MiRKAT Package

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1 Overview

MiRKAT package (v0.02) has functions to test an association between a microbiome community and continuous/binary phenotypes via a kernel metric, where the kernel can be constructed using phylogenetic or non-phylogenetic distance metrics.

2 Changes from v0.01

1) We incorporated the source code of CompQuadForm and BiasedUrn into MiRKAT. Therefore, MiRKAT0.02 doesn't rely on these packages.

2) When the outcome is binary and the sample size is extremely small ($n < 50$), p-values from single kernel-MiRKAT using davies method can be conservative. We recommend using permutation method instead. In the new version, we permute the kernels instead of the residuals.

3)The Omnibus p-value is obtained by permuting the kernel matrices, instead of permuting the residuals.

3 Testing an association between microbiome composition and phynotypes

3.1 Example Dataset

The throat data in GUniFrc contains 60 subjects with 28 smokers and 32 nonsmokers. Microbiome data were collected from right and left nasopharynx and oropharynx region to form an OTU table with 856 OTUs. We want to evaluate whether smoking can affect the microbiome composition in the upper respiratory tract, taking into consideration additional covariates including gender and antibiotic use within 3 months.

```
> library(MiRKAT)
> library(GUniFrac)
> data(throat.tree)
> data(throat.otu.tab)
> data(throat.meta)
> attach(throat.meta)
```

3.2 Prepare the data

```
> set.seed(123)
> Male = (Sex == "Male")**2
> Smoker =(SmokingStatus == "Smoker") **2
> anti =  (AntibioticUsePast3Months_TimeFromAntibioticUsage != "None")^2
> cova = cbind(Male,  anti)
```

3.3 Create the UniFrac Distances

```
> otu.tab.rff <- Rarefy(throat.otu.tab)$otu.tab.rff
> unifracs <- GUniFrac(otu.tab.rff, throat.tree, alpha=c(0, 0.5, 1))$unifracs
> D.weighted = unifracs[,,"d_1"]
> D.unweighted = unifracs[,,"d_UW"]
> D.BC= as.matrix(vegdist(otu.tab.rff , method="bray"))
```

3.4 Convert Distances to kernel matrices

```
> K.weighted = D2K(D.weighted)
> K.unweighted = D2K(D.unweighted)
> K.BC = D2K(D.BC)
```

3.5 Testing using a single kernel

```
> MiRKAT(y = Smoker, Ks = K.weighted, X = cbind(Male, anti),out_type = "D",
+        method = "davies")

[1] 0.004757438
```

"Method" indicates which method to use to compute kenel specific p-value. "davies" represents an exact method that computes the p-value by inverting the characteristic function of the mixture chisq. We adopt an exact variance component tests because most of the studies concerning microbiome compositions have modest sample size. "permutation" represents a residual permutation approach with nperm number of permutations. "moment" represents an approximation method that matches the first two moments. When out_type = "C" (continuous outcome y), the "moment" method is the Satterthwaite approaximation. When out_type = "D" (dichotomous outcome), the "moment" method is the small sample adjustment in Lee et al (2012). When sample size is modest(n $<$ 100 for continuous or n $<$ 200 for dichotomous outcome), the "moment" method

can be inflated at very small size (such as $\alpha = 0.001$), although the type I error at $\alpha = 0.05$ is usually sustained. Therefore, we suggest using "davies" or permutation approach for such situations.

One thing to note is that the "method" only concerns with the way that a kernel specific p-value is produced.

3.6 Testing using multiple kernels

```
> Ks = list(K.weighted, K.unweighted, K.BC)
> MiRKAT(y = Smoker, Ks = Ks, X = cbind(Male, anti),out_type = "D" ,
+        nperm = 9999, method = "davies")

$indivP
[1] 0.004757438 0.014189982 0.002043271

$omnibus_p
[1] 0.0066
```

This function outputs p-values for associatiion using each single kernel and an omnibus p-value considering all three kernels. The omnibus p-value is obtained through residual permutation where the minimum p-values from each of the individual tests are used as test statistics.

4 Reference

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