

User Manual: PAMAM

PAMAM is an online tool to calculate sample size and power for admixture mapping approaches to detect the disease susceptibility loci in the recently admixed populations. PAMAM is featured with power and sample size analysis for two-way and three-way admixed population and for both qualitative (dichotomous) and quantitative phenotype with different model assumptions.

In this manual, we provide description of terminologies used, a step-by-step guide to run PAMAM for the desired sample size or power analysis using examples and screenshot images, and important formulas used in the analysis. It is divided into four sections - 1) Terminology, 2) Interface Overview, 3) Step-by-step guideline, and 4) Downloading results.

1) Terminology

Admixture Level	
Two-way	Admixed population is an admixture of two ancestral population
Three-way	Admixed population is an admixture of three ancestral population
Model Building	
Phenotype	Disease or trait of interest - Two possible types: Dichotomous = Disease or trait which is categorized as presence or absent Quantitative = Disease or trait with measurable attribute and varies along a continuum
Risk Factor	Disease risk factor – Three possible values: AOR (Ancestry Odds Ratio) – positive numeric value GRR (Genotype Risk Ratio) – positive numeric value PRR (Parental Risk Ratio) – positive numeric value
Admixture Process	Process describing the flow of genetic materials in admixed population – Two possible types – Hybrid Isolation (HI) or Continuous Gene Flow (CGF)
Study Design	Type of Study – Two possible types: Case = Study using case subjects only CC (Case-control) = Study using both case and control subjects

Inheritance Mode	Mode of disease inheritance – Four possible modes: Mul = Multiplicative Add = Additive Rec = Recessive Dom = Dominant
Effect Statistics	Measurement of the effect of ancestry on quantitative phenotype – For two-way admixture: Slope = Coefficient of ancestry variable in the linear regression model for quantitative trait, a real number Cor. Coeff = Correlation coefficient between the quantitative phenotype and ancestry – a numeric value between -1 and 1 For three-way admixture: No selection is needed as multiple R^2 is the only effect statistics used

Parameters	
Admixture Prop.	Population-wise average proportions of genome of the ancestral populations– comma separated numeric value in the interval (0,1)
AOR	Ancestry Odds Ratio – positive numeric value
GRR	Genotype Risk Ratio – positive numeric value
PRR	Parental Risk Ratio – positive numeric value
Risk Allele Freq	Frequencies of risk allele in the ancestral populations, comma separated numerical values between 0 and 1.
Generation	Number of generations since admixture – Integer value ≥ 1
Recombination	Recombination rate - numeric value in the interval [0, 0.5]
Ancestry SD	Standard deviation of ancestry variable - a positive real number
Error SD	Standard deviation of residuals - a positive real number
Inflation	Inflation factor defined as the multiple R^2 between the ancestry variable and covariates in the linear regression model – numeric value in the interval [0,1)

Corr. Coef.	Correlation coefficient between the quantitative phenotype and ancestry – a numeric value between -1 and 1
Multiple R²	Multiple R ² from the multiple regression model under null and alternate hypothesis - an ordered pair of numeric values between 0 and 1 (null R2, alt R2); alt R2 ≥ null R2.

Statistical Analysis	
Analysis Type	Type of desired analysis: Select 'Power' or 'Sample Size' analysis
Type I Error	Level of significance (α) in the hypothesis testing: usually a positive number ≤ 0.05 . We recommend using α with appropriate multiple testing adjustment.
Side	Index variable whether a hypothesis test is one-sided or two sided test – One-side is default
Sample Size	Number of subjects: For a case-only study, Sample size = # of cases For a case-control study, Sample size = # of cases = # of control For a quantitative trait, Sample size = # of subjects
Power	Desired power of the test: A positive value ≤ 1

2) Interface overview

The PAMAM homepage layout shows three sections - **Admixture Level**, **Model selection** and **Statistical Analysis**, three clickable buttons - **Submit**, **Reset**, and **Summarize**, and 4 examples link - **Example 1** and **Example 2** for **two-way admixture mapping**, **Example 3** and **Example 4** for **three-way admixture mapping**. At the beginning, users will always start PAMAM by selecting the Admixture Level followed by 'Phenotype Category' under **Model Selection** and complete the process as described in the section 3 ([Step-by-step guideline](#)). **Submit** allow users to submit the input information and run the analysis, while **Reset** will clear all the input information. **Summarize** will produce a summary table of all the input information provided along with the result. **Note that users must Submit the input information to run the analysis.**



Home

Manual

FAQ

Contact

Login/Signup



PAMAM - Power Analysis in Multi-ancestry Admixture Mapping

Admixture Level

Two-way

Three-way

Model Selection

Phenotype Category

Risk Factor

Admixture Process

Study Design

Inheritance Mode

Statistical Analysis

Type I Error

Side

Analysis Type

Submit

Reset

Summarize

Examples

- Two-way examples ([Example 1](#)) ([Example 2](#))
- Three-way examples ([Example 3](#)) ([Example 4](#))

Geography of Users



Example links run the sample analyses.

- Example 1: Two-way discrete trait
- Example 2: Two-way quantitative trait
- Example 3: Three-way discrete trait
- Example 4: Three-way quantitative trait

re, 2018

Users can click one of the link of the examples to run the sample example. By clicking on the **Example 1** link, information will be populated in the required fields for the dichotomous (discrete) trait with genotype risk ratio as the risk factor. Similarly, **Example 2** link will show an example for quantitative trait. Then, clicking the **Submit** bottom will run the analysis and generate the results and graphs. By clicking on the **Summarize** bottom will generate table of the input information which can be downloaded as a csv or an excel file.

Admixture Level Two-way Three-way

Model Selection

Phenotype Category:

Risk Factor:

Admixture Process:

Study Design:

Inheritance Mode:

Parameters

GRR: Admix Proportion: Risk Allele Frequencies:

Statistical Analysis

Type I Error:

Side:

Analysis Type:

Sample Size(Cases):

Sample Size(Controls):

Examples

- Two-way examples ([Example 1](#)) ([Example 2](#))
- Three-way examples ([Example 3](#)) ([Example 4](#))

Geography of Users

© Cincinnati Children's Hospital Medical Centre, 2018

3) Step-by-step guideline

Scenario 1: Power and sample size calculation for dichotomous (discrete) phenotype in two-way admixed population

Scenario Example: An investigator want to estimate the power of a two-way admixture mapping case-control study with $n_1 = 500$ cases and $n_2 = 750$ controls at the significance level of $\alpha = 2.5 \times 10^{-5}$ (type I error rate) to detect true risk variants with genotype risk ratio (GRR) = 2.5. The samples are from a recently admixed population of two ancestries X and Y respectively with the admixture proportion of population X is 60%. For a risk variant under investigation, the risk allele frequencies in the ancestral population X and Y are $p_x = 0.05$ and $p_y = 0.40$ respectively.

In the following, we describe the step-wise process to calculate required power. Step 1 is the selection of admixture level. Step 2 through Step 6 constitute the 'Model Building Step' of PAMAM. Required inputs will be provided in the Step 6. Step 7 and Step 8 show the selection of statistical analysis.

- ❖ Step 1 - Admixture Level
 - Select the admixture level: In the PAMAM input window, select the admixture level as 'Two-way'.
- ❖ Step 2 - Model selection
 - Select the phenotype category: In the PAMAM input window, select the phenotype category as 'Dichotomous'.

- Select the risk ratio: The investigator has the information of the genotype risk ratio. In the PAMAM input window under 'Dichotomous' phenotype, select 'Risk Ratio' as 'GRR'.
- Select the admixture process: With the genotype risk ratio, the default and the only available admixture process is the 'Hybrid Isolation (HI)'. [For GRR, it is the default and only available option].
- Select the study design: The investigator wants to conduct a case-control study, so he/she will select 'Case-Control' study design.
- Select mode of inheritance: With the genotype risk ratio, the analysis will be performed under the multiplicative mode of inheritance and will be selected by default.

❖ Step 3 - Parameters Selection

- Entering Parameters: Once Step 1 through Step 4 are completed, new 'Parameter' panel will appear. In this panel, the investigator will enter the following information in the boxes sequentially -- GRR = 2.5, Admix. Proportion = 0.6, Risk Allele Frequencies = 0.05, 0.40.

❖ Step 4 - Statistical Analysis

- Entering the analysis specific information: The investigator will provide the following analysis specific information in the appropriate boxes - Type I error, side of the test used (say one-sided test).
 - Type I error = 2.5×10^{-5} [Note: This is equivalent of Bonferroni adjustment (with $\alpha = 0.05$) when 2000 independent tests are performed. For the admixture mapping the adjustment for multiple testing correction is very liberal than the association test]
 - # of cases = 500
 - # of controls = 750
 - Side = 'One-sided' [Note: Side = 'Two-sided' if two-sided test is chosen]
- Selecting the analysis type: In the 'Statistical Analysis' panel, the type of analysis wanted will be selected. The investigator wants the power calculation, he/she will choose 'Power' option.

❖ Step 5 - Submit

- Once the Step 1 through Step 4 are performed, the investigator will submit the data using the **Submit** bottom. The output from the analysis will appear subsequently.

The screenshot shows the PAMAM web application interface. At the top, there is a navigation bar with links for Home, Manual, FAQ, Contact, and Login/Signup. The main title is "PAMAM - Power Analysis in Multi-ancestry Admixture Mapping".

The interface is divided into several sections:

- Admixture Level:** Radio buttons for "Two-way" (selected) and "Three-way".
- Model Selection:** Dropdown menus for Phenotype Category (Dichotomous), Risk Factor (Genotype risk ratio), Admixture Process (Hybrid Isolation (HI)), Study Design (Case-control), and Inheritance Mode (Multiplicative).
- Statistical Analysis:** Input fields for Type I Error (0.000025), Side (One-sided), Analysis Type (Power), Sample Size(Cases) (500), and Sample Size(Controls) (750).
- Parameters:** Input fields for GRR (2.5), Admix Proportion (0.6), and Risk Allele Frequencies (0.05,0.4).
- Buttons:** Submit, Reset, and Summarize.
- Examples:** Links for Two-way examples (Example 1, Example 2) and Three-way examples (Example 3, Example 4).
- Results:** A section for "Results (Two-way)".

Red annotations indicate the following steps:

- Step 1:** Points to the "Two-way" radio button.
- Step 2:** Points to the dropdown menus in the "Model Selection" section.
- Step 3:** Points to the "Parameters" input fields.
- Step 4:** Points to the "Statistical Analysis" input fields.
- Step 5:** Points to the "Submit" button.

© Cincinnati Children's Hospital Medical Centre, 2018

- ❖ Output – Once the information submitted, the software will perform the power analysis (specified in Step 4) for the case-control study design. The results show the power = 0.799 of the study. Two graphs, one for cases vs power with fixed control (= 750) and one for the control vs. power with fixed cases (= 500) are drawn. The graph can be downloaded by clicking on the graph.

Results (Two-way)



© Cincinnati Children's Hospital Medical Centre, 2018

Scenario 2: Power and sample size calculation for quantitative (continuous) phenotype

Scenario Example: An investigator want to estimate sample size required for 80% power under the significance level of $\alpha = 2.5 \times 10^{-5}$ (type I error rate) to detect the all ancestral variants on a quantitative phenotype in an admixed population. Assume the variant explains approximately 1% of the variance of the phenotype.

- ❖ Step 1 - Admixture Level
 - In the PAMAM input window, select the admixture level as 'Two-way'.
- ❖ Step 2 - Model Selection:
 - Select the phenotype category 'Quantitative'
 - Select 'Effect Statistics' as 'Correlation'. The investigator has the information on the percentage of the variance explained (r^2) = 1%, which is equivalent of the squared correlation in simple regression analysis. For a simple linear model with no covariates, correlation is the square root of proportion of the variance explained.
- ❖ Step 3 - Parameters:
 - With the selection of 'Effect Statistics' as 'Correlation' in Step 2, a new 'Parameter' panel will appear. In this panel, the investigator will enter the following information- 'Correlation

Coeff.'. Since $r^2 = 0.01$ (as 1% of the variance is explained) $\rightarrow r = 0.1$ (positive square root of the r^2), the investigator will input Correlation Coeff. = 0.1

❖ Step 4 - Selecting the analysis:

- Entering the analysis specific information. The investigator will the type I error and side of the test used (say one-sided test).
- In the 'Statistical Analysis' panel, the type of analysis wanted will be selected. The investigator wants the sample size calculation, he/she will choose 'Sample Size' option.
- Once the investigator selects 'Sample Size' option, the investigator will be asked to provide the desired power to determine the sample size.

Type I error = 2.5×10^{-5} [Note: This is equivalent of Bonferroni adjustment (with $\alpha = 0.05$) when 2000 independent tests are performed. For the admixture mapping the adjustment for multiple testing correction is very liberal than the association test]

Power = 80 [80% power is desired]

Side = 'Two-sided' (for two-sided test) [Note: Side = 'One-sided' if one-sided test is chosen]

❖ Step 5 - Submit

- Once the Step 1 through Step 4 are performed, the investigator will submit the data using the **Submit** bottom. The output from the analysis will appear subsequently.

The screenshot shows the PAMAM web application interface. At the top, there is a navigation bar with links for Home, Manual, FAQ, Contact, and Login/Signup. The main title is "PAMAM - Power Analysis in Multi-ancestry Admixture Mapping".

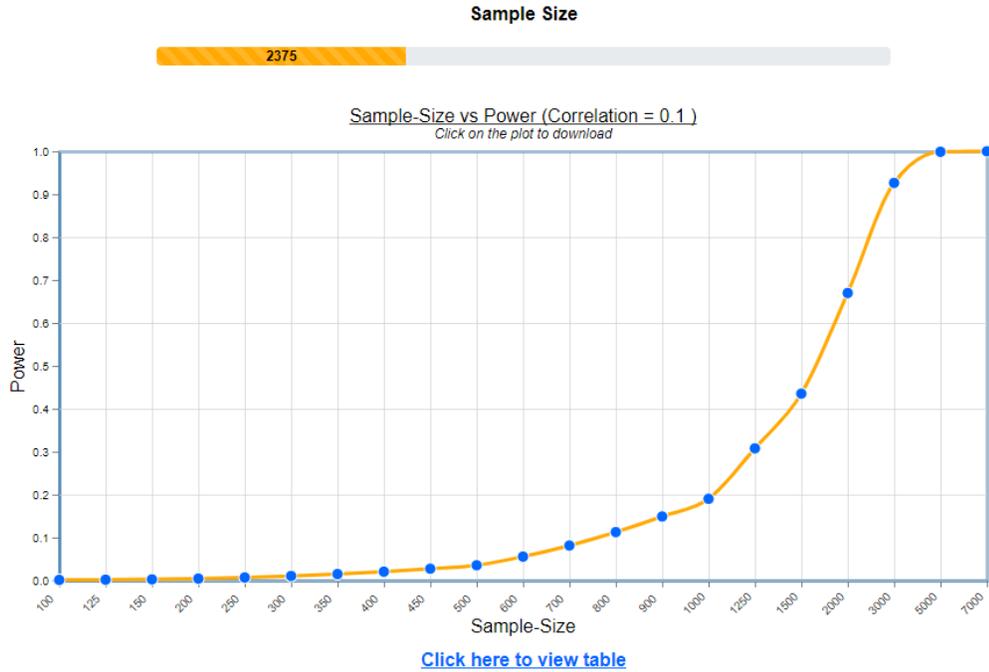
The interface is divided into several sections:

- Admixture Level:** Radio buttons for "Two-way" (selected) and "Three-way". A red "Step 1" annotation is placed over the "Two-way" button.
- Model Selection:** Dropdown menus for "Phenotype Category" (set to "Quantitative") and "Effect Statistics" (set to "Correlation"). A red "Step 2" annotation is placed over the "Effect Statistics" dropdown.
- Parameter:** A text input field for "Correlation Coeff." set to "0.1". A red "Step 3" annotation is placed over the input field.
- Statistical Analysis:** Input fields for "Type I Error" (0.000025), "Side" (set to "One-sided"), "Analysis Type" (set to "Sample Size"), and "Power(%)" (set to 80). A red "Step 4" annotation is placed over the "Side" and "Analysis Type" dropdowns.
- Buttons:** "Submit" (with a red "Step 5" annotation), "Reset", and "Summarize".
- Examples:** Links for "Two-way examples" (Example 1, Example 2) and "Three-way examples" (Example 3, Example 4).
- Geography of Users:** A world map icon.

- ❖ Output – Once the information are submitted, the software will calculate the sample size (specified in Step 4) for the quantitative study. The results will show the required sample size = 2375. The

associate graph shows the sample size vs power for different sample sizes. The graph can be downloaded by clicking on the graph.

Results (Two-way)



4) Downloading Results

Downloading summary of input information - Once the input information are submitted for analysis, the summary information can be generated using **Summarize** bottom.

Download Options

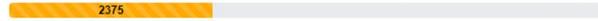
Summary of Example1

Option	Value
Admixture Process	Hybrid Isolation(HI)
Admixture Proportion	0.8
Allele Freq(X)	0.05
Allele Freq(Y)	0.4
Analysis Type	Power
GRR	2.5
Inheritance Mode	Multiplicative
Phenotype Category	Dichotomous
Power	0.932
Risk Factor	Genotype Risk Ratio(GRR)

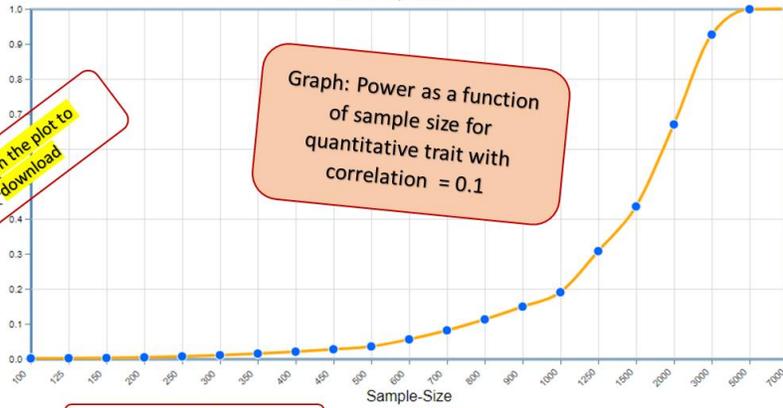
Summary

Results (Two-way)

Sample Size



Sample-Size vs Power (Correlation = 0.1)
Click on the plot to download



Click on the plot to download

Graph: Power as a function of sample size for quantitative trait with correlation = 0.1

Download the table [Click here to view table](#)