

# Package ‘StageWise’

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**Title** Two-stage analysis of multi-environment trials for genomic selection and GWAS

**Version** 1.13

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**Description** Fully efficient, two stage analysis of multi-environment trials, including directional dominance and multi-trait genomic selection

**Depends** R (>= 4.0)

**License** GPL-3

**RoxygenNote** 7.3.2

**Encoding** UTF-8

**Imports** Matrix (>= 1.5-0), ggplot2, methods, ggrepel, rlang, ggpubr, SpATS, spam, AGHmatrix, MASS, CVXR, ggforce, data.table

**Suggests** knitr, rmarkdown, asreml

**Collate** 'Stage1.R'  
'Stage2.R'  
'blup.R'  
'blup\_prep.R'  
'class\_geno.R'  
'class\_genoD.R'  
'class\_prep.R'  
'class\_var.R'  
'corr.R'  
'dominance.R'  
'gain.R'  
'gwas\_threshold.R'  
'inbreeding.R'  
'manhattan\_plot.R'  
'predict.geno.R'  
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'quantile.geno.R'  
'read\_geno.R'  
'remove\_spatialtrend.R'  
'summary.var.R'  
'uniplot.R'  
'wheat-data.R'

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blup	<i>BLUP</i>
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Description

BLUP

Usage

```
blup(data, geno = NULL, what, index.coeff = NULL, gwas.ncore = 0L)
```

Arguments

data	one object, or list of objects, of <a href="#">class_prep</a> from <a href="#">blup_prep</a>
geno	object of <a href="#">class_geno</a> from <a href="#">read_geno</a>
what	One of the following: AV, BV, GV, AM, DM. See Details.
index.coeff	named vector of index coefficients for the locations or traits
gwas.ncore	Integer indicating number of cores to use for GWAS (default is 0 for no GWAS).

## Details

The argument `what` takes 5 possible values: "AV" (additive value), "BV" (breeding value), "GV" (genotypic value), "AM" (additive marker effect), and "DM" (dominance marker effect). "Values" refer to predictions for individuals, as opposed to markers. Predicted values include the average fixed effect of the environments, whereas predicted marker effects do not. Argument `index.coeff` is a named vector (matching the names of the locations or traits), and the values are interpreted for standardized traits.

When multiple objects of `class_prep` are used for data, they must be based on the same marker data and genetic model. Also, reliabilities are not computed.

## Value

Data frame of BLUPs

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blup_prep	<i>Prepare data for BLUP</i>
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## Description

Prepare data for BLUP

## Usage

```
blup_prep(data, vcov = NULL, geno = NULL, vars, mask = NULL, method = NULL)
```

## Arguments

<code>data</code>	data frame of BLUEs from Stage 1
<code>vcov</code>	list of variance-covariance matrices for the BLUEs
<code>geno</code>	object of <code>class_geno</code> from <code>read_geno</code>
<code>vars</code>	object of <code>class_var</code> from <code>Stage2</code>
<code>mask</code>	(optional) data frame with possible columns "id", "env", "loc", "trait"
<code>method</code>	(optional) "MME", "Vinv", NULL (default). see Details

## Details

The `method` argument can be used to control how the linear system is solved. "MME" leads to inversion of the MME coefficient matrix, while "Vinv" leads to inversion of the overall var-cov matrix for the response vector. If NULL, the software uses whichever method involves inverting the smaller matrix. If the number of random effects ( $m$ ) is less than the number of BLUEs ( $n$ ), "MME" is used.

For the multi-location model, if all of the environments for a location are masked, the average of the other locations is used when computing average fixed effects.

## Value

Object of `class_prep`

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class_geno-class	<i>S4 class for marker genotype data</i>
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### Description

S4 class for marker genotype data

### Slots

ploidy If mixed ploidy, then a vector equal to pop size; otherwise a single integer  
 map Marker map positions  
 coeff Coefficients of the marker effects (dim: indiv x marker)  
 scale Scaling factor between markers and indiv, vector of length equal to pop size  
 G Additive relationship matrix (from markers and potentially also pedigree)  
 eigen.G list of eigenvalues and eigenvectors

---

class_genoD-class	<i>S4 class for marker genotype data with dominance</i>
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---

### Description

S4 class for marker genotype data with dominance

### Slots

ploidy ploidy  
 map Marker map positions  
 coeff Coefficients of the additive marker effects (dim: indiv x marker)  
 scale Scaling factor between markers and indiv for additive effects  
 G Additive relationship matrix (from markers and potentially also pedigree)  
 eigen.G list of eigenvalues and eigenvectors for G  
 coeff.D coefficients of the dominance marker effects (dim: indiv x marker)  
 scale.D Scaling factor between markers and indiv for dominance effects  
 D Dominance relationship matrix  
 eigen.D list of eigenvalues and eigenvectors for D  
 Fg genomic inbreeding coefficient (based on dominance)

---

class_prep-class	<i>S4 class to prepare for blup</i>
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---

**Description**

S4 class to prepare for blup

**Slots**

id genotype identifiers  
 ploidy ploidy  
 var.u variance of random effects  
 var.umat variance of BLUPs  
 avg.env average fixed effect of the environments  
 heterosis regression coefficients for inbreeding  
 fixed.marker fixed marker effects  
 B var-cov matrix for fixed effects  
 random random effect estimates  
 geno1.var first var-cov matrix from [class\\_var](#)  
 geno2.var second var-cov matrix from [class\\_var](#)  
 model model from [class\\_var](#)

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class_var-class	<i>S4 class for variances</i>
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---

**Description**

S4 class for variances

**Slots**

geno1 first genetic effect  
 geno2 second genetic effect  
 model 0=no markers, 1=add, 2=add+g.resid, 3=add+dom  
 resid residual  
 vars variances for reporting  
 B var-cov matrix of fixed effects for gain  
 fix.eff.marker names of fixed effect markers

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corr	<i>Trait correlations</i>
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---

### Description

Trait correlations

### Usage

```
corr(vars, traits = NULL, effect = NULL)
```

### Arguments

vars	object of <code>class_var</code> from <code>Stage2</code>
traits	pair of traits
effect	name of effect

### Details

Use either the argument `traits` or `effect`, not both. Using `traits` leads to a partitioning of the total correlation between those two traits, based on path analysis, assuming no correlation between the effects of the Stage 2 model. Using `effect` displays the correlation between all traits for that effect. Use the `summary` command to see the names of the possible effects.

### Value

matrix

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dominance	<i>Report dominance parameters</i>
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### Description

Report dominance parameters

### Usage

```
dominance(params, index.coeff = NULL, gamma = 0)
```

### Arguments

params	list returned by <code>Stage2</code>
index.coeff	merit index coefficients
gamma	contribution of non-additive values for genetic merit

## Details

The dominance variance ( $V_d$ ) and baseline heterosis ( $b$ ) are quantified relative to additive variance ( $V_a$ ) and std. dev. ( $SD_a$ ), respectively. As of v1.11, the variances are scaled to the population (previously, it was just the variance components). For a multi-trait/loc model, `index.coeff` specifies the coefficients of the standardized true values (see also [blup](#)), with `gamma` indicating the relative weight of non-additive to additive genetic merit for the standardization (see also [gain](#)).

## Value

data frame with estimates

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gain	<i>Genetic gain</i>
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## Description

Genetic gain calculations

## Usage

```
gain(
  input,
  merit = NULL,
  desired = NULL,
  restricted = NULL,
  traits = NULL,
  gamma = NULL,
  solver = "ECOS",
  ...
)
```

## Arguments

<code>input</code>	either object of <a href="#">class_prep</a> or <code>quad.mat</code> returned by this function
<code>merit</code>	named vector of merit coefficients, in genetic standard deviation units
<code>desired</code>	named vector of desired gains, in genetic standard deviation units
<code>restricted</code>	data frame of restricted traits, see Details
<code>traits</code>	optional vector with exactly 2 trait names, to plot elliptical response
<code>gamma</code>	contribution of non-additive values for genetic merit
<code>solver</code>	name of convex solver (default is "ECOS")

## Details

Either `merit` or `desired` can be used, not both. The former specifies the relative contribution of each trait to genetic merit, while the latter specifies the relative desired gain in genetic standard deviation units. All traits must be specified. Optional argument `restricted` is a data frame with columns "trait" and "sign", where the options for sign are "=", ">", "<", representing equal to zero, non-negative, and non-positive. When `desired` is used, the `restricted` argument is ignored.

The argument `gamma` controls the definition of genetic merit. (See notation in the journal publication.) The default is `NULL`, which implies breeding values. For purely additive values, use `gamma = 0`. For total genotypic value, use `gamma = 1`.

Note that this function assumes a selection index of BLUPs, not phenotypes.

### Value

List containing

**quad.mat** quadratic matrix for the ellipsoid

**plot** ellipse plot

**table** data frame with the response and index coefficients for all traits

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<code>gwas_threshold</code>	<i>Compute GWAS discovery threshold</i>
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### Description

Compute GWAS discovery threshold

### Usage

```
gwas_threshold(geno, alpha = 0.05, exclude.chrom = NULL, n.core = 1)
```

### Arguments

<code>geno</code>	object of <code>class_geno</code>
<code>alpha</code>	genome-wide significance level
<code>exclude.chrom</code>	chromosomes to exclude
<code>n.core</code>	number of cores to use

### Details

Uses a Bonferroni-type correction based on an effective number of markers that accounts for LD (Moskvina and Schmidt, 2008).

### Value

$-\log_{10}(p)$  threshold

### References

Moskvina V, Schmidt KM (2008) On multiple-testing correction in genome-wide association studies. *Genetic Epidemiology* 32:567-573. doi:10.1002/gepi.20331



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inbreeding	<i>Genomic inbreeding coefficient</i>
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**Description**

Genomic inbreeding coefficient

**Usage**

```
inbreeding(geno)
```

**Arguments**

geno	object of <code>class_geno</code>
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**Details**

Under the additive model, the inbreeding coefficient comes from the diagonal elements of the G matrix according to  $F = (G-1)/(ploidy-1)$ . For dominance, the inbreeding coefficient is the scaled row-sum of the dominance coefficient matrix.

**Value**

data frame with F[G] and (when dominance is present) F[D]

---

manhattan_plot	<i>Create Manhattan plot</i>
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**Description**

Create Manhattan plot

**Usage**

```
manhattan_plot(data, chrom = NULL, thresh = NULL, rotate.label = FALSE)
```

**Arguments**

data	data frame with columns for marker, chrom, position, and score
chrom	optional, to plot only one chromosome
thresh	optional, to include horizontal line at discovery threshold
rotate.label	TRUE/FALSE whether to rotate x-axis labels to be perpendicular

**Details**

Assumes position in bp

**Value**

ggplot2 object

---

predict	<i>Predict individual values from marker effects</i>
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**Description**

Predict individual values from marker effects

**Arguments**

object                    object of `class_geno`  
marker.effects   data frame with columns "marker" and "effect"

**Details**

Use the `blup` function with `what="AM"` or `"DM"` to generate the data frame for `marker.effects`.

**Value**

data frame with columns "id" and "value"

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quantile	<i>G matrix quantile</i>
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---

**Description**

G matrix quantile

**Arguments**

x                        object of `class_geno`  
prob                    probability

**Details**

Unlike the S3 method, `prob` must have `length = 1`

**Value**

data frame with the quantile of the G matrix coefficients for each id

---

read_genotype	<i>Read marker genotype data</i>
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---

## Description

Read marker genotype data

## Usage

```
read_genotype(
  filename,
  ploidy,
  map,
  min.minor.allele = 5,
  w = 1e-05,
  ped = NULL,
  dominance = FALSE,
  pop.file = NULL
)
```

## Arguments

filename	Name of CSV file with marker allele dosage
ploidy	2,4,6,etc. (even numbers)
map	TRUE/FALSE
min.minor.allele	threshold for marker filtering (see Details)
w	blending parameter (see Details)
ped	optional, pedigree data frame with 3 or 4 columns (see Details)
dominance	TRUE/FALSE whether to include dominance covariance (see Details)
pop.file	CSV file defining populations

## Details

When map=TRUE, first three columns of the file are marker, chrom, position. When map=FALSE, the first column is marker. Subsequent columns contain the allele dosage for individuals/clones, coded 0,1,2,...ploidy (fractional values are allowed). The input file for diploids can also be coded using -1,0,1 (fractional values allowed). Additive coefficients are computed by subtracting the population mean from each marker, and the additive (genomic) relationship matrix is computed as  $G = \text{tcrossprod}(\text{coeff})/\text{scale}$ . The scale parameter ensures the mean of the diagonal elements of G equals 1 under panmictic equilibrium. Missing genotype data is replaced with the population mean.

G can be blended with the pedigree relationship matrix (A) by providing a pedigree data frame in ped and blending parameter w. The blended relationship matrix is  $H = (1-w)G + wA$ . The first three columns of ped are id, parent1, parent2. Missing parents must be coded NA. An optional fourth column in binary (0/1) format can be used to indicate which ungenotyped individuals should be included in the H matrix, but this option cannot be combined with dominance. If there is no fourth column, only genotyped individuals are included. If a vector of w values is provided, the function returns a list of [class\\_genotype](#) objects.

If the A matrix is not used, then G is blended with the identity matrix (times the mean diagonal of G) to improve numerical conditioning for matrix inversion. The default for w is 1e-5, which is somewhat arbitrary and based on tests with the vignette dataset. The D matrix is also blended with the identity matrix using 1e-5 for numerical conditioning.

When dominance=FALSE, non-additive effects are captured using a residual genetic effect, with zero covariance. If dominance=TRUE, a (digenic) dominance covariance matrix is used instead.

The argument min.minor.allele specifies the minimum number of individuals that must contain the minor allele. Markers that do not meet this threshold are discarded.

Optional argument pop.file gives the name of a CSV file with two columns: id,pop. If the populations have different ploidy, this is indicated using a named vector for ploidy.

### Value

Variable of class `class_geno`.

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remove_spatialtrend	<i>Remove spatial trend</i>
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---

### Description

Removes spatial trend to prepare for multi-trait Stage 1

### Usage

```
remove_spatialtrend(filename, traits, spline, effects = NULL)
```

### Arguments

filename	Name of CSV input file
traits	trait names
spline	vector of variable names for 2D spline with SpATS
effects	data frame specifying other effects in the model (see Details)

### Details

SpATS used to remove 2D spatial trend for each field experiment, labeled with column 'expt' in the input file. Genotype labels are in column 'id' and modeled as i.i.d random effect. Argument effects is used to specify other i.i.d. effects besides genotype and has three columns: name, fixed, factor. The "name" column is a string that must match a column in the input file. The fixed column is a logical variable to indicate whether the effect is fixed (TRUE) or random (FALSE). The factor column is a logical variable to indicate whether the effect is a factor (TRUE) or numeric (FALSE).

Argument traits is a character vector of trait names. Single-trait analyses are performed for each trait, and the results are combined in the output.

### Value

Data frame with adjusted phenotypes

## Stage1

*Stage 1 analysis of multi-environment trials***Description**

Computes genotype BLUEs for each experiment

**Usage**

```
Stage1(
  filename,
  traits,
  effects = NULL,
  solver = "asreml",
  spline = NULL,
  silent = TRUE,
  workspace = c("500mb", "500mb"),
  max.iter = 30
)
```

**Arguments**

filename	Name of CSV file
traits	trait names (see Details)
effects	data frame specifying other effects in the model (see Details)
solver	one of the following: "asreml", "spats"
spline	vector of variable names for 2D spline with SpATS
silent	TRUE/FALSE, whether to suppress REML output
workspace	memory limits for ASRreml-R
max.iter	maximum number of iterations for ASRreml-R

**Details**

The input file must have one column labeled "id" for the individuals and one labeled "env" for the environments. The data for each environment are analyzed independently with a linear mixed model. Although not used in Stage1, to include a genotype x location effect in [Stage2](#), a column labeled "loc" should be present in the input file.

Argument effects is used to specify other i.i.d. effects besides genotype and has three columns: name, fixed, factor. The "name" column is a string that must match a column in the input file. The fixed column is a logical variable to indicate whether the effect is fixed (TRUE) or random (FALSE). The factor column is a logical variable to indicate whether the effect is a factor (TRUE) or numeric (FALSE).

Argument solver specifies which software to use for REML. Current options are "asreml" and "spats". For "spats", the argument spline must be a vector of length two, with the names of the x and y variables (respectively) for the 2D spline.

The heritability and residuals in the output are based on a random effects model for id. When asreml is used, the plot-based h2 is reported. When spats is used, it is entry-mean h2.

Missing response values are omitted for single-trait analysis but retained for multi-trait analysis (unless both traits are missing), to allow for prediction in Stage 2.

Argument `workspace` is a vector of length two containing the workspace and `pworkspace` limits for ASReml-R, with default values of 500mb. If you get an error about insufficient memory, try increasing the appropriate value (workspace for variance estimation and `pworkspace` for BLUE computation).

For multiple traits, only "asreml" is supported, and only the BLUE model is run, so the returned object does not contain H2.

If the input file has a column "expt", this allows for the use of separate spatial models for multiple experiments within an environment (only for single trait): each experiment is first analyzed separately, and then the BLUEs from all experiments per env are jointly analyzed to compute a single BLUE per env. The estimation errors from each experiment are propagated into the multi-expt model using ASReml-R. The situation is different with multi-trait analysis, as all experiments are analyzed jointly per env, with a fixed effect for expt but a common residual model. Any additional cofactors (e.g., block) that are nested within expt need to be explicitly nested!

### Value

List containing

**blues** data frame of BLUEs

**vcov** list of variance-covariance matrices for the BLUEs, one per env

**fit** data frame with broad-sense H2 and/or AIC

**resid** For single trait, list of diagnostic plots and data frame of residuals. For multi-trait, list of resid var-cov matrices.

---

Stage2

*Stage 2 analysis of multi-environment trials*

---

### Description

Stage 2 analysis of multi-environment trials

### Usage

```
Stage2(
  data,
  vcov = NULL,
  geno = NULL,
  fix.eff.marker = NULL,
  silent = TRUE,
  workspace = "500mb",
  non.add = "g.resid",
  max.iter = 20,
  covariates = NULL,
  pairwise = FALSE
)
```

## Arguments

<code>data</code>	data frame of BLUEs from Stage 1 (see Details)
<code>vcov</code>	named list of variance-covariance matrices for the BLUEs
<code>geno</code>	output from <a href="#">read_geno</a>
<code>fix.eff.marker</code>	markers in <code>geno</code> to include as additive fixed effect covariates
<code>silent</code>	TRUE/FALSE, whether to suppress ASReml-R output
<code>workspace</code>	Memory limit for ASReml-R variance estimation
<code>non.add</code>	one of the following: "none", "g.resid", "dom"
<code>max.iter</code>	maximum number of iterations for asreml
<code>covariates</code>	names of other covariates in data
<code>pairwise</code>	TRUE/FALSE should multi-trait analysis proceed pairwise

## Details

Stage 2 of the two-stage approach described by Damesa et al. 2017, using ASReml-R for variance component estimation. The variable `data` has three mandatory column: `id`, `env`, `BLUE`. Optionally, `data` can have a column labeled `"loc"`, which changes the main effect for genotype into a separable genotype-within-location effect, using a FA2 covariance model for the locations. Optionally, `data` can have a column labeled `"trait"`, which uses an unstructured covariance model. The multi-location and multi-trait analyses cannot be combined. Missing data are allowed in the multi-trait but not the single-trait analysis. The argument `geno` is used to partition genetic values into additive and non-additive components. Any individuals in `data` that are not present in `geno` are discarded.

The argument `vcov` is used to partition the macro- and micro-environmental variation, which are called GxE and residual in the output. `vcov` is a named list of variance-covariance matrices for the BLUEs within each environment, with `id` for rownames (single trait) or `id:trait`. The order in `vcov` and `data` should match. Both `data` and `vcov` can be created using the function [Stage1](#).

Because ASReml-R can only use relationship matrices defined in the global environment, this function creates and then removes global variables when either `vcov` or `geno` is used. By default, the workspace memory for ASReml-R is set at 500mb. If you get an error about insufficient memory, try increasing it. ASReml-R version 4.1.0.148 or later is required.

The `covariates` option is only available for single trait/loc analysis.

Argument `pairwise` was added in package version 1.04, which specifies that multi-trait analysis is performed as multiple bivariate analyses, which often converges better. The returned object is a list of the results from the bivariate analyses, as well as `"vars"` for all traits, which is needed for [blup\\_prep](#).

## Value

List containing

**aic** AIC

**vars** variance components for [blup\\_prep](#), as variable of class `class_var`

**params** Estimates and SE for fixed effects and variance components

**random** Random effect predictions

**loadings** scaled loadings for the FA2 multi-loc model

## References

Damesa et al. 2017. *Agronomy Journal* 109: 845-857. doi:10.2134/agronj2016.07.0395

---

summary.var	<i>Displays variances and correlations</i>
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---

### Description

Displays variances and correlations

### Arguments

object	object of <a href="#">class_var</a>
digits	number of digits for rounding
index.coeff	merit index coefficients
gamma	contribution of non-additive values for genetic merit
separate.loc	TRUE/FALSE controls output for multi-location

### Details

For a single trait, the 'var' output is a data frame with two columns of information for the various effects: the first is the variance and the second is the proportion of variance explained (PVE), excluding the environment effect. For multiple locations or traits, the 'cor' output is the correlation matrix for additive effects (does not include fixed effect markers).

For multiple traits, the variance and PVE results are returned as separate data frames, unless `index.coeff` is used to create an index. To create a similar output with multiple locations, use `separate.loc=TRUE`.

The `index.coeff` are the coefficients of the standardized true values (see also [blup](#)). The argument `gamma` is the relative weight for non-additive to additive genetic merit (see also [gain](#)).

### Value

List output that varies depending on the situation (see Details)

---

uniplot	<i>Uniplot for multi-location models</i>
---------	------------------------------------------

---

### Description

Displays scaled loadings of the FA2 model

### Usage

```
uniplot(loadings, nudge = 0.1)
```

### Arguments

loadings	scaled factor loadings, from <a href="#">Stage2</a> .
nudge	distance to nudge labels



**Details**

The squared radius for each point is the proportion of genetic variance explained by the latent factors. For points on the unit circle, the cosine of the subtended angle equals the correlation.

**Value**

ggplot2 object

---

wheat.data

*Genomic prediction from secondary traits in wheat*

---

**Description**

Canopy temperature (CT) measurements collected during grain fill; used for genomic prediction of grain yield (GY) in wheat. Data come from the drought and extreme drought environments of Rutkoski et al. (2016). The CT phenotype was dated 3/7/2014. Stage 1 BLUEs were computed using rep(trial) as a random effect.

**Usage**

data(wheat)

**Format**

wheat.geno is object of [class\\_geno](#). wheat.blues and wheat.vcov are output from [Stage1](#).

**References**

Rutkoski et al. (2016) G3 (Bethesda) 6:2799–2808. <https://doi.org/10.1534/g3.116.032888>

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