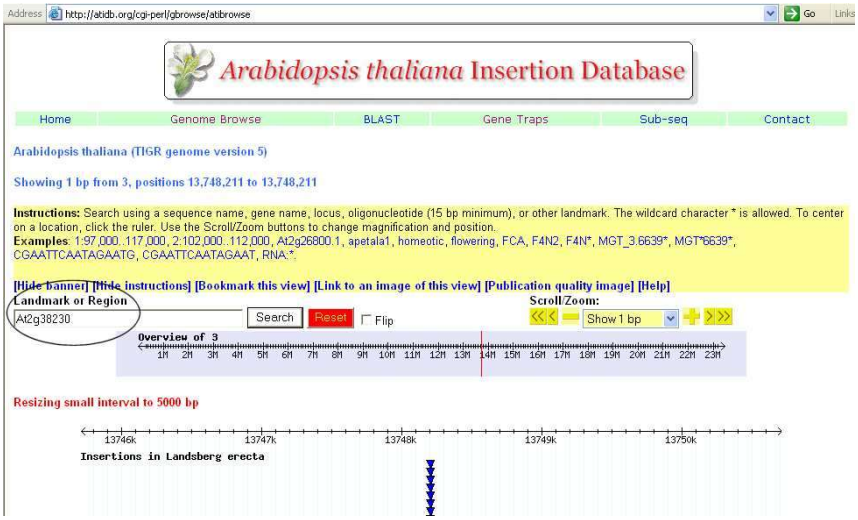
TRANSPOSON INSERTION SITE VERIFICATION

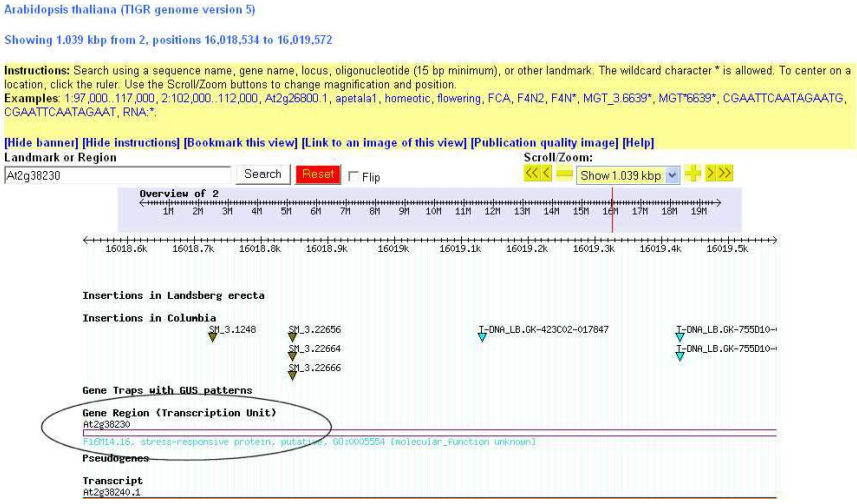
Transposon and T-DNA insertion in Arabidopsis genes can be identified using the Arabidopsis thaliana Insertion Database (ATIdb) (http://atidb.org/cgi-perl/gbrowse/atibrowse). There is, as yet, no publicly available insertion site verification data

VERIFICATION OF INSERTIONS SITES

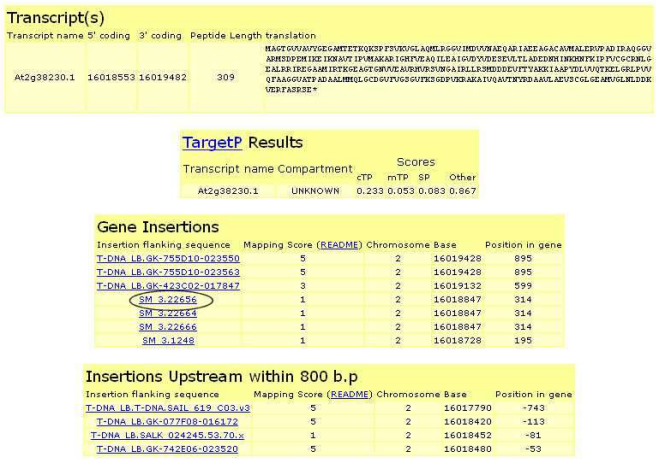
- Enter the gene id into the **Landmark or Region** window (in this case At2g38230) (http://atidb.org/cgi-perl/gbrowse/atibrowse) press the **Search** button.



- Click on the bar in the **Gene Region** field. This links to a reference page in ATIdb that give you details of the gene and the insertions;

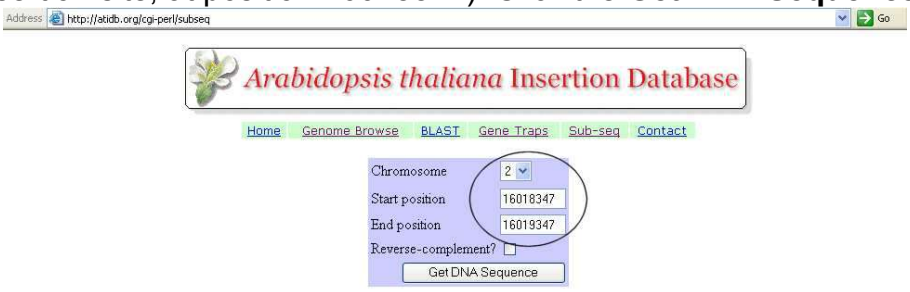


Select an insertion line from the **Gene Insertions** field. Click on the line number. This links in to a second reference page in ATIdb that gives you details of the bp position of the insertion relative to the psuedochromosome (in this case, SM_3.22656 is located on Chromosome 2 position 16018847)

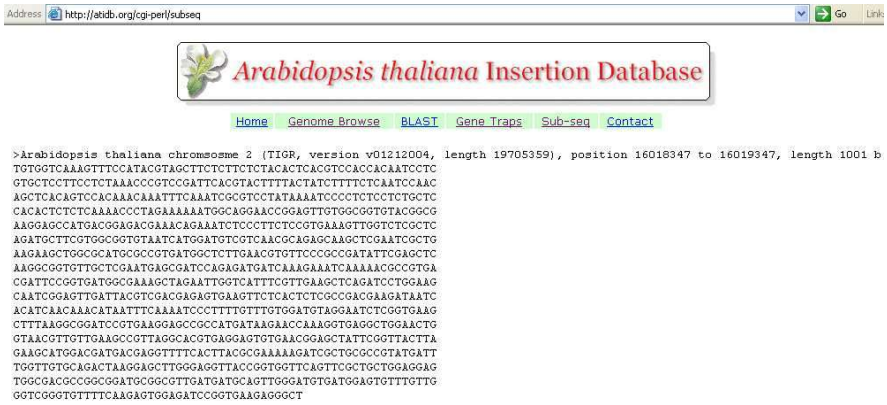


- Select **Sub-Seq** from the toolbar (http://atidb.org/cgi-perl/subseq). This links to a third page in ATIdb which runs the Sub-Seq tool which allows you to extract the genomic sequence surrounding the insertion;

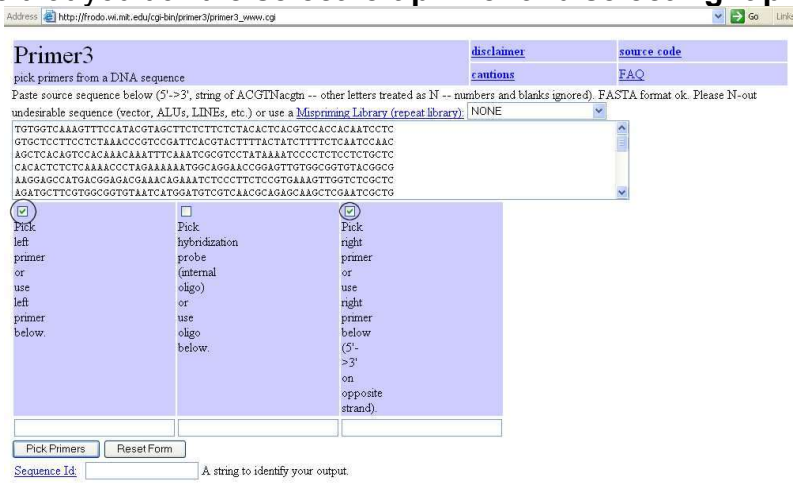
- Enter the details of the insertion site into the fields within Sub-Seq. (In this case Chromosome 2; 16018347 to16019347 (500bp 5' + 500bp 3' to the insertion site, at position 16018847). Click the **Get DNA Sequence** button;



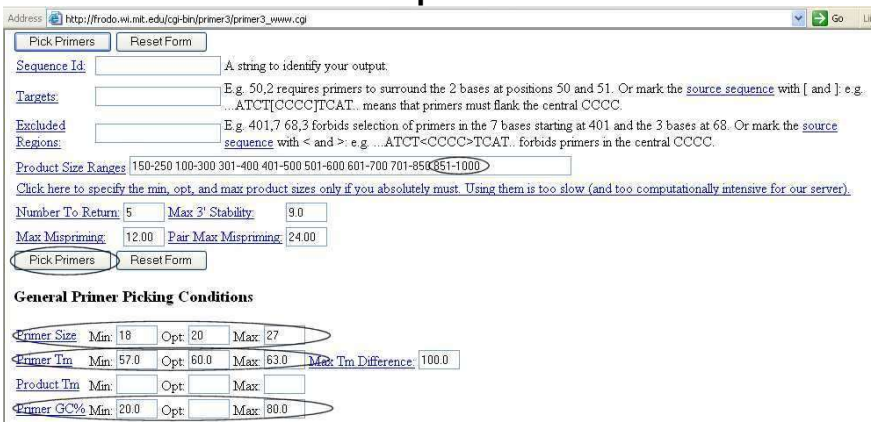
- Sub-Seq will retrieve the DNA sequence in a FASTA Format suitable for using in sequence analysis packages;



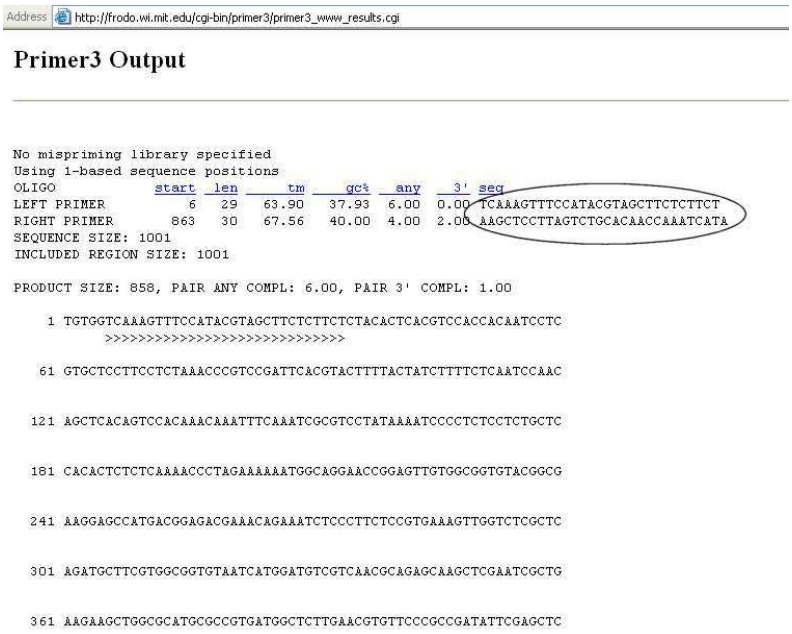
- Copy this sequence and paste it into a primer design package (we recommend Primer 3: http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Ensure that you tick the **select left primer** and **select right primer** boxes;



- Select the product size range 850-1000 from the **select product size range** field (Delete the other size ranges). Enter the following parameters:
- **Primer size:** 27 min **29 opt** 31 max
- **Primer tm:** 60 min **65opt** 70 max
- **Primer GC content:** 30 min **35 opt** 40 max



- Press the **Pick Primers** button



Address http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www_results.cgi

Primer3 Output

```
No mispriming library specified
Using 1-based sequence positions
OLIGO            start  len      tm     gc%   any   3'  seq
LEFT PRIMER          6   29   63.90   37.93  6.00  0.00  TCAAAGTTTCCATACGTAGCTTCTCTTCT
RIGHT PRIMER       863   30   67.56   40.00  4.00  2.00  AAGCTCCTTAGTCTGCACAACCAAATCATA
SEQUENCE SIZE: 1001
INCLUDED REGION SIZE: 1001

PRODUCT SIZE: 858, PAIR ANY COMPL: 6.00, PAIR 3' COMPL: 1.00

    1 TGTGGTCAAAGTTTCCATACGTAGCTTCTCTTCTCTACACTCACGTCCACCACAATCCTC
        >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>

   61 GTGCTCCTTCCTCTAAACCCGTCCGATTCACGTACTTTTACTATCTTTTCTCAATCCAAC

  121 AGCTCACAGTCCACAAACAAATTTCAAATCGCGTCCTATAAAATCCCCTCTCCTCTGCTC

  181 CACACTCTCTCAAAACCCTAGAAAAAATGGCAGGAACCGGAGTTGTGGCGGTGTACGGCG

  241 AAGGAGCCATGACGGAGACGAAACAGAAATCTCCCTTCTCCGTGAAAGTTGGTCTCGCTC

  301 AGATGCTTCGTGGCGGTGTAATCATGGATGTCGTCAACGCAGAGCAAGCTCGAATCGCTG

  361 AAGAAGCTGGCGCATGCGCCGTGATGGCTCTTGAACGTGTTCCCGCCGATATTCGAGCTC
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- Primer 3 will generate a number of primer pair options. We recommend that you BLAST the primer sequences against the Arabidopsis genome sequence to confirm their specificity for the target region;
- The insertion site specific primers designed (in this case SMF & SMR) will be used in a 3 primer PCR reaction. This will verify the insertion site and to confirm if the line is homozygous for the insertion.

John Innes Centre
Genome
LABORATORY

INSERTION SITE PCR

Design a pair of insertion site specific primers (SMF/SMR) that flank the insertion site and amplify a fragment of approx 900bp using the parameters defined:

Primer design features

- 29 mer ± 2
- tm 65°C ± 5
- GC% 35 ± 5

You will need an additional primer specific to the transposon (dSpm or Ds)

For SM lines Transposon specific primer (3' dSpm)
Spm32 (29-mer)
5'-TAC GAA TAA GAG CGT CCA TTT TAG AGT GA-**3'**

For AT, ET, GT, MET, MGT, MT (GMT) lines Transposon specific primer (3' Ds)
Ds3-1 (20 mer)
5'-ACC CGA CCG GAT CGT ATC GGT**-3'**

3 PRIMER PCR REACTION
SMF, SMR & Spm32 or Ds3-1

PCR REACTION.
5.0µl DNA
2.0µl x10 PCR BUFFER
2.5µl 2mM dNTP's
0.5µl 10µM SMF
0.5µl 10µM SMR
0.5µl 10µM Spm32 or Ds3-1
0.5µl TAQ
8.5µl dH_2O
For the negative control the PCR reaction was carried out as above but with wild-type DNA.
A positive control is also carried out as above using wild-type DNA but used both the left and right primers with no Transposon specific primer (Spm32/Ds3-1).

PCR CONDITIONS.
94°C for 2mins
94°C for 30sec }
55°C for 45sec } X 30 CYCLES
72°C for 1mins }
72°C for 5mins
4°C forever
Run out the PCR products from all reactions on a 1% agarose gel and photograph.

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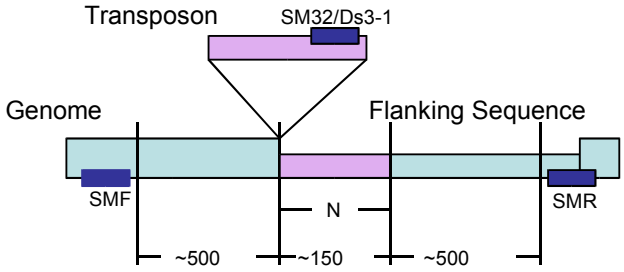
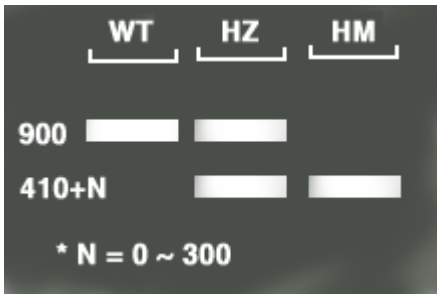
SMF with SMR & Spm32/Ds3-1 will generate:

1. an approx 900bp product (homozygous wild-type **WT**);
2. an approx 900bp product (wild-type) & a <500bp product (heterozygous for insertion **HZ**);
3. a single <500bp product (homozygous for insertion **HM**).





N – Transposon sequence amplified in addition to flanking sequence.

Please let me know how you get on. We are trying to collate information on confirmed insertions sites in the SM collections to define a confirmed unigene set. The JGL will update ATIdb and NASC with information on which lines have been verified (your details will be treated in confidence).

INSERTION SITE VERIFICATION FOR T-DNA LINES

For Salk T-DNA lines we recommend you use the protocols from SIGnAL (http://signal.salk.edu/tdnaprimers.html)

For SAIL T-DNA lines we recommend you use the protocols from SIGnAL (http://signal.salk.edu/tdnaprimers.html)