# *qcCHIP* User's Guide

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## Contents



## Introduction

Clonal hematopoiesis (CH) is a molecular biomarker associated with various adverse outcomes in healthy and disease individuals. Detecting CHs usually involves genomic sequencing of individual blood samples followed by rigorous bioinformatics data filtering. We report an R package qcCHIP, a bioinformatics pipeline to identify CH from sequencing data by implementing a series of quality control filters and a permutation-based parameter optimization.

## Install package

Install *qcCHIP* package via *devtools*.

```
library(devtools)
devtools::install_github("https://github.com/tenglab/qcCHIP.git",force=T)
#> -- R CMD build -------------------------------------------------------------
#>      checking for file '/private/var/folders/3s/9r3z6h_n0z3889fx5pn38svw0021h2/T/Rtmp1BPsHb/remotes1
#>   -  preparing 'qcCHIP':
```

```
#>   v  checking DESCRIPTION meta-information
#>   -  checking for LF line-endings in source and make files and shell scripts
#>   -  checking for empty or unneeded directories
#>   -  building 'qcCHIP_0.0.0.9000.tar.gz'
#>      Warning: invalid uid value replaced by that for user 'nobody'
#>
#>
```

## Getting Started

Load the package in R.

```
library(qcCHIP)
library(GenomicRanges)
```

## Preparing Input files

qcCHIP requires an annotated text file with specific column names:

All empty value should be noted as ".".

1. Chr: chromosome of variant. Exp: chr1, chr2,chrX.
2. Start: start posation of variant.
3. End: end posation of variant.
4. Ref: reference allele.
5. Alt: alternative allele.
6. TLOD: TLOD or Qual Info from vcf file.
7. SOR: SOR Info from vcf file.
8. AD_alt: Allelic depths for the alt alleles from vcf file.
9. AF: AF or VAF from blood sample vcf file.
10. DP: DP from vcf file.
11. SAF: SAF info from vcf file.
12. SAR: SAR info from vcf file.
13. SampleID: sample ID or variant.
14. Func.refGene: function annotation from refGene.
15. ExonicFunc.refGene: exonic function annotation from refGene. (nonsynonymous SNV and synoymous SNV values need to be named as "nonsynonymous SNV" and"synonymous SNV")
16. cosmic70: if the variant is exist in cosmic database. (empty value needs to be ".")
17. tumor_AF: optional, AF or VAF from tumor sample vcf file.
18. non_cancer_AF_popmax: optional, non cancer AF value from gnomad database.
19. Alt_dpGAP_PopFreq: optional, ALT population frequency from dpGAP databse.

There are two ways to prepare the input file.

1. Using in-house converting tool to convert raw VCF or annotated VCF files.
2. Manually create the input file with the columns described above.

```r
# example input file
input_path<- system.file("extdata","demo_input.txt",package="qcCHIP")
in_f <- read.table(input_path,sep="\t",header=T)

# name of each variables
colnames(in_f)
#>  [1] "Chr"                  "Start"                "End"
#>  [4] "Ref"                  "Alt"                  "TLOD"
#>  [7] "SOR"                  "AD_alt"               "AF"
#> [10] "DP"                   "SAF"                  "SAR"
#> [13] "tumor_AF"             "SampleID"             "Func.refGene"
#> [16] "Gene.refGene"         "GeneDetail.refGene"   "ExonicFunc.refGene"
#> [19] "AAChange.refGene"     "cosmic70"             "non_cancer_AF_popmax"
#> [22] "ALT_dpGAp"            "Ref_dpGAP_PopFreq"    "Alt_dpGAP_PopFreq"

# value format of each variables
head(in_f)
#>     Chr     Start       End  Ref Alt   TLOD   SOR AD_alt    AF  DP SAF SAR
#> 1 chr16   3728301   3728304 CGCT   C  17.34 0.768      9 0.021 463   5   4
#> 2 chr17  31169974  31169974    C   A    3.3 4.975     11 0.051 142   0  11
#> 3  chrX  15820243  15820243    C   T 228.43 1.483     72 0.984  72  47  25
#> 4  chr7 140734494 140734494    T  TA    7.8 0.919     14 0.051 227   6   8
#> 5 chr21  43093000  43093000    G  GT   6.93 0.356      8 0.085  95  16   0
#> 6  chr7 102257421 102257422   GA   G   5.28 0.899      7 0.059 121   9   7
#>   tumor_AF   SampleID Func.refGene  Gene.refGene
#> 1    0.018 sample_155       exonic        CREBBP
#> 2    0.025 sample_155       exonic           NF1
#> 3    0.996 sample_155       exonic         ZRSR2
#> 4    0.000 sample_155         UTR3          BRAF
#> 5    0.141 sample_155         UTR3 U2AF1;U2AF1L5
#> 6    0.000 sample_155         UTR3          CUX1
#>
#> 1
#> 2
#> 3
#> 4
#> 5 NM_006758:c.*102C>AC;NM_001025203:c.*102C>AC;NM_001025204:c.*102C>AC;NM_001320650:c.*102C>AC;NM_00
#> 6
#>           ExonicFunc.refGene
#> 1 nonframeshift substitution
#> 2          nonsynonymous SNV
#> 3             synonymous SNV
#> 4                          .
#> 5                          .
#> 6                          .
#>                                                                                                                                A
#> 1    CREBBP:NM_001079846:exon30:c.6629_6632delinsG:p.Q2210del,CREBBP:NM_004380:exon31:c.6743_6746del
#> 2 NF1:NM_000267:exon5:c.C563A:p.A188E,NF1:NM_001042492:exon5:c.C563A:p.A188E,NF1:NM_001128147:exon5:
#> 3                                                                                                ZRSR2:NM_005089:exon10:
#> 4
#> 5
#> 6
#>   cosmic70 non_cancer_AF_popmax ALT_dpGAp Ref_dpGAP_PopFreq Alt_dpGAP_PopFreq
```

```
#> 1        .             2.03E-05        .                .                .
#> 2        .                    .        .                .                .
#> 3        1               0.6222        .                .                .
#> 4        .                    .        .                .                .
#> 5        .                    .        .                .                .
#> 6        .                    .        .                .                .
```

## Using VCF files as input

Tool vcf2input can convert raw or annotated VCF files to the input file that can be used for qcCHIP.

### Raw VCF files

Below are the examples of how to convert raw VCF files to input file.

```
# Path of vcf files
raw_vcf_path<- system.file("extdata/raw_vcf",package="qcCHIP")

# Create data.frame of samples
sample_df <- data.frame(blood=list.files(raw_vcf_path)[! list.files(raw_vcf_path) %in%
                                                         grep("*_tumor\\.vcf\\.gz",list.files(raw_vcf_pa
                        tumor=grep("*_tumor\\.vcf\\.gz",list.files(raw_vcf_path),value=T))
head(sample_df)
#>              blood                   tumor
#> 1  sample_1.vcf.gz  sample_1_tumor.vcf.gz
#> 2 sample_10.vcf.gz sample_10_tumor.vcf.gz
#> 3  sample_2.vcf.gz  sample_2_tumor.vcf.gz
#> 4  sample_3.vcf.gz  sample_3_tumor.vcf.gz
#> 5  sample_4.vcf.gz  sample_4_tumor.vcf.gz
#> 6  sample_5.vcf.gz  sample_5_tumor.vcf.gz

# 1. No paired tumor sample and no annotation of other database
input_1 <- vcf2input(vcf_path = raw_vcf_path,
                     sample_list=sample_df$blood)
head(input_1)
#>        SampleID  Chr    Start      End Ref Alt   TLOD   SOR AD_alt    AF  DP
#> 1 sample_1_st_g chr1  1786850  1786850   G   T   4.08 0.375      3 0.103  30
#> 2 sample_1_st_g chr1  1786996  1786996   C   G  43.36 0.333     17 0.469  36
#> 3 sample_1_st_g chr1 36471458 36471458   A   G 187.69  0.43     72 0.500 148
#> 4 sample_1_st_g chr1 64833405 64833405   A   C  18.22 0.593      8 0.532  14
#> 5 sample_1_st_g chr1 64837976 64837976   C   T  14.64 1.244      7 0.269  30
#> 6 sample_1_st_g chr1 64841324 64841324   G   T   3.08 1.142      3 0.062  76
#>   SAF SAR
#> 1   2   1
#> 2   9   8
#> 3  36  36
#> 4   3   5
#> 5   5   2
#> 6   1   2

# 2. With paired tumor sample and no annotation of other database
input_2 <- vcf2input(vcf_path = raw_vcf_path,
```

```
                  sample_list=sample_df$blood,
                  tumor=T,
                  tumor_path=raw_vcf_path,
                  tumor_list=sample_df$tumor)
head(input_2)
#>    Chr    Start Ref Alt      SampleID      End   TLOD   SOR AD_alt    AF  DP
#> 1 chr1  1786850   G   T sample_1_st_g  1786850   4.08 0.375      3 0.103  30
#> 2 chr1  1786996   C   G sample_1_st_g  1786996  43.36 0.333     17 0.469  36
#> 3 chr1 36471458   A   G sample_1_st_g 36471458 187.69  0.43     72 0.500 148
#> 4 chr1 64833405   A   C sample_1_st_g 64833405  18.22 0.593      8 0.532  14
#> 5 chr1 64837976   C   T sample_1_st_g 64837976  14.64 1.244      7 0.269  30
#> 6 chr1 64841324   G   T sample_1_st_g 64841324   3.08 1.142      3 0.062  76
#>   SAF SAR    SampleID_t tumor_AF
#> 1   2   1             .        .
#> 2   9   8 sample_1_st_t    0.216
#> 3  36  36 sample_1_st_t     0.16
#> 4   3   5 sample_1_st_t    0.534
#> 5   5   2 sample_1_st_t    0.483
#> 6   1   2             .        .
```

Note that, raw vcf files don't have the required information of Func.refGene, ExonicFunc.refGene, and cosmic70 for qcCHIP. Users need to manually adding the corresponding columns to the resulting table converted from raw VCF.

### Annotated VCF files

Currently, qcCHIP can take the following annotation information: 1. Function name, gene name, exonic function, AAchange, and GeneDetail information from refGene database; 1. COSMIC info from COSMIC database (e.g. COSMIC70); 1. Non cancer AF population max from gnomad database.

Function name, exonic function, and COSMIC are required annotation information for qcCHIP.

```
# Path of vcf files
annot_vcf_path<- system.file("extdata/annot_vcf",package="qcCHIP")

# Create data.frame of samples
sample_df <- data.frame(blood=list.files(annot_vcf_path)[! list.files(annot_vcf_path) %in%
                                                           grep("*_tumor\\.hg38_multianno\\.vcf",list.files(a
                        tumor=grep("*_tumor\\.hg38_multianno\\.vcf",list.files(annot_vcf_path),value=T))
head(sample_df)
#>                          blood                                  tumor
#> 1  sample_1.hg38_multianno.vcf  sample_1_tumor.hg38_multianno.vcf
#> 2 sample_10.hg38_multianno.vcf sample_10_tumor.hg38_multianno.vcf
#> 3  sample_2.hg38_multianno.vcf  sample_2_tumor.hg38_multianno.vcf
#> 4  sample_3.hg38_multianno.vcf  sample_3_tumor.hg38_multianno.vcf
#> 5  sample_4.hg38_multianno.vcf  sample_4_tumor.hg38_multianno.vcf
#> 6  sample_5.hg38_multianno.vcf  sample_5_tumor.hg38_multianno.vcf

# example of annotated VCF files
library(vcfR)
exp_vcf <- read.vcfR(system.file("extdata/annot_vcf","sample_1.hg38_multianno.vcf",package="qcCHIP"))
#> Scanning file to determine attributes.
#> File attributes:
```

```
#>   meta lines: 3468
#>   header_line: 3469
#>   variant count: 213
#>   column count: 10
#> Meta line 1000 read in.Meta line 2000 read in.Meta line 3000 read in.Meta line 3468 read in.
#> All meta lines processed.
#> gt matrix initialized.
#> Character matrix gt created.
#>   Character matrix gt rows: 213
#>   Character matrix gt cols: 10
#>   skip: 0
#>   nrows: 213
#>   row_num: 0
#> Processed variant: 213
#> All variants processed

# check name of annotation information
strsplit(head(exp_vcf@fix,n=1)[,8],";")
#> $INFO
#>  [1] "AS_SB_TABLE=19,8|2,1"
#>  [2] "DP=32"
#>  [3] "ECNT=1"
#>  [4] "FS=0"
#>  [5] "MBQ=30,20"
#>  [6] "MFRL=182,144"
#>  [7] "MMQ=60,60"
#>  [8] "MPOS=10"
#>  [9] "POPAF=7.3"
#> [10] "SOR=0.375"
#> [11] "TLOD=4.08"
#> [12] "ANNOVAR_DATE=2020-06-08"
#> [13] "Func.refGeneWithVer=UTR3"
#> [14] "Gene.refGeneWithVer=GNB1"
#> [15] "GeneDetail.refGeneWithVer=NM_001282538.1:c.*213C>A\\x3bNM_001282539.1:c.*213C>A\\x3bNM_002074.
#> [16] "ExonicFunc.refGeneWithVer=."
#> [17] "AAChange.refGeneWithVer=."
#> [18] "cosmic70=."
#> [19] "AF=."
#> [20] "AF_popmax=."
#> [21] "AF_male=."
#> [22] "AF_female=."
#> [23] "AF_raw=."
#> [24] "AF_afr=."
#> [25] "AF_sas=."
#> [26] "AF_amr=."
#> [27] "AF_eas=."
#> [28] "AF_nfe=."
#> [29] "AF_fin=."
#> [30] "AF_asj=."
#> [31] "AF_oth=."
#> [32] "non_topmed_AF_popmax=."
#> [33] "non_neuro_AF_popmax=."
#> [34] "non_cancer_AF_popmax=."
```

```
#> [35] "controls_AF_popmax=."
#> [36] "ALLELE_END"

# Annotated VCF of sample and paired tumor sample. Annotated with refGene, cosmic, and gnomad.
input_3 <- vcf2input(vcf_path = annot_vcf_path,
                     sample_list=sample_df$blood,
                     tumor=T,
                     tumor_path=annot_vcf_path,
                     tumor_list=sample_df$tumor,
                     refGene=T,
                     refGene_func_name="Func.refGeneWithVer",
                     refGene_gene_name="Gene.refGeneWithVer",
                     refGene_Exonicfunc_name="ExonicFunc.refGeneWithVer",
                     refGene_AAchange_name=NA,
                     refGene_GeneDetail_name=NA,
                     cosmic=T,
                     cosmic_name="cosmic70",
                     gnomad=T,
                     gnomad_name="non_cancer_AF_popmax")

head(input_3)
#>    Chr    Start Ref Alt      SampleID      End   TLOD   SOR AD_alt    AF  DP
#> 1 chr1  1786850   G   T sample_1_st_g  1786850   4.08 0.375      3 0.103  30
#> 2 chr1  1786996   C   G sample_1_st_g  1786996  43.36 0.333     17 0.469  36
#> 3 chr1 36471458   A   G sample_1_st_g 36471458 187.69  0.43     72 0.500 148
#> 4 chr1 64833405   A   C sample_1_st_g 64833405  18.22 0.593      8 0.532  14
#> 5 chr1 64837976   C   T sample_1_st_g 64837976  14.64 1.244      7 0.269  30
#> 6 chr1 64841324   G   T sample_1_st_g 64841324   3.08 1.142      3 0.062  76
#>   SAF SAR    SampleID_t tumor_AF Func.refGene Gene.refGene ExonicFunc.refGene
#> 1   2   1             .        .         UTR3         GNB1                  .
#> 2   9   8 sample_1_st_t    0.216         UTR3         GNB1                  .
#> 3  36  36 sample_1_st_t     0.16       exonic        CSF3R     synonymous_SNV
#> 4   3   5 sample_1_st_t    0.534         UTR3         JAK1                  .
#> 5   5   2 sample_1_st_t    0.483       exonic         JAK1     synonymous_SNV
#> 6   1   2             .        .       exonic         JAK1           stopgain
#>   cosmic70 non_cancer_AF_popmax Alt_dpGAP_PopFreq
#> 1        .                    .                 .
#> 2        .                    .                 .
#> 3        .               0.7273                 .
#> 4        .                    .                 .
#> 5        .               0.1083                 .
#> 6        .                    .                 .
```

This output table can be directly used to find CH.

```
# find CH with default metrics
ch_candidate <- CHIPfilter(input_3,tumor_sample=T)
#> [1] "Perform population metrics"
#> [1] "Perform technique metrics"
#> [1] "Perform individual metrics with paired tumor sample"
#> [1] "Perform functional metrics"
#> [1] "Perform not nonsunonymous metrics"
#> [1] "Perform gnomad metrics only"
#> [1] "No blacklist region bed file find, skip"
```

```
head(ch_candidate)
#>       Chr     Start  Ref Alt       SampleID       End   TLOD   SOR AD_alt    AF
#> 347  chr5 150068242 GAGC   G sample_10_st_g 150068245  18.15 1.508      8 0.036
#> 391  chrX  48792153    G   C sample_10_st_g  48792153 205.62 0.716     85 0.296
#> 2170 chr7 102255385    C CAA  sample_9_st_g 102255385  31.03 0.284     49 0.187
#> 2176 chr7 102257898    C  CT  sample_9_st_g 102257898  14.91 0.565     19 0.233
#>       DP SAF SAR     SampleID_t tumor_AF Func.refGene Gene.refGene
#> 347  291   6   2 sample_10_st_t    0.013       exonic       SETBP1
#> 391  299  40  45 sample_10_st_t    0.026       exonic       BCORL1
#> 2170 240 125  85  sample_9_st_t    0.113       exonic        SRSF2
#> 2176  79   4  33  sample_9_st_t    0.142       exonic         JAK3
#>      ExonicFunc.refGene                                      cosmic70
#> 347   nonsynonymous_SNV                                             .
#> 391      synonymous_SNV                                             .
#> 2170     synonymous_SNV ID\\x3dCOSM4130674\\x3bOCCURENCE\\x3d1(thyroid)
#> 2176     synonymous_SNV                                             .
#>      non_cancer_AF_popmax Alt_dpGAP_PopFreq                 v_name
#> 347                0.2566                 . chr5:150068242:GAGC:G
#> 391                     .                 .     chrX:48792153:G:C
#> 2170                    1                 .  chr7:102255385:C:CAA
#> 2176                    .                 .   chr7:102257898:C:CT
#>                 loci                            mut_sample
#> 347  chr5:150068242 chr5:150068242:GAGC:G|sample_10_st_g
#> 391   chrX:48792153     chrX:48792153:G:C|sample_10_st_g
#> 2170 chr7:102255385   chr7:102255385:C:CAA|sample_9_st_g
#> 2176 chr7:102257898    chr7:102257898:C:CT|sample_9_st_g
```

## Basic Usage of *qcCHIP*

In this section, we use *qcCHIP* to test the results of select CHIP candidate with different setting of VAF, DP, or population. The resulting figures and comparision summary file will help user diceide the optimal VAF, DP, or population metric for their dataset.

### *Run qcCHIP with change of minimum VAF*

This section demonstrates the usage of *qcCHIP* when use different setting of minimum VAF.

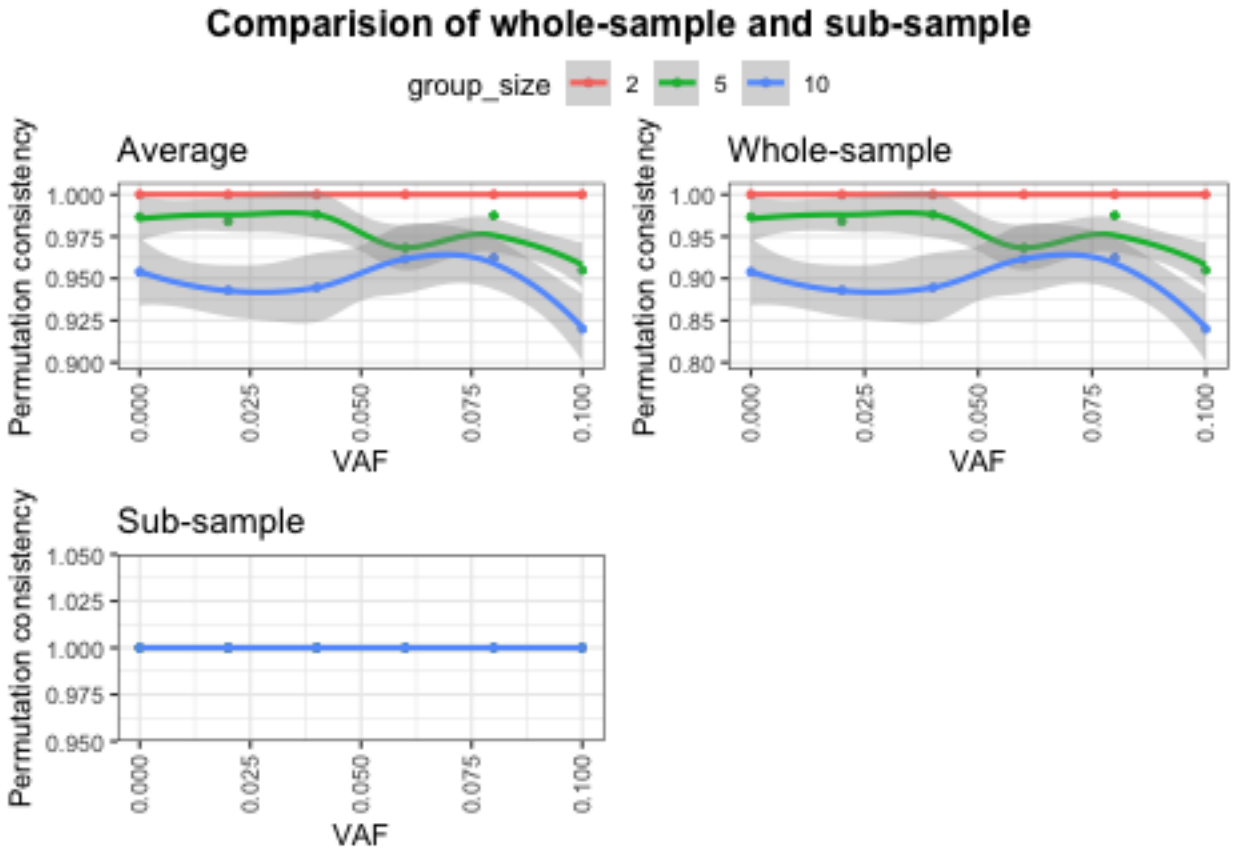
```
# input file
input_path<- system.file("extdata","demo_input.txt",package="qcCHIP")
in_f <- read.table(input_path,sep="\t",header=T)

# create test directory
out_dir <- paste0(getwd(),"/vaf_test")
vaf_permut <- qcCHIP(in_f,out_path = out_dir
                     ,metric_min = 0,
                     metric_step = 0.02,
                     metric_max = 0.1,
                     core=1,
                     show_info = F)

# example of comparision summary output
```

```
head(vaf_permut$summary_df)
#>   metric_name metric_setting group_size permut_index var_n_whole var_n_sub
#> 1         VAF              0          2            1         221       221
#> 2         VAF              0          2            2         221       221
#> 3         VAF              0          2            3         221       221
#> 4         VAF              0          2            4         221       221
#> 5         VAF              0          2            5         221       221
#> 6         VAF              0          2            6         221       221
#>   union_n common_n whole_only sub_only common_whole common_sub
#> 1     221      221          0        0            1          1
#> 2     221      221          0        0            1          1
#> 3     221      221          0        0            1          1
#> 4     221      221          0        0            1          1
#> 5     221      221          0        0            1          1
#> 6     221      221          0        0            1          1

# permutation consistency plot
vaf_permut$figs
```




***Run qcCHIP with change of minimum DP***

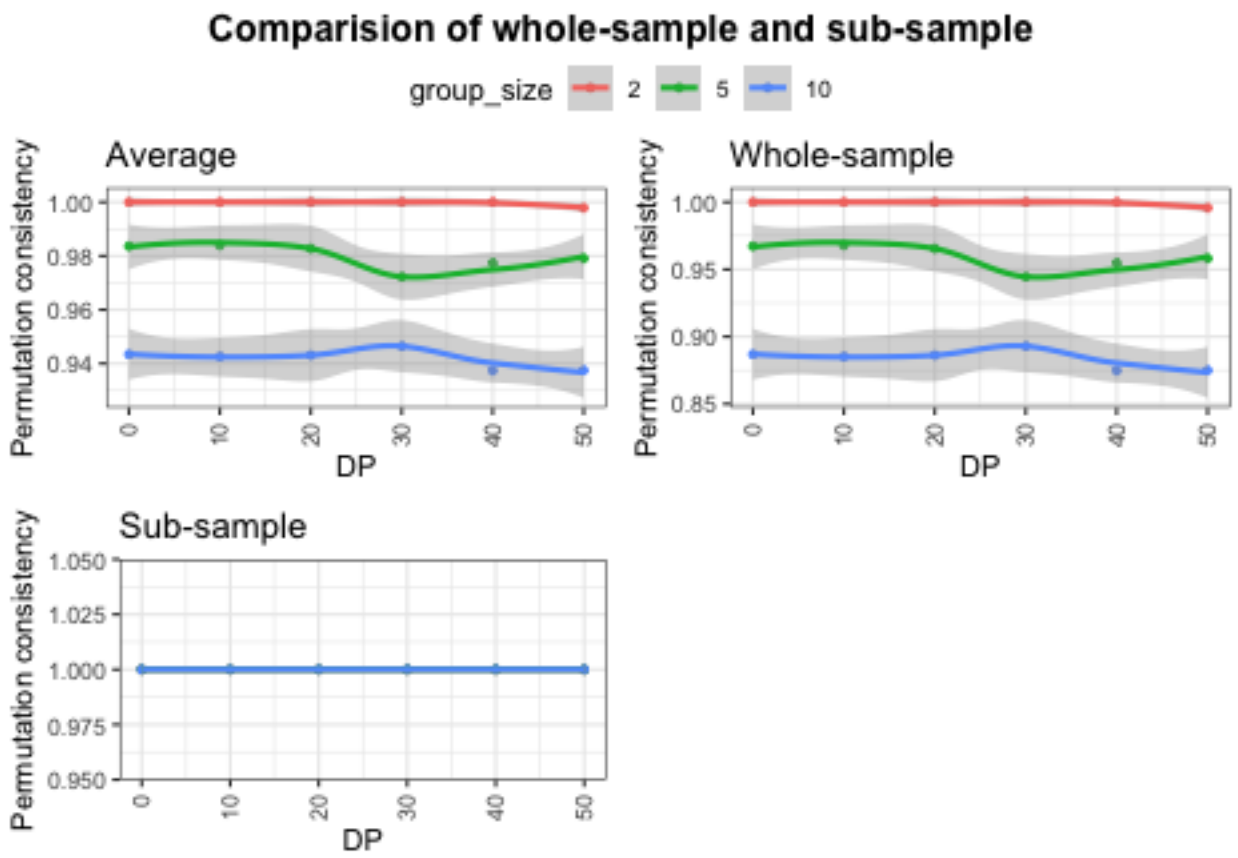
This section demonstrates the usage of *qcCHIP* when use different setting of minimum DP.

```r
# input file
input_path<- system.file("extdata","demo_input.txt",package="qcCHIP")
in_f <- read.table(input_path,sep="\t",header=T)

# create test directory
out_dir <- paste0(getwd(),"/DP_test")
DP_permut <- qcCHIP(in_f,out_path = out_dir,permut_metrics = "DP",
                    metric_min = 0,
                    metric_step = 10,
                    metric_max = 50,
                    core=1,
                    show_info = F)

# example of comparision summary output
head(DP_permut$summary_df)
#>   metric_name metric_setting group_size permut_index var_n_whole var_n_sub
#> 1          DP              0          2            1         148       148
#> 2          DP              0          2            2         148       148
#> 3          DP              0          2            3         148       148
#> 4          DP              0          2            4         148       148
#> 5          DP              0          2            5         148       148
#> 6          DP              0          2            6         148       148
#>   union_n common_n whole_only sub_only common_whole common_sub
#> 1     148      148          0        0            1          1
#> 2     148      148          0        0            1          1
#> 3     148      148          0        0            1          1
#> 4     148      148          0        0            1          1
#> 5     148      148          0        0            1          1
#> 6     148      148          0        0            1          1

# permutation consistency plot
DP_permut$figs
```




***Run qcCHIP with change of maximum population percentage***

This section demonstrates the usage of *qcCHIP* when use different setting of maximum population percentage.

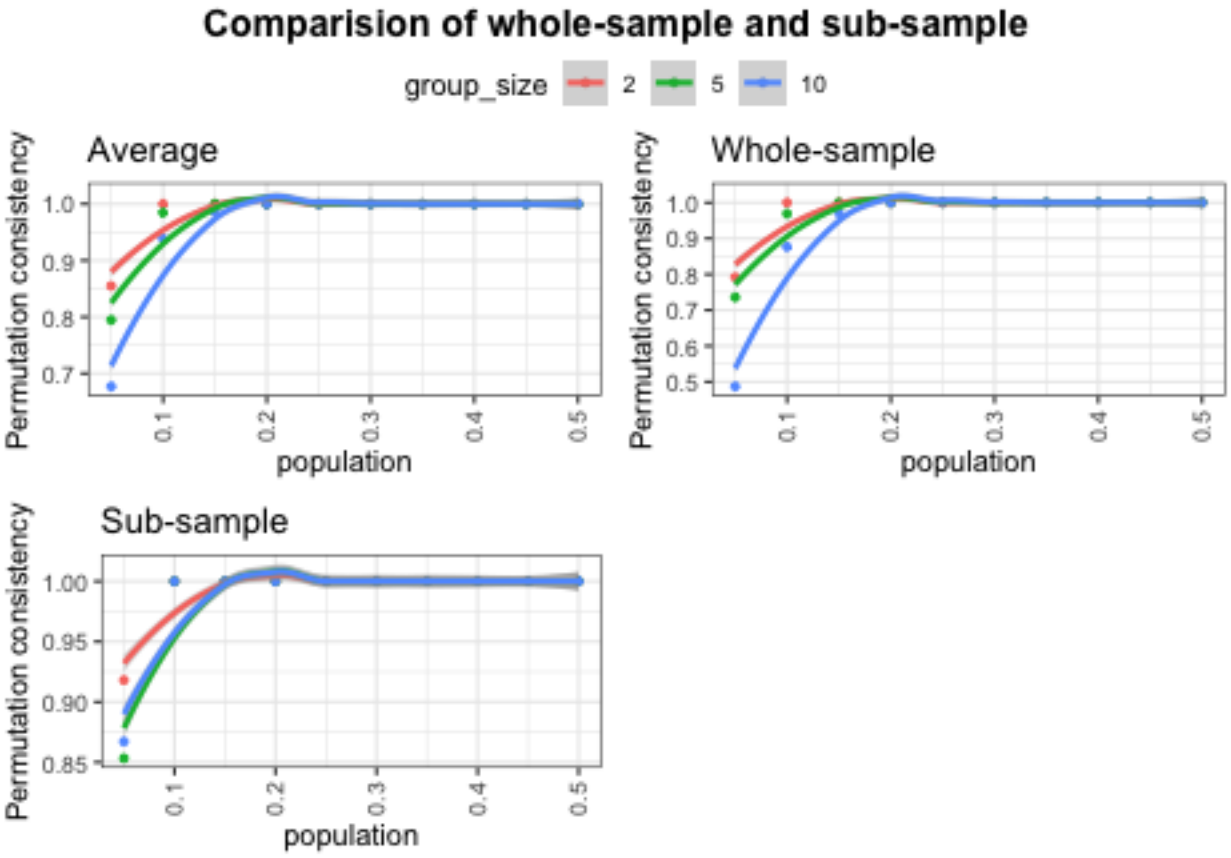
```
# input file
input_path<- system.file("extdata","demo_input.txt",package="qcCHIP")
in_f <- read.table(input_path,sep="\t",header=T)

# create test directory
out_dir <- paste0(getwd(),"/population_test")
pop_permut <- qcCHIP(in_f,out_path = out_dir,permut_metrics = "population",
                     metric_min = 0.05,
                     metric_step = 0.05,
                     metric_max = 0.5,
                     core=1,
                     show_info = F)

# example of comparision summary output
head(pop_permut$summary_df)
#>   metric_name metric_setting group_size permut_index var_n_whole var_n_sub
#> 1  population           0.05          2            1         103        79
#> 2  population           0.05          2            2         103        77
#> 3  population           0.05          2            3         103        87
#> 4  population           0.05          2            4         103        94
```

```
#> 5  population           0.05          2            5         103         90
#> 6  population           0.05          2            6         103         95
#>   union_n common_n whole_only sub_only common_whole common_sub
#> 1     110       72         31        7        0.699      0.911
#> 2     105       75         28        2        0.728      0.974
#> 3     108       82         21        5        0.796      0.943
#> 4     110       87         16        7        0.845      0.926
#> 5     112       81         22        9        0.786      0.900
#> 6     107       91         12        4        0.883      0.958

# permutation consistency plot
pop_permut$figs
```




## Basic Usage of *CHIPfilter*

In this section, we use *CHIPfilter* to get the result of select CHIP candidate based on variety of selection matrics (detailed in the man page of *CHIPfilter*). The output will be a subset of input file which pass the selection. Users can directly use this function without runing *qcCHIP*. Some features of *CHIPfilter* are described below.

```
# input file
input_path<- system.file("extdata","demo_input.txt",package="qcCHIP")
in_f <- read.table(input_path,sep="\t",header=T)
```

```r
# blacklist region to exclude
bf_path<- system.file("extdata","demo_blacklist.bed",package="qcCHIP")

bl_f <- read.table(bf_path,sep = "\t",header=F)

# run default setting
out_1 <- CHIPfilter(in_f)
#> [1] "Perform population metrics"
#> [1] "Perform technique metrics"
#> [1] "No paired tumor sample, skip"
#> [1] "Perform functional metrics"
#> [1] "Perform not nonsunonymous metrics"
#> [1] "Perform gnomad metrics only"
#> [1] "No blacklist region bed file find, skip"

# change different metrics
out_2 <- CHIPfilter(in_f,max_percent=0.02,DP_min = 40,VAF_min=0.002,info=F)

# with paired tumor sample
out_3 <- CHIPfilter(in_f,tumor_sample = T,tumor_VAF_min = 0.02,info=F)

# with gnomad or dpGAP reference file
out_4 <- CHIPfilter(in_f,gnomad = F,dpGAP = F,info=F)

# with blacklist region
out_5 <- CHIPfilter(in_f,blacklist_f = bl_f,info=F)

# check the number of CHIP
length(unique(out_1$mut_sample))
#> [1] 148
length(unique(out_2$mut_sample))
#> [1] 71
length(unique(out_3$mut_sample))
#> [1] 103
length(unique(out_4$mut_sample))
#> [1] 148
length(unique(out_5$mut_sample))
#> [1] 145
```