Appendix A. User guideline for our developed FastMtQTL R package and SAS program (integrated SAS and R codes), and the existing PROC QTL SAS add-in package

1. Example data files

R dataset: The simulated R dataset (SimData.RData) for multi-trait QTL analysis is available at www.bbcba.org/FastMtQTL

CSV file: The simulated dataset in CSV format (SimData.csv) is available at www.bbcba.org/FastMtQTL

2. FastMtQTL R package

1. First install the qtl (version: 1.46-2) and FastMtQTL (our developed package) R-packages in R-3.6.3 using the following codes. The binary (.zip) file of FastMtQTL package is available at www.bbcba.org/FastMtQTL

   ```
   install.packages("qtl")
   install.packages("E:/MVN-based_QTL_Mapping/pgm/FastMtQTL_0.01.zip"
              , repos = NULL, type="source")
   ```

   Note that the location "E:/MVN-based_QTL_Mapping/pgm/FastMtQTL_0.01.zip" may need to be changed depending at which location you store the "FastMtQTL_0.01.zip" file.

2. Now, load the qtl and FastMtQTL libraries in your R session using the following codes.

   ```
   library(qtl)
   library(FastMtQTL)
   ```

3. Now, read/load the example R QTL dataset (SimData.RData) into R using the following codes.

   ```
   load("E:/FastMtQTL/Appendix/data files/SimData.RData")
   ```

   Note that the location of the data file may need to change depending on at which you store the data file.

4. Now, run the bcMVNmtQTL(inData, phenoCols, Chrs, markerInterval=1.0) function of FastMtQTL R-package using the following codes.

LODscores <- bcMVNmtQTL(inData=SimData,phenoCols=c(1:5),
Chrs=c(1:13), markerInterval=1.0)

plot(LODscores)

That is all to calculate LOD statistic and create LOD score plot.

3. SAS program (integrated SAS and R codes)

1. Set the environment to run R from SAS (i.e., set up environmental variable for R to enable R for calling it from SAS).
2. Make sure that the qtl and FastMtQTL R-packages are installed in R software.
3. Run the following codes to calculate LOD statistic using the following SAS codes integrated with R codes.

```
PROC IML;
  TITLE "Statistic in R (integration with SAS)";

  SUBMIT/R;
    #_____Begin of R code_____

    #install.packages("qtl", lib=rLibLoc)

    #Load required R packages
    library(qtl)
    library(FastMtQTL)


    #Load the simulated data into R
    load("E:/FastMtQTL/Appendix/data files/SimData.RData")



    #Perform the analysis to calculate LOD statistic
    MVN_LODscores<-bcMVNmtQTL(inData=SimData,phenoCols=c(1:5),
                        Chrs=c(1:13), markerInterval=1.0)

    #Save the final count dataset permanently
    save(MVN_LODscores, file = "E:/FastMtQTL/Appendix/data files/MVN_LODscores.RData")

        #_____End of R code_____

  ENDSUBMIT;
QUIT; /*Use Quit command to end the IML procedure*/
```

4. PROC QTL SAS add-in package

First install the PROC QTL package in your Windows based system.

Data preparation

Read the CSV data file (SimData.csv) into SAS using the following SAS codes. Note that you have to change the location/path of the data file according to your data file location. SimData.csv file contains phenotypic data and genotypic data along with the genetic map.

```
PROC IMPORT OUT=WORK.BC_SimData
     DATAFILE="E:\FastMtQTL\Appendix\data files\SimData.csv"
     DBMS=CSV REPLACE;
     GETNAMES=YES;
     DATAROW=2;
   RUN;
```

Now, create a SAS dataset (Bc_simdata_map_final) of the genetic map using the BC_SimData SAS dataset using the following SAS codes.

```
DATA Bc_simdata_map;
  SET Bc_simdata(DROP=Pheno1--Pheno5);
  IF _N_ > 2 THEN DELETE;
RUN;

PROC TRANSPOSE DATA=Bc_simdata_map OUT=Bc_simdata_map_trns;
  VAR _ALL_;
RUN;

DATA Bc_simdata_map_final(DROP=_NAME_ COL1 COL2);
  SET Bc_simdata_map_trns;

  marker=_NAME_;
  position = INPUT(COL2,BEST12.);
  chromosom = INPUT(COL1,BEST12.);
RUN;
```

Now, create dataset (Bc_simdata_Genopheno) that contains the phenotypic and genotypic information using the following SAS codes.

```
DATA Bc_simdata_Genopheno;
  SET Bc_simdata;
  IF _N_ IN(1,2) THEN DELETE;
RUN;
```

Maximum likelihood (ML) based multi-trait QTL analysis

The followings are the SAS codes for genome-wide QTL searching at each 1 cM (STEP=1.0) flanking marker interval using ML method (METHOD='ml') of multi-trait QTL analysis.

```
PROC QTL DATA=Bc_simdata_Genopheno
         MAP=Bc_simdata_map_final
         OUT=result_ML
         METHOD='ml' STEP=1.0;
  MODEL Pheno1 Pheno2 Pheno3 Pheno4 Pheno5=;
  MATINGTYPE 'BC';
  GENOTYPE A1A1='A' A1A2='H';
  ESTIMATE 'additive'=1 0;
RUN;
QUIT;
```

Now calculate log of odds (LOD) score as follows.

```
DATA result_ML_LOD;
  SET result_ML;

  index=_N_;
  LOD = 0.2171*LRT;
RUN;
```

Least squares (LS) based multi-trait QTL analysis

The followings are the SAS codes for genome-wide QTL searching at each 1 cM (STEP=1.0) flanking marker interval using LS method (METHOD='ls') of multi-trait QTL analysis.

```
PROC QTL DATA=Bc_simdata_Genopheno
         MAP=Bc_simdata_map_final
         OUT=result_Reg
         METHOD='ls' STEP=1.0;
  MODEL Pheno1 Pheno2 Pheno3 Pheno4 Pheno5=;
  MATINGTYPE 'BC';
  GENOTYPE A1A1='A' A1A2='H';
  ESTIMATE 'additive'=1 0;
RUN;
QUIT;
```

Now calculate log of odds (LOD) score as follows.

```
DATA result_reg_LOD;
  SET result_Reg;

  index=_N_;
  LOD = 0.2171*LRT;
RUN;
```