

SomaticSeq Documentation

Li Tai Fang

June 10, 2016

1 Introduction

SomaticSeq is a flexible post-somatic-mutation-calling workflow for improved accuracy. We have incorporated multiple somatic mutation caller(s) to obtain a combined call set, and then it uses machine learning to distinguish true mutations from false positives from that call set. We have incorporated the following somatic mutation caller: MuTect/Indelocator, VarScan2, JointSNVMix, SomaticSniper, VarDict, MuSE, LoFreq, and Scalpel. You may incorporate some or all of those callers into your own pipeline with SomaticSeq.

The manuscript, An ensemble approach to accurately detect somatic mutations using SomaticSeq, is published in Genome Biology 2015, 16:197. The SomaticSeq project is located at <http://bioinform.github.io/somaticseq/>. The data described in the manuscript is also described at <http://bioinform.github.io/somaticseq/data.html>. There have been some major improvements since the publication.

SomaticSeq.Wrapper.sh is a bash script that calls a series of scripts to combine the output of the somatic mutation caller(s), after the somatic mutation callers are run. Then, depending on what R scripts are fed to SomaticSeq.Wrapper.sh, it will either 1) train the call set into a classifier, 2) predict high-confidence somatic mutations from the call set based on a pre-defined classifier, or 3) simply label the calls (i.e., PASS or REJECT) based on majority vote of the tools.

1.1 Dependencies

- Python 3, plus regex, pysam, numpy, and scipy libraries. All the .py scripts are written in Python 3 (Yes, Python 3. I deserve brownie points from Python developers).
- R, plus the ada package in R.
- BEDTools (if there is an inclusion and/or an exclusion region file)
- Optional: Free GATK (We use GATK CombineVariants to merge VCF files. You may do it other ways.)
- Optional: dbSNP and COSMIC files in VCF format (if you want to use these features as a part of the training).
- At least one of MuTect/Indelocator, VarScan2, JointSNVMix, SomaticSniper, VarDict, MuSE, LoFreq, and/or Scalpel. Those are the tools we have incorporated in SomaticSeq. If there are other somatic tools that may be good addition to our list, please make the suggestion to us.

2 To use SomaticSeq.Wrapper.sh

The SomaticSeq.Wrapper.sh is a wrapper script that calls a series of programs and procedures. It can be easily run if all the dependencies are met. Some optional parameters are hard-coded as defaults, but can be easily edited by advanced users. In the next section, we also described the workflow in more detail, so you are not dependent on this wrapper script. You can either modify this wrapper script or create your own workflow.

2.1 To train data set into a classifier

To create a trained classifier, ground truth files are required for the data sets. There is also an option to include a list of regions to include and/or exclude. The exclusion or inclusion regions can be VCF or BED files. An inclusion region may be subset of the call sets where you have validated their true/false mutation status, so that only those regions will be used for training. An exclusion region can be regions where the “truth” is ambiguous.

All the output VCF files from individual callers are optional. Those VCF files can be bgzipped if they have .vcf.gz extensions. It is imperative that the parameters used for the training and prediction are identical.

```
1 # For training, truth file and the correct R script are required.
3 SomaticSeq.Wrapper.sh \
4   --mutect MuTect/variants.snp.vcf \
5   --mutect2 MuTect2/variants.vcf \
6   --indelocator Indelocator/variants.indel.vcf \
7   --varscan-snv VarScan2/variants.snp.vcf \
8   --varscan-indel VarScan2/variants.indel.vcf \
9   --jsm JointSNVMix2/variants.snp.vcf \
10  --sniper SomaticSniper/variants.snp.vcf \
11  --vardict VarDict/variants.vcf \
12  --muse MuSE/variants.snp.vcf \
13  --lofreq-snv LoFreq/variants.snp.vcf \
14  --lofreq-indel LoFreq/variants.indel.vcf \
15  --scalpel Scalpel/variants.indel.vcf \
16  --normal-bam matched_normal.bam \
17  --tumor-bam tumor.bam \
18  --ada-r-script ada_model_builder.R \
19  --genome-reference human_b37.fasta \
20  --cosmic cosmic.b37.v71.vcf \
21  --dbSNP dbSNP.b37.v141.vcf \
22  --gatk $PATH/TO/GenomeAnalysisTK.jar \
23  --exclusion-region ignore.bed \
24  --inclusion-region validated.bed
25  --truth-snv truth.snp.vcf \
26  --truth-indel truth.indel.vcf \
27  --output-dir $OUTPUT_DIR
```

SomaticSeq.Wrapper.sh supports any combination of the somatic mutation callers we have incorporated into the workflow. SomaticSeq will run based on the output VCFs you have provided. It will train for SNV and/or INDEL if you provide the truth.snp.vcf and/or truth.indel.vcf file(s) as well as the proper R script (ada_model_builder.R). Otherwise, it will fall back to the simple caller consensus mode.

2.2 To predict somatic mutation based on trained classifiers

Make sure the classifiers and the proper R script (ada_model_predictor.R) are supplied, Without either of them, it will fall back to the simple caller consensus mode.

```
1 # The *.RData files are trained classifier from the training mode.
SomaticSeq.Wrapper.sh \
3 --mutect MuTect/variants.snp.vcf \
  --mutect2 MuTect2/variants.vcf \
5 --indelocator Indelocator/variants.indel.vcf \
  --varscan-snv VarScan2/variants.snp.vcf \
7 --varscan-indel VarScan2/variants.indel.vcf \
  --jsm JointSNVMix2/variants.snp.vcf \
9 --sniper SomaticSniper/variants.snp.vcf \
  --vardict VarDict/variants.vcf \
11 --muse MuSE/variants.snp.vcf \
  --lofreq-snv LoFreq/variants.snp.vcf \
13 --lofreq-indel LoFreq/variants.indel.vcf \
  --scalpel Scalpel/variants.indel.vcf \
15 --normal-bam matched_normal.bam \
  --tumor-bam tumor.bam \
17 --ada-r-script ada_model_predictor.R \
  --genome-reference human_b37.fasta \
19 --cosmic cosmic.b37.v71.vcf \
  --dbsnp dbSNP.b37.v141.vcf \
21 --snpeff-dir $PATH/TO/DIR/snpSift \
  --gatk $PATH/TO/GenomeAnalysisTK.jar \
23 --exclusion-region ignore.bed \
  --inclusion-region validated.bed
25 --classifier-snv sSNV.Classifier.RData \
  --classifier-indel sINDEL.Classifier.RData \
27 --output-dir $OUTPUT_DIR
```

If both MuTect2 and MuTect/Indelocator VCF files are provided, the script is written such that it will use MuTect2 and ignore MuTect.

2.3 To classify based on simple majority vote

Same as the command previously, but not including the R script or the ground truth files. Without those information, SomaticSeq will fall back into a simple majority vote.

3 The step-by-step SomaticSeq Workflow

We'll describe the workflow here, so you may modify the workflow and/or create your own workflow instead of using the wrapper script described previously.

3.1 Combine the call sets

We use GATK CombineVariants to combine the VCF files from different callers, although it does not matter what tools are used to merge VCF files. We GATK CombineVariants because it's quite fast. To make them compatible with GATK, the VCF files are modified.

A simple alternative method is to use GNU sort and uniq in Linux to list all the unique first-5-column (i.e., CHROM, POS, ID, REF, and ALT) from all the VCF files, and then fill the remaining required VCF columns with whatever string and sort it according to the reference. That VCF file will do the job just fine.

For our GATK CombineVariants procedure:

1. Modify MuTect and/or Indelocator output VCF files. Since MuTect's output VCF do not always put the tumor and normal samples in the same columns, the script will determine that information based on either the BAM files (the header has sample name information), or based on the sample information that you tell it, and then determine which column belongs to the normal, and which column belongs to the tumor.

```
# Modify MuTect and Indelocator's output VCF based on BAM files
1 modify_MuTect.py -infile input.vcf -outfile output.vcf -nbam normal.bam -tbam
  tumor.bam

4 # Based on the sample name you supply:
  modify_MuTect.py -infile input.vcf -outfile output.vcf -nsm NormalSampleName -
    tsm TumorSampleName
```

2. Modify VarScan's output VCF files to be rigorously concordant to VCF format standard, and to attach the tag 'VarScan2' to somatic calls.

```
1 # Do it for both the SNV and indel
  modify_VJSD.py -method VarScan2 -infile input.vcf -outfile output.vcf
```

3. JointSNVMix2 does not output VCF files. In our own workflow, we have already converted its text file into a basic VCF file with an 2 awk one-liners, which you may see in the Run_5-callers directory, which are:

```
# To avoid text files in the order of terabytes, this awk one-liner keeps
  entries where the reference is not "N", and the somatic probabilities are
  at least 0.95.
2 awk -F "\t" 'NR!=1 && $4!="N" && $10+$11>=0.95'

4 # This awk one-liner converts the text file into a basic VCF file
  awk -F "\t" '{print $1 "\t" $2 "\t.\t" $3 "\t" $4 "\t.\t.\tAAAB=" $10 " ";AABB="
    $11 "\tRD:AD\t" $5 ":" $6 "\t" $7 ":" $8}'

6

8 ## The actual commands we've used in our workflow:
  echo -e '##fileformat=VCFv4.1' > unsorted.vcf
10 echo -e '##INFO=<ID=AAAB,Number=1,Type=Float,Description="Probability of Joint
  Genotype AA in Normal and AB in Tumor">' >> unsorted.vcf
  echo -e '##INFO=<ID=AABB,Number=1,Type=Float,Description="Probability of Joint
  Genotype AA in Normal and BB in Tumor">' >> unsorted.vcf
12 echo -e '##FORMAT=<ID=RD,Number=1,Type=Integer,Description="Depth of reference
  supporting bases (reads1)">' >> unsorted.vcf
  echo -e '##FORMAT=<ID=AD,Number=1,Type=Integer,Description="Depth of variant-
  supporting bases (reads2)">' >> unsorted.vcf
14 echo -e '#CHROM\tPOS\tID\tREF\tALT\tQUAL\tFILTER\tINFO\tFORMAT\tNORMAL\tTUMOR'
  >> unsorted.vcf

16 python $PATH/TO/jsm.py classify joint_snv_mix_two genome.GRCh37.fa normal.bam
  tumor.bam trained.parameter.cfg /dev/stdout | awk -F "\t" 'NR!=1 && $4!="N"
  " && $10+$11>0.95' | awk -F "\t" '{print $1 "\t" $2 "\t.\t" $3 "\t" $4 "\t
  .\t.\tAAAB=" $10 " ";AABB=" $11 "\tRD:AD\t" $5 ":" $6 "\t" $7 ":" $8}' >>
  unsorted.vcf
```

After that, you'll also want to sort the VCF file. Now, to modify that basic VCF into something that will be compatible with other VCF files under GATK CombineVariants:

```
modify_VJSD.py -method JointSNVMix2 -infile input.vcf -outfile output.vcf
```

4. Modify SomaticSniper's output:

```
1 modify_VJSD.py -method SomaticSniper -infile input.vcf -outfile output.vcf
```

5. VarDict has both SNV and indel, plus some other variants in the same VCF file. Our script will create two files, one for SNV and one for indel, while everything else is ignored for now. By default, LikelySomatic/StrongSomatic and PASS calls will be labeled VarDict. However, in our SomaticSeq paper, based on our experience in DREAM Challenge, we implemented two custom filters to relax the VarDict tagging criteria.

```
1 # Default VarDict tagging criteria, only PASS (and Likely or Strong Somatic):  
modify_VJSD.py -method VarDict -infile input.vcf -outfile output.vcf  
3  
4 # When running VarDict, if var2vcf_paired.pl is used to generate the VCF file,  
   you may relax the tagging criteria with -filter paired  
5 modify_VJSD.py -method VarDict -infile input.vcf -outfile output.vcf -filter  
   paired  
7 # When running VarDict, if var2vcf_somatic.pl is used to generate the VCF file  
   , you may relax the tagging criteria with -filter somatic  
modify_VJSD.py -method VarDict -infile input.vcf -outfile output.vcf -filter  
   somatic
```

In the SomaticSeq paper, -filter somatic was used because var2vcf_somatic.pl was used to generate VarDict's VCF files. In the SomaticSeq.Wrapper.sh script, however, -filter paired is used because VarDict authors have since recommended var2vcf_paired.pl script to create the VCF files. While there are some differences (different stringencies in some filters) in what VarDict labels as PASS between the somatic.pl and paired.pl scripts, the difference is miniscule after applying our custom filter (which relaxes the filter, resulting in a difference about 5 calls out of 15,000).

The output files will be snp.output.vcf and indel.output.vcf.

6. MuSE was not a part of our analysis in the SomaticSeq paper. We have implemented it later.

```
modify_VJSD.py -method MuSE -infile input.vcf -outfile output.vcf
```

7. LoFreq does not require modification. It has no sample columns anyway.
8. Finally, with the VCF files modified, you may combine them with GATK CombineVariants: one for SNV and one for indel separately. There is no particular reason to use GATK CombineVariants. Other combiners should also work. The only useful thing here is to combine the calls and GATK CombineVariants does it well and pretty fast.

```

1 # Combine the VCF files for SNV. Any or all of the VCF files may be present.
2 # -nt 6 means to use 6 threads in parallel
3 java -jar $PATH/TO/GenomeAnalysisTK.jar -T CombineVariants -R genome.GRCh37.fa
   -nt 6 --setKey null --genotypemergeoption UNSORTED -V mutect.vcf -V
   varscan.snp.vcf -V jointsvnmix.vcf -V snp vardict.vcf -V muse.vcf --out
   CombineVariants.snp.vcf
4 java -jar $PATH/TO/GenomeAnalysisTK.jar -T CombineVariants -R genome.GRCh37.fa
   -nt 6 --setKey null --genotypemergeoption UNSORTED -V indelocator.vcf -V
   varscan.snp.vcf -V indel.vardict.vcf --out CombineVariants.indel.vcf

```

3.2 For model training: process and annotate the VCF files (union of call sets)

This step may be needed for model training. The workflow in SomaticSeqWrapper.sh allows for inclusion and exclusion region. An inclusion region means we will only use calls inside these regions. An exclusion region means we do not care about calls inside this region. DREAM Challenge had exclusion regions, e.g., blacklisted regions, etc.

```

1 # In the DREAM_Stage_3 directory, we have included an exclusion region BED file as
   an example
2 # This command uses BEDtools to rid of all calls in the exclusion region
   intersectBed -header -a BINA_somatic.snp.vcf -b ignore.bed -v > somatic.snp.
   processed.vcf
3 intersectBed -header -a BINA_somatic.indel.vcf -b ignore.bed -v > somatic.indel.
   processed.vcf
4
5 # Alternatively (or both), this command uses BEDtools to keep only calls in the
   inclusion region
6 intersectBed -header -a BINA_somatic.snp.vcf -b inclusion.bed > somatic.snp.
   processed.vcf
7 intersectBed -header -a BINA_somatic.indel.vcf -b inclusion.bed > somatic.indel.
   processed.vcf
8

```

3.3 Convert the VCF file, annotated or otherwise, into a tab separated file

This script works for all VCF files. It extracts information from BAM files as well as some VCF files created by the individual callers. If the ground truth VCF file is included, a called variant will be annotated as a true positive, and everything will be annotated as a false positive.

```

1 # SNV
   SSeq_merged.vcf2tsv.py -ref genome.GRCh37.fa -myvcf somatic.snp.processed.vcf -
   truth Ground.truth.snp.vcf -mutect MuTect/variants.snp.vcf.gz -varscan VarScan2
   /variants.snp.vcf -jsm JSM2/variants.vcf -sniper SomaticSniper/variants.vcf -
   vardict VarDict/snp.variants.vcf -muse MuSE/variants.vcf -lofreq LoFreq/
   variants.snp.vcf -dedup -tbam tumor.bam -nbam normal.bam -outfile Ensemble.sSNV
   .tsv

```

That was for SNV, and indel is almost the same thing. After version 2.1, we have replaced all information from SAMtools and HaplotypeCaller with information directly from the BAM files. The accuracy differences are negligible with significant improvement in usability and resource requirement.

```

# INDEL:
2 SSeq-merged.vcf2tsv.py -ref genome.GRCh37.fa -myvcf somatic.indel.processed.vcf -
  truth Ground.truth.indel.vcf -varscan VarScan2/variants.snp.vcf -vardict
  VarDict/indel.variants.vcf -lofreq LoFreq/variants.indel.vcf -scalpel Scalpel/
  variants.indel.vcf -tbam tumor.bam -nbam normal.bam -dedup -outfile Ensemble.
  sINDEL.tsv

```

At the end of this, Ensemble.sSNV.tsv and Ensemble.sINDEL.tsv are created.

All the options for SSeq-merged.vcf2tsv.py are listed here. They can also be displayed by running SSeq-merged.vcf2tsv.py -help.

```

2 -myvcf      Input VCF file of the merged calls [REQUIRED]
  -ref       Genome reference fa/fastq file [REQUIRED]
4 -nbam      BAM file of the matched normal sample [REQUIRED]
  -tbam      BAM file of the tumor sample [REQUIRED]
6 -ref       Genome reference fa/fastq file [REQUIRED]
  -truth     Ground truth VCF file. Every other position is a False Positive.
8 -dbSNP     dbSNP VCF file
  -cosmic    COSMIC VCF file
10 -mutect    VCF file from either MuTect2, MuTect, or Indelocator
  -sniper    VCF file from SomaticSniper
12 -varscan   VCF file from VarScan2
  -jsm       VCF file from Bina's workflow that contains JointSNVMix2
14 -vardict   VCF file that contains only SNV or only INDEL from VarDict
  -muse      VCF file from MuSE
16 -lofreq    VCF file from LoFreq
  -scalpel   VCF file from Scalpel
18 -dedup     A flag to consider only primary reads
  -minMQ     Minimum mapping quality for reads to be considered (Default = 1)
20 -minBQ     Minimum base quality for reads to be considered (Default = 5)
  -mincaller Minimum number of caller classification for a call to be considered
  (Use 0.5 to consider some LowQual calls. Default = 0).
22 -scale     The options are phred, fraction, or None, to convert numbers to
  Phred scale or fractional scale. (default = None, i.e., no conversion)
  -outfile   Output TSV file name

```

Note: Do not worry if Python throws a warning like this.

```

RuntimeWarning: invalid value encountered in double_scalars
2 z = (s - expected) / np.sqrt(n1*n2*(n1+n2+1)/12.0)

```

This is to tell you that scipy was attempting some statistical test with empty data. That's usually due to the fact that normal BAM file has no variant reads at that given position. That is why lots of values are NaN for the normal.

3.4 Model Training or Mutation Prediction

You can use Ensemble.sSNV.tsv and Ensemble.sINDEL.tsv files either for model training (provided that their mutation status is annotated with 0 or 1) or mutation prediction. This is done with stochastic boosting algorithm we have implemented in R.

Model training:

```

# Training:
R --no-save --args Ensemble.sSNV.tsv < ada_model_builder.R
R --no-save --args Ensemble.sINDEL.tsv < ada_model_builder.R

```

Ensemble.sSNV.tsv.Classifier.RData and Ensemble.sINDEL.tsv.Classifier.RData will be created from model training.

Mutation prediction:

```

# Mutation prediction:
R --no-save --args Ensemble.sSNV.tsv.Classifier.RData Ensemble.sSNV.tsv Trained
.sSNV.tsv < ada_model_predictor.R
R --no-save --args Ensemble.sINDEL.tsv.Classifier.RData Ensemble.sINDEL.tsv Trained
.sINDEL.tsv < ada_model_predictor.R

```

After mutation prediction, if you feel like it, you may convert Trained.sSNV.tsv and Trained.sINDEL.tsv into VCF files. Use -tools to list ONLY the individual tools used to have appropriately annotated VCF files. Accepted tools are CGA (for MuTect/Indelocator), VarScan2, JointSNVMix2, SomaticSniper, VarDict, MuSE, LoFreq, and/or Scalpel. To list a tool without having run it, the VCF will be annotated as if the tool was run but did not identify that position as a somatic variant.

```

# Probability above 0.7 labeled PASS (-pass 0.7), and between 0.1 and 0.7 labeled
  LowQual (-low 0.1):
# Use -all to include REJECT calls in the VCF file
# Use -phred to convert probability values (between 0 to 1) into Phred scale in the
  QUAL column in the VCF file
SSeq.tsv2vcf.py -tsv Trained.sSNV.tsv -vcf Trained.sSNV.vcf -pass 0.7 -low 0.1
  -tools CGA VarScan2 JointSNVMix2 SomaticSniper VarDict MuSE LoFreq -all -phred
SSeq.tsv2vcf.py -tsv Trained.sINDEL.tsv -vcf Trained.sINDEL.vcf -pass 0.7 -low 0.1
  -tools CGA VarScan2 VarDict LoFreq Scalpel -all -phred

```

4 Release Notes

Make sure training and prediction use the same version. Otherwise the prediction is not valid.

4.1 Version 1.0

Version used to generate data in the manuscript and Stage 5 of the ICGC-TCGA DREAM Somatic Mutation Challenge, where SomaticSeq's results were #1 for INDEL and #2 for SNV.

In the original manuscript, VarDict's var2vcf_somatic.pl script was used to generate VarDict VCFs, and subsequently "-filter somatic" was used for SSeq_merged.vcf2tsv.py. Since then (including DREAM Challenge Stage 5), VarDict recommends var2vcf_paired.pl over var2vcf_somatic.pl, and subsequently "-filter paired" was used for SSeq_merged.vcf2tsv.py. The difference in SomaticSeq results, however, is pretty much negligible.

4.2 Version 1.1

Automated the SomaticSeq.Wrapper.sh script for both training and prediction mode. No change to any algorithm.

4.3 Version 1.2

Have implemented the following improvement, mostly for indels:

- SSeq_merged.vcf2tsv.py can now accept pileup files to extract read depth and DP4 (reference forward, reference reverse, alternate forward, and alternate reverse) information (mainly for indels). Previously, that information can only be extracted from SAMtools VCF. Since the SAMtools or HaplotypeCaller generated VCFs hardly contain any indel information, this option improves the indel model. The SomaticSeq.Wrapper.sh script is modified accordingly.
- Extract mapping quality (MQ) from VarDict output if this information cannot be found in SAMtools VCF (also mostly benefits the indel model).
- Indel length now positive for insertions and negative for deletions, instead of using the absolute value previously.

4.4 Version 2.0

- Removed dependencies for SAMtools and HaplotypeCaller during feature extraction. SSeq_merged.vcf2tsv.py extracts those information (plus more) directly from BAM files.
- Allow not only VCF file, but also BED file or a list of chromosome coordinate as input format for SSeq_merged.vcf2tsv.py, i.e., use -mybed or -mypos instead of -myvcf.
- Instead of a separate step to annotate ground truth, that can be done directly by SSeq_merged.vcf2tsv.py by supplying the ground truth VCF via -truth.
- SSeq_merged.vcf2tsv.py can annotate dbSNP and COSMIC information directly if BED file or a list of chromosome coordinates are used as input in lieu of an annotated VCF file.
- Consolidated feature sets, e.g., removed some redundant feature sets coming from different resources.

4.5 Version 2.0.2

- Incorporated LoFreq.
- Used getopt to replace getopts in the SomaticSeq.Wrapper.sh script to allow long options.

4.6 Version 2.1.2

- Properly handle cases when multiple ALT's are calls in the same position. The VCF files can either contain multiple calls in the ALT column (i.e., A,G), or have multiple lines corresponding to the same position (one line for each variant call). Some functions were significantly re-written to allow this.
- Incorporated Scalpel.
- Deprecated HaplotypeCaller and SAMTools dependencies completely as far as feature generation is concerned.

- The Wrapper script removed SnpSift/SnpEff dependencies. Those information can be directly obtained during the SSeq_merged.vcf2tsv.py step. Also removed some additional legacy steps that has become useless since v2 (i.e., score_Somatic.Variants.py). Added a step to check the correctness of the input. The v2.1 and 2.1.1 had some typos in the wrapper script, so only describing v2.1.2 here.

4.7 Version 2.2

- Added MuTect2 support.

4.8 Version 2.2.1

- InDel_3bp now stands for indel counts within 3 bps of the variant site, instead of exactly 3 bps from the variant site as it was previously (likewise for InDel_2bp).
- Collapse MQ0 (mapping quality of 0) reads supporting reference/variant reads into a single metric of MQ00 reads (i.e., tBAM_MQ0 and nBAM_MQ0). From experience, the number of MQ00 reads is at least equally predictive of false positive calls, rather than distinguishing if those MQ0 reads support reference or variant.
- Obtain SOR (Somatic Odds Ratio) from BAM files instead of VarDict's VCF file.
- Fixed a typo in the SomaticSeq.Wrapper.sh script that did not handle inclusion region correctly.

5 To do: planned improvement

- Develop a version for deep sequencing cfDNA/ctDNA.

6 Contact Us

For suggestions, bug reports, or technical support, please post in the github issues page, or email li.tai.fang@bina.roche.com.