

# Guide for Users

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## Pacakge Installation

*AnceTran* is an *R* package that performs analyses of transcriptome evolution based on *RNA-seq* expression data or *ChIP-seq* TF binding data. Here, we use HNF4A-binding data for 4 mice species as an example to show how *AnceTran* works. A convenient way to install package from github is through *devtools* package:

```
install.packages('devtools')
devtools::install_github("jingwyang/AnceTran")
```

After installation, *AnceTran* can be loaded in the usual way:

```
library('AnceTran')
```

## Input Format:

*AnceTran* package takes binding score data in certain format:

- Binding score file should be a text file in the matrix shape, Rows correspond to orthologous. Columns correspond to sample names. Sample names are in format of “TaxaName\_SubtaxaName\_ReplicatesName”.

The example files are included in the *AnceTran* package, which can be found in `extdata` folder in the package. One can load them in to take a look:

```
BindingScore.table = read.table(system.file('extdata', 'HNF4A_meanIntensity_4Mouse.txt', package = 'AnceTran'))
head(BindingScore.table[,1:5])
```

```
##           GeneID BL6_HNF4A CAST_HNF4A SPRET_HNF4A CAR_HNF4A
## 1 ENSMUSG00000000001 244.6250 338.4167 159.0 96.5000
## 2 ENSMUSG00000000003 0.0000 41.0000 0.0 0.0000
## 3 ENSMUSG00000000028 184.5000 199.6875 289.4 107.0000
## 4 ENSMUSG00000000037 0.0000 0.0000 41.0 20.0000
## 5 ENSMUSG00000000049 224.2632 179.7917 191.5 120.1875
## 6 ENSMUSG00000000056 266.2500 317.0769 141.4 204.8333
```

## Construction:

The construction function `TFconstruct` loads in the `BindingScore` data file, and wraps them in a list of *taxonTF* objects (one *taxaTF* object).

```
library('AnceTran')
taxa.objects = tTFConstruct(BSFile=system.file('extdata', 'HNF4A_meanIntensity_4Mouse.txt', package = 'AnceTran'))
```

The construction process takes **several minutes** on a desktop computer depending on data size and hardware performance. Specify “**taxa**” and “**subtaxa**” options in the function when using partial of your data. The construction process will be faster. If you are hesitated to test the *AnceTran*, the package has already bundled a constructed object and you can load the object through:

```
data(TF.objects)
```

## Data filtering and normalization

We excluded genes whose TF binding score equals to 0 in all species. To account for differences in sequencing depths between species, we quantile-normalized these binding score values across species and also log-transformed the values for the further analysis.

```
library('limma')
TF_table = TFtab(objects = TF.objects, taxa = "all", tf = "all", rowindex = NULL, filtering = FALSE, norm = "log")
keep<-rowSums((TF_table == 0)) < ncol(TF_table)
TF_table<-TF_table[keep,]
TF_table<-data.frame(log2(normalizeQuantiles(TF_table[,])+1))
```

## Distance matrix

First, we generate an TF-binding distance matrix of these mice species using *sOU* method:

```
library('ape')
dismat <- TFdist.sou(bsMat = TF_table)
colnames(dismat)=colnames(TF_table)
rownames(dismat)=colnames(dismat)
dismat
```

```
##          BL6_HNF4A CAST_HNF4A SPRET_HNF4A CAR_HNF4A
## BL6_HNF4A 0.0000000 0.0000000 0.0000000          0
## CAST_HNF4A 0.3588558 0.0000000 0.0000000          0
## SPRET_HNF4A 0.4497102 0.4869901 0.0000000          0
## CAR_HNF4A 0.6862219 0.7693106 0.6649105          0
```

## TF-binding tree building

After the TF-binding distance matrix is created, you can construct character tree by Neighbor-Joining method, and bootstrap values based on re-sampling orthologous genes with replacements can also be generated by `boot.phylo` function:

```
tf_tree <- NJ(dismat)
tf_tree <- root(tf_tree, outgroup = "CAR_HNF4A", resolve.root = T)
tf_tree <- no0br(tf_tree)

f <- function(xx) {
  mat <- TFdist.sou(t(xx))
  # the distance metrics here should be the same as you specified
  # when you created the TF-binding distance matrix
```

```

colnames(mat) <- rownames(xx)
rownames(mat) <- colnames(mat)

root(NJ(mat), "CAR_HNF4A", resolve.root = T)
}
bs <- boot.phylo(tf_tree, t(TF_table), f, B = 100)

```

```

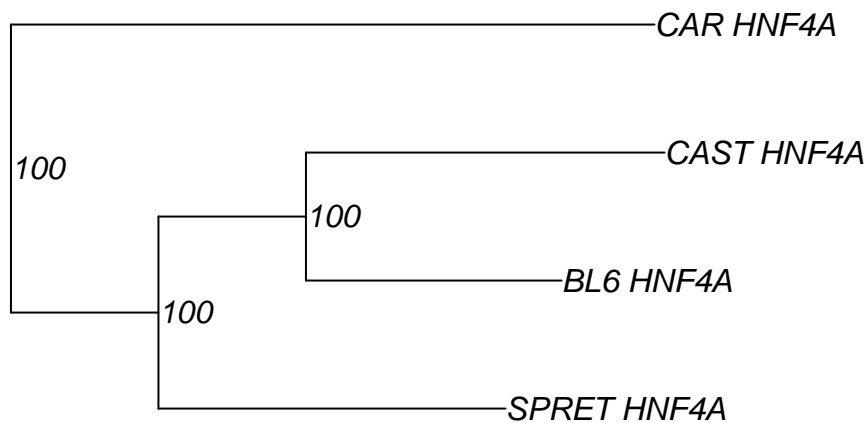
##
Running bootstraps:      100 / 100
## Calculating bootstrap values... done.

```

```

tf_tree$node.label = bs
plot(tf_tree, show.node.label = TRUE)

```



By now, an TF-binding character tree is successfully constructed.

### Creating variance co-variance matrix

```

var_mat <- varMatInv(dismat,TF_table,phy = tf_tree)

```

### Ancestral TF-binding state estimation

Here, we extract the TF-binding values of gene *MUP20* as an example:

```

mup20_binding <- TF_table[which(rownames(TF_table) == "ENSMUSG00000078672"),]

```

Then we infer the TF-binding scores at ancestral nodes of the TF-binding tree:

```

mup20_anc <- aee(mup20_binding, tf_tree, var_mat, select = "all")

```

Finally, we map these estimations on the 4 mice species tree to give a direct presentation of these values:

```
tf_tree$node.label <- sprintf("%.4f",mup20_anc$est)
tf_tree$tip.label <- paste0(tf_tree$tip.label, " ", sprintf("%.4f", mup20_binding))
plot(tf_tree, edge.color = "grey80", edge.width = 4,show.node.label = T,align.tip.label = T,main="Ancest
```

## Ancestral HNF4A–Binding Estimation of Gene MUP20

