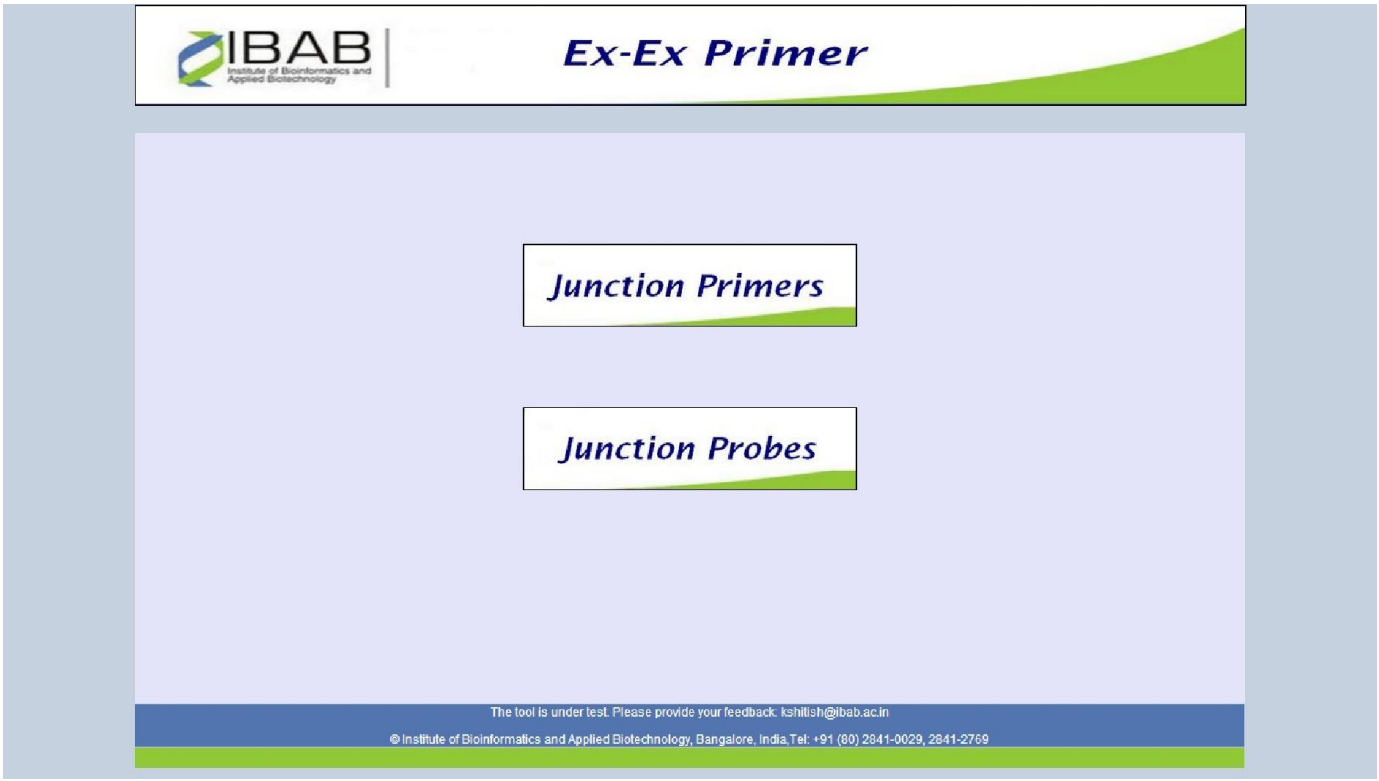
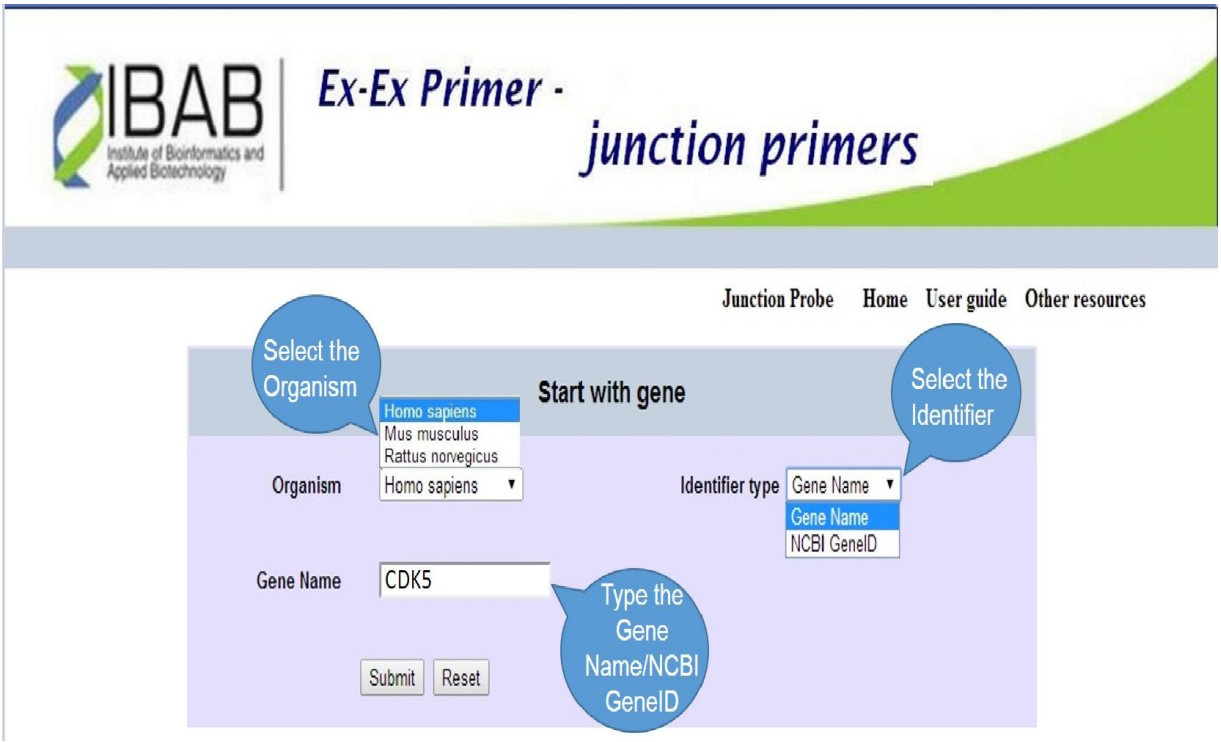
Home page of Ex-Ex primer:

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



1. Start with gene:



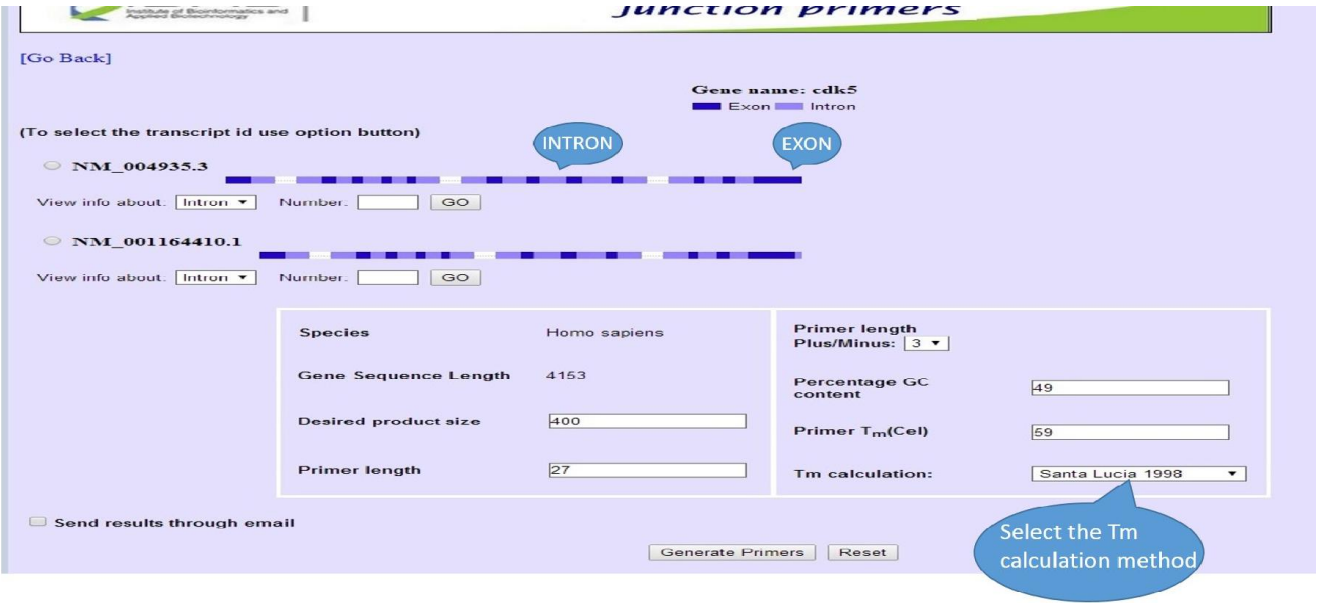
This option consist of following parameters:

a. **Organism**: select the interest of organism from drop down menu.

b. **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.

c. **Gene Name**: type the appropriate ID depending on the type of identifier chosen.

d. Click on the submit button for results.



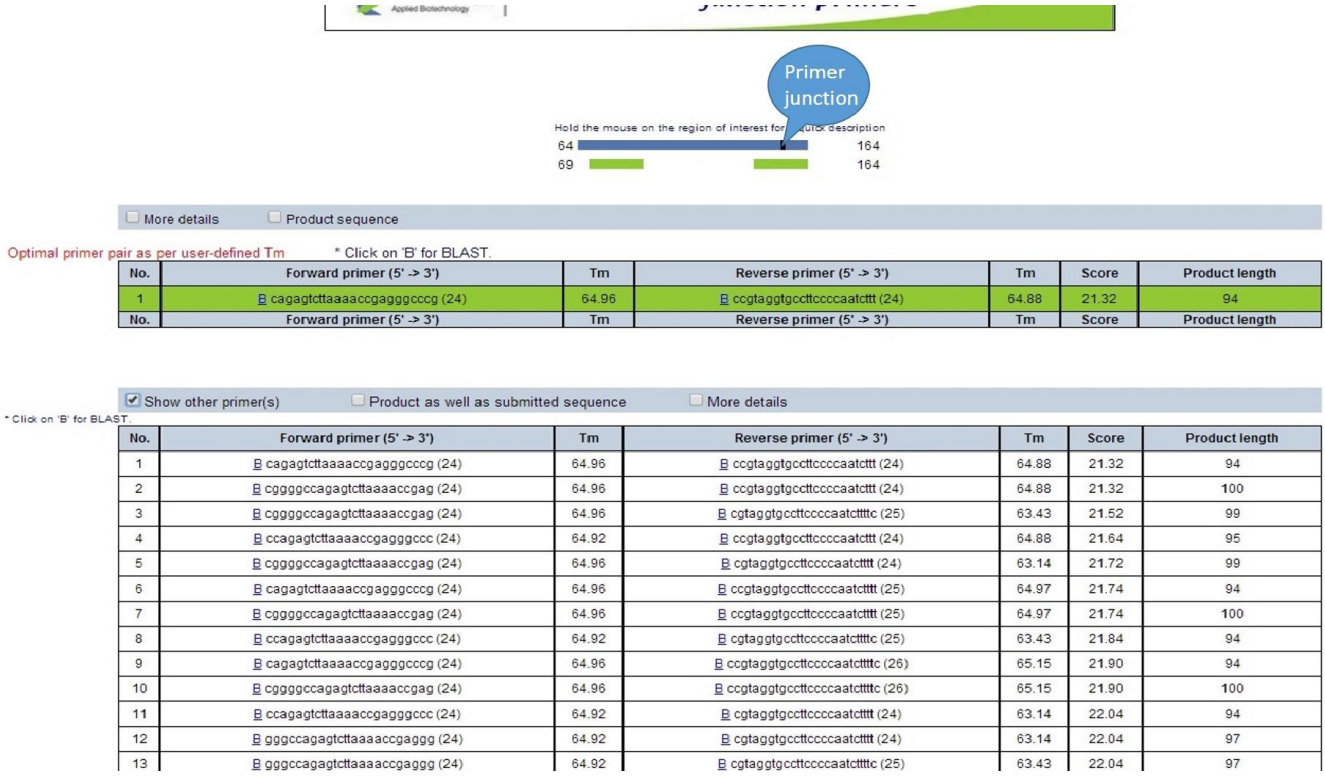
Results:

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 Tm of primer is 59, user can enter the Tm of primer as per requirement.
8. Default Tm calculation method is Santa Lucia 1998, user can choose different Tm 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 option will appear on bottom of the page.

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.



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

No.	Forward primer (5' -> 3')	Tm	Reverse primer (5' -> 3')	Tm	Score	Product length
1	B cagagtcttaaaaccgagggcccg (24)	64.96	B ccgtaggtgccttccccaatcttt (24)	64.88	21.32	94
2	B cggggccagagtcttaaaaccgag (24)	64.96	B ccgtaggtgccttccccaatcttt (24)	64.88	21.32	100
3	B cggggccagagtcttaaaaccgag (24)	64.96	B cgtaggtgccttccccaatctttc (25)	63.43	21.52	99
4	B ccagagtcttaaaaccgagggccc (24)	64.92	B ccgtaggtgccttccccaatcttt (24)	64.88	21.64	95
5	B cggggccagagtcttaaaaccgag (24)	64.96	B cgtaggtgccttccccaatcttt (24)	63.14	21.72	99
6	B cagagtcttaaaaccgagggcccg (24)	64.96	B ccgtaggtgccttccccaatcttt (25)	64.97	21.74	94
7	B cggggccagagtcttaaaaccgag (24)	64.96	B ccgtaggtgccttccccaatcttt (25)	64.97	21.74	100
8	B ccagagtcttaaaaccgagggccc (24)	64.92	B cgtaggtgccttccccaatctttc (25)	63.43	21.84	94
9	B cagagtcttaaaaccgagggcccg (24)	64.96	B ccgtaggtgccttccccaatctttc (26)	65.15	21.90	94
10	B cggggccagagtcttaaaaccgag (24)	64.96	B ccgtaggtgccttccccaatctttc (26)	65.15	21.90	100
11	B ccagagtcttaaaaccgagggccc (24)	64.92	B cgtaggtgccttccccaatcttt (24)	63.14	22.04	94
12	B gggccagagtcttaaaaccgaggg (24)	64.92	B cgtaggtgccttccccaatcttt (24)	63.14	22.04	97
13	B gggccagagtcttaaaaccgaggg (24)	64.92	B cgtaggtgccttccccaatctttc (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.

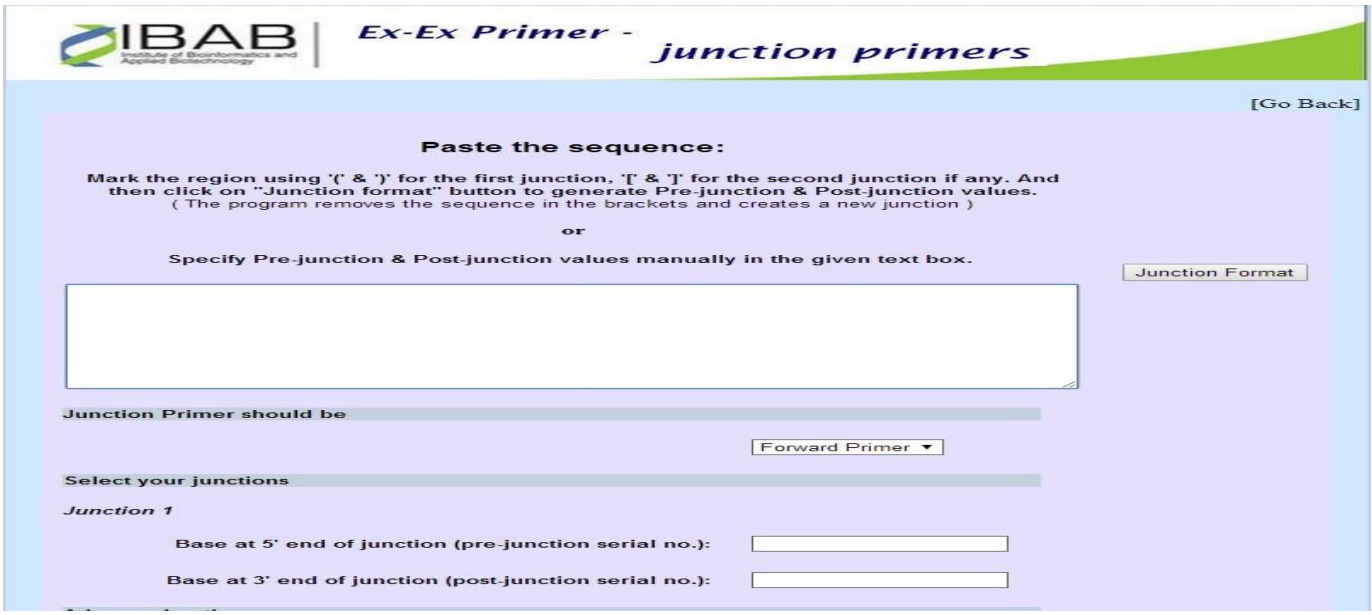
2. Top box showing the best primer pair, it has the primer details other information. Lower the score the better primer pair.

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:



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).

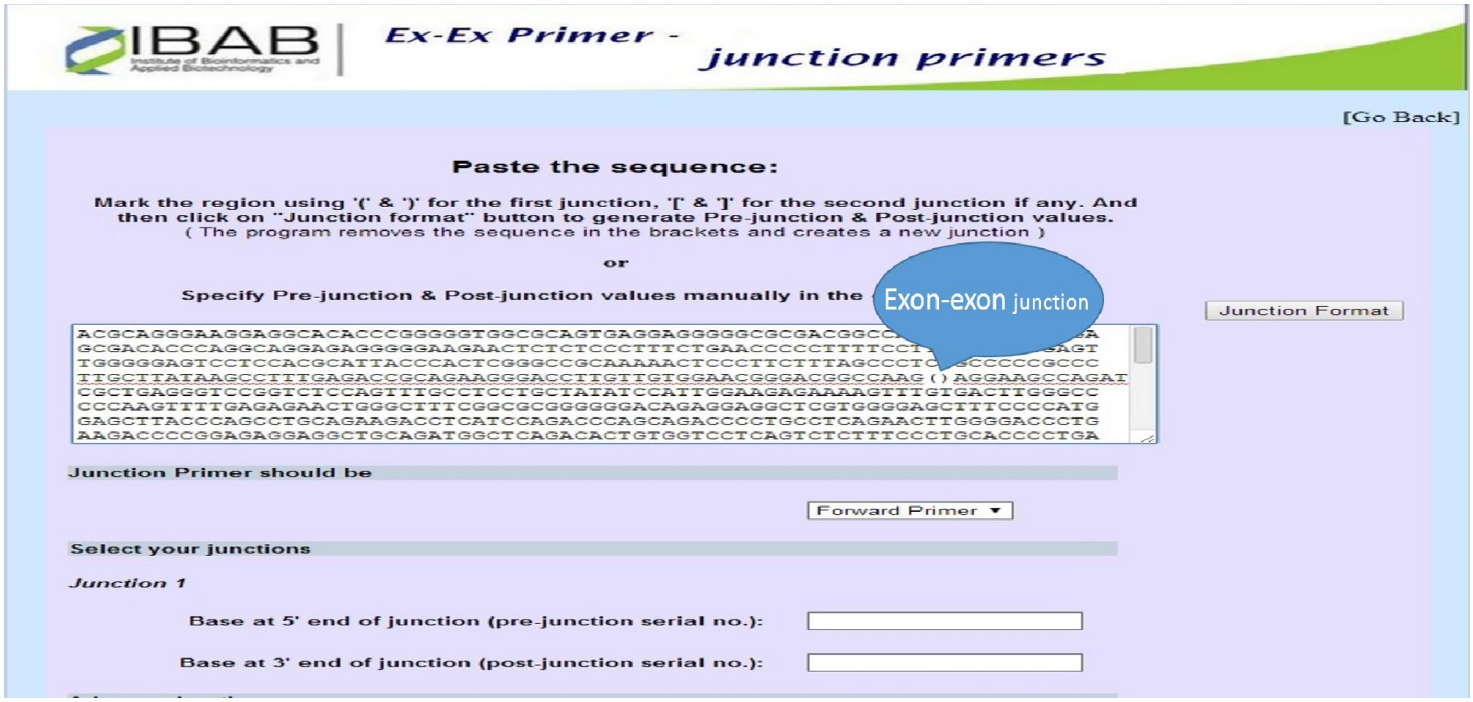
3. Click on the "Junction Format" to generate pre and post junction serial number.

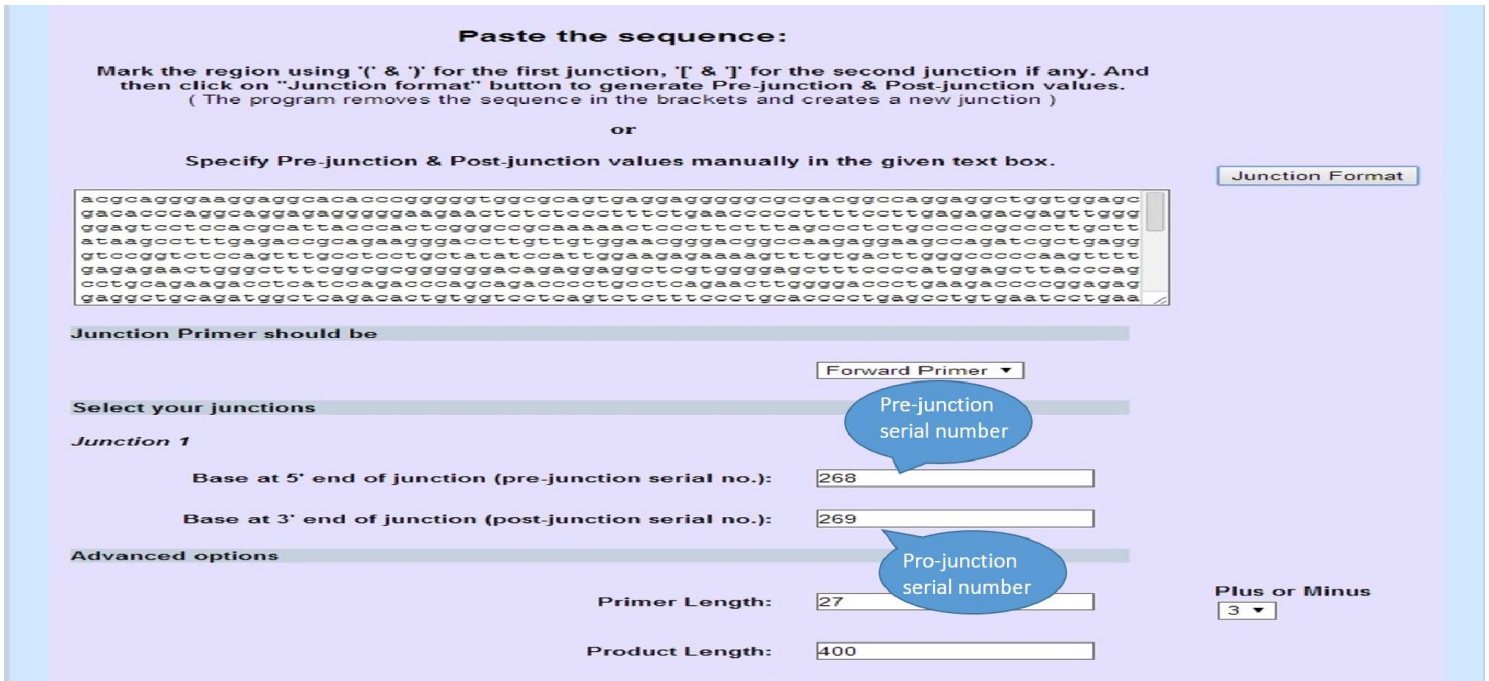
4. Follow the step 11 onwards from "Results" (above) to generate the primers.

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.

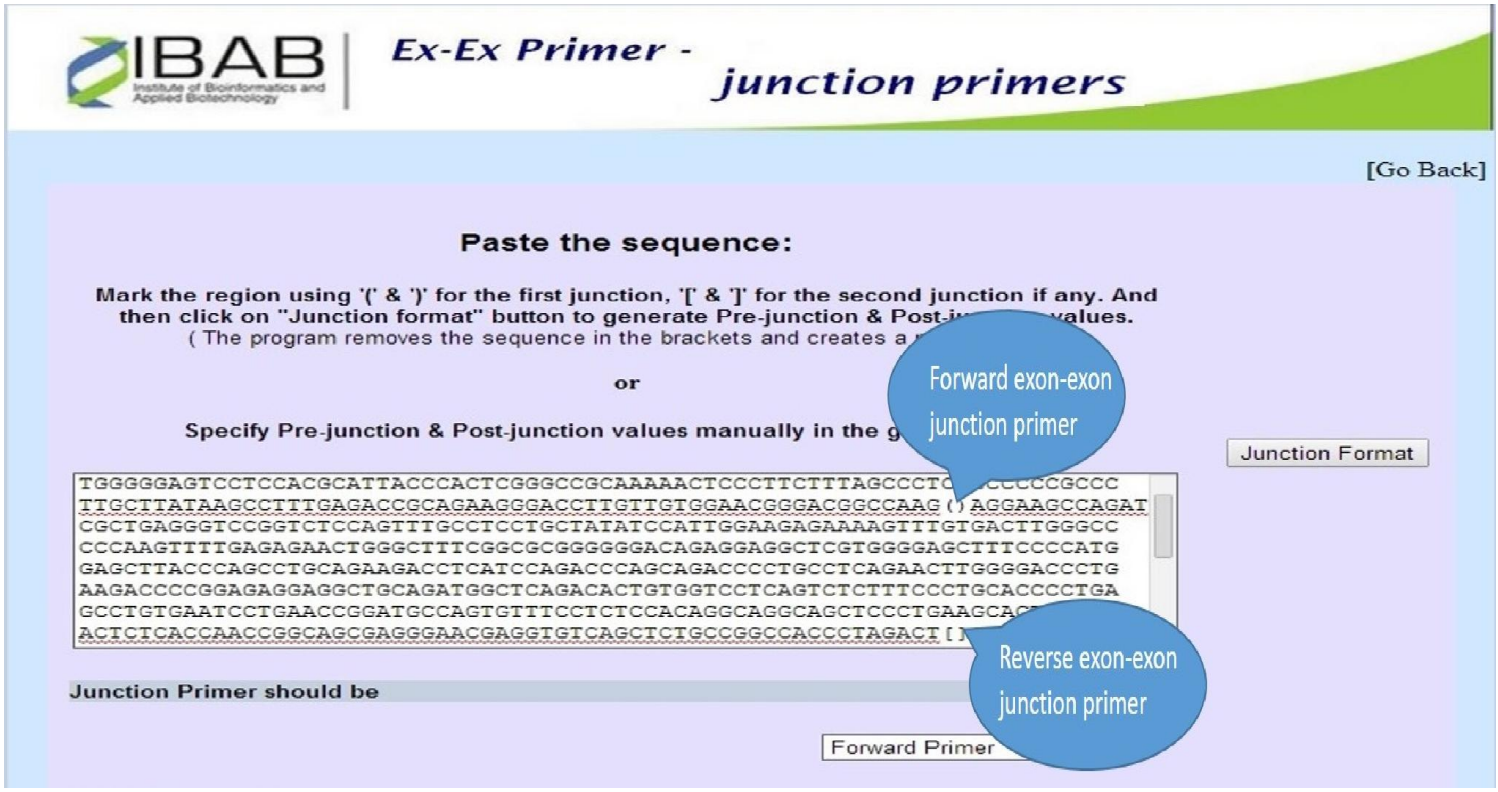
6. Follow the step 11 onwards from “Results” (above) to generate the primers.

One side exon-exon junction primer design





Both side exon-exon junction primer




or
Specify Pre-junction & Post-junction values manually in the given text box.
Junction Format
Junction Primer should be
Both
Select your junctions
Junction 1
Pre and pro junction 1 serial number's
Base at 5' end of junction (pre-junction serial no.):
268
Base at 3' end of junction (post-junction serial no.):
269
Junction 2
Pre and pro junction 2 serial number's
Base at 5' end of junction (pre-junction serial no.):
688
Base at 3' end of junction (post-junction serial no.):
689