

SALK or SAIL tDNA insert lines

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9:09 AM

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1. Go to <http://signal.salk.edu/>
2. Click on SIGnAL T-DNA Express
2. Under Search in the Query box enter the At# of the gene you want to find the insert in, ex At1g10760
3. Once it bring up your gene of interest zoom in on it using the arrows in the upper right hand corner
4. Once zoomed in, you will see all the tDNA insets and their approximate location below your gene
5. Once you have found the insert you are interested in, ex: SALK_077211, click on iSct Primers under Tools on the right hand side of the screen.
6. When you page down you will be presented a screen to fill in with your primer requirements fill in as you would primer3 use example below and put in the name of the mutant you are looking for

Primer size - Opt: 21	Min: 18	Max: 28
Primer TM - Opt: 60	Min: 55	Max: 70
GC Content - Min: 40	Max: 60	Clamp: 0
Max N:300	Ext5: 300	Ext3: 300
Primer Zone: 200		BPos: 110
Data Type:Insertion		Format:

Please paste your list: like

```
Salk_000002  
SAIL_155_D07  
GABI_756F01  
FLAG_270B05  
RATM15-1976-1_G  
WiscDsLox289_292P9  
SM_3_19088
```

Salk_077211

7. It will then give you a LP and RP sequence for primers, these are the primers that span the insertion site.

This is similar to a forward and reverse primer but it takes into account the direction of the gene and the insert so that the LB primer for the inset will work with the RP primers in the genomic DNA

8. If you are using going after a SALK insert use the LB primer 1.3 ATTTTGCCGATTTCCGGAAC
9. You can submit the sequences that it gives you directly to the oligonucleotide synthesis facility (IDT)

Sex1-8 Salk_077211

```
SALK_077211.27.50.x PRODUCT_SIZE 1017 PAIR_ANY_COMPL 0.00 PAIR_3'_COMPL 0.00 DIFF_TM  
0.08 LP TCCGGTATGACAAGTCGAATC Len 21 TM 59.95 GC 47.62 SELF_ANY_COMPL 0.00 3'_COMPL  
0.00 RP GTCAGTCTATCCTGCGCTTTG Len 21 TM 60.03 GC 52.38 SELF_ANY_COMPL 0.00 3'_COMPL  
0.00 Insertion chr1 3586831 BP+RP_PRODUCT_SIZE 464-764
```

Sex4-3 Salk_102567

SALK_102567.51.65.x PRODUCT_SIZE 1159 PAIR_ANY_COMPL 0.00 PAIR_3'_COMPL 0.00 DIFF_TM
0.15 LP AAGGCAACGATCTAGCTGTGA Len 21 TM 60.03 GC 47.62 SELF_ANY_COMPL 0.00 3'_COMPL
0.00 RP TCCCCAACATCCTCACATAA Len 21 TM 60.18 GC 42.86 SELF_ANY_COMPL 0.00 3'_COMPL
0.00 Insertion chr3 19362544 BP+RP_PRODUCT_SIZE 539-839