM\_001111,NM\_000111

- OR -

Enter gene sequence or sequence of interest  
(multiple sequences in FASTA format, positive orientation, maximum 10)

```
CAAGTATTTTCAGCCCCAGCCGGCCACACAGCTCGGATCTCCTC
CTGTGGATCCCCCAGCTCTGCGATGATGGCAGAAGAGCACACA
GATCTCGAGGCCAGATCGTCAAGGATATCCACTGCAAGGAGAT
TGACCTGGTGAACCGAGACCCCAAGAACATTAACGAGGACATAG
TCAAGgtaggctctgcaggcctgctcggcgggcgagagtgatcaggttgag
acgtggcgcttgccaggggagggtatctgctgcagacacagttccctgaaaaga
gacttggtctgtgtctctgatcaccccccaaccccccccccaaaaaagg
```

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# Setting Global Options for Target Locations

On the Target Locations page, you can make general selections that affect all Gene Target Sequence Results.

**Note** For selections that affect all Results, you must set Global Target Selections **first**, then make selections for Individual Target Results.

## Option One - Setting Global Target Selections

**Note** If you enter your own sequence information, the Target Selection box does not show CDS options.

Gene Entry | **Target Locations** | Primer Design | Login Quick Start Guide

### Global Target Selection

 Exons  Introns  
 5'UTR  CDS  3'UTR

### Primer Design Criteria

Amplicon Size Minimum  Forward Primer

Amplicon Size Optimum  Reverse Primer

Amplicon Size Maximum

### Target Sequence Segment Selection

Target 1: NM\_001234

1 50  
CAAGTATTTTCAGCCCCAGCCGCCACACAGCTCGGATCTCCTCCTGTGG

(Select custom target region above)

■ Exon ■ Selected Exon  
■ Intron ■ Selected Intron  
— Untranscribed Genome

NM_001234 Exon and Intron Locations				
	Exon	Position	Intron	Position
<input type="checkbox"/> 5' UTR	<input type="checkbox"/> Exon 1 - 5'UTR	1-67	<input type="checkbox"/> Intron 1	182-11716
<input type="checkbox"/> CDS	<input type="checkbox"/> Exon 1 - CDS	68-181	<input type="checkbox"/> Intron 2	12065-12160
<input type="checkbox"/> 3'UTR	<input type="checkbox"/> Exon 2 - CDS	11717-12058		
	<input type="checkbox"/> Exon 2 - 3'UTR	12059-12064		
	<input type="checkbox"/> Exon 3	12161-12956		

NM\_001234 Specific Product Locations

Start Position  End Position

1 On the **Target Locations** page, in the **Global Target Selection** box, place a **check** in the check box in front of the selections you want:

- a **Exons** - Checking the Exons box places check marks in all of the Exon boxes for all results.
- b **Introns** - Checking the Introns box places check marks in all of the Intron boxes for all results.
- c **5'UTR** - Checking the 5' UTR box places check marks in all of the 5' UTR related boxes for all results.
- d **3'UTR** - Checking the 3' UTR box places check marks in all of the 3' UTR related boxes for all results.
- e **CDS** - Checking the CDS box places check marks in all of the CDS related boxes for all results.

### Global Target Selection

 Exons  Introns  
 5'UTR  CDS  3'UTR

## Option Two - Selecting Primer Design Criteria

1 To set Primer Design Criteria for all results, in the **Primer Design Criteria** box, enter the **information** you want to select for all results:

- a **Amplicon Size Minimum** - enter the minimum number for your Amplicon size.
- b **Amplicon Size Optimum** - enter the optimum number for your Amplicon size.
- c **Amplicon Size Maximum** - enter the maximum number for your Amplicon size.
- d **Forward Primer** - Select from the drop down menu the Forward Primer you want.
- e **Reverse Primer** - Select from the drop down menu the Reverse Primer you want.

### Primer Design Criteria

Amplicon Size Minimum  Forward Primer

Amplicon Size Optimum  Reverse Primer

Amplicon Size Maximum

2 To reset the Primer Design Criteria to the default selections, in the **Primer Design Criteria** box, click **Reset Defaults**.

**Note** For more information on Individual Primer Design Criteria, refer to "Setting Criteria for Individual Primer Designs".

## Setting Criteria for Individual Primer Designs

In addition to selecting Primer Design Criteria on the Target Locations page, you can make detailed selections for each Individual Parameter on the Primer Design page.

- 1 To access the Primer Design page, at the top of the 454 FusionPrimers page, click **Primer Design**.

### Gene Entry | Target Locations | **Primer Design**

- 2 On the **Primer Design** page, click any of the **tabs** to see individual pages for Target Specific Parameters, FusionPrimer Parameters or Amplicon Primer Parameters. Or, you can click **Show All** to view all the Parameters at once (as shown in this example).
- 3 Under **Target Specific Parameters**, to change any of the numbers, click in the **corresponding box** and highlight the **existing number**, then type the **number** you want.
- 4 Under **454 FusionPrimers Parameters**, for **Forward and Reverse Primers**, select an **option** from the list provided.
- 5 To make additional changes, click in the **corresponding box** and highlighting the **existing number**, then type the **number** you want.
- 6 Under **Amplicon Parameters**, click in the **corresponding box** and highlight the **existing number**, then type the **number** you want.
- 7 For **Discrete Selections**, select **Yes or No** from the list.
- 8 When you have completed all your selections, click **Design**.
- 9 To reset all selections to the default, click **Reset Defaults**.

Gene Entry | Target Locations | **Primer Design**

Show All | Target Specific | Fusion Primer | Amplicon

Design

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**Target Specific Parameters**

Max Sequence Repeat	<input type="text" value="8"/>		
Oligo Concentration	<input type="text" value="200"/>	nM	
Na <sup>+</sup> Concentration	<input type="text" value="100"/>	mM	
Mg <sup>++</sup> Concentration	<input type="text" value="0"/>	mM	
dNTPs Concentration	<input type="text" value="0"/>	mM	

			<b>Free Energy Thresholds</b>
Hairpin	<input type="text" value="-3"/>	kcal/mole	
Homo Dimer	<input type="text" value="-6"/>	kcal/mole	
Hetero Dimer	<input type="text" value="-4"/>	kcal/mole	

	Min	Optimum	Max
Oligo Length	<input type="text" value="12"/>	<input type="text" value="19"/>	<input type="text" value="28"/>
Melting Temperature	<input type="text" value="55"/> °C	<input type="text" value="60"/> °C	<input type="text" value="65"/> °C
GC Content	<input type="text" value="35"/> %	<input type="text" value="50"/> %	<input type="text" value="65"/> %

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**Fusion Primer Parameters**

Forward Primer	<input type="text" value="Primer A"/>	
Reverse Primer	<input type="text" value="Primer B"/>	
Max Sequence Repeat	<input type="text" value="5"/>	

			<b>Free Energy Thresholds</b>
Hairpin	<input type="text" value="-4"/>	kcal/mole	
Homo Dimer	<input type="text" value="-6"/>	kcal/mole	
Hetero Dimer	<input type="text" value="-5"/>	kcal/mole	

	Min	Optimum	Max
Melting Temperature	<input type="text" value="63"/> °C	<input type="text" value="72"/> °C	<input type="text" value="80"/> °C
GC Content	<input type="text" value="35"/> %	<input type="text" value="50"/> %	<input type="text" value="65"/> %

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**Amplicon Parameters**

	Min	Optimum	Max
Amplicon Length	<input type="text" value="90"/>	<input type="text" value="100"/>	<input type="text" value="140"/>
Min Amplicon Overlap	<input type="text" value="20"/>		
Discrete Selections	<input type="text" value="Yes"/>		

Reset Defaults      Design

## Reviewing Target Sequence Segments

On the Target Locations page, you can examine Sequence information using two quick methods. With either method, remember that **Exons appear in uppercase character format** and **Introns appear in lowercase character format**.

- 1 To view any section of the Target Sequence Segment, click the **arrows at either end** of the Gene Target Sequence.
- 2 For a faster selection, click the **Triangle** shown just below the Target Sequence Segment line and drag it to the **location on the line** that corresponds with the area of the Gene Target Sequence you want to see.

**Target Sequence Segment Selection**

Target 1: NM\_001234

2687      2736

↑ tacaccaggtgggctgcagggagatgggaaagaggatcccacgggagaa ↑

(Select custom target region above)

Legend:  
█ Exon    █ Selected Exon  
█ Intron    █ Selected Intron  
█ Untranscribed Genome

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## Setting Individual Target Options

On the Target Locations page, after setting Global Target Selections for your Results, you can also make the following Individual Target selections:

- Select a Custom Target Region for an Individual Target Sequence
- Set specific Start and End positions for a Target Sequence
- Make Individual Selections for a Specific Target Sequence Result

### Option One - Selecting an Individual Custom Target Region

- Under **Target Sequence Segment Selection**, click in the **Gene Target display**, select the **area** you want, then click **Commit Selected Area**.

**Target Sequence Segment Selection**

Target 1: NM\_001234

1 50

CAAGTATTTTCAGCCCCAGCCGGCCACACAGCTCGGATCTCCTCCTGTGG

Commit Selected Area Start: 12 End: 40

Legend: Exon (blue), Selected Exon (red), Intron (grey), Selected Intron (red), Untranscribed Genome (brown)

### Option Two - Setting Numeric Start and End Positions for Target Sequences

**Note** Custom Target Sequence sites are numbered sequentially with the 5' most base position beginning with the number "1".

- Under **Specific Product Locations**, in the **Start Position** box, type the **Start Position Number** for the Sequence.

**NM\_001234 Specific Product Locations**

Start Position	End Position	Remove
12	40	Remove
50	150	Remove
125	250	Remove

- In the **End Position** box, type the **End Position Number** for the Target Sequence.

**Note** As you complete numbers for Start/End Positions, a new set of boxes appear to allow for additional entries.

- If additional boxes do not appear, click the **Lock** in front of the previous set of boxes to trigger additional entry boxes.
- To remove a Specific Product Location, click the **Remove** button at the far right of the Location you want to remove.

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## Setting Individual Target Options - Continued

### Option Three - Making Individual Selections for a Specific Target Sequence Result

- 1 To make Individual Selections for a Specific Target Sequence Result, under that **Result**, place a check in any additional check boxes for selections that have not already been checked under the Global Target Selection process.

Ex: Global Target Selections were Exons and CDS. To add Intron 1 and Intron 2 for Target 1, place checks in those boxes.

The screenshot displays the 'Global Target Selection' and 'Primer Design Criteria' sections. Under 'Global Target Selection', 'Exons' and 'CDS' are checked. Under 'Primer Design Criteria', 'Amplicon Size Minimum' is 90, 'Amplicon Size Optimum' is 100, and 'Amplicon Size Maximum' is 140. The 'Target Sequence Segment Selection' section shows a sequence for Target 1: NM\_001234 with a custom target region highlighted. Below this is a table of 'NM\_001234 Exon and Intron Locations' with checkboxes for 'Exon' and 'Intron' and their respective positions.

	Exon	Position	Intron	Position
<input type="checkbox"/> 5' UTR	<input type="checkbox"/> Exon 1 - 5'UTR	1-67	<input type="checkbox"/> Intron	
<input checked="" type="checkbox"/> CDS	<input checked="" type="checkbox"/> Exon 1 - CDS	68-181	<input checked="" type="checkbox"/> Intron 1	182-11716
	<input checked="" type="checkbox"/> Exon 2 - CDS	11717-12058		
<input type="checkbox"/> 3'UTR	<input type="checkbox"/> Exon 2 - 3'UTR	12059-12064	<input checked="" type="checkbox"/> Intron 2	12065-12160
	<input type="checkbox"/> Exon 3	12161-12956		

### Completing Global and Individual Selections for Target Sequence Results

- 1 When you have completed your selections for global and target areas, at the bottom of the **Results** page, click **Design**.



- 2 To receive an email notification rather than waiting for the Search calculation Results, on the **Results Processing** page, click **Email Notification**.
- 3 On the **Email Notification** page, enter the **Email Address and Job Name**. You will receive an email with a link to access the request.

The screenshot shows the 'Results Processing...' page with a message: 'Please wait while your request processes. This process may take several minutes. You may request an email notification when this process completes if you do not wish to wait:'. There are buttons for 'Email Notification' and 'View Completed Tasks'. Below the message, it says 'Task 2 of 5 is completed. You may view the completed tasks or wait until all tasks are completed.'

The screenshot shows the 'Email Notification' page with a message: 'Please enter the email address you want this notification to be sent and the job name you wish to use for identification. When the process is completed you will receive an email with a link to access the request.' There are input fields for 'Enter Email Address:' and 'Enter Job Name:', and an 'Email Notification' button.

- 4 If you choose to wait for Results, you may see a message on the Processing page indicating some (but not all) of the tasks have been completed. To view the tasks that have been completed, click **View Completed Tasks**.

**Note** If you choose to view the partial Tasks that have been completed (before all the Tasks have been processed), you will need to wait for the Processing page to indicate all Tasks have completed before you can view all the Results.

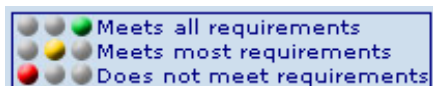
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# Reviewing Gene Results and Selecting Your Order

Results for your Gene Sequence selections appear on the Results page. The Results page defaults to the Sequence view for your first Result, but you can also view Sequence Details, Amplicon Details and Parameters.

## Understanding the Results Grid

The following color coded grid provides an assessment of the criteria for each Result and the sets you selected:



Color	Primer / Probe Indicator
Green	The Result met all of the requirements you selected.
Yellow	The Result met all but one of the requirements you selected.
Red	The Result did not meet two or more of the requirements you selected.

**Fusion Primer Result NM\_001234**

→ ← Set 1  
→ ← Set 2

Gene coded on '+' strand of genome

[View Sequence](#) | [View Details](#) | [View Amplicon Details](#) | [View Parameters](#)

Assessment	Fusion Primer	
	Universal Segment	Target Specific Segment
<input type="checkbox"/> ●●● Set 1		
●●● Forward	GCCTCCCTCGCGCCATCAG	AGTATTTTCAGCCCCAGCC
●●● Reverse	GCCTTGCCAGCCCGCTCAG	CCTTGACTATGTCTCGTTAATGT
<input type="checkbox"/> ●●● Set 2		
●●● Forward	GCCTCCCTCGCGCCATCAG	GGCAGAAGAGCACACAGAT
●●● Reverse	GCCTTGCCAGCCCGCTCAG	TCTCGCAAACCTGACACTC

## Selecting a Specific Result

- To view Results for a specific Gene Entry, on the **Results** page, under **Select to View Result**, click the **Result** you want to see.

[Gene Entry](#) | [Target Locations](#) | **[Results](#)** | [Login](#)

Select to View Result: ◀ 1 of 5  
NM\_001234 | NM\_001235

## Viewing Details for a Result

- To view Details for a selected Result, on the **Results** page, click **View Details**.

[View Sequence](#) | **[View Details](#)** | [View Amplicon Details](#) | [View Parameters](#)

Assessment	Positions		Fusion Primer						Target Specific				
	Start	End	Len	T <sub>M</sub> (°C)	GC (%)	ΔG (kcal/mole)			T <sub>M</sub> (°C)	GC (%)	ΔG (kcal/mole)		
						Hairpin	Hetero-dimer	Homo-dimer			Hairpin	Hetero-dimer	Homo-dimer
<input type="checkbox"/> ●●● Set 1													
●●● Forward	3	21	19	76.7	63.2	-0.58	-8.58	-7.96	59.9	52.6	-0.43	-3.63	-4.48
●●● Reverse	182	159	24	75.9	55.8	-0.16	-8.58	-11.20	59.5	41.7	-0.16	-3.63	-5.48
<input type="checkbox"/> ●●● Set 2													
●●● Forward	73	91	19	77.5	63.2	-2.91	-9.94	-14.81	59.7	52.6	-0.62	-4.97	-4.48
●●● Reverse	232	214	19	76.8	63.2	-0.76	-9.94	-11.94	59.5	52.6	0.00	-4.97	-2.90
<input type="checkbox"/> ●●● Set 3													
●●● Forward	11650	11670	21	76.1	57.5	-0.69	-5.86	-9.64	60.0	42.9	-0.69	-3.52	-6.80
●●● Reverse	11826	11807	20	76.9	61.5	-1.58	-5.86	-10.58	60.2	50.0	-0.94	-3.52	-6.56

The View Details page appears and you can review the details for each set.

## Viewing Amplicon Details for a Result

- To view the Amplicon Details for a selected Result, on the **Results** page, click **View Amplicon Details**.  
The Amplicon Details page appears.

View Sequence | View Details | **View Amplicon Details** | View Parameters

Assessment	Amplicon Region			Selected Region			
	Start	End	Length	Start	End	Length	Selected
<input type="checkbox"/> <b>Set 1</b>	22	158	137	68	181	114	Exon 1 - CDS
Forward							
Reverse							
<input type="checkbox"/> <b>Set 2</b>	92	213	122	68	181	114	Exon 1 - CDS
Forward							
Reverse							

## Viewing Parameters for a Result

- To view Parameters for a selected Result, on the **Results** page, click **View Parameters**.  
For more information on setting Parameters, return to the Target Locations page and then refer to "Setting Criteria for Individual Primer Designs".

View Sequence | View Details | View Amplicon Details | **View Parameters**

Target Specific Parameters			
Max Sequence Repeat	8	Hairpin	-3 kcal/mole
Oligo Concentration	200 nM	Homo Dimer	-6 kcal/mole
Salt Concentration	100 mM	Hetero Dimer	-4 kcal/mole
	Min	Opt	Max
Oligo Length	12	19	28
Melting Temperature	55 °C	60 °C	65 °C
GC Content	35 %	50 %	65 %
Fusion Primer Parameters			
Forward Primer		Hairpin	-4 kcal/mole
Reverse Primer		Homo Dimer	-6 kcal/mole
Max Sequence Repeat	5	Hetero Dimer	-5 kcal/mole
	Min	Opt	Max
Melting Temperature	63 °C	72 °C	80 °C
GC Content	35 %	50 %	65 %
Amplicon Parameters			
Amplicon Length	90	Opt	140
Min Amplicon	20		
Overlap			
Discrete Selections	Yes		

## Returning to the Default Sequence Detail View

- To return to the default Sequence Details view, on the **Results** page, click **View Details**.  
The Details Sequence page appears and you can proceed with your order.

View Sequence | **View Details** | View Amplicon Details | View Parameters

Assessment	Positions		Fusion Primer			Target Specific							
	Start	End	Len	ΔG (kcal/mole)			T <sub>M</sub> (°C)	GC (%)	ΔG (kcal/mole)				
				Hairpin	Hetero-dimer	Homo-dimer			Hetero-dimer	Homo-dimer			
<input type="checkbox"/> <b>Set 1</b>													
Forward	3	21	19	76.7	63.2	-0.58	-8.58	-7.96	59.9	52.6	-0.43	-3.63	-4.48
Reverse	182	159	24	75.9	55.8	-0.16	-8.58	-11.20	59.5	41.7	-0.16	-3.63	-5.48
<input type="checkbox"/> <b>Set 2</b>													
Forward	73	91	19	77.5	63.2	-2.91	-9.94	-14.81	59.7	52.6	-0.62	-4.97	-4.48
Reverse	232	214	19	76.8	63.2	-0.76	-9.94	-11.94	59.5	52.6	0.00	-4.97	-2.90

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## Saving Results for Use at a Later Time

You can save Results you have selected and then review and order them at a later time.

- 1 To save Results for review and order at a later time, on the **Results** page, under **View Sequence**, click the **check box** in front of each **Set** you want for your Order
- 2 At the bottom of the **Results** page, click **Save**.



[View Sequence](#) | [View Details](#) | [View Amplicon Details](#) | [View Parameters](#)

Assessment	Fusion Primer	
	Universal Segment	Target Specific Segment
<input checked="" type="checkbox"/> <b>Set 1</b>		
Forward	GCCTCCCTCGCGCCATCAG	AGTATTTTCAGCCCCAGCC
Reverse	GCCTTGCCAGCCCGCTCAG	CCTTGACTATGTCCTCGTTAATGT
<input checked="" type="checkbox"/> <b>Set 2</b>		
Forward	GCCTCCCTCGCGCCATCAG	GGCAGAAGAGCACACAGAT
Reverse	GCCTTGCCAGCCCGCTCAG	TCTCGCAAACCTGACACTC

**Note** If you have not Logged In, you will be prompted to do so at this time.

- 3 On the **Save Job** page, in the **Enter Job Name** box, enter a **name** for the Job (Order) you are saving, then click **Save**.  
Your selections are saved for review at a later time.

### Save Job

Please enter the job name you wish to use for identification.

Enter Job Name:



## Accessing Your Saved Results

- 1 To access your Saved Results, **log in** to **IDT's web site**, then select **SCITools** and **454 FusionPrimers**.
- 2 On the **Fusion Primer** page, on the **menu** at the top, click the **Saved Requests** tab.

[Gene Entry](#) | [Target Locations](#) | [Results](#) | [Saved Requests](#)

- 3 On the **Saved Requests** page, find the **saved job** you want to see, then click **View**.

[Gene Entry](#) | [Saved Requests](#)

### Select Desired Job

Job Name	Date Created		
1 Fusion Primer Order	9/25/2008	Delete	View

- 4 To delete a **Saved Job**, click **Delete**.
- 5 To complete the deletion of the selected job, on the **Saved Requests Warning** page, click **Delete**.
- 6 To cancel deletion of the selected job, click **Cancel**.

[Gene Entry](#) | [Saved Requests](#)

**Warning: This action will permanently delete all information from "Fusion Primer Order"**

Do you wish to proceed?



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# Placing a 454 FusionPrimers Order

On the Results page you can select All Sets from a Result or choose Individual Sets from a Result. You can also make selections for each Result and save them for ordering at a later time.

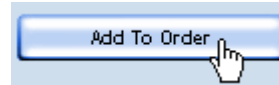
## Selecting Result Sets and Purification

- 1 On the **Results** page, under **Select to View Result**, click the **Result** you want to view.

**Gene Entry | Target Locations | Results | Saved Requests**

Select to View Result: NM\_001234 | NM\_001235 | NM\_001236

- 2 Under **View Sequence**, make any **selections** for the Sets you want, then click **Add to Order** at the bottom of the page.



**Note** If you have not logged in at this point, you will be prompted to do so.

- 3 On the **Order** page, in the **Select Details for the Order** box, under **Purification**, select **one** of the options.

**Results | Order**

Select Details for the Order

Scale: 100 nmole 454 FusionPrimer Purification: HPLC Purification

Notes: HPLC Purification Standard Desalting

Cancel Add And Return To Design View Add And Checkout

- 4 To add the selections you have made for **this Result** to your Order and then return to the Design View to make selections for other Results, click **Add and Return to Design View**.

- 5 Then, on the **Results** page, select your **next Result** and repeat the procedure outlined in "Reviewing Gene Results and Selecting Your Order".
- 6 To cancel any selections you have made for a Result, on the **Order** page, click **Cancel**.
- 7 When you have made all the selections for the Results you want to Order, to complete your Order, on the **Order** page, click **Add and Checkout**.
- 8 On the **Shopping Cart** page, review your **Order**, then click **Checkout**.

### Shopping Cart

Current Order as of 9/26/2008 9:22:49 AM (CDT)

#	Item	Product	Usually Ships In:	Guaranteed Yield:	Length:	Price
#1	NM_001234.Set2.For	100 nmole 454 FusionPrimer	3-4 business days	1 ODs = 2.8 nmoles = 32.2 µgrams	38	\$45.00
		Purification: HPLC Purification				
		Sequence: 5'- GCC TCC CTC GCG CCA TCA GGG CAG AAG AGC ACA CAG AT -3'				
#2	NM_001234.Set2.Rev	100 nmole 454 FusionPrimer	3-4 business days	1 ODs = 3 nmoles = 34.3 µgrams	38	\$45.00
		Purification: HPLC Purification				
		Sequence: 5'- GCC TTG CCA GCC CGC TCA GTC TCG CAA ACC TGA CAC TC -3'				
<input checked="" type="radio"/> Ship order when complete (single shipment)						
<input type="radio"/> Ship items as available						
Promo Code <input type="text"/> <input type="button" value="Go"/>					SubTotal	\$90.00 USD
					Shipping and Handling	Inquire
					Tax	\$0.00 USD
					Total	Inquire

- 9 To save your Order selections for a later time, click **Save to Wish List**.
- 10 To continue Shopping, click **Continue Shopping**.



~ End of Document ~

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