**Coding lines for the proposed bioinformatics analysis steps.**

#Find and instal the package

*chooseCRANmirror()*

*install.packages("BiocManager")*

*source("*[*https://bioconductor.org/biocLite.R*](https://bioconductor.org/biocLite.R)*")*

*BiocManager::install("ChAMP")*

*abiocLite("ChAMP")*

*library("ChAMP")*

#choose the sample sheet directory

*testDir <- "C:/Users/~"*

#This line code upload the raw data and apply filters. 450k parameter can be changed by EPIC for the newest version of the beadchip.

*myLoad <- champ.load(testDir,arraytype="450k")*

*myLoad$pd*

*champ.QC()*

#Performs imputation using the KNN method as default parameter

*myImpute<-champ.impute()*

*library("doParallel")*

*Ncores <- detectCores() - 1*

#Performs BMIQ normalization as a default parameter

*myNorm <- champ.norm()*

*write.csv(myNorm, file = "C:/Users/~/normalized\_data.csv")*

#Batch effects evaluation by Singular Value Decomposition Analysis

*champ.SVD()*

#Estimation of cell type

*myRefBase <- champ.refbase(beta = myNorm, arraytype = "450k")*

*head (myRefBase)*

*write.csv(myRefBase$CorrectedBeta, file = "C:/Users/~/corrected\_and\_normalized\_data.csv")*

*write.csv(myRefBase$CellFraction, file = "C:/Users/~/cell\_fractions.csv")*

*myNorm <- myRefBase*

#Differential Methylation Analysis.

*DMP<- champ.DMP(beta = myRefBase$CorrectedBeta,*

 *pheno = myLoad$pd$Sample\_Group,*

 *compare.group = c("II","III"),*

 *adjPVal = 1,*

 *adjust.method = NULL,*

 *arraytype = "450k")*

*DMP.GUI()*

*write.csv(DMP, file = "C:/Users/~/DMPs.csv")*

\*To obtain trait related CpGs change the parameter Sample\_Group for the desired column from the metadata provided in the sample sheet.