

SVAPLSseq: An R package to correct for hidden sources of variability in differential gene expression studies based on RNAseq data

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1 Overview

The R package *SVAPLSseq* contains functions that are intended for the extraction and correction of different types of hidden biological and technical variables that could potentially generate latent heterogeneity in RNAseq data on gene expression. The complexity of the sequencing workflow creates a number of technical artefacts along with the inherent biological variability stemming from the unknown gene and sample profiles. The package aims to provide the users with a flexible and generalized framework to identify these hidden effects and adjust for them in order to re-estimate the primary signals of group-specific differential gene expression with higher power and accuracy. The underlying method operates by implementing a non-linear partial least squares regression algorithm on two multivariate random matrices constructed from the data. To that end two methodological variants are provided in this package: (1) Unsupervised SVAPLSseq and (2) Supervised SVAPLSseq. Both these variants yield a set of surrogate variables that are then tested for statistical significance in order to detect the important signatures of latent variability in the data. The package also provides an added functionality

in terms of incorporating these extracted signatures in a linear regression framework and estimating the group-specific differential expression effects. For this purpose two different options are provided: (a) Wald test that uses the R packages “edgeR” and “limma” and (b) Likelihood ratio test.

This document provides a tutorial to use the package for:

- Formatting the data for use in the package.
- Extracting the signatures of the hidden effects in the data.
- Using the estimated hidden effect signatures to detect the truly differentially expressed genes.

2 Formatting the data for use in the package

The starting step for using the package is to set up the RNAseq expression data in an appropriate format. The input data should be in the form of either a count matrix object or a ‘SummarizedExperiment’ or a ‘DGEList’ object. The object will contain a feature matrix that will list the features (genes/transcripts) along the rows and samples along the columns. This matrix will contain the read count values for the features corresponding to the different samples. In addition, a separate factor variable should be designed that will keep track of the group each sample belongs to (e.g. “treated” and “untreated”, “Normal” and “Cancer”). This variable will enable the estimation of the primary signal for group-specific differential expression of the features.

```
> library(SummarizedExperiment)
> library(SVAPLSseq)
> library(edgeR)
> data(sim.dat)
> dat = SummarizedExperiment(assays = SimpleList(counts = sim.dat))
> dat = DGEList(counts = sim.dat)
> sim.dat[1:6, c(1:3, 11:13)]

      [,1] [,2] [,3] [,4] [,5] [,6]
[1,]  180  180  183  594  555  585
[2,]  278  275  269  357  359  350
[3,]  182  196  205  439  454  444
[4,]  438  430  345 2818 2790 2888
[5,]  238  226  215 1413 1452 1471
[6,]  156  158  180  486  527  455
```

3 Extracting the signatures of the hidden effects in the data

The package contains a function `svplsSurr` that extracts the signatures of latent variability (surrogate variables) in the data by using a multivariate non-linear partial least squares (NPLS) algorithm

(Boulesteix and Strimmer 2007). The function takes the original read count matrix of feature expression values along with a factor variable indicating the group of each sample as input. Moreover, it allows the user to specify a certain number of surrogate variables (`max.surrs`) that will be extracted from the data. These variables are further tested for statistical significance to generate an optimal set of significant surrogate variables capturing the latent variation in the data. The function returns a matrix with these variables along the columns and a vector containing the proportions of the total variance in the data space that are explained by them.

The function provides the user with two methodological variants: (1) The Unsupervised SVAPLSseq and (2) The Supervised SVAPLSseq. Details on these two variants and their usage on an RNAseq gene expression data are provided below:

3.1 The Unsupervised SVAPLSseq

This version of the method regresses the primary signal corrected residual matrix on the original gene expression data matrix via NPLS. The estimated scores in the data space are considered as the surrogate variables that are further tested for statistical significance. Setting the `controls` argument of the function to `NULL` starts this version.

```
> data(sim.dat)
> group = as.factor(c(rep(1, 10), rep(-1, 10)))
> sim.dat.se = SummarizedExperiment(assays = SimpleList(counts = sim.dat))
> sim.dat.dg = DGEList(counts = sim.dat)
> sv <- svplsSurr(dat = sim.dat, group = group, max.surrs = 3, controls = NULL)
> sv <- svplsSurr(dat = sim.dat.se, group = group, max.surrs = 3, controls = NULL)
> sv <- svplsSurr(dat = sim.dat.dg, group = group, max.surrs = 3, controls = NULL)
> print(sv)
```

An object of class "svplsSurr"

Slot "surr":

	Comp 1	Comp 2	Comp 3
1	-40.926156	-0.8084875	4.224341
2	-40.055960	-0.4950606	3.898370
3	-42.367566	-1.5554843	5.060546
4	-42.233553	-1.3974685	4.856386
5	-41.106307	-0.9151236	4.329619
6	7.970834	17.1226899	-1.939348
7	12.782978	18.5159304	-1.274102
8	4.759267	16.2514642	-2.199716
9	7.035339	16.7946280	-1.952845
10	11.272798	18.0598937	-1.490734
11	-9.475664	-9.6107729	-6.147861
12	-8.877108	-9.4874725	-6.302462
13	-8.538197	-9.4380389	-6.117937

```
14 -6.817952 -9.4635581 -6.029625
15 -10.096205 -9.4816131 -6.184044
16 40.686615 -6.9212112 3.270012
17 38.179231 -7.1881869 2.481356
18 43.294054 -6.5935848 4.070858
19 38.161401 -7.1118876 2.432756
20 46.352152 -6.2766559 5.014430
```

```
Slot "prop.vars":
```

```
Comp 1 Comp 2 Comp 3
0.87480779 0.10710424 0.01808797
```

```
> surr(sv)
```

```
Comp 1 Comp 2 Comp 3
1 -40.926156 -0.8084875 4.224341
2 -40.055960 -0.4950606 3.898370
3 -42.367566 -1.5554843 5.060546
4 -42.233553 -1.3974685 4.856386
5 -41.106307 -0.9151236 4.329619
6 7.970834 17.1226899 -1.939348
7 12.782978 18.5159304 -1.274102
8 4.759267 16.2514642 -2.199716
9 7.035339 16.7946280 -1.952845
10 11.272798 18.0598937 -1.490734
11 -9.475664 -9.6107729 -6.147861
12 -8.877108 -9.4874725 -6.302462
13 -8.538197 -9.4380389 -6.117937
14 -6.817952 -9.4635581 -6.029625
15 -10.096205 -9.4816131 -6.184044
16 40.686615 -6.9212112 3.270012
17 38.179231 -7.1881869 2.481356
18 43.294054 -6.5935848 4.070858
19 38.161401 -7.1118876 2.432756
20 46.352152 -6.2766559 5.014430
```

```
> prop.vars(sv)
```

```
Comp 1 Comp 2 Comp 3
0.87480779 0.10710424 0.01808797
```

3.2 The Supervised SVAPLSseq

In this variant a submatrix of the original gene expression data is first created corresponding to a set of available control probes that do not have any differential expression between the two groups. Hence, this submatrix is only expected to contain the signatures of the hidden effects in the data. This matrix is

then regressed on the original data to extract the surrogate variables for the underlying latent variation. This variant is called by setting the `controls` argument of the function to the collection of the control probes.

```
> data(sim.dat)
> controls = c(1:nrow(sim.dat)) > 400
> group = as.factor(c(rep(1, 10), rep(-1, 10)))
> sim.dat.se = SummarizedExperiment(assays = SimpleList(counts = sim.dat))
> sim.dat.dg = DGEList(counts = sim.dat)
> sv <- svplsSurr(dat = sim.dat, group = group, max.surrs = 3, controls = controls)
> sv <- svplsSurr(dat = sim.dat.se, group = group, max.surrs = 3, controls = controls)
> sv <- svplsSurr(dat = sim.dat.dg, group = group, max.surrs = 3, controls = controls)
> print(sv)
```

An object of class "svplsSurr"

Slot "surr":

	Comp 1	Comp 2	Comp 3
Sample1	-31.926628	-3.208735	-0.03536976
Sample2	-31.272906	-2.904747	-0.14619953
Sample3	-32.891642	-3.941770	0.28247933
Sample4	-32.853971	-3.871881	0.26042255
Sample5	-31.989481	-3.260728	0.03723828
Sample6	5.474165	3.098831	0.45612324
Sample7	9.194147	2.585913	0.63804992
Sample8	3.014986	3.316163	0.58915161
Sample9	4.717672	3.192498	0.62023566
Sample10	8.012877	2.745850	0.59885841
Sample11	-7.207680	3.575643	-0.52382734
Sample12	-6.747954	3.643430	-0.53557381
Sample13	-6.530576	3.560833	-0.51574318
Sample14	-5.160810	3.581938	-0.44025060
Sample15	-7.682810	3.548411	-0.66007413
Sample16	32.286081	-3.013467	-0.12416276
Sample17	30.302523	-2.336366	0.07454508
Sample18	34.293006	-3.629939	-0.22964625
Sample19	30.285908	-2.312349	0.04708990
Sample20	36.683092	-4.369528	-0.39334663

Slot "prop.vars":

	Comp 1	Comp 2	Comp 3
	0.9800041257	0.0196787967	0.0003170775

```
> surr(sv)
```

	Comp 1	Comp 2	Comp 3
Sample1	-31.926628	-3.208735	-0.03536976
Sample2	-31.272906	-2.904747	-0.14619953

```
Sample3 -32.891642 -3.941770 0.28247933
Sample4 -32.853971 -3.871881 0.26042255
Sample5 -31.989481 -3.260728 0.03723828
Sample6 5.474165 3.098831 0.45612324
Sample7 9.194147 2.585913 0.63804992
Sample8 3.014986 3.316163 0.58915161
Sample9 4.717672 3.192498 0.62023566
Sample10 8.012877 2.745850 0.59885841
Sample11 -7.207680 3.575643 -0.52382734
Sample12 -6.747954 3.643430 -0.53557381
Sample13 -6.530576 3.560833 -0.51574318
Sample14 -5.160810 3.581938 -0.44025060
Sample15 -7.682810 3.548411 -0.66007413
Sample16 32.286081 -3.013467 -0.12416276
Sample17 30.302523 -2.336366 0.07454508
Sample18 34.293006 -3.629939 -0.22964625
Sample19 30.285908 -2.312349 0.04708990
Sample20 36.683092 -4.369528 -0.39334663
```

```
> prop.vars(sv)
```

```
Comp 1      Comp 2      Comp 3
0.9800041257 0.0196787967 0.0003170775
```

4 Using the estimated hidden effect signatures to detect the truly differentially expressed genes

The package contains another function `svplsTest` that incorporates the significant surrogate variables estimated by the function `svplsSurr` inside a regression framework in order to test for the genes that are truly differentially expressed between the two groups. The function provides the user with two testing options: (1) Wald test based on the regression coefficients of the primary signal effects (group effects) after incorporating the surrogate variables in a linear model and (2) Likelihood ratio test (LRT) comparing two different regression models: one containing primary signal effects as well as the surrogate variables and the other including only the surrogate variables. A list is returned as the output that contains the genes detected to be differentially expressed between the two groups (`sig.genes`, the uncorrected pvalues from the test (`pvs.unadj`) and the corresponding FDR adjusted pvalues (`pvs.adj`).

```
> data(sim.dat)
> group = as.factor(c(rep(1, 10), rep(-1, 10)))
> sv = svplsSurr(dat = sim.dat, group = group)
> surr = surr(sv)
> sim.dat.se = SummarizedExperiment(assays = SimpleList(counts = sim.dat))
> sim.dat.dg = DGEList(counts = sim.dat)
```

```
> fit = svplsTest(dat = sim.dat, group = group, surr = surr, test = "Wald")
> fit = svplsTest(dat = sim.dat.se, group = group, surr = surr, test = "Wald")
> fit = svplsTest(dat = sim.dat.dg, group = group, surr = surr, test = "Wald")
> sig.genes(fit)

[1] "13" "73" "210" "211" "246" "267" "311" "321" "347" "368" "371" "381" "395" "925"

> pvs.unadj(fit)

      1      2      3      4      5      6
3.014261e-01 4.796025e-01 5.695433e-01 5.758314e-01 4.261951e-02 6.291970e-01
      7      8      9     10     11     12
1.533013e-02 6.196252e-01 1.585284e-01 1.960720e-01 5.620965e-01 7.506645e-01
     13     14     15     16     17     18
1.890925e-04 8.812975e-01 2.491662e-02 1.997496e-01 5.494928e-01 2.095962e-02
     19     20     21     22     23     24
9.132696e-01 3.131585e-01 9.642487e-01 5.550774e-01 7.823371e-01 3.202374e-01
     25     26     27     28     29     30
5.675575e-01 5.193929e-01 2.382217e-01 1.976546e-01 2.453857e-02 6.833366e-01
     31     32     33     34     35     36
4.526750e-03 6.219588e-01 1.403786e-01 7.446261e-01 8.480906e-02 2.533287e-01
     37     38     39     40     41     42
2.730402e-01 8.705380e-01 2.433785e-02 2.147033e-01 4.715889e-01 3.950975e-02
     43     44     45     46     47     48
7.593842e-01 8.685858e-01 5.710237e-02 4.327231e-01 4.897303e-01 8.494247e-02
     49     50     51     52     53     54
3.104139e-01 3.953672e-01 9.172999e-01 1.029676e-01 5.626289e-02 5.507234e-01
     55     56     57     58     59     60
5.156423e-01 5.030969e-01 6.445932e-01 3.592109e-01 9.821898e-01 5.316723e-01
     61     62     63     64     65     66
6.741478e-02 7.597194e-03 1.742699e-01 5.670300e-02 2.644249e-01 6.520203e-01
     67     68     69     70     71     72
6.741126e-01 7.182892e-03 4.843172e-01 1.575505e-02 2.804980e-01 4.324877e-02
     73     74     75     76     77     78
2.258966e-04 8.881456e-01 7.150193e-01 7.246354e-03 8.185911e-01 1.176236e-01
     79     80     81     82     83     84
5.764380e-01 1.788935e-01 2.019131e-01 1.432802e-01 5.817406e-01 5.763965e-01
     85     86     87     88     89     90
4.099495e-02 1.020773e-01 2.031208e-01 5.394007e-01 2.360643e-01 6.553808e-01
     91     92     93     94     95     96
6.651853e-03 1.708780e-01 2.755178e-01 1.242173e-03 8.823303e-01 6.499922e-01
     97     98     99    100    101    102
9.177030e-01 7.521183e-01 8.321848e-03 1.277577e-02 6.959816e-01 9.236069e-01
    103    104    105    106    107    108
4.463025e-03 2.337050e-01 4.521204e-01 2.114998e-02 6.837446e-01 9.975591e-01
    109    110    111    112    113    114
```

9.355124e-01	4.782297e-02	3.432122e-01	1.980548e-01	1.994431e-01	9.224227e-01
115	116	117	118	119	120
8.396360e-03	7.358687e-01	2.456568e-01	1.191554e-02	1.710796e-02	9.121816e-01
121	122	123	124	125	126
1.651429e-01	4.507083e-02	2.631089e-02	1.455229e-01	3.602302e-01	7.472321e-01
127	128	129	130	131	132
6.659646e-01	5.856624e-01	2.980434e-01	8.899840e-01	2.597737e-01	4.344713e-01
133	134	135	136	137	138
9.686205e-01	3.068335e-01	2.267240e-02	2.810722e-01	8.748837e-01	4.837649e-01
139	140	141	142	143	144
5.188322e-01	1.230208e-03	7.708964e-01	7.279723e-03	3.714957e-01	2.562310e-01
145	146	147	148	149	150
2.881637e-01	9.201401e-03	1.570499e-02	3.769022e-01	6.547095e-01	3.330224e-02
151	152	153	154	155	156
6.194566e-02	7.850738e-01	6.167033e-02	4.899539e-02	2.340495e-02	9.378677e-03
157	158	159	160	161	162
1.085890e-03	6.372875e-03	1.513101e-02	3.424752e-01	4.030207e-01	2.316368e-01
163	164	165	166	167	168
2.050591e-01	9.981873e-01	7.010178e-01	7.853664e-01	1.074607e-02	7.945379e-01
169	170	171	172	173	174
7.350670e-01	1.941206e-01	1.526000e-01	1.154233e-02	7.954814e-02	7.972488e-02
175	176	177	178	179	180
4.193635e-01	8.479089e-01	1.678819e-01	3.632338e-01	1.981754e-01	6.890816e-01
181	182	183	184	185	186
2.679278e-02	5.371849e-01	5.378731e-01	1.443694e-01	1.865869e-02	1.212830e-01
187	188	189	190	191	192
8.597783e-01	8.276049e-01	4.558031e-01	5.920464e-01	5.624297e-01	4.231607e-01
193	194	195	196	197	198
4.755165e-01	7.701399e-01	5.340620e-01	4.302948e-01	6.628691e-01	3.247952e-01
199	200	201	202	203	204
5.667435e-01	6.863541e-01	7.700647e-02	2.857562e-01	6.609133e-01	1.427450e-02
205	206	207	208	209	210
1.427111e-01	9.579575e-01	5.090425e-01	9.084815e-01	5.926177e-01	6.227310e-04
211	212	213	214	215	216
1.211629e-04	7.972167e-03	4.328486e-01	1.490321e-01	4.105117e-01	1.286076e-01
217	218	219	220	221	222
4.511548e-01	8.971585e-01	9.417585e-01	1.207143e-03	6.832578e-01	3.377431e-01
223	224	225	226	227	228
5.634313e-01	1.171892e-01	8.091100e-01	8.342700e-01	2.249621e-01	4.163084e-02
229	230	231	232	233	234
4.353725e-01	5.618317e-01	8.364380e-01	9.541648e-01	2.124681e-01	7.827780e-01
235	236	237	238	239	240
2.277823e-01	7.960302e-01	3.329818e-01	3.219657e-02	2.809008e-01	1.442074e-01
241	242	243	244	245	246
9.708559e-01	3.211792e-01	1.022415e-01	3.737944e-01	9.463642e-01	5.739411e-05

247	248	249	250	251	252
3.551511e-03	8.102844e-01	5.950282e-01	5.399978e-02	1.219517e-01	1.621688e-02
253	254	255	256	257	258
3.062092e-02	6.520014e-01	6.549634e-01	4.391680e-01	1.121206e-01	5.049483e-02
259	260	261	262	263	264
3.657969e-01	6.060412e-01	3.139200e-01	5.569980e-01	8.818397e-01	2.250581e-02
265	266	267	268	269	270
3.285575e-01	8.955073e-03	9.866340e-07	3.022918e-01	2.320209e-02	7.519378e-01
271	272	273	274	275	276
7.875097e-01	4.445122e-01	1.743715e-01	1.116445e-02	1.411827e-01	4.718807e-01
277	278	279	280	281	282
6.653047e-01	6.333764e-02	3.314076e-01	2.568518e-01	7.891735e-01	2.374489e-01
283	284	285	286	287	288
3.895449e-01	3.276284e-01	7.082934e-01	2.366130e-01	7.186317e-01	6.187758e-01
289	290	291	292	293	294
4.856183e-01	7.964545e-01	9.372287e-02	1.778966e-03	6.055129e-02	7.313456e-01
295	296	297	298	299	300
2.304326e-02	2.187624e-01	1.684374e-01	2.630169e-01	7.940024e-01	4.787256e-01
301	302	303	304	305	306
2.058437e-02	8.267972e-01	5.770612e-01	1.599661e-03	5.063473e-01	7.766337e-01
307	308	309	310	311	312
1.750777e-03	9.618564e-01	2.389263e-02	1.057180e-01	4.995911e-06	2.657109e-01
313	314	315	316	317	318
1.028845e-03	8.304688e-01	6.485786e-01	1.608580e-02	7.247144e-01	1.616595e-02
319	320	321	322	323	324
2.232938e-01	9.748692e-01	1.995526e-05	1.617538e-01	5.862926e-01	3.737406e-01
325	326	327	328	329	330
3.353866e-01	4.532597e-01	5.420227e-01	6.599062e-01	5.132774e-01	8.659980e-01
331	332	333	334	335	336
9.785800e-02	4.889175e-01	8.104640e-01	8.772126e-01	5.614657e-01	3.654762e-01
337	338	339	340	341	342
2.890634e-01	3.844619e-01	6.958796e-01	3.557833e-01	7.296530e-01	6.458883e-01
343	344	345	346	347	348
3.017340e-02	7.137902e-01	4.988790e-01	7.180922e-01	2.537685e-04	6.187653e-01
349	350	351	352	353	354
5.890155e-01	1.277671e-01	1.496073e-01	8.159943e-01	8.094584e-02	1.324460e-03
355	356	357	358	359	360
1.152180e-01	3.374424e-02	1.796969e-01	3.174945e-01	6.459570e-01	1.915817e-01
361	362	363	364	365	366
7.021831e-01	7.133959e-01	6.122107e-02	4.740982e-03	2.120181e-03	2.160872e-01
367	368	369	370	371	372
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0.288689087 0.971482416 0.137174228 0.971482416 0.694083663 0.971482416 0.556876250
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71	72	73	74	75	76	77
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78	79	80	81	82	83	84
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0.768267766	0.987645908	0.186585774	0.971482416	0.816135656	0.220658074	0.240957253
120	121	122	123	124	125	126
0.987645908	0.727501825	0.387666788	0.291225839	0.696281882	0.900575536	0.971482416
127	128	129	130	131	132	133
0.971482416	0.964297024	0.874027701	0.985680037	0.829948036	0.930283112	0.987645908
134	135	136	137	138	139	140
0.881207506	0.285426214	0.853459414	0.985229339	0.955420023	0.960723638	0.062108646
141	142	143	144	145	146	147
0.971482416	0.177554225	0.908185867	0.828377154	0.857630050	0.195389111	0.231669683
148	149	150	151	152	153	154
0.910296457	0.971482416	0.320213831	0.463889424	0.973145579	0.463889424	0.410479342
155	156	157	158	159	160	161
0.285426214	0.195389111	0.062108646	0.172239875	0.231669683	0.885835104	0.918335243
162	163	164	165	166	167	168
0.801511295	0.771217169	0.998910167	0.971482416	0.973145579	0.219307456	0.973145579
169	170	171	172	173	174	175
0.971482416	0.768267766	0.699999812	0.220658074	0.546060844	0.546060844	0.921678078
176	177	178	179	180	181	182
0.979802909	0.729166229	0.905819861	0.768267766	0.971482416	0.291225839	0.960723638
183	184	185	186	187	188	189
0.960723638	0.694083663	0.255598505	0.662780940	0.979802909	0.977101396	0.938626319
190	191	192	193	194	195	196
0.966749924	0.960723638	0.923931690	0.955420023	0.971482416	0.960723638	0.929362482
197	198	199	200	201	202	203
0.971482416	0.881207506	0.961141314	0.971482416	0.546060844	0.855557365	0.971482416
204	205	206	207	208	209	210
0.231669683	0.694083663	0.987645908	0.960723638	0.987645908	0.966749924	0.044480787
211	212	213	214	215	216	217

0.019491028	0.185399225	0.929550617	0.696772424	0.918335243	0.680463299	0.938009157
218	219	220	221	222	223	224
0.987645908	0.987645908	0.062108646	0.971482416	0.883417512	0.960723638	0.657114932
225	226	227	228	229	230	231
0.977101396	0.979435637	0.786580773	0.376496427	0.930283112	0.960723638	0.979435637
232	233	234	235	236	237	238
0.987645908	0.783917199	0.973145579	0.793497269	0.973145579	0.881207506	0.318363375
239	240	241	242	243	244	245
0.853459414	0.694083663	0.987645908	0.881207506	0.604979428	0.908185867	0.987645908
246	247	248	249	250	251	252
0.011478823	0.121983933	0.977101396	0.967525518	0.431998257	0.662780940	0.231669683
253	254	255	256	257	258	259
0.312458323	0.971482416	0.971482416	0.934399896	0.642756738	0.417312619	0.907299710
260	261	262	263	264	265	266
0.971482416	0.881207506	0.960723638	0.985325509	0.285426214	0.881207506	0.194675489
267	268	269	270	271	272	273
0.000986634	0.874498145	0.285426214	0.971482416	0.973145579	0.934904404	0.732921676
274	275	276	277	278	279	280
0.220658074	0.694083663	0.953294254	0.971482416	0.469167700	0.881207506	0.828377154
281	282	283	284	285	286	287
0.973145579	0.807531097	0.916749183	0.881207506	0.971482416	0.807531097	0.971482416
288	289	290	291	292	293	294
0.971482416	0.955420023	0.973145579	0.579859080	0.071158658	0.463889424	0.971482416
295	296	297	298	299	300	301
0.285426214	0.785407644	0.729166229	0.836787727	0.973145579	0.955420023	0.278167103
302	303	304	305	306	307	308
0.977101396	0.961141314	0.069550488	0.960723638	0.973145579	0.071158658	0.987645908
309	310	311	312	313	314	315
0.287863007	0.614639823	0.002497956	0.838204600	0.062108646	0.978560840	0.971482416
316	317	318	319	320	321	322
0.231669683	0.971482416	0.231669683	0.786245689	0.990720705	0.006651754	0.722115304
323	324	325	326	327	328	329
0.964297024	0.908185867	0.882596339	0.938425973	0.960723638	0.971482416	0.960723638
330	331	332	333	334	335	336
0.981440843	0.597014623	0.955420023	0.977101396	0.985325509	0.960723638	0.907299710
337	338	339	340	341	342	343
0.857754835	0.915027126	0.971482416	0.898442745	0.971482416	0.971482416	0.312458323
344	345	346	347	348	349	350
0.971482416	0.957541286	0.971482416	0.023069864	0.971482416	0.966749924	0.679612272
351	352	353	354	355	356	357
0.696772424	0.977101396	0.550652003	0.063069535	0.650949016	0.321373697	0.745630391
358	359	360	361	362	363	364
0.881207506	0.971482416	0.767957983	0.971482416	0.971482416	0.463889424	0.139440640
365	366	367	368	369	370	371
0.081327072	0.785407644	0.981440843	0.021010282	0.971482416	0.987645908	0.033794544

372	373	374	375	376	377	378
0.987645908	0.698219486	0.816135656	0.971482416	0.966749924	0.760278947	0.696772424
379	380	381	382	383	384	385
0.728924220	0.783917199	0.010420715	0.960723638	0.987645908	0.312458323	0.569228505
386	387	388	389	390	391	392
0.874498145	0.981119026	0.960723638	0.853459414	0.987645908	0.694083663	0.716308382
393	394	395	396	397	398	399
0.881207506	0.971482416	0.024893801	0.922184489	0.377760757	0.671417923	0.960723638
400	401	402	403	404	405	406
0.231669683	0.971482416	0.979802909	0.971482416	0.987645908	0.971482416	0.971482416
407	408	409	410	411	412	413
0.888204010	0.987645908	0.055351314	0.971482416	0.910296457	0.960723638	0.579859080
414	415	416	417	418	419	420
0.229784305	0.997795708	0.971482416	0.728908836	0.971482416	0.132724273	0.971482416
421	422	423	424	425	426	427
0.916749183	0.987645908	0.694083663	0.985680037	0.896809199	0.981440843	0.971482416
428	429	430	431	432	433	434
0.971482416	0.569228505	0.694083663	0.927208509	0.960723638	0.807811565	0.921678078
435	436	437	438	439	440	441
0.793497269	0.918335243	0.977101396	0.971482416	0.987645908	0.881207506	0.969145551
442	443	444	445	446	447	448
0.278461117	0.971482416	0.960723638	0.828377154	0.955420023	0.918335243	0.971482416
449	450	451	452	453	454	455
0.969145551	0.918335243	0.900277974	0.961141314	0.955420023	0.865503880	0.987645908
456	457	458	459	460	461	462
0.960723638	0.971482416	0.987645908	0.977101396	0.971482416	0.987645908	0.987645908
463	464	465	466	467	468	469
0.971482416	0.777227965	0.981440843	0.908185867	0.938009157	0.973145579	0.961141314
470	471	472	473	474	475	476
0.971482416	0.918335243	0.987645908	0.849348037	0.694083663	0.483876927	0.987645908
477	478	479	480	481	482	483
0.987645908	0.987645908	0.230496040	0.881207506	0.739025096	0.853459414	0.979435637
484	485	486	487	488	489	490
0.786580773	0.971482416	0.881207506	0.960723638	0.755093360	0.694083663	0.671417923
491	492	493	494	495	496	497
0.816541774	0.874498145	0.928224593	0.604979428	0.960723638	0.694083663	0.821639951
498	499	500	501	502	503	504
0.977101396	0.811970466	0.987645908	0.908185867	0.849348037	0.997795708	0.546060844
505	506	507	508	509	510	511
0.918335243	0.971482416	0.987645908	0.979802909	0.977101396	0.971482416	0.971482416
512	513	514	515	516	517	518
0.971482416	0.987645908	0.703404851	0.884576704	0.987645908	0.883417512	0.987645908
519	520	521	522	523	524	525
0.785407644	0.828377154	0.732921676	0.694083663	0.938009157	0.971482416	0.881207506
526	527	528	529	530	531	532

0.987645908	0.987645908	0.987645908	0.960723638	0.971482416	0.987645908	0.881207506
533	534	535	536	537	538	539
0.786245689	0.961141314	0.971482416	0.910296457	0.971482416	0.918335243	0.960723638
540	541	542	543	544	545	546
0.777227965	0.714119766	0.971482416	0.987645908	0.987645908	0.732921676	0.980607100
547	548	549	550	551	552	553
0.977101396	0.985325509	0.960723638	0.998023998	0.987645908	0.955420023	0.160337782
554	555	556	557	558	559	560
0.955420023	0.431998257	0.387666788	0.828377154	0.981440843	0.318609936	0.977101396
561	562	563	564	565	566	567
0.696772424	0.960723638	0.163663100	0.960723638	0.768267766	0.987645908	0.977101396
568	569	570	571	572	573	574
0.881207506	0.642756738	0.753443761	0.929550617	0.985325509	0.971482416	0.910545435
575	576	577	578	579	580	581
0.918335243	0.960723638	0.979802909	0.694083663	0.291225839	0.291225839	0.821639951
582	583	584	585	586	587	588
0.971482416	0.069041388	0.971482416	0.971482416	0.971482416	0.732921676	0.918335243
589	590	591	592	593	594	595
0.971482416	0.971482416	0.960723638	0.960723638	0.987645908	0.918335243	0.843436340
596	597	598	599	600	601	602
0.712086958	0.977101396	0.767957983	0.971482416	0.971482416	0.881207506	0.960723638
603	604	605	606	607	608	609
0.881207506	0.971482416	0.335358498	0.987645908	0.987645908	0.971482416	0.318363375
610	611	612	613	614	615	616
0.971482416	0.944296301	0.955420023	0.971482416	0.977101396	0.881207506	0.786245689
617	618	619	620	621	622	623
0.971482416	0.971482416	0.960723638	0.556876250	0.971482416	0.573981959	0.987645908
624	625	626	627	628	629	630
0.556876250	0.973145579	0.955420023	0.985680037	0.971482416	0.987645908	0.979802909
631	632	633	634	635	636	637
0.971482416	0.422197328	0.971482416	0.987645908	0.971482416	0.971482416	0.934904404
638	639	640	641	642	643	644
0.955420023	0.915027126	0.971482416	0.857630050	0.971482416	0.997795708	0.881207506
645	646	647	648	649	650	651
0.971482416	0.908185867	0.816135656	0.694083663	0.969145551	0.971482416	0.987645908
652	653	654	655	656	657	658
0.960723638	0.694083663	0.556876250	0.786245689	0.979802909	0.967525518	0.998910167
659	660	661	662	663	664	665
0.938626319	0.987170893	0.910296457	0.231669683	0.960723638	0.987645908	0.953294254
666	667	668	669	670	671	672
0.971482416	0.885835104	0.699999812	0.971482416	0.290403321	0.918335243	0.987645908
673	674	675	676	677	678	679
0.961141314	0.865503880	0.760278947	0.971482416	0.918335243	0.727501825	0.997795708
680	681	682	683	684	685	686
0.315981643	0.971482416	0.971482416	0.977101396	0.971482416	0.664924913	0.425141341

687	688	689	690	691	692	693
0.971482416	0.960723638	0.971482416	0.987645908	0.977101396	0.546060844	0.971482416
694	695	696	697	698	699	700
0.971482416	0.979802909	0.961141314	0.971482416	0.881207506	0.886526168	0.978560840
701	702	703	704	705	706	707
0.694083663	0.895674825	0.895674825	0.977101396	0.918335243	0.231669683	0.849348037
708	709	710	711	712	713	714
0.597014623	0.973145579	0.950874611	0.936799204	0.785407644	0.974874576	0.767957983
715	716	717	718	719	720	721
0.979802909	0.883417512	0.996068481	0.987645908	0.853459414	0.920968340	0.573981959
722	723	724	725	726	727	728
0.971482416	0.865503880	0.977101396	0.081327072	0.885835104	0.979435637	0.998910167
729	730	731	732	733	734	735
0.629028520	0.971482416	0.971482416	0.920968340	0.662780940	0.463889424	0.819363669
736	737	738	739	740	741	742
0.997795708	0.960723638	0.971482416	0.918335243	0.971482416	0.933176207	0.979802909
743	744	745	746	747	748	749
0.971482416	0.987645908	0.613708222	0.785306877	0.971482416	0.971482416	0.569141838
750	751	752	753	754	755	756
0.987645908	0.960723638	0.971482416	0.890467849	0.934904404	0.971482416	0.987645908
757	758	759	760	761	762	763
0.896809199	0.920968340	0.849348037	0.987645908	0.307054769	0.927208509	0.987645908
764	765	766	767	768	769	770
0.973145579	0.604979428	0.971482416	0.997795708	0.987645908	0.934904404	0.997795708
771	772	773	774	775	776	777
0.558270210	0.960723638	0.900277974	0.312458323	0.955420023	0.255598505	0.727501825
778	779	780	781	782	783	784
0.961141314	0.955420023	0.915027126	0.971482416	0.960723638	0.971482416	0.121983933
785	786	787	788	789	790	791
0.569141838	0.881207506	0.960723638	0.979802909	0.969145551	0.973145579	0.971482416
792	793	794	795	796	797	798
0.987645908	0.971482416	0.662780940	0.971482416	0.973145579	0.961141314	0.987645908
799	800	801	802	803	804	805
0.769396818	0.376496427	0.881207506	0.987645908	0.971482416	0.881207506	0.955420023
806	807	808	809	810	811	812
0.546060844	0.853459414	0.971482416	0.971482416	0.773748256	0.971482416	0.955420023
813	814	815	816	817	818	819
0.985680037	0.977101396	0.971482416	0.960723638	0.923931690	0.768267766	0.973145579
820	821	822	823	824	825	826
0.971482416	0.977101396	0.598569723	0.979802909	0.969145551	0.918335243	0.985680037
827	828	829	830	831	832	833
0.771217169	0.960723638	0.971482416	0.979802909	0.220658074	0.971482416	0.973145579
834	835	836	837	838	839	840
0.971482416	0.376100488	0.987645908	0.979802909	0.979802909	0.803688065	0.971482416
841	842	843	844	845	846	847

0.960723638	0.960723638	0.694083663	0.950874611	0.971482416	0.696772424	0.814846007
848	849	850	851	852	853	854
0.387666788	0.987645908	0.979435637	0.971482416	0.966749924	0.987645908	0.974874576
855	856	857	858	859	860	861
0.985325509	0.987645908	0.881207506	0.971482416	0.971482416	0.971482416	0.964297024
862	863	864	865	866	867	868
0.998910167	0.971482416	0.979802909	0.987645908	0.971482416	0.971482416	0.960723638
869	870	871	872	873	874	875
0.920968340	0.971482416	0.960723638	0.874498145	0.918335243	0.881207506	0.662780940
876	877	878	879	880	881	882
0.955420023	0.918335243	0.973145579	0.971482416	0.971482416	0.971482416	0.961141314
883	884	885	886	887	888	889
0.918335243	0.960723638	0.985325509	0.934904404	0.881207506	0.960723638	0.960723638
890	891	892	893	894	895	896
0.732921676	0.291225839	0.785407644	0.915027126	0.979802909	0.546060844	0.987170893
897	898	899	900	901	902	903
0.487881308	0.410479342	0.971482416	0.960723638	0.971482416	0.960723638	0.979802909
904	905	906	907	908	909	910
0.955420023	0.979802909	0.927208509	0.918335243	0.881207506	0.971482416	0.960723638
911	912	913	914	915	916	917
0.971482416	0.836787727	0.961141314	0.979802909	0.977101396	0.444708100	0.971482416
918	919	920	921	922	923	924
0.971482416	0.696772424	0.642756738	0.974874576	0.955420023	0.908185867	0.748834667
925	926	927	928	929	930	931
0.019491028	0.953294254	0.950874611	0.694083663	0.318363375	0.987645908	0.979802909
932	933	934	935	936	937	938
0.979802909	0.908185867	0.971482416	0.960723638	0.955420023	0.973145579	0.971482416
939	940	941	942	943	944	945
0.987645908	0.971482416	0.938009157	0.973145579	0.973145579	0.987645908	0.231669683
946	947	948	949	950	951	952
0.977101396	0.985680037	0.960723638	0.971482416	0.960723638	0.971482416	0.971482416
953	954	955	956	957	958	959
0.821639951	0.971482416	0.908185867	0.916749183	0.964297024	0.918335243	0.918335243
960	961	962	963	964	965	966
0.546060844	0.938626319	0.220658074	0.987645908	0.979802909	0.755093360	0.971482416
967	968	969	970	971	972	973
0.881207506	0.987645908	0.971482416	0.771217169	0.955420023	0.768954459	0.953294254
974	975	976	977	978	979	980
0.960723638	0.786245689	0.934904404	0.971482416	0.971482416	0.955420023	0.971482416
981	982	983	984	985	986	987
0.971482416	0.768267766	0.556876250	0.971482416	0.971482416	0.996068481	0.231669683
988	989	990	991	992	993	994
0.973145579	0.971482416	0.920968340	0.960723638	0.105404445	0.979802909	0.855557365
995	996	997	998	999	1000	
0.291914137	0.987645908	0.907299710	0.987170893	0.881207506	0.849348037	

References

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