```
/*Supplemental material : SAS codes for analyzing linkage mapping populations
for marker main- and marker × treatment (environment) interactions as well as
epistasis (marker × marker) and epistasis × treatment (environment)
interaction. In following codes, the word "treatment" represents
"environment".  */

/* Create the simulated data files and install the marcos  */


/***************************************************************************/
            /*Declare variables used to define simulation scenarios */
/***************************************************************************/

%let No_Genotypes=250;                         /*Specify the number of tested
genotypes in the estimation set*/

%let No_SNP=5;                         /*Specify the number of all SNP markers
to simulate*/


/***************************************************************************/
                       /*Simulation of datasets*/
/***************************************************************************/

/*Simulate vector u of dimension p (No of SNP) of marker effects*/

/********* See also supplementary material Piepho HP, Ogutu JO, Schulz-Streeck
T, Estaghvirou B, Gordillo A, Technow F. 2012. Efficient Computation of Ridge-
Regression Best Linear Unbiased Prediction in Genomic Selection in Plant
Breeding. Crop Science 52, 1093-1104. ***/
/******************** for the simulation of the test data
*********************************************************/


data u;
array u u1-u&No_SNP;
do i=1 to &No_SNP;
  u[i]=normal(59752)*sqrt(1/&No_SNP);
/*var(u)=var(e)/No_SNP*/
end;
output;
run;

/*Simulate genotypic values (g) and phenotypic values (y =genotypic
effect+random effect) for the estimation
set, i.e. part of the data set with only the n tested genotypes*/

data g;
set u;
array SNP SNP1-SNP&No_SNP;
array u u1-u&No_SNP;
do j=1 to &No_Genotypes;
  g=0;
  do i=1 to &No_SNP;
    x=ranbin(69752,1,0.5);
    SNP[i]=2*x-1;
    g=g+SNP[i]*u[i];
    y=g+normal(174265);
  end;
```

```sas
   keep  SNP1-SNP&No_SNP g y ;
   output;
end;
run;
Data g; set g;
     Genotype=_N_;
Run;
data g_treat;
set u;
array SNP SNP1-SNP&No_SNP;
array u u1-u&No_SNP;
do j=1 to &No_Genotypes;
  g=0;
  do i=1 to &No_SNP;
    x=ranbin(123456,1,0.5);
    SNP[i]=2*x-1;
    g=g+SNP[i]*u[i];
    y=g+normal(987654);
  end;
  keep  SNP1-SNP&No_SNP g y ;
  output;
end;
run;
Data g_treat; set g_treat;
     Genotype=_N_; Treatment=1;
Run;
Data g_treat_1; set g;
     Genotype=_N_; Treatment=2;
Run;
Proc append Base=g_treat data=g_treat_1 force; run ;
proc delete data = work.g_treat_1; run;
proc delete data = work.u; run;


    /************************************************************************
*/

                        /* Macros for analyzing Marker-trait
associations */


    /************************************************************************
*/

%macro nobs(ds);
     DATA _NULL_;
          IF _N_ = 0 THEN DO;
               I = 1;
               SET &ds POINT=I NOBS=NOBS;
          END;
          CALL SYMPUT('NOBS',TRIM(LEFT(PUT(NOBS,6.))));
          STOP;
     RUN;
%mend nobs;


%macro marker_main_effect;
```

```
proc delete data = work.Marker_Main_effect; run;

%do j=1 %to   &No_SNP  ;

        %let marker=marker_&j;

        /*********** Hide log information ***********************/
        %if &j gt 3  %then %do;
                proc printto log=outlog;              run;
                proc options;                                  run;
                proc printto print = outdoc;  run;
                ODS Results OFF;
            %end;
        /***********************************************************/

        /************** Model; change for your experimental design ***/


        proc mixed data=G;
            class genotype SNP&j;
            model y= SNP&j / htype=1 solution;
            ods output   tests1=SNP_test_Anova;
        run;


        /***********************************************************/


        Data SNP_test_Anova; set SNP_test_Anova;
            informat   effect $25.  Marker $25.  probf 30.28;
            format         effect $25.  Marker $25.  probf 30.28;
            Marker="&Marker";
        run;
        %if &j eq 1 %then %do;
            Data Marker_Main_effect; set SNP_test_Anova;  run;
        %end;
        %else %do;
            Proc append Base=Marker_Main_effect data=SNP_test_Anova
force;      run ;
        %end;
    %end;
    Proc sort data=Marker_Main_effect; by ProbF; run;
    proc delete data = work.SNP_test_Anova; run;

    /*********** Calculation of FDR probability ******************/

    proc multtest inpvalues(ProbF)=Marker_Main_effect OUT=Marker_Main_effect
FDR; run;

    /***********************************************************/

    /*************** Print Log file information again ************/

    Proc printto; run;

    /********************************************************/

%Mend marker_main_effect;
```

```
%macro marker_Treatment_interaction;
      proc delete data = work.Marker_treat_interaction; run;
      proc delete data = work.Marker_main_effect_in_treat; run;

      %do j=1 %to   &No_SNP  ;

            %let marker=marker_&j;

            /*********** Hide log information ************************/
            %if &j gt 3  %then %do;
                        proc printto log=outlog;            run;
                        proc options;                                run;
                        proc printto print = outdoc;  run;
                        ODS Results OFF;
                  %end;
            /***************************************************************/



            /************** Model; change according to your experimental
design ***/


                  proc mixed data=g_treat;
                  class genotype treatment SNP&j;
                  model y= treatment SNP&j treatment*SNP&j / htype=1 solution;
                  ods output   tests1=SNP_by_treat_test_Anova;
                  run;

            /************************************************************/

            Data SNP_by_treat_test_Anova;       set SNP_by_treat_test_Anova;
                  informat    effect $25.  Marker $25.  probf 30.28;
                  format           effect $32.  Marker $25.  probf 30.28;
                  Marker="&Marker";
            run;
            Data SNP_treat_interaction;  set SNP_by_treat_test_Anova;  If
effect eq  "Treatment*SNP&j"; run;
            Data SNP_effect;                      set SNP_by_treat_test_Anova;
If effect eq  "SNP&j"; run;
            %if &j le 2 %then %do;
                  Data Marker_treat_interaction; set SNP_treat_interaction;
run;
                  Data Marker_main_effect_in_treat; set SNP_effect;  run;
            %end;
            %else %do;
                  Proc append Base=Marker_treat_interaction
      data=SNP_treat_interaction force;   run ;
                  Proc append Base=Marker_main_effect_in_treat
      data=SNP_effect force;  run ;
            %end;
      %end;

      /*********** Calculation of FDR probability ******************/
```

```sas
      proc multtest inpvalues(ProbF)=Marker_treat_interaction
OUT=Marker_treat_interaction FDR; run;
      proc multtest inpvalues(ProbF)=Marker_main_effect_in_treat
OUT=Marker_main_effect_in_treat FDR; run;


      /**************************************************************/


      Data Marker_main_effect_in_treat; set Marker_main_effect_in_treat;
Main_NumDF=NumDF; Main_DenDF=DenDF; Main_FValue=FValue; Main_ProbF=ProbF;
Main_FDR_p=fdr_p;
            Drop NumDF DenDF FValue  ProbF fdr_p;
      run;
      Data Marker_treat_interaction; set Marker_treat_interaction;
Treat_NumDF=NumDF; Treat_DenDF=DenDF; Treat_FValue=FValue; Treat_ProbF=ProbF;
Treat_FDR_p=fdr_p;
            Drop NumDF DenDF FValue  ProbF fdr_p;
      run;
      Proc sort data=Marker_treat_interaction; by Marker; run;
      Proc sort data=Marker_main_effect_in_treat; by Marker; run;
      data Marker_treat_interaction; merge Marker_main_effect_in_treat
Marker_treat_interaction; by Marker; run;

      Proc sort data=Marker_treat_interaction; by Treat_ProbF; run;

      proc delete data = work.SNP_treat_interaction; run;
      proc delete data = work.SNP_effect; run;
      proc delete data = work.SNP_by_treat_test_Anova; run;
      proc delete data = work.Marker_main_effect_in_treat; run;

      /***************** Print Log file information again ************/

      Proc printto; run;

      /**************************************************************/

%Mend marker_Treatment_interaction;


%macro Epistatic_effect;


            /*********** Hide results as the system would be slow otherwise
*************************/

                  ods graphics off;
                  ods html close;


      /***********+++++++++++++++++++++++++++++++++++++++++++++++++++********
*****************/

      proc delete data = work.Epistatic_effect; run;

      %do i=1 %to &No_SNP -1  ;
            %do j=&i+1 %to  &No_SNP  ;
                  %let marker_a=marker_&i;     %let marker_b=marker_&j;

            /*********** Hide log information ************************/
```

```sas
                %if &marker_a eq marker_1  %then %do;
                     %if &marker_b eq marker_3 %then %do;
                          proc printto log=outlog;           run;
                          proc options;                                    run;
                          proc printto print = outdoc;  run;
                          ODS Results OFF;
                     %end;
                %end;
          /************************************************************/

                Data SNP_test_Anova;
                     informat    Effect $32.  Marker_a $25. Marker_b $25.
probf 30.28;
                     format              Effect $32.  Marker_a $25. Marker_b
$25. probf 30.28;
                     length              Effect $32;
                run;

        /************** Model; change according to your experimental
design ***/

                proc mixed data=G;
                     class genotype SNP&i SNP&j;
                     model y= SNP&i*SNP&j / htype=1 solution;
                     ods output   tests1=SNP_test_Anova;
                run;




    /**********************************************************************/
                Data SNP_test_Anova; set SNP_test_Anova;
                     informat    Effect $32.  Marker_a $25. Marker_b $25.
probf 30.28;
                     format              Effect $32.  Marker_a $25. Marker_b
$25. probf 30.28;
                     length              Effect $32;
                     Marker_a="&marker_a"; Marker_b="&marker_b";
                run;
                %if &i eq 1 and &j eq 2 %then %do;
                     Data Epistatic_effect; set SNP_test_Anova;  run;
                %end;
                %else %do;
                     Proc append Base=SNP_test_Anova data=Epistatic_effect
force;      run ;
                     Data Epistatic_effect; set SNP_test_Anova; run;
                %end;
          %end;
     %end;
     ODS Results on; ods html;
     Proc sort data=Epistatic_effect; by ProbF; run;
     proc delete data = work.SNP_test_Anova; run;

     /*********** Calculation of FDR probability ******************/

     proc multtest inpvalues(ProbF)=Epistatic_effect OUT=Epistatic_effect
FDR; run;
```

```
      /*************************************************************/

      /**************** Print Log file information again ************/

      Proc printto; run;

      /*************************************************************/

%Mend Epistatic_effect;


%macro Epistatic_Treatment_interaction;

          /*********** Hide results as the system would be slow otherwise
************************/

              ods graphics off;
              ods html close;


      /***********++++++++++++++++++++++++++++++++++++++++++++++++++++*********
*****************/

      proc delete data = work.Epistatic_treat_interaction; run;
      proc delete data = work.Epistatic_effect_in_treat; run;

      %do i=1 %to &No_SNP -1  ;
          %do j=&i+1 %to  &No_SNP  ;
              %let marker_a=marker_&i;     %let marker_b=marker_&j;

              /*********** Hide log information
************************/
              %if &marker_a eq marker_1  %then %do;
                  %if &marker_b eq marker_3 %then %do;
                      proc printto log=outlog;          run;
                      proc options;                           run;
                      proc printto print = outdoc;  run;
                      ODS Results OFF;
                  %end;
              %end;

      /*************************************************************/

          /************** Model; change according to your experimental
design ***/


              proc mixed data=G_treat;
                  class genotype Treatment SNP&i SNP&j;
                  model y= Treatment SNP&i*SNP&j Treatment*SNP&i*SNP&j/
htype=1 solution;
                  ods output   tests1=SNP_by_treat_test_Anova;
              run;

          /*************************************************************/

              Data SNP_by_treat_test_Anova; set SNP_by_treat_test_Anova;
```

```
                              informat     Effect $32.    Marker_a $25. Marker_b $25.
probf 30.28;

                     format              Effect $32.    Marker_a $25. Marker_b
$25. probf 30.28;
                     Marker_a="&marker_a"; Marker_b="&marker_b";
            run;
            Data Epi_effect;        set SNP_by_treat_test_Anova; where
effect contains  "SNP&i"; run;
            Data Epi_effect_treat;  set Epi_effect; where effect contains
"Treatment"; run;
            Data Epi_effect;         set Epi_effect; where effect not
contains  "Treatment"; run;
            %if i eq 1 and j eq 2 %then %do;
                 Data Epistatic_treat_interaction; set
Epi_effect_treat;  run;
                 Data Epistatic_effect_in_treat; set Epi_effect;  run;
            %end;
            %else %do;
                 Proc append Base=Epi_effect_treat
data=Epistatic_treat_interaction force;   run ;
                 Proc append Base=Epi_effect
data=Epistatic_effect_in_treat force;     run ;
                 Data Epistatic_treat_interaction; set
Epi_effect_treat; run;
                 Data Epistatic_effect_in_treat; set Epi_effect; run;
            %end;
       %end;
    %end;
    ODS Results on; ods html;


    /*********** Calculation of FDR probability *****************/

    proc multtest inpvalues(ProbF)=Epistatic_treat_interaction
OUT=Epistatic_treat_interaction FDR; run;
    proc multtest inpvalues(ProbF)=Epistatic_effect_in_treat
OUT=Epistatic_effect_in_treat FDR; run;

    /**************************************************************/

    Data Epistatic_effect_in_treat; set Epistatic_effect_in_treat;
Epi_NumDF=NumDF; Epi_DenDF=DenDF; Epi_FValue=FValue; Epi_ProbF=ProbF;
Epi_FDR_p=fdr_p;
          Drop NumDF DenDF FValue  ProbF fdr_p;
    run;
    Data Epistatic_treat_interaction; set Epistatic_treat_interaction;
Epi_Treat_NumDF=NumDF; Epi_Treat_DenDF=DenDF; Epi_Treat_FValue=FValue;
Epi_Treat_ProbF=ProbF; Epi_Treat_FDR_p=fdr_p;
          Drop NumDF DenDF FValue  ProbF fdr_p;
    run;
    Proc sort data=Epistatic_effect_in_treat;      by marker_a marker_b;
run;
    Proc sort data=Epistatic_treat_interaction; by marker_a marker_b; run;
    data Epistatic_treat_interaction; merge Epistatic_effect_in_treat
Epistatic_treat_interaction; by marker_a marker_b; run;

    Proc sort data=Epistatic_treat_interaction;    by Epi_Treat_ProbF; run;
```

```sas
        proc delete data = work.Epistatic_effect_in_treat; run;
        proc delete data = work.SNP_by_treat_test_Anova; run;
        proc delete data = work.Epi_effect; run;
        proc delete data = work.Epi_effect_treat; run;

        /**************** Print Log file information again ************/

        Proc printto; run;

        /************************************************************/

%Mend Epistatic_Treatment_interaction;


/*************************************************************************

Start the macro by calling it using %(the given) macro:

Macro for Marker-trait associations/Epistasis use data corrected by population
structure and/or kinship:

%marker_main_effect;

%Epistatic_effect;

Macro for Marker-trait associations/Epistasis including treatment
(environment: fixed factor) interactions use data corrected by population
structure and/or kinship:

%marker_Treatment_interaction;

%Epistatic_Treatment_interaction;

Macro for Marker-trait associations/Epistasis including treatment calculates
the main effect and the treatment (environment) interaction effect as the same
time
and results are presented in the same table e. g. Main_ProbF and Treat_ProbF
or Epi_ProbF and Epi_Treat_ProbF.


*************************************************************************/




%Macro cross_validation;
            %let randomini=123345;
            %do cv=1 %to &No_CV_reps  ;
                  %let marker=marker_&j;

                  data Crossvalidation_dataset Crossvalidation_dataset_1;  set
g;
                        Keep SNP&j y genotype;
                  run;
                  Proc sort data=Crossvalidation_dataset_1; by genotype; run;
                  data Crossvalidation_dataset_1; set
Crossvalidation_dataset_1; by genotype;
```

```sas
                if first.genotype;
        run;
        Proc sort data=Crossvalidation_dataset_1; by SNP&j; run;
        data Crossvalidation_dataset_2; set
Crossvalidation_dataset_1; by SNP&j;
                if first.SNP&j;
        run;
        %let nobs=0; %nobs(Crossvalidation_dataset_2);

        %do i=1 %to &nobs  ;
                data Crossvalidation_dataset_3; set
Crossvalidation_dataset_2; If _N_ eq &i; run;
                proc sql noprint;
                        select SNP&j into :Cross_val_allele  from
Crossvalidation_dataset_3;
                quit; run;
                Data Crossvalidation_allele; set
Crossvalidation_dataset_1; if SNP&j eq &Cross_val_allele;
                run;
                %let nobs=0; %nobs(Crossvalidation_allele);
                %let randomini=%sysevalf(&randomini + 10);

                data Unif(keep= m);
                call streaminit(%sysevalf(&randomini));
                a = -1; b = 1;
                Min = 1; Max = 5;
                do i = 1 to &NObs;
                  u = rand("Uniform");    /* U[0,1] */
                  m = min + floor((1+Max-Min)*u); /* uniform integer
in Min..Max */
                  output;
                end;
                run;
                Data Crossvalidation_allele; merge
Crossvalidation_allele unif; if m ne 1; drop m; Select=1; run;
                %if &i eq 1 %then %do;
                        Data CV_data; set Crossvalidation_allele; run;
                %end;
                %else %do;
                        Proc append Base=CV_data
data=Crossvalidation_allele force ; run ;
                %end;
        %end;
        Proc sort data=Crossvalidation_dataset; by genotype; run;
        Proc sort data=CV_data; by genotype; run;

        Data g_CV; merge Crossvalidation_dataset CV_data ; by
genotype; if select eq 1; drop select; run;
        proc delete data = work.Crossvalidation_dataset; run;
        proc delete data = work.Crossvalidation_dataset_1; run;
        proc delete data = work.Crossvalidation_dataset_2; run;
        proc delete data = work.Crossvalidation_dataset_3; run;
        proc delete data = work.Crossvalidation_allele; run;
        proc delete data = work.Crossvalidation_allele_1; run;
        proc delete data = work.Unif; run;
        proc delete data = work.Cv_data; run;
```

```sas
                    /************** Model; change for your experimental design
***/


                    proc mixed data=G_cv;
                         class genotype SNP&j;
                         model y= SNP&j / htype=1 solution;
                         ods output   tests1=SNP_test_Anova;
                    run;



      /***************************************************************/


                    Data SNP_test_Anova; set SNP_test_Anova;
                         informat    effect $32.  Marker $25. probf 30.28;
                         format          effect $25.  Marker $25. probf 30.28;
Marker="&Marker";
                    run;
                    %if &cv eq 1 %then %do;
                         Data Marker_Main_effect_CV; set SNP_test_Anova;  run;
                    %end;
                    %else %do;
                         Proc append Base=Marker_Main_effect_CV
data=SNP_test_Anova force;    run ;
                    %end;
                    proc delete data = work.SNP_test_Anova; run;
            %end;
            proc MEANS NOPRINT mean std
                    data=Marker_Main_effect_CV;
                    class Marker;
                    var Fvalue probF ;
                    output out=CVprobf      mean= ;
                    output out=cvprobfstd   stddev=;
            run;
            data cvprobF; set cvprobF;  if _type_ eq 1; CVfvalue=fvalue;
CVprobf=probf; format Marker $25. CVprobf 30.28;  keep Marker CVfvalue CVprobf
; run;
            data cvprobfstd; set cvprobfstd; CVstdfvalue=fvalue;
CVstdprobf=probf;  if _type_ eq 1; format Marker $25. CVstdprobf 30.28; keep
Marker CVstdfvalue CVstdprobf; run;
            data cvprobF; merge cvprobf cvprobfstd; run;
            proc delete data = work.cvprobfstd; run;

            %if &j eq 1 %then %do;
                    Data Marker_Main_cvprobF; set cvprobF;  run;
            %end;
            %else %do;
                    Proc append Base=Marker_Main_cvprobF data=cvprobF force;
     run ;
            %end;
            proc delete data = work.cvprobF; run;

%Mend cross_validation;


%macro marker_main_and_Cross_Val;
```

```sas
        proc delete data = work.Marker_Main_effect; run;

    %do j=1 %to   &No_SNP  ;

            %let marker=marker_&j;

            /*********** Hide log information ************************/
            %if &j gt 3  %then %do;
                        proc printto log=outlog;              run;
                        proc options;                              run;
                        proc printto print = outdoc;  run;
                        ODS Results OFF;
                %end;
            /*************************************************************/

            /************** Model; change for your experimental design ***/


            proc mixed data=G;
                    class genotype SNP&j;
                    model y= SNP&j / htype=1 solution;
                    ods output   tests1=SNP_test_Anova;
            run;


            /*************************************************************/


            Data SNP_test_Anova; set SNP_test_Anova;
                    informat effect $32  Marker $25.  probf 30.28;
                    format   effect $32  Marker $25.  probf 30.28;
Marker="&Marker";
            run;

            proc sql noprint;
                    select ProbF into :Cross_val_ProbF  from SNP_test_Anova;
            quit; run;

            %if &j eq 1 %then %do;
                    Data Marker_Main_effect; set SNP_test_Anova;  run;
            %end;
            %else %do;
                    Proc append Base=Marker_Main_effect data=SNP_test_Anova
force;      run ;
            %end;

            %if &Cross_val_ProbF le &CV_Prob %then %do;
                    %cross_validation;
            %end;

    %end;
    %let nobs=0; %nobs(Marker_Main_cvprobF);
    %if &nobs ge 1 %then %do;
            Proc sort data=Marker_Main_effect; by Marker; run;
            Proc sort data=Marker_Main_cvprobF; by Marker; run;
            data Marker_Main_effect; merge Marker_Main_effect
Marker_Main_cvprobF; by Marker; run;
            proc delete data = work.Marker_Main_cvprobF; run;
```

```
        %end;

        Proc sort data=Marker_Main_effect; by ProbF; run;
        proc delete data = work.SNP_test_Anova; run;
        proc delete data = work.Marker_Main_effect_cv; run;
        proc delete data = work.G_cv; run;

        /*********** Calculation of FDR probability ******************/

        proc multtest inpvalues(ProbF)=Marker_Main_effect OUT=Marker_Main_effect
FDR; run;

        /**********************************************************/

        /**************** Print Log file information again ************/

        Proc printto; run;

        /**********************************************************/

%Mend marker_main_and_Cross_Val;

/*  Start the macro by calling it using %(the given) macro:

Example for cross_validation in connection with Marker-trait associations
data:

Define probability of applying the Cross_Validation and number of runs:

%let CV_Prob=0.05; %let No_CV_reps=20;

%marker_main_and_Cross_Val;

*/
```