Tutorial

This package is used for estimating viral transmission bottleneck sizes using different methods.

1. Install package and dataset

First step, install the package "ViralBottleneck"

1.1 Example data

Some datasets is associated with the R package, which could be imported directly using

```
ViralBottleneck::
```

A window would pop up which contains all the things exported from package including function and example dataset. All the example datasets is named start with `Example_`.

1.2. Test dataset

The folder of test dataset (`test_dataset`) on Github (https://github.com/BowenArchaman/ViralBottleneck/) contains two classes of dataset: H1N1 dataset which is a realistic dataset and Simulated dataset which is created by the simulation in associated publication. In simulated dataset folder, `Publicated_simulated_dataset` contains all the datasets that could help users to recover the publicated results. `Example_dataset` is used for this tutorial. `H1N1_dataset` would be used for raising an example that how to use this package using users' own dataset.

2. Create transmission object

Second step, download the `test_dataset/Simulated_dataset/Example_dataset`. It could be download using "https://download-directory.github.io". The transmission object need to be created before bottleneck size estimation. To create transmission object, the working directory need to meet two requirements: transmission pairs table and sample files used for estimation. This package would extract sample files according to the transmission pairs table the users input. The sample files for this tutorial is in folder `Example_dataset`.

Transmission pairs is a table which contains the names of donors in the first column and recipients in the second column.You could see the example via following code:

```
ViralBottleneck::Example_TansmissionPairs
```

The results:

donor	recipient
donor_3000	50_0_All_r1
donor_3000	50_3_All_r1

donor_3000	50_6_All_r1
donor_3000	50_9_All_r1
donor_3000	50_12_All_r1

Note: Do not put the "-" in name of sample.

After making sure the sample files all exist according to the transmission pairs, start to create transmission object. Here we directly import data of transmission pair from the package using `ViralBottleneck::` (details in 1.1 Example data) to craete transmission object, example code:

```
Sim_trans = ViralBottleneck::Example_TansmissionPairs
Sim_ob = CreateTransmissionObject(Sim_trans)
```

Transmission object is an R object class which contains the transmission pair ID that is created by linking the donor and recipient sample names with a "-" character, and two "sample" R object classes: donor and recipient. The "sample" data structure stores the sample ID and the variant sites table containing the following information in columns: position along the genome, viral genome segment name, frequencies of the four bases (A, C, G, T), and whether the allele of the variant site are synonymous or non-synonymous mutations. You could see the example vis following code:

```
ViralBottleneck::Example_ob
```

2.1 Subset transmission object

The transmission object could be used as list.

```
# Get first 3 transmission object

Sim_ob_subset = Sim_ob[1:2]
```

3. Summary transmission object

After creating transmission object, the `Summary_ob` function would provide the information of shared sites (the sites belong to shared sites should be sequenced both in donor and recipient.) for users. Example code:

```
Summary_Sim = Summary_ob(Sim_ob)
```

The result (it is also stored in Example data using `ViralBottleneck::Example_summaryOutput`):

Donors	Recipients	number.of.shared.sites
donor_3000	50_0_All_r1	13158
donor_3000	50_3_All_r1	13158
donor_3000	50_6_All_r1	13158
donor_3000	50_9_All_r1	13158
donor_3000	50_12_All_r1	13158

4. Transmission bottleneck size estimation

Finally, start to calculate transmission bottleneck size using transmission object. There are currently six methods provided in `ViralBottleneck`, including: `KL` method (Emmett et al., 2015), `Presence-Absence` method (Sacristán et al., 2011), `Binomial` method (Leonard et al., 2017), `Beta_binomial_Approximate`

method (Leonard et al., 2017) and `Beta_binomial_Exact` method (Leonard et al., 2017) and `Wight-Fisher` method (Poon et al., 2016). In the future, more methods would be integrated into the package.(Note: if you want to access the original publication for each methods, you could click the *Publication link* after each methods)

4.1 Output of `Bottleneck_size_Calculation` function

Take calculation using Beta-binomial method approximate version as an example:

```
BB_App_output =
  Bottleneck_size_Calculation(
            transmission_ob = Sim_ob,
            method ="Beta_binomial_Approximate",
            variant_calling = 0.03,
            error_filtering = 0
            Nbmin = 1,
            Nbmax = 200,
            donor_depth_threshold = 0,
            recipient_depth_threshold = 0
            )
```

Output like (it is also stored in Example data using `ViralBottleneck::Example_output`):

donor	recipient	transmission_bottleneck_size	CI_low	CI_high
donor_3000	50_0_All_r1	70	64	70
donor_3000	50_3_All_r1	45	30	64
donor_3000	50_6_All_r1	28	20	39
donor_3000	50_9_All_r1	34	23	47
donor_3000	50_12_All_r1	47	31	67

4.2 Specify transmission pairs during estimation

This package provide a chance that if user need to specify some transmission pairs for estimation. Here we used example data to import the data.

```
subset_transmission_pairs = ViralBottleneck::
```

4.3 Plot

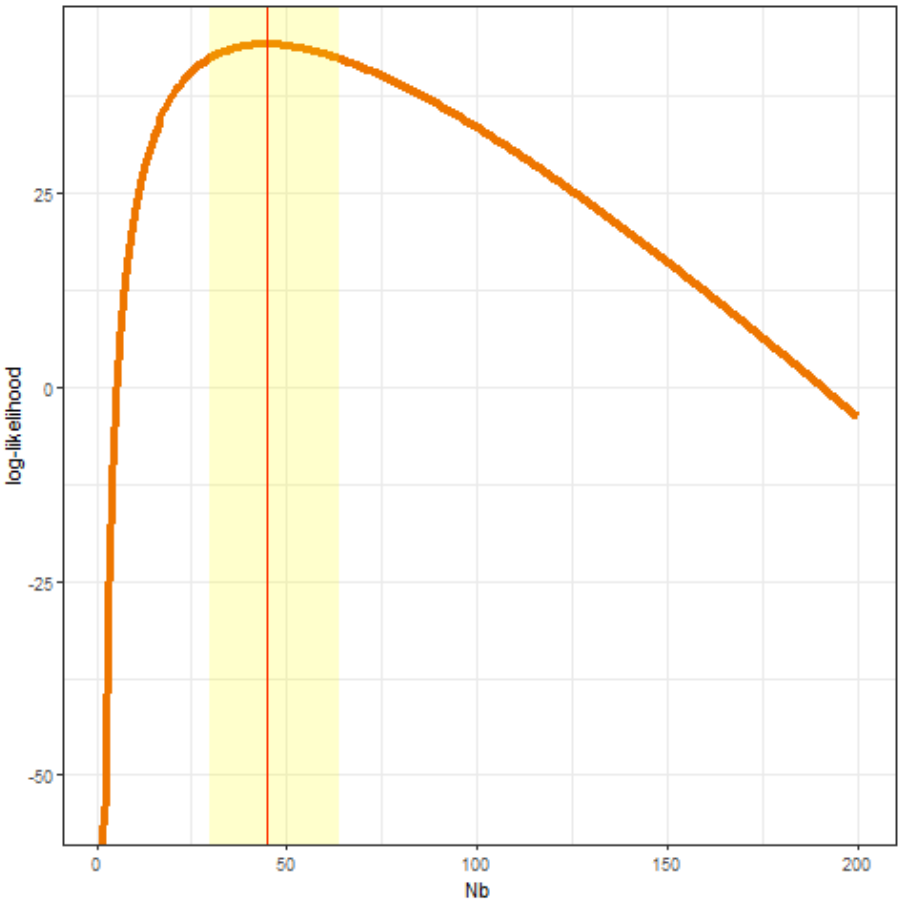
`Bottleneck_size_Calculation` could create plot of likelihood curve for each transmission pairs and save the output as csv file in working directory. However, this argument just used for the methods using maximum likelihoods estimation, including `KL` method , `Presence-Absence` method, `Binomial` method, `Beta_binomial_Approximate` method and `Beta_binomial_Exact` method. Using `show_table` and `plot` options could help to save output and obtain the plots of likelihood curve for each transmission pairs.

The program would create individual folder for each transmission pair to store the plot. Example code for creating plot:

```
BB_App_output_plot =
            Bottleneck_size_Calculation(
            transmission_ob = Sim_ob,
            method = "Beta_binomial_Approximate",
            variant_calling = 0.03,
            error_filtering = 0
            Nbmin = 1,
```

```
            Nbmax = 200,
            donor_depth_threshold = 0,
            recipient_depth_threshold = 0,
            show_table = FALSE,
            plot= TRUE
            )
```

The plot of likelihood curve for one transmission pairs (donor_3000-50_3_All_r1) is below:



4.4 Log file

`Bottleneck_size_Calculation` could create log file containing number of variant used in calculation and number of variant filtered before calculation in working directory.

Example code:

```
BB_App_output_log =
            Bottleneck_size_Calculation(
            transmission_ob = Sim_ob,
            method = "Beta_binomial_Approximate",
            variant_calling = 0.03,
            error_filtering = 0
            Nbmin = 1,
            Nbmax = 200,
            donor_depth_threshold = 0,
```

```
            recipient_depth_threshold = 0,
            log= TRUE
            )
```

Output of `log` argument:

donor	recipient	donor_used	donor_unused	recipient_used	recipient_unused
donor_3000	50_0_All_r1	193	12965	193	12965
donor_3000	50_3_All_r1	193	12965	193	12965
donor_3000	50_6_All_r1	193	12965	193	12965
donor_3000	50_9_All_r1	193	12965	193	12965
donor_3000	50_12_All_r1	193	12965	193	12965

4.5 Methods comparison

Given that one major purpose of the package is to compare calculation of bottleneck sizes across methods on the same data set, it would be nice to illustrate this. For example, compare all methods (except Wright-Fisher, see below) on a single pair, Sim_ob[1]:

```
all_methods <-
  c("KL", "Presence-Absence", "Binomial", "Beta_binomial_Approximate", "Beta_binomial_Exact")

compare_methods <-
  t(sapply(all_methods, function(m){
    Bottleneck_size_Calculation(Sim_ob[1], method = m)
  }))

compare_methods
```

5.Example of using H1N1 dataset

An example using the realistic H1N1 dataset in the folder `test_dataset`. After downloading the `H1N1_dataset` and set up working directory to the path to `H1N1_dataset`, the code could be used. In this case, we import information of transmission pairs from external csv file, which is similar with users use their own datasets. The basic rule is set the correct working directory and make sure you have transmission pairs file and related host files in this directory. The code below could be applied all the methods on one transmission pair:

```
library(ViralBottleneck)
# Set working directory and make sure you have
#               transmission pairs file and related host files in this directory.

setwd("your working directory")

# Create transmission object.

transmission_pairs = read.csv("H1N1_transmission_pairs.csv", sep = ",")
ob_H1N1 = ViralBottleneck::CreateTransmissionObject(transmission_pairs)

# Applying all methods on one transmission pair.

all_methods <-
```

```
 c("KL", "Presence-Absence", "Binomial", "Beta_binomial_Approximate", "Beta_binomial_Exact")

compare_methods <-
  t(sapply(all_methods, function(m){
    Bottleneck_size_Calculation(ob_H1N1[1],
                                variant_calling = 0.03,
                                error_filtering = 0,
                                Nbmin = 1,Nbmax = 400,
                                donor_depth_threshold = 0,
                                recipient_depth_threshold = 0 ,
                                method = m)
  }))

# Save results as csv file.

write.csv(compare_methods,"compare_methods.csv")
```

result:

method	donor	recipient	transmission_bottleneck_size	CI_low	CI_high
KL	681_1_H1N1_donor	681_1_H1N1_recipient	21	14	30
Presence-Absence	681_1_H1N1_donor	681_1_H1N1_recipient	13	9	19
Binomial	681_1_H1N1_donor	681_1_H1N1_recipient	66	66	67
Beta_binomial_Approximate	681_1_H1N1_donor	681_1_H1N1_recipient	50	30	78
Beta_binomial_Exact	681_1_H1N1_donor	681_1_H1N1_recipient	49	30	78

Reference:

Emmett, K. J., Lee, A., Khiabanian, H., & Rabadan, R. (2015) High-resolution genomic surveillance of 2014 Ebolavirus using shared subclonal variants. PLOS Currents Outbreaks 7, ecurrents.outbreaks.

Sacristán, S., Malpica, J. M., Fraile, A., & García-Arenal, F. (2003) Estimation of population bottlenecks during systemic movement of tobacco mosaic virus in tobacco plants. Journal of Virology77(18), 9906–9911.

Poon, L. L. M., Song, T., Rosenfeld, R., Lin, X., Rogers, M. B., Zhou, B., Sebra, R., Halpin, R.,Guan, Y., Twaddle, A., DePasse, J., Stockwell, T., Wentworth, D., Holmes, E., Greenbaum, B.,Peiris, J. S. M., Cowling, B. J., & Ghedin, E. (2016) Quantifying influenza virus diversity andtransmission in humans. Nature Genetics 48(2), 195–200.

Sobel Leonard, A., Weissman, D. B., Greenbaum, B., Ghedin, E., & Koelle, K. (2017) Transmission bottleneck size estimation from pathogen deep-sequencing data, with an application to human influenza A virus. Journal of Virology 91(14), e00171-17.