



The “Primers” Page

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Purpose

The BankIt submission tool “Primers” page is where you will provide the PCR primers you used to *amplify* the nucleic acid that you sequenced. The information we ask for in this page includes:

- The sequence of the forward and reverse PCR primers for each primer reaction set
- The names of the forward and reverse PCR primers for each primer reaction set

Do NOT submit sequencing primers in this page. This page is for entering the PCR primers used to amplify the nucleic acid that you sequenced.

Note: PCR primers are optional and are not required for a submission.

Single Sequence Submissions

If you submitted a single sequence in the “Nucleotide” page of the form, when you get to the “Primers” page, you will see primer sequence examples followed by text/name boxes for your primers (Figure 18):

Starting with the text box on the far left:

1. Place the **sequence** of your forward (fwd) primer in the **first** box.
2. Place the **name** of the forward primer in the **second** box.
3. Place the **sequence** of your reverse (rev) primer in the **third** box.
4. Place the **name** of the reverse primer in the **fourth** box.

Note: The primers you enter in these text boxes are PCR primers for nucleic acid amplification, and NOT sequencing primers.

Additional Primer Pairs for the Same Reaction Set

If you have additional pair(s) of forward and reverse primers for your PCR reaction mixture, click the “Add” button to get text boxes for another pair of forward and reverse primers (Figure 19). You can click the “Add” button as many times as you have primer pairs for this reaction mixture.

Additional PCR Reaction Sets for Your Sequence

- If you have another PCR reaction for the sequence you are submitting, click the “Add Another Primer Reaction Set” to get a set of boxes where you can add the forward and reverse primers for the additional reaction set (Figure 20).
- You can click the “Add” button to get text boxes for another primer set for this new reaction. You can click the “Add” button as many times as you have primer pairs for this reaction mixture.

GenBank Submissions

Contact Reference Nucleotide Submission Category Source Modifiers **Primers** Features Review and Correct

Submission # 1449155

PCR Primers (Optional)

Set PCR primers for sequence

- Each table represents a **Reaction Set**.
- To indicate multiple primers within a reaction, add appropriate number of rows.
- To indicate multiple reactions, click on **'Add Another Primer Reaction Set'**.
- Below is an example for multiple primers within a reaction.

fwd_primer_seq	rev_primer_seq
gtgacacgtacacgt	atcgcacgtacacgt
accaccacacactacac	caaccacagactacac

Enter the **SEQUENCE** for your PCR forward primer here

Enter the **SEQUENCE** for your PCR reverse primer here

Reaction Set #1

fwd_primer_seq	fwd_primer_name	rev_primer_seq	rev_primer_name
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

Add additional primers for this reaction set.

Add Another Primer Reaction Set

Continue

Enter the **NAME** for your PCR forward primer here

Enter the **NAME** for your PCR reverse primer here

Figure 18: The “Primers” page when a single sequence is submitted. Figure text gives instructions for entering primer sequences and names.

- Once you have finished entering the primer pairs for this new reaction, if you have any additional reaction mixtures for this sequence, click the “Add Another Primer Reaction Set” button as many times as you have PCR reaction sets for this sequence that you need to add.

Check Your Primer Sequences before Continuing

Once you have finished entering the PCR primers for the sequence you are submitting, click the “Continue” button” to refresh the static table at the bottom of the “Primers” page with the sequences you have entered. Once the table has refreshed, check your primer sequences to be sure they are correct. Once you have verified that your primer sequences are correct, click the “Continue” button again to go to the next page of the form.

Multiple Sequence Submissions

If you submitted multiple sequences in the “Nucleotide” page of the form, when you get to the “Primers” page, you will be given a choice of uploading a Primers Table File or using the BankIt form to enter your primers. (Figure 21)

GenBank Submissions

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Submission # 1449155

PCR Primers (Optional)

Set PCR primers for sequence

- Each table represents a **Reaction Set**.
- To indicate multiple primers within a reaction, add appropriate number of rows.
- To indicate multiple reactions, click on '**Add Another Primer Reaction Set**'.
- Below is an example for multiple primers within a reaction.

fwd_primer_seq	rev_primer_seq
gtgacacgtacacgt	atcgcacgtacacgt
accaccacacactacac	caaccacagactacac

Click the "Add" button as many times as you need to create additional boxes you can use for entering more primers in this reaction set

Reaction Set #1

fwd_primer_seq	fwd_primer_name	rev_primer_seq	rev_primer_name
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

additional primers for this reaction set.

Figure 19: The "Primers" page when a single sequence is submitted and the "Add" button is clicked twice. Figure text gives instructions for using the "Add" button to enter more primers in a reaction set.

If the primer sets are different for each of the sequences:

1. Select the "Upload Primers Table File" button. When you do, a text box and "Browse" button will appear.
2. Use the "Browse" button to select the Primers Table File from your local computer.
3. Click the "Continue" button to load your Primers Table File.
4. The primers you submitted in your Primers Table File will be displayed in the "Primers" table at the bottom of the page.
5. Check the Primers table at the bottom of the page to verify that the primer sequences displayed are correct.
6. Click the "Continue" button to go to the next page of the form.

Instructions for creating a Primers Table File are available from BankIt Help.

If the Primer Sets are the Same for all the Sequences:

Select the "Input PCR Primers" button. When you do, primer sequence examples followed by text/name boxes for your primers will appear (Figure 22):

GenBank Submissions

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Submission # 1449155

PCR Primers (Optional)

Set PCR primers for sequence

- Each table represents a **Reaction Set**.
- To indicate multiple primers within a reaction, add appropriate number of rows.
- To indicate multiple reactions, click on **'Add Another Primer Reaction Set'**.
- Below is an example for multiple primers within a reaction.

fwd_primer_seq	rev_primer_seq
gtgacacgtacacgt	atcgcacgtacacgt
accaccacacactacac	caaccacagactacac

Reaction Set #1

fwd_primer_seq	fwd_primer_name	rev_primer_seq	rev_primer_name
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

additional primers for this reaction set.

Reaction Set #2

fwd_primer_seq	fwd_primer_name	rev_primer_seq	rev_primer_name
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

additional primers for this reaction set.

Click the "Add Another Primer Reaction Set" button as many times as you need if you have additional PCR reactions for the sequence you are submitting.



Figure 20: The "Primers" page when a single sequence is submitted and the "Add Another Primer Reaction Set" button is clicked once. Figure text gives instructions for using the "Add Another Primer Reaction Set" button to enter more PCR reaction sets for a sequence.

Starting with the text box on the far left:

1. Place the **sequence** of your forward (fwd) primer in the **first** box.
2. Place the **name** of the forward primer in the **second** box.
3. Place the **sequence** of your reverse (rev) primer in the **third** box.
4. Place the **name** of the reverse primer in the **fourth** box.

If you have additional primer pairs for the same reaction set, see the instructions in the single sequence submission section for entering additional primer pairs in the form.

If you have additional PCR reaction sets for your sequences see the instructions in the single sequence submission section for entering additional PCR reaction sets in the form.

Check Your Primer Sequences before Continuing

Once you have finished entering the PCR primers for the sequence you are submitting, click the "Continue" button to refresh the static table at the bottom of the "Primers" page with the sequences you have entered. Once

Click this button if the primer set(s) are different for each of the sequences

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PCR Primers (Optional)

Set different PCR primers for sequences

Upload Primers Table File
How do I create a [primers table](#) file?

Set same PCR primers for all sequences

Input PCR primers

Continue

Click this button if the primer set(s) are the same for all of the sequences

Figure 21: The “Primers” page when multiple sequences are submitted. Figure text gives hints for selecting a primer submission method.

the table has refreshed, check your primer sequences to be sure they are correct. Once you have verified that your primer sequences are correct, click the “Continue” button again to go to the next page of the form.

Common Mistakes Made While Filling Out the “Primers” Page

- **Mistake: Entering the primer sequences and names in the wrong text boxes.**

Fix: Be sure to read the text boxes carefully before entering your data. Starting on the left, you enter the forward primer sequence in the first box and the name for this primer in the second box. Then you enter the reverse primer sequence in the third box and the name for this primer in the fourth box.

- **Mistake: Entering sequencing primers instead of PCR primers.**

Fix: This page is for entering PCR primers used to amplify the nucleic acid that you sequenced. Do not enter sequencing primers on this page.

GenBank Submissions

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Submission # 1449155

PCR Primers (Optional)

Set different PCR primers for sequences

Upload Primers Table File
How do I create a [primers table](#) file?

Set same PCR primers for all sequences

Input PCR primers

- Each table represents a **Reaction Set**.
- To indicate multiple primers within a reaction, add appropriate number of rows.
- To indicate multiple reactions, click on '**Add Another Primer Reaction Set**'.
- Below is an example for multiple primers within a reaction.

fwd_primer_seq	rev_primer_seq
gtgacacgtacacgt	atcgacgtacacgt
accaccacacactacac	caaccacagactacac

Enter the **SEQUENCE**
for your PCR forward
primer here

Enter the **SEQUENCE**
for your PCR reverse
primer here

Reaction Set #1

fwd_primer_seq	fwd_primer_name	rev_primer_seq	rev_primer_name
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

additional primers for this reaction set.

Enter the **NAME**
for your PCR forward
primer here

Enter the **NAME**
for your PCR reverse
primer here

Figure 22: The “Primers” page when multiple sequences are submitted and the “Input PCR primers” button is selected. Figure text gives instructions for entering primer sequences and names.