Laboratory 6: PERMANOVA with extensions.

Objectives:

- experiment with PERMANOVA analysis in R;
- test the limits of PERMANOVA and explore alternatives;
- implement permutation testing;

1. Load the RData image for Nature 488, pp. 621-626 data.
2. Use `distance()` function to compute JSD distance of the normalized microbiome data.
3. Use `adonis()` function of the ade4 package to:
   I. Test for difference between the levels of Location variable.
   II. Test for difference between the levels of Treatment variable.
   III. Test for difference between the levels of Treatment variable stratified by Location.
4. Implement post hoc procedure for finding pairwise differences between Treatment levels in model 3.III. Compare these to post hoc tests without stratification.
5. Within the subset of fecal samples compute the PERMANOVA analysis of Antibiotic (all types) vs. Control.
6. Compare the significance values obtained in #5 to a simulated result obtained by running this code:

```
simulate.ps = function(){
  n1 = 10; x1 = rnorm(n1, mean=0, sd = 10)
  n2 = 36; x2 = rnorm(n2, mean=0, sd = 1)
  x=c(x1,x2)
  f=factor(c(rep(1,n1), rep(2,n2)))
  dm = dist(x)
  c(adonis.p = adonis(dm~f)$aov.tab[1,6],
  tev.p = t.test(x1, x2, var.equal = T)$p.value,
  tuv.p = t.test(x1, x2, var.equal = F)$p.value)
}

set.seed(20160726)
simulate.ps()
sim = t(replicate(1000, simulate.ps()))
colSums(sim<0.05)/1000
```

   Are you still confident in result in #5?
7. Given Tw2 statistic, implement permutation testing procedure and apply it to #5.

```
WT = function(dm, f){
  if(nlevels(f) != 2) return(NULL)
  lev = levels(f)
  ns = table(f)
  N = sum(ns)
  dd = as.matrix(dm)
```

```
  dd[upper.tri(dd)]=0 ##
  SST = sum(dd^2)/N
  SSW1 = sum(dd[f==lev[1],f==lev[1]]^2)/ns[1]
  SSW2 = sum(dd[f==lev[2],f==lev[2]]^2)/ns[2]
  SSW = SSW1 + SSW2

  s1 = SSW1/(ns[1]-1)
  s2 = SSW2/(ns[2]-1)
  if(SST < SSW)
    t.stat = 0
  else
    t.stat =
sqrt((ns[1]+ns[2])/(ns[1]*ns[2]))*sqrt(SST-
SSW)/sqrt(s1/ns[1] + s2/ns[2])
  t.stat
}
```

8. BONUS: Experiment with PERMANOVA-S, source code available at https://medschool.vanderbilt.edu/tang-lab/software/miProfile.