Package ‘Oscope’

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Type Package

Title Oscope - A statistical pipeline for identifying oscillatory genes in unsynchronized single cell RNA-seq

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Suggests BiocStyle

Description Oscope is a statistical pipeline developed to identifying and recovering the base cycle profiles of oscillating genes in an unsynchronized single cell RNA-seq experiment. The Oscope pipeline includes three modules: a sine model module to search for candidate oscillator pairs; a K-medoids clustering module to cluster candidate oscillators into groups; and an extended nearest insertion module to recover the base cycle order for each oscillator group.

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  'Opt2Shift.R' 'SineOptim.R' 'Pipe.R' 'ImpShift.R'
  'PipeShiftCDF.R' 'scanK.R' 'NISFun.R' 'CalcMV.R' 'OscopeKM.R'
  'OscopeENI.R' 'OscopeSine.R' 'FlagCluster.R' 'PermuCut.R'

BuildVignettes yes

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Description

Oscope is a statistical pipeline developed to identifying and recovering the base cycle profiles of oscillating genes in an unsynchronized single cell RNA-seq experiment. The Oscope pipeline includes three modules: a sine model module to search for candidate oscillator pairs; a K-medoids clustering module to cluster candidate oscillators into groups; and an extended nearest insertion module to recover the base cycle order for each oscillator group.

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Author(s)

Ning Leng

Maintainer: Ning Leng <lengning1@gmail.com>

References

Leng et al. Oscope - A statistical pipeline for identifying oscillatory genes in unsynchronized single cell RNA-seq, accepted
AbsCor

Description

Calculate absolute correlations among gene pairs

Usage

AbsCor(DataIn, method="pearson", diagNA=TRUE)

Arguments

DataIn input data, gene-by-sample matrix
method "pearson" or "spearman"; default is "pearson"
diagNA whether replace diagonal values to NA’s

Value

Output is a gene-by-gene matrix; the i, j th entry shows the absolute correlation of the ith and jth gene.

Author(s)

Ning Leng

Examples

AbsCor(matrix(rnorm(10),ncol=5))

CalcMV

Calculate estimated mean and variance of RNA-Seq data

Description

Calculate estimated mean and variance of RNA-Seq data

Usage

CalcMV(Data, Sizes=NULL, NormData=FALSE, MeanCutLow=100, MeanCutHigh=NULL, ApproxVal=10^-6, Plot=}
FlagCluster

Flag gene clusters with small within-cluster phase differences and/or small within-cluster sine scores

**Description**

Flag gene clusters with small within-cluster phase differences and/or small within-cluster sine scores

**Usage**

FlagCluster(SineRes, KMRes, Data, qt, thre=pi/4, qtincluster=.5, qtinpermu=.9, Seed=1)
FormatSineOut

Arguments

- **SineRes**: output of `Os scopeSine()` function
- **KMRes**: output of `KMRes()` function
- **Data**: a gene-by-sample (isoform-by-sample) matrix indicating the rescaled expression of genes/isoforms. All values should be between [-1, 1].
- **qt, thre**: Define a gene pair's linear score as min(eta, pi-eta), in which eta is defined as phase shift mod pi. A cluster will be flagged if the qt th quantile of within-cluster linear score is less than thre.
- **qtincluster, qtinpermu**: To define clusters with small within-cluster sine scores, for each cluster we generate permuted data of these genes (different cell permutation for each gene). We calculate the within-cluster sine scores within the cluster of permuted genes, then infer whether the sine scores in the cluster of interest are greater than those generated by the permuted genes. A cluster will be flagged if its qtincluster th quantile in the original data is less than its qtinpermu th quantile in permuted data.
- **Seed**: seed

Value

Output: `RemoveID`: a vector of cluster numbers that are flagged; `SineCompreList`: sine score and permuted sine score for each cluster; `LinearList`: linear score of each cluster.

Author(s)

Ning Leng

Examples

```r
aa <- sin(seq(0,1,.1))
bb <- sin(seq(0.5,1.5,.1))
cc <- sin(seq(0.9,1.9,.1))
tmp <- matrix(sin(rnorm(330)),ncol=11)
rownames(tmp) <- paste0("tmp",1:30)
Dat <- rbind(aa, bb, cc, tmp)
res1 <- Os scopeSine(Dat)
res2 <- Os scopeKM(res1, quan=.8, maxK=5)
res <- FlagCluster(res1, res2, Dat)
```

Description

Format SinFun outputs from lists to matrix

Usage

`FormatSineOut(result, DataInSc, ShiftRg=pi/4)`
ImpShift

Arguments

| result | Output from SineFun |
| DataInSc | a gene-by-sample (isoform-by-sample) matrix indicating the rescaled expression of two genes/isoforms. All values should be between [-1, 1]. |
| ShiftRg | phase shift cutoff. |

Value

Output is a list with 4 sublists, each shows a N-by-N matrix, in which N is the total number of genes (isoforms). SimiMat: similarity matrix (sine scores); the sine scores are calculated by -log10(epsilon^2). DiffMat: dissimilarity matrix; shown are epsilon^2 for each gene pair. ShiftMat: optimal phase shift estimate for each pair of genes.

Author(s)

Ning Leng

Examples

```r
aa <- sin(seq(0,1,.1))
bb <- sin(seq(0.5,1.5,.1))
cc <- sin(seq(0.9,1.9,.1))
DataInSc <- rbind(aa,bb,cc)
NumGene <- nrow(DataInSc)
Res <- sapply(1:(NumGene-1),function(i)SineFun(DataInSc, i),simplify=FALSE)
Out <- FormatSineOut(Res, DataInSc)
```

---

ImpShift

*Search for the optimal sample order by using the Extended Nearest Insertion*

Description

Search for the optimal sample order by using the Extended Nearest Insertion

Usage

```r
ImpShift(Data, Seq=NULL, NChun=4, RdmStart=FALSE, Ndg=3)
```

Arguments

| Data | gene-by-sample matrix or isoform-by-sample matrix. It should be rescaled to values between [-1,1]. |
| Seq | NULL or a vector indicates the sample order. If specified, the samples will be first reordered by this vector. |
| NChun | number of starting points for polynomial fitting. |
| RdmStart | whether the start points are randomly selected. |
| Ndg | degree of polynomial. |
Value

This function performs the extended nearest insertion (ENI). The ENI algorithm searches for the optimal sample order which minimizes the MSE of sliding polynomial regression (SPR). This function will call PipeShiftCDF() function, which fits SPR to each row of the data. For each gene/isoform, SPR fits NChun polynomial curves with different starting points (samples). The samples with smaller order than the start point will be appended to follow the last sample when fitting. So each fitting consider same number of samples. If RdmStart = TRUE, the start points are randomly selected. Otherwise they are evenly sampled along the sample order. The aggregated MSE of a fit (using a specific start point) is defined as the summation of the MSEs of all genes/isoforms considered here. The MSE of the SPR is defined as the largest aggregated MSE across fits using different start points. The output returns the optimal order which provides the smallest SPR MSE.

Author(s)

Ning Leng

Examples

```r
aa <- sin(seq(0,1,.1))
bb <- sin(seq(0.5,1.5,.1))
cc <- sin(seq(0.9,1.9,.1))
res <- ImpShift(rbind(aa,bb,cc), NChun=2)
```

---

**NISFun**

Run Extended Nearest Insertion and 2-opt on a gene cluster identified by OscopeKM function

Description

Run Extended Nearest Insertion and 2-opt on a gene cluster identified by OscopeKM function

Usage

```r
NISFun(ClusterList, DataIn, i, Ndg=3, NChun=4, RdmStart=FALSE, N=20000, NCThre=1000)
```

Arguments

- **ClusterList**: a list of gene clusters. Each sublist contains a vector of gene names.
- **DataIn**: gene-by-sample matrix or isoform-by-sample matrix. It should be rescaled to values between [-1,1].
- **i**: the cluster of interest. If the second cluster in ClusterList is of interest, specify i=2.
- **Ndg**: degree of polynomial.
- **NChun**: number of starting points for polynomial fitting.
- **RdmStart**: whether the start points are randomly selected.
- **N,NCThre**: The 2-opt algorithm will stop if N iterations has been performed or if the optimal order remains unchanged for over NCThre iterations.
This function performs the extended nearest insertion (ENI) and 2-opt algorithm to a particular cluster identified by OscopeKM function. The ENI algorithm searches for the optimal sample order which minimizes the MSE of sliding polynomial regression (SPR). This function will call PipeShiftCDF() function, which fits SPR to each row of the data. For each gene/isoform, SPR fits NChun polynomial curves with different starting points (samples). The samples with smaller order than the start point will be appended to follow the last sample when fitting. So each fitting consider same number of samples. If RdmStart = TRUE, the start points are randomly selected. Otherwise they are evenly sampled along the sample order. The aggregated MSE of a fit (using a specific start point) is defined as the summation of the MSEs of all genes/isoforms considered here. The MSE of the SPR is defined as the largest aggregated MSE across fits using different start points. The output of PipeShiftCDF() returns the optimal order which provides the smallest SPR MSE. The 2-opt algorithm is then applied to improve the optimal order searching of the ENI. In each iteration, 2-opt algorithm will randomly choose two points (samples), the flip the samples between these two points. The new order will be adapted if it provides smaller SPR MSE. The output returns the optimal order for the cluster of interest.

Author(s)
Ning Leng

Examples
```r
aa <- sin(seq(0,1,.1))
b <- sin(seq(0.5,1.5,.1))
c <- sin(seq(0.9,1.9,.1))
res <- NISFun(list(c("aa","bb"),"cc"), rbind(aa,bb,cc),i=1, NChun=2, N=50)
```

### Description
Rescale the gene/isoform expression matrix

### Usage
```
NormForSine(Data, qt1=.05, qt2=.95)
```

### Arguments
- **Data**: input gene-by-sample matrix or isoform-by-sample matrix
- **qt1, qt2**: thresholds for outlier adjustment. For each gene/isoform, values <= qt1 th quantile (>= qt2 th quantile) will be pushed to qt1 th quantile (qt2 th quantile) prior to the scaling. default values are 0.05 and 0.95.

### Value
The output will be a gene-by-sample or isoform-by-sample matrix. For each gene/isoform, the expressions will be scaled linearly to [-1,1]
Run the 2-opt algorithm to improve the optimal order searching of the Extended Nearest Insertion

Usage

Opt2Shift(Data,N=20000,Seq,Ndg=3,NChun=4, NCThre=1000, RdmStart=FALSE)

Arguments

Data gene-by-sample matrix or isoform-by-sample matrix. It should be rescaled to values between [-1,1].
N,NCThre The 2-opt algorithm will stop if N iterations has been performed or if the optimal order remains unchanged for over NCThre iterations.
Seq a vector indicates the sample order obtained from the ENI.
Ndg degree of polynomial.
NChun number of starting points for polynomial fitting.
RdmStart whether the start points are randomly selected.

Value

This function performs the the 2-opt algorithm to improve the optimal order searching of the Extended Nearest Insertion (ENI). In each iteration, the function will randomly choose two points (samples), the flip the samples between these two points. The new order will be adapted if it provides smaller SPR MSE. The output returns the optimal order and its SPR MSE.

Author(s)

Ning Leng

Examples

aa <- sin(seq(0,1,.1))
bb <- sin(seq(0.5,1.5,.1))
cc <- sin(seq(0.9,1.9,.1))
res <- ImpShift(rbind(aa,bb,cc), NChun=2)
res2 <- Opt2Shift(rbind(aa,bb,cc), NChun=2, N=50, Seq=res)
OsscopeENI

Search for the optimal sample order for different gene clusters

Description
Search for the optimal sample order for different gene clusters

Usage
OsscopeENI(KMRes, Data, ClusterUse=NULL, Ndg=3, NChun=4, RdmStart=FALSE,
N=20000, NCThre=1000, parallel=FALSE, parallelParam=NULL)

Arguments
KMRes
output of OscopeKM() function.

Data
gene-by-sample matrix or isoform-by-sample matrix. It should be rescaled to
values between [-1,1].

ClusterUse
a vector indicating what clusters are of interest. For example, by setting Clus-
terUse = c(1,2,3), only the top 3 clusters will be considered while recovering the
base cycle order. If ClusterUse=NULL, all clusters will be used.

Ndg
degree of polynomial.

NChun
number of starting points for polynomial fitting.

RdmStart
whether the start points are randomly selected.

N,NCThre
The 2-opt algorithm will stop if N iterations has been performed or if the optimal
order

parallel
whether apply parallel computing. If it is TRUE, BiocParallel will be called.

parallelParam
a SnowParam object to specify the clusters. If it is NULL, the default will be
set as SnowParam(workers = 5, type = "SOCK") remains unchanged for over
NCThre iterations.

Value
This function performs the extended nearest insertion (ENI) and 2-opt algorithm to all clusters (or
a subset of picked clusters) identified by OscopeKM function. The function will recover independent
orders to each of the clusters. For each cluster, the ENI algorithm will be applied to search for
the optimal sample order which minimizes the MSE of sliding polynomial regression (SPR).
This function will call PipeShiftCDF() function, which fits SPR to expression of each gene/isoform
within a cluster. For each gene/isoform, SPR fits NChun polynomial curves with different starting
points (samples). The samples with smaller order than the start point will be appended to follow the
last sample when fitting. So each fitting consider same number of samples. If RdmStart = TRUE,
the start points are randomly selected. Otherwise they are evenly sampled along the sample order.
The aggregated MSE of a fit (using a specific start point) is defined as the summation of the MSEs
of all genes/isoforms considered here. The MSE of the SPR is defined as the largest aggregated
MSE across fits using different start points. The output of PipeShiftCDF() returns the optimal order
which provides the smallest SPR MSE. The 2-opt algorithm will then be applied to improve the
optimal order searching of the ENI. In each iteration, the 2-opt algorithm will randomly choose two
points (samples), the flip the samples between these two points. The new order will be adapted if it
provides smaller SPR MSE. The output returns the optimal order for each cluster of interest. It is a
list with multiple sublists, in which each sublist includes the recovered order of the corresponding
cluster in ClusterUse. If ClusterUse is not specified, the k th sublist shows the recovered order in KMRes

Author(s)

Ning Leng

Examples

aa <- sin(seq(0,1,.1))
bb <- sin(seq(0.5,1.5,.1))
ce <- sin(seq(0.9,1.9,.1))
dd <- sin(seq(1.2,2.2,.1))
res <- OscopeENI(list(c1=c("aa","bb"),c2=c("cc","dd")), rbind(aa,bb,ce,dd), NChun=2, N=50)

Description

Simulated gene level data set with 600 genes and 30 cells.

Format

GeneExampleData is a matrix with 600 genes (rows) and 30 cells (columns).

Examples

data(GeneExampleData)
str(GeneExampleData)

Description

Os cope K medoid module

Usage

OscopeKM(SineRes, quan=.95,cut=TRUE,maxK=NULL,minSize=0, maxSize=200, fixK=NULL, rawscale=TRUE)
Arguments

quan: only gene pairs with similarity score >= quan th quantile will be considered in the clustering analyses. Default is 0.95.
cut: pre-defined cutoff. Gene pairs with similarity score >= cut will be considered in cluster analyses. If cut is defined, quan will be ignored.
maxK: max number of clusters to consider (scan). if numbC=NULL, it will be calculated as [number of gene considered]/10
minSize,maxSize: Only clusters with minSize<= cluster size <= maxSize are reported in output.
fixK: if fixK is specified, the k-medoids algorithm will be applied with fixK clusters.
rawscale: Recall the input is the similarity matrix (-log10(distance from the sine model)). the k-medoids clustering will be applied using (-Input) as distance. If rawscale is defined as TRUE, the k-medoids clustering will be applied using -10^Input as distance.

Value

OscopeKM() calls scanK() function, which runs k-medoid clustering with varying number of clusters (k). The k is varied from 2 to maxK. The input should be the output of OscopeSine() function. scanK() function will cluster genes in gene pairs with high similarity score (the threshold can be defined using parameter quan). To select the top genes, the function first calculate the max similarity score for each gene, then select the genes with high max score.

The output object shows members in each cluster. clusters are sorted by median similarity score within cluster.

Author(s)
Ning Leng

Examples

aa <- sin(seq(0,1,.1))
bb <- sin(seq(0.5,1.5,.1))
cc <- sin(seq(0.9,1.9,.1))
tmp <- matrix(sin(rnorm(330)),ncol=11)
rownames(tmp) <- paste0("tmp",1:30)
Dat <- rbind(aa, bb, cc, tmp)
res1 <- OscopeSine(Dat)
res2 <- OscopeKM(res1, quan=.8, maxK=5)

OscopeSine
Apply sine model on the full set of genes or isoforms

Description

Apply sine model on the full set of genes or isoforms
Usage

OscopeSine(DataInSc, parallel=FALSE, parallelParam=NULL)

Arguments

DataInSc a gene-by-sample (isoform-by-sample) matrix indicating the rescaled expression of two genes/isoforms. all values should be between [-1, 1].
parallel whether apply parallel computing. if it is TRUE, BiocParallel will be called.
parallelParam a SnowParam object to specify the clusters. If it is NULL, the default will be set as SnowParam(workers = 5, type = "SOCK") remains unchanged for over NCThre iterations.

Value

Output is a list with 4 sublists, each shows a N-by-N matrix, in which N is the total number of genes (isoforms). SimiMat: similarity matrix (sine scores); the sine scores are calculated by -log10(epsilon^2). DiffMat: dissimilarity matrix; shown are epsilon^2 for each gene pair. ShiftMat: optimal phase shift estimate for each pair of genes.

Author(s)

Ning Leng

Examples

aa <- sin(seq(0,1,.1))
bb <- sin(seq(0.5,1.5,.1))
cc <- sin(seq(0.9,1.9,.1))
OscopeSine(rbind(aa,bb,cc))

---

PermuCut

Define sine score cutoff using permuted data

Usage

PermuCut(Data, NumPermu=1000)

Arguments

Data a gene-by-sample (isoform-by-sample) matrix indicating the rescaled expression of genes/isoforms. all values should be between [-1, 1].
NumPermu number of permuted genes to generate.

Value

Output contains a vector of numbers. Each number presents max sine score of a given permuted gene.
Author(s)

Ning Leng

Examples

aa <- sin(seq(0,1,.1))
bb <- sin(seq(0.5,1.5,.1))
cc <- sin(seq(0.9,1.9,.1))
tmp <- matrix(sin(rnorm(330)),ncol=11)
rownames(tmp) <- paste0("tmp",1:30)
Dat <- rbind(aa, bb, cc, tmp)
res1 <- PermuCut(Dat,100)

---

**PipeR**

*Calculate residual of polynomial fit*

Description

Calculate residual of polynomial fit

Usage

`PipeR(Data,Ndg=3,Method="Poly")`

Arguments

- `Data` : gene-by-sample matrix or isoform-by-sample matrix. It should be rescaled to values between [-1,1].
- `Ndg` : degree of polynomial.
- `Method` : only polynomial fitting ("Poly") is available now.

Value

The function will fit polynomial curve to each row of the data. The output returns the MSE of each row (gene/isoform).

Author(s)

Ning Leng

Examples

aa <- sin(seq(0,1,.1))
bb <- sin(seq(0.5,1.5,.1))
cc <- sin(seq(0.9,1.9,.1))
res <- PipeR(rbind(aa,bb,cc))
PipeShiftCDF

Calculate residual of the sliding polynomial regression

Description

Calculate residual of the sliding polynomial regression

Usage

PipeShiftCDF(Data, Ndg=3, NChun=4, RdmStart=FALSE)

Arguments

- **Data**: gene-by-sample matrix or isoform-by-sample matrix. It should be rescaled to values between [-1,1].
- **Ndg**: degree of polynomial.
- **NChun**: number of starting points for polynomial fitting.
- **RdmStart**: whether the start points are randomly selected.

Value

The function will fit sliding polynomial regression (SPR) to each row of the data. For each gene/isoform, SPR fits NChun polynomial curves with different starting points (samples). The samples with smaller order than the start point will be appended to follow the last sample when fitting. So each fitting consider same number of samples. If RdmStart = TRUE, the start points are randomly selected. Otherwise they are evenly sampled along the sample order. The aggregated MSE of a fit (using a specific start point) is defined as the summation of the MSEs of all genes/isoforms considered here. The output returns the MSE of the SPR, which is the largest aggregated MSE across fits using different start points.

Author(s)

Ning Leng

Examples

```r
aa <- sin(seq(0,1,.1))
bb <- sin(seq(0.5,1.5,.1))
cc <- sin(seq(0.9,1.9,.1))
res <- PipeShiftCDF(rbind(aa,bb,cc), NChun=2)
```
scanK  

Run k-medoid algorithm with varying k on similarity matrix

Description
Run k-medoid algorithm with varying k on similarity matrix

Usage

scanK(SimiMatIn, quan=.95, cut=NULL, maxK=NULL, minSize=0, maxSize=200, fixK=NULL, rawscale=FALSE)

Arguments

SimiMatIn  
gene-by-gene similarity matrix
quan  
only gene pairs with similarity score >= quan th quantile will be considered in the cluster analyses. Default is 0.95.
cut  
pre-defined cutoff. Gene pairs with similarity score >= cut will be considered in cluster analyses. If cut is defined, quan will be ignored.
maxK  
max number of clusters to consider (scan). if numbC=NULL, it will be calculated as [number of gene considered]/10.
minSize,maxSize  
Only clusters with minSize<= cluster size <= maxSize are reported in output.
fixK  
if fixK is specified, the k-medoids algorithm will be applied with fixK clusters.
rawscale  
Recall the input is the similarity matrix (-log10(distance from the sine model)). the k-medoids clustering will be applied using (-Input) as distance. If rawscale is defined as TRUE, the k-medoids clustering will be applied using -10^Input as distance.

Value

scanK() function runs k-medoid clustering with varying number of clusters (k). The k is varied from 2 to maxK. The input of scanK() function should be a similarity matrix. scanK() function will cluster genes in gene pairs with high similarity score (the threshold can be defined using parameter quan). To select the top genes, the function first calculate the max similarity score for each gene, then select the genes with high max score.

The output object is a list with 4 sublists: membOut: members in each cluster. clusters are sorted by median similarity score within cluster; MedCor: median similarity score for each cluster; Mat: input similarity matrix; filteredMat: similarity matrix, only showing the top genes used in clustering; Kcluster: cluster indicator of each top gene.

Author(s)
Ning Leng
Examples

aa <- sin(seq(0,1,.1))
bb <- sin(seq(0.5,1.5,.1))
cc <- sin(seq(0.9,1.9,.1))
tmp <- matrix(sin(rnorm(330)),ncol=11)
rownames(tmp) <- paste0("tmp",1:30)
Dat <- rbind(aa, bb, cc, tmp)
res1 <- OscopeSine(Dat)
res2 <- scanK(res1$SimiMat, quan=.8, maxK=5)

SineFun

Apply sine model on one particular gene vs. other genes

Description

Apply sine model on one particular gene vs. other genes

Usage

SineFun(DataInSc,i)

Arguments

DataInSc a gene-by-sample (isoform-by-sample) matrix indicating the rescaled expression of two genes/isoforms. all values should be between -1, 1.

i the gene (isoform) of interest. The function will apply the sine model on gene (isoform) i vs. gene (isoform) j for all j > i. Gene (isoform) i (j) is defined as the gene (isoform) shown in the i (j) th row. i should be smaller than the total number of genes (isoforms).

Value

Output is a list with two sublists, each shows the optimal phi’s (shift) and epsilon’s (value). N-i entries will be included in each sublist (N is the total number of genes/isoforms). The kth entry indicates results of gene (isoform) i vs. i+k.

Author(s)

Ning Leng

Examples

aa <- sin(seq(0,1,.1))
bb <- sin(seq(0.5,1.5,.1))
cc <- sin(seq(0.9,1.9,.1))
SineFun(rbind(aa,bb,cc), 1)
SineOptim

Function for searching optimal phase shift

Description
Function for searching optimal phase shift

Usage
SineOptim(Pairdata)

Arguments
Pairdata  a sample-by-2 matrix indicating the rescaled expression of two genes/isoforms. all values should be between [-1, 1].

Value
Output provides the optimal phi (shift) and its corresponding epsilon^2 (value) of the sine model.

epsilon_{g1,g2}^2 = \sum_s [X_{g1,s}^2 + X_{g2,s}^2 - 2X_{g1,s}X_{g2,s}\cos(\phi_{g1,g2}) - \sin^2(\phi_{g1,g2})]^2

Author(s)
Ning Leng

Examples
aa <- sin(seq(0,1,.1))
bb <- sin(seq(0.5,1.5,.1))
SineOptim(cbind(aa,bb))
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