Most possible mechanism:

It's clearly evident that transposase carried the 18bp from some other part of the genome and recognized the ~32bp TIR, since it has the homology with the 18bp. Then, this 18bp either inserted at the region after 93bp from the ~32bp TIR

Transposase carrying the element 5'AGTACCATCTCCACAGAA TGTTNNNN 3' TCATGGTACAGGAGTCTTACAANNNN

(Total 1191bp--- (18bp+18bpTIR+ (4bp+4bpITSD) +1147bp internal region)) from some where else of the genome)

Recognizing the Same TIR and considering TC as a target site after ~90bp

(This schematic diagram focused on 5' terminal)

Insertion after ~90bp at both 5' and 3' terminals

ACTGTAATCAGTACCATCTCCACAGAAACAATTGTA-----93bp-----TC(Target site)

ACTGTAATCAGTACCATCTCCACAGAAACAATTGTA------93bp------5TCAGTACCATCTCCACAGAATGTTNNNN

3' TCATGGTACAGGAGTCTTACAANNNN

5'TCAGTACCATCTCCACAGAATGTTNNNN 3' TCATGGTACAGGAGTCTTACAANNNN

5'TCAGTACCATCTCCACAGAATGTTNNNN

3'AGTCATGGTACAGGAGTCTTACAANNNN

The organization of internal transposon will be:

 $5'\ \ 2bp\ TSD\ +18bpTIR+4bp\ ITSD+internal\ 1147bp\ region+4bp\ ITSD+18bpTIR+2bpTSD\ 3'$

Total length of internal transposon: 1191bp+ 2+2bpTSD-----1195bp

Now the total length of this nested non autonomous DNA transposon:

253bp (already existing transposon)+1191(newly inserted)+2bp+2bp(TSD upon insertion at both end)= 1448bp

Organization of this nested non autonomous DNA transposon:

 $4 \text{bpTSD } 32 \text{bpTIR} + 93 \text{bp} + 2 \text{bpTSD } 18 \text{bpTIR} + 4 \text{bpITSD} \\ --1147 \text{bp} -- \\ 4 \text{bpITSD} + 18 \text{bpTIR } 2 \text{bpTSD} + 89 \text{bp} + 31 \text{bpTIR } 4 \text{bpTSD} \\ -- \\ 147 \text{bp} -- \\ 4 \text{bpITSD} + 18 \text{bpTIR } 2 \text{bpTSD} + 89 \text{bp} + 31 \text{bpTIR } 4 \text{bpTSD} \\ -- \\ 147 \text{bp} -- \\ 4 \text{bpITSD} + 18 \text{bpTIR } 2 \text{bpTSD} + 89 \text{bp} + 31 \text{bpTIR } 4 \text{bpTSD} \\ -- \\ 147 \text{bp} -- \\ 147 \text{bp$

Or

The 18bp enlarged up to ~32bp upon the selective insertion of this 18bp TIR+2bpTSD on12bp TIR, which leads to the formation of ~32bp TIR with 4bp TSD.

<mark>TC</mark>AGTACCATCTCCACAGAA<mark>TGTT</mark>

18bpTIR

Selections of TSD and TIR from 18bpTIR transposable element and leaving ITSD for the Insertion on the destination (14bp TIR already having 4bpTSD) by Transposase?

Insertion of 18bp on 12bp TIR transposon, which is already having 4bpTSD

ACTGTAAACAATTGTA
12bp ______

ACTG<u>TA<mark>ATC</mark>AGTACCATCTCCACAGAA</u>ACAATTGTA

~32 bp TIR

(Now ~32 bp TIR formed with 4bp TSD externally, besides the 18bp TIR with 2bp TSD)