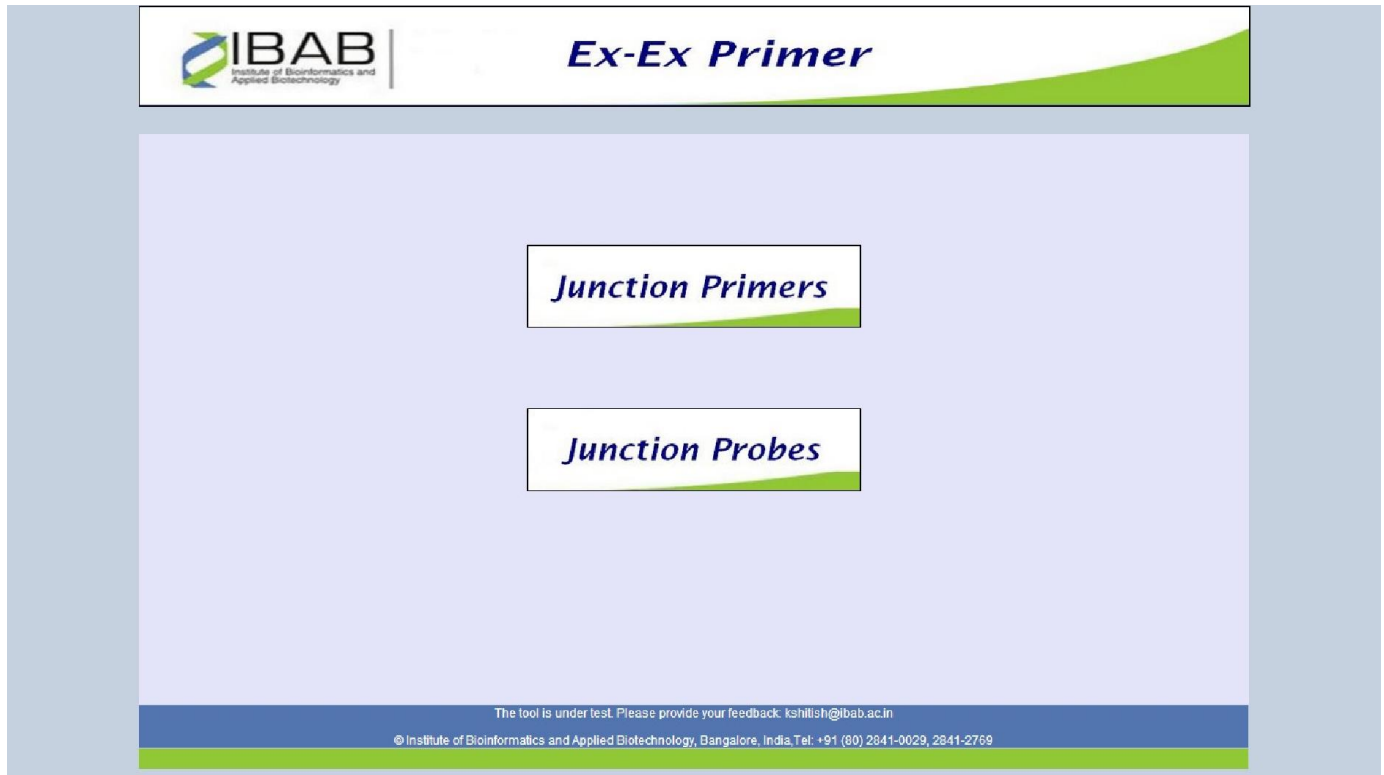


Home page of Ex-Ex primer:

User can choose the any one of the option depending on the requirement from home page.



1. Start with gene:

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Ex-Ex Primer - junction primers

Junction Probe Home User guide Other resources

Select the Organism

Start with gene

Organism: Homo sapiens (dropdown menu)

Identifier type: Gene Name (dropdown menu)

Gene Name: CDK5 (text input)

Type the Gene Name/NCBI GeneID

Select the Identifier

Submit Reset

This option consist of following parameters:

- Organism:** select the interest of organism from drop down menu.
- Identifier type:** choose type of ID you're going provide as a identifier, this option consist of two types of identifiers viz. Gene Name and NCBI GeneID.
- Gene Name:** type the appropriate ID depending on the type of identifier chosen.
- Click on the submit button for results.

junction primers

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Gene name: **cdk5**
■ Exon ■ Intron

(To select the transcript id use option button)

NM_004935.3 ■ ■ INTRON EXON

View info about: Number:

NM_001164410.1 ■ ■

View info about: Number:

Species	Homo sapiens	Primer length Plus/Minus:	<input type="text" value="3"/>
Gene Sequence Length	4153	Percentage GC content	<input type="text" value="49"/>
Desired product size	<input type="text" value="400"/>	Primer T _m (Cel)	<input type="text" value="59"/>
Primer length	<input type="text" value="27"/>	T _m calculation:	<input type="text" value="Santa Lucia 1998"/>

Send results through email

Select the T_m calculation method

Results:

1. Display the available Transcript ID's in the NCBI for the gene.
2. Exon and Intron's are highlighted in dark and light blue respectively.
3. Move cursor on the dark or light blue to get the information about the exon or intron.
4. Default Product size is 400bp, user can define the product size accordingly.
5. Default primer length is 27bp, user can choose the length of the primer.
6. Default GC content is 49%, user can define the GC percentage accordingly.
7. Default T_m of primer is 59, user can enter the T_m of primer as per requirement.
8. Default T_m calculation method is Santa Lucia 1998, user can choose different T_m calculation method from drop down menu.
9. User can get the results through e-mail by clicking on the "send results through email" option.

10. Choose the transcript ID for which primers can be designed, after selection few options will appear on bottom of the page.

NM_001164410.1

View info about: Number:

Species	Homo sapiens	Primer length Plus/Minus:	<input type="text" value="3"/>
Gene Sequence Length	4153	Percentage GC content	<input type="text" value="49"/>
Desired product size	<input type="text" value="400"/>	Primer T _m (Cel)	<input type="text" value="59"/>
Primer length	<input type="text" value="27"/>	Tm calculation:	<input type="text" value="Santa Lucia 1998"/>

Transcript ID chosen:

Junction primer should be:

Exon at 5' of the junction should be (pre-junction exon):

Exon at 3' of the junction should be (post-junction exon):

The other primer should be from:

Send results through email

11. **Junction primer should be:** user can choose the exon-exon primer type i.e. Right Primer (Reverse primer) or Left Primer (Forward primer) or Both Primer's from drop menu.

12. **Pre-junction exon:** user can choose the any 5' exon of the transcript as 1st partner for exon-exon primer design, default is exon 1.

13. **Post-junction exon:** user can select the 3' exon of the transcript as 2nd partner for exon-exon primer, default is exon 2.

14. The other primer should be from: user can select other primer pair designed from intron or exon reason by choosing the “Intron only” or “Normal” option from drop down menu, default is “Normal” i.e. exon.

15. Click on the “Generate Primers” to get the primers.



More details Product sequence

Optimal primer pair as per user-defined Tm * Click on 'B' for BLAST.

No.	Forward primer (5' -> 3')	Tm	Reverse primer (5' -> 3')	Tm	Score	Product length
1	B cagagtcctaaaaaccgagggcccg (24)	64.96	B ccgtaggtgcctcccacatctt (24)	64.88	21.32	94
No.	Forward primer (5' -> 3')	Tm	Reverse primer (5' -> 3')	Tm	Score	Product length

Show other primer(s) Product as well as submitted sequence More details

* Click on 'B' for BLAST.

No.	Forward primer (5' -> 3')	Tm	Reverse primer (5' -> 3')	Tm	Score	Product length
1	B cagagtcctaaaaaccgagggcccg (24)	64.96	B ccgtaggtgcctcccacatctt (24)	64.88	21.32	94
2	B cggggccagagtcctaaaaaccgag (24)	64.96	B ccgtaggtgcctcccacatctt (24)	64.88	21.32	100
3	B cggggccagagtcctaaaaaccgag (24)	64.96	B cgtaggtgcctcccacatctt (25)	63.43	21.52	99
4	B ccagagtcctaaaaaccgagggccc (24)	64.92	B ccgtaggtgcctcccacatctt (24)	64.88	21.64	95
5	B cggggccagagtcctaaaaaccgag (24)	64.96	B cgtaggtgcctcccacatctt (24)	63.14	21.72	99
6	B cagagtcctaaaaaccgagggcccg (24)	64.96	B ccgtaggtgcctcccacatctt (25)	64.97	21.74	100
7	B cggggccagagtcctaaaaaccgag (24)	64.96	B ccgtaggtgcctcccacatctt (25)	64.97	21.74	100
8	B ccagagtcctaaaaaccgagggccc (24)	64.92	B cgtaggtgcctcccacatctt (25)	63.43	21.84	94
9	B cagagtcctaaaaaccgagggcccg (24)	64.96	B ccgtaggtgcctcccacatctt (26)	65.15	21.90	94
10	B cggggccagagtcctaaaaaccgag (24)	64.96	B ccgtaggtgcctcccacatctt (26)	65.15	21.90	100
11	B ccagagtcctaaaaaccgagggccc (24)	64.92	B cgtaggtgcctcccacatctt (24)	63.14	22.04	94
12	B gggccagagtcctaaaaaccgaggg (24)	64.92	B cgtaggtgcctcccacatctt (24)	63.14	22.04	97
13	B gggccagagtcctaaaaaccgaggg (24)	64.92	B cgtaggtgcctcccacatctt (25)	63.43	22.04	97

Primer results:

1. Top of the page graphical representation of the primer binding and the exon-exon junction primer reason is highlighted in dark blue.
2. Top box showing the best primer pair, it has the primer details other information. Lower the score the better primer pair.
3. To check the primer specificity click on B (blast), it will display the results in new web page.
4. To see the more primer pair combinations click on the “show more primer(s)” option.

5. To visualize the start and end of the product click on the “Product as well as submitted sequences”.

2. Paste the sequence:

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Paste the sequence:

Mark the region using '(' & ')' for the first junction, '[' & ']' for the second junction if any. And then click on "Junction format" button to generate Pre-junction & Post-junction values. (The program removes the sequence in the brackets and creates a new junction)

or

Specify Pre-junction & Post-junction values manually in the given text box.

Junction Format

Junction Primer should be

Forward Primer ▾

Select your junctions

Junction 1

Base at 5' end of junction (pre-junction serial no.):


Base at 3' end of junction (post-junction serial no.):

To demonstrate how to use paste the sequence option example nucleotide used is "AF094760".

1. Paste the sequence in the box.
2. Mark the exon-exon junction for the primer design using "(" at end of the 1st exon and begin of the second exon to design primers for one side exon-exon junction (see below images).
3. Click on the "Junction Format" to generate pre and post junction serial number.
4. Follow the step 11 onwards from "Results" (above) to generate the primers.
5. To design exon-exon junction primer from both the side (see below image "both side"), follow the step 2 from above to mark the forward exon-exon primer and to design reverse exon-exon junction primer mark using "[" at the end of the exon 4 and beginning of the exon 5.

6. Follow the step 11 onwards from “Results” (above) to generate the primers.

One side exon-exon junction primer design



Ex-Ex Primer - junction primers

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Paste the sequence:

Mark the region using '(' & ')' for the first junction, '[' & ']' for the second junction if any. And then click on "Junction format" button to generate Pre-junction & Post-junction values.
(The program removes the sequence in the brackets and creates a new junction)

or

Specify Pre-junction & Post-junction values manually in the Exon-exon junction

ACGCAGGGGAAGGAGGCACACCCGGGGGTGGCGCAGTGAGGAGGGGGCGCGACGGCC...
 GCGACACCCAGGCAGGAGAGGGGGGAAGAACTCTCTCCCTTTCTGAACCCCTTTTCC...
 TGGGGGAGTCCCTCCACGCATTACCCACTCGGGCCCGCAAAAACCTCCCTTCTTTAGCCCTC...
 TTGCTTATAAGCCCTTGGAGACCCGAGAAGGGACCTTGTGGGAACGGGACGGCCCAAG () AGGAAGCCAGAT...
 CCTGAGSSSTCCGGTCTCCASTTTGGCTTCTGCTATATCCATTGGAAGAGAAAAGTTTGTGACTTGGGCC...
 CCCAAGTTTTGAGAGAACTGGGCTTTCCGGCCGGGGGACAGAGGAGGCTCGTGGGGAGCTTCCCCAIG...
 GAGCTTACCCASCTSCAGASACTCATCCAGACCCAGCAGACCCCTCTCAGAACTTGGGACCCG...
 AAGACCCCGGAGAGGGCTGCAGATGGCTCAGACACTGTGGTCTCAGTCTCTTTCCCTGCACCCCTGA


Junction Primer should be

Select your junctions

Junction 1

Base at 5' end of junction (pre-junction serial no.):

Base at 3' end of junction (post-junction serial no.):



Ex-Ex Primer - junction primers

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Paste the sequence:

Mark the region using '(' & ')' for the first junction, '[' & ']' for the second junction if any. And then click on "Junction format" button to generate Pre-junction & Post-junction values.
(The program removes the sequence in the brackets and creates a new junction)

or

Specify Pre-junction & Post-junction values manually in the given text box.

acgcaggggaaggagggcacacccccgggggtggcgcagtgaggagggggcgcgacggcc...
 gacaccccaggcaggagaggggggagaactctctctccctttctgaaccccttttcc...
 ggagtcootccaacgcaataccccactcggggccgcaaaaactccctctcttagccctctt...
 ataggcoottgagacccgagaagggaccttgggtgggaacgggaggggcaagaggaagccgat...
 gtcgggtctccagctttgcootcootgctatccatgggaagagaaagtttggacttgggccc...
 gagagaactctgggctttcggcggggggggacagagaggctctggggagctttccccaatggagct...
 cctgcagagaagacctcatccagaccccagcagaccccctcagacctcggggacctgaagacccc...
 gaggctcgcagatggctcagacaactgtggtctctcagttctttccctgcaaccccctgagcct...
 ggaatccctgaa

Junction Primer should be

Select your junctions

Junction 1

Base at 5' end of junction (pre-junction serial no.): Pre-junction serial number

Base at 3' end of junction (post-junction serial no.):

Advanced options

Primer Length: Pre-junction serial number

Product Length:

Both side exon-exon junction primer



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Paste the sequence:

Mark the region using '(' & ')' for the first junction, '[' & ']' for the second junction if any. And then click on "Junction format" button to generate Pre-junction & Post-junction values.

(The program removes the sequence in the brackets and creates a new sequence)

or

Specify Pre-junction & Post-junction values manually in the given text box

Forward exon-exon junction primer

Junction Format

```
TGGGGGAGTCCTCCACGCATTACCCACTCGGGCCGCAAAAACCTCCCTTCTTTAGCCCTC  
TTGCTTATAAGCCTTTGAGACCCGAGAAAGGGACCTTGTGTTGGAAACGGGACGGCCAAG ( ) AGGAAGCCAGAT  
CGCTGAGGGTCCGGTCTCCAGTTTGCCTCCTGCTATATCCATTGGAAGAGARRAGTTTGTGACTTGGGCC  
CCCAAGTTTTGAGAGAACTGGGCTTTTCGGCCGCGGGGGACAGAGGAGGCTCGTGGGGAGCTTTCCTCATG  
GAGCTTACCCAGCCTGCAGAAGACCTCATCCAGACCCAGCAGACCCCTGCCTCAGAACTTGGGGACCCCTG  
AAGACCCCGGAGAGGAGGCTGCAGATGGCTCAGACACTGTGGTCCTCAGTCTCTTTCCCTGCACCCCTGA  
GCCTGTGAATCCTGAACCGGATGCCAGTGTTCCTCTCCACAGGCAGGCAGCTCCCTGAAGCACT  
ACTCTCACCAACCGGCAGCGAGGGAACGAGGIGTCAGCTCTGCCGGCCACCCCTAGACT [ ]
```

Reverse exon-exon junction primer

Junction Primer should be

Forward Primer

