Origin of 18bp TIR transposon element :

AGTACCATCTCCACAGAATGTTTTCTGTGGAGATGGTATG

ITSD formation due to the insertion of region to be carried; here the normal 18bp flanking 4bp inverted repeat to become a non autonomous transposon

AGTACCATCTCCACAGAATGTTNNNNNNNTGTTTTCTGTGGAGATGGTATG

*ITSD (TGTT) formation separately mentioned

Transposase recognition of homology

Stepwise origin of 32bp Transposable element:

```
 603 TAATCAGTACCATCTCCACAGAAACAATTGTA 634
     ||| ||| || ||||||||||||||| |||||
2042 ATTTGTC-TGTTAGAGGTGTCTTTGTAAACAT 2012
```

AGTACCATCTCCACAGAAACAATTGTA TACAAATGTTTCTGTGGAGATTGTCTG

Insertion1 of 27bp on target site and creates TC/TT TSD , single base mutated

TCAGTACCATCTCCACAGAAACAATTGTA TACAAATGTTTCTGTGGAGATTGTCTGTT

Again **Insertion2** on target site TAA and creates TSD TAA

TAATCAGTACCATCTCCACAGAAACAATTGTA TACAAATGTTTCTGTGGAGATTGTCTGTTTAA

Insertion2 (here it seems tourist like MITES due to the presence of TAA TSD . It was possibly existed before with the range of **246bp including TAA TSD**)

Insertion 3

It perhaps again inserted from other region of genome to the gene portion and creates ACTG/AGTG or CTG/GTG duplications, it's not so clear due to spontaneous mutation on TSD

ACTGTAATCAGTACCATCTCCACAGAAACAATTGTA TACAAATGTTTCTGTGGAGATTGTCTGTTTAAGTG

Now the total size **253bp including 4bp TSD**

Final insertion on insertion on target site TC and formation of TC (TSD) at both sides

AGTACCATCTCCACAGAATGTTNNNNNNNTGTTTTCTGTGGAGATGGTATG

TCAGTACCATCTCCACAGAATGTTNNNNNNNTGTTTTCTGTGGAGATGGTATGTC

Finally it became the size of 1448bp

If we remove the internal transposon of AsDRF1, the size will become 253bp as follows:
This was possibly existed (perhaps MITES) before the insertion of internal transposon.

32bpTIR

TCAGTACCATCTCCACAGAATGTT → 18bpTIR (belongs to Internal transposon)

ACTGTAATCAGTACCATCTCCACAGAAACAATTGTACCAGTTGTTCTGCTCACATGAC
CAGGTTCCTTCACTGGATTGGGACTTGGGAGTGATGTTTATGGCGCCTAGCATAAAA
TTTGTTAAATTCATCGCAAGGAAGTTTATATGTCCCTGTTCCTGTACATCTGTTCCTGT
GGGGATGGTCTGTTTGTGTACATTTAGTAACAGTTTAATCGTTT
TACAAATGTTTCTGTGGAGATTGTCTGTTTAAGTG → ~32bpTIR

TGTTTTCTGTGGAGATGGTATGTC → 18bpTIR (belongs to Internal transposon)

18bpTIR ---18bpTIR trasposon-transposase complex

Homology recognition

Insertion after ~90 bp

5'---------32bpTIR----------32bpTIR------3'

↓

5' ------32bp----------18bpTIR ---18bpTIR----------32bp------3'

<u>Homology comparison between 18bp and 32bp TIR in AsDRF1</u>

ACTGTAATCAGTACCATCTCCACAGAAACAATTGTA ~32bp (3+1mismatch)+ 4bpTSD(single mismatchACTG)

TCAGTACCATCTCCACAGAATGTT 18bp TIR (2mismatch AG) + 2bp TSD (TC) + ITSD-TGTT

TTACAAATGTTTCTGTGGAGATTGTCTGTTTAAGTG ~31bpTIR(3+1mismatch)+4bpTSD (single mismatchAGTG)

TGTTTTCTGTGGAGATGGTATGTC 18bp TIR (2mismatch TG)+ 2bp TSD (TC)+ ITSD-TGTT

32bp TIR showing 3 mismatches and 1 deletion at 3'.

```
 603 TAATCAGTACCATCTCCACAGAAACAATTGTA 634
     ||| ||| || ||||||||||||||| |||||
2042 ATTTGTC-TGTTAGAGGTGTCTTTGTAAACAT 2012
```

Homology: 18bp TIR has a similarity with 18bp of ~32bp TIR at 5',
but 16bp are similar of ~32bp TIR at 3' end