

Agilp Manual Vignette

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Introduction

This package was developed to provide a pipeline for the low-level analysis of gene expression microarray data, primarily focused on the Agilent platform, but which also provides utilities which may be useful for other platforms. Agilp v2.0 is made up of five processing functions, which are described briefly below and in more detail in the individual help files for each function. The package is designed for people with little previous experience of R, or of microarray analysis. The package was developed for use in the Innate2Adaptive Immunity Laboratory, Division of Infection and Immunity, UCL, London run by Benny Chain and Maddy Noursadeghi. A number of references describing the microarray work from the laboratory are given at the end of this document.

A note on default paths in agilp functions

The default folders for all the functions in agilp use folders input, input1 and output within the agilp folder. The user files to be processed (e.g. array scanner files or the output of agilp functions themselves) should be placed within the input or input1 folders as instructed in the help files. Note that after installing the package these folders will initially contain a dummy file called toberemoved.txt in order to avoid the installation process removing empty folders. These dummy files should be deleted before use. To locate the path of the agilp folder on your computer type

```
> file.path(system.file(package="agilp"))
```

```
[1] "/private/var/folders/v1/y6dg5h4n163dzmrf16t_r5480000gp/T/RtmpBjkTKh/Rinstbaa81f30b1d9/agilp"
```

Alternatively, the user can create their own input and output directories, and alter the path description in the function call as appropriate.

Workflow

A typical workflow for using the package is described below. The examples are designed to be implemented sequentially.

- **AAProcess. Extract and save the red and green median expression data from Agilent scanner arrays, averaging replicate probes.** The raw (unprocessed) expression data is first extracted from the scanner files which are in tab delimited .txt format. This step is platform specific, and uses the column *gMedianSignal* or *rMedianSignal* to identify the data to use. The expression values for replicate probes are averaged. The function can use single or two colour outputs, and treats each colour independently. Output is one spreadsheet per microarray per colour, saved as tab delimited text files to a directory of choice. Each file contains all probe names (first column) and raw (untransformed, unnormalized) expression levels (second column).

Examples. This example extracts expression data from two sample array scanner files which are found in the folder "scanner" within the agilp package extdata folder and places them in the folder output within the agilp package folder.

```
> library(agilp)
> inputdir<-file.path(system.file(package="agilp"),"extdata","scanner/", "", fsep = .Platform$file.sep)
> outputdir<-file.path(system.file(package="agilp"),"output/", "", fsep = .Platform$file.sep)
> AAProcess(input = inputdir, output = outputdir, s = 9)
```

To remove these files again and empty the output directory use :

```
> unlink(file.path(system.file(package="agilp"),"output", "", "*. *" ,
+ fsep = .Platform$file.sep), recursive=FALSE)
```

The number of lines header to be skipped from scanner array files can be changed from the default value of 9 by setting variable s. AAProcess(input = inputdir, output = outputdir, s = 10)

- **filenamex. Copy extracted raw file names to template.** Typically, the array file names are stored in a database. We use a simple spreadsheet for this (e.g. Excel or other) which contains details such as the date the experiments were run, the types of samples, the experimenter, and any other important experimental detail (see *sample_template.txt* in the *agilp* *extdata* folder for an example). However, a major source of documentation error occurs while entering the array file names into the template file (array file names are often very long arbitrary alphanumeric sequences). For this reason we use *filenamex* which outputs a text file containing the names of all the files in a directory; this list can be easily copied and pasted directly into a template file.

Examples. This example makes a list of the names of all the files in the folder *agilp/extdata/raw* and saves it in a file called *names.txt* (tab delimited) in the folder *agilp/output*.

```
> library(agilp)
> inputdir<-file.path(system.file(package="agilp"),"extdata","raw/", "", fsep = .Platform$file.sep)
> outputdir<-file.path(system.file(package="agilp"),"output/", "", fsep = .Platform$file.sep)
> filenamex(input=inputdir,output=outputdir)
```

To remove these files again and empty the output directory use :

```
> unlink(file.path(system.file(package="agilp"),"output", "", "*. *" ,
+ fsep = .Platform$file.sep), recursive=FALSE)
```

- **Baseline. Generate a normalisation baseline file.**

The *AALoess* normalisation function (see below) requires a ‘baseline’ file which contains the mean value for each probe from a number of different arrays against which to normalise each new array. *Baseline* generates such a baseline from a set of arrays. The set can be either all arrays in a chosen directory, or selected from a template file (see help file for more details). By default, all array data is base 2 log transformed before calculating the mean. If the files to be analysed contain different numbers of identifiers, the output will contain only the data for the IDs common to the whole set.

Examples. Takes all the files of unprocessed data in folder *agilp/extdata/raw* , calculates the mean expression value for each probe, and saves as *agilp/output/testbase.txt*.

```
> library(agilp)
> inputdir<-file.path(system.file(package="agilp"),"extdata","raw/", "", fsep = .Platform$file.sep)
> outputbase<-file.path(system.file(package="agilp"),"output", "testbase.txt", fsep = .Platform$file.sep)
> template<-file.path(system.file(package="agilp"),"extdata","sample_template.txt", fsep = .Platform$file.sep)
> Baseline(NORM="LOG",allfiles="TRUE",r=2,A=2,B=3,input=inputdir, baseout=outputbase, t = template)
```

Alternatively the following example uses only those data files defined in column 2, rows 2-5 of the template file. In addition, this example does not log transform the data.

```
> Baseline(NORM="FALSE",allfiles="FALSE",r=2,A=2,B=5,input=inputdir, baseout=outputbase, t = template)
```

To remove these files again and empty the output directory use :

```
> unlink(file.path(system.file(package="agilp"),"output", "", "*. *" ,
+ fsep = .Platform$file.sep), recursive=FALSE)
```

- **Loader. Select set of processed array files from directory according to criteria from a template file.** It is often necessary to select a series of expression array data files (for example generated by *AAProcess*) from a directory containing many such files (our repository has around 1000 array data sets so far) according to some criteria in the template file. For example, one might want to analyze all samples from particular dates, or tissue type etc. *Loader* inputs a template file in which each row contains the details for a separate array, and which has previously been sorted according to some predefined criteria (see *sample_template.txt* in the *agilp/extdata* for an example). It uses the array file names for a set of rows of the template (first and last row chosen by user, but must be contiguous), and either (if *f* = “TRUE”) transfers the chosen files to a different directory, or outputs a single file (by default saved as *alldata.txt*) in which each column contains one array set of data.

Examples. This will copy the files with names given in column 2, rows 2:3 of the sample template file from the input folder to output folder.

```
> inputdir<-file.path(system.file(package="agilp"),"extdata","raw/", "", fsep = .Platform$file.sep)
> outputdir<-file.path(system.file(package="agilp"),"output/", "", fsep = .Platform$file.sep)
> template<-file.path(system.file(package="agilp"),"extdata","sample_template.txt",
```

```
+ fsep = .Platform$file.sep)
> Loader(input=inputdir,output=outputdir,t=template,f="TRUE",r=2,A=2,B=5)
```

Alternatively this will output a single file all_data.txt with the same data in a merged file. The file is also output back to R as object dataout

```
> Loader(input=inputdir,output=outputdir,t=template,f="FALSE",r=2,A=2,B=5)
```

```
gRaw_arrays_scanner_file1.txt
(+)E1A_r60_1      84.76562
(+)E1A_r60_3      86.65625
(+)E1A_r60_a104   85.42188
(+)E1A_r60_a107   84.73438
(+)E1A_r60_a135   84.98438
(+)E1A_r60_a20    90.53125
(+)E1A_r60_a22    99.98438
(+)E1A_r60_a97    83.65625
(+)E1A_r60_n11    87.15625
(+)E1A_r60_n9     85.90625
(+)eQC-39         83.25000
(+)eQC-40         80.20000
(+)eQC-41         83.15000
(+)eQC-42         84.55000
3xSLv1           84.34967
A_23_P100001     1244.00000
A_23_P100011     145.00000
A_23_P100022     134.00000
A_23_P100056     288.50000
A_23_P100074     1641.00000
A_23_P100092     372.00000
A_23_P100103     173.00000
A_23_P100111     128.00000
A_23_P100127     489.00000
A_23_P100133     184.00000
A_23_P100141     562.00000
A_23_P100156     253.00000
A_23_P100177     116.00000
A_23_P100189      99.00000
A_23_P100196    1209.00000
A_23_P100203    2508.00000
A_23_P100220    1887.50000
A_23_P100240     140.00000
A_23_P10025      191.00000
A_23_P100263    1572.50000
A_23_P100278     110.00000
A_23_P100292    9570.00000
A_23_P100315     3100.00000
A_23_P100326     4399.50000
A_23_P100341    10593.50000
A_23_P100344     1922.00000
A_23_P100355     2719.00000
A_23_P100386      92.50000
A_23_P100392     804.50000
A_23_P100408      80.50000
A_23_P100413     239.00000
A_23_P100420    3512.50000
A_23_P100441    1324.00000
A_23_P100455     328.00000
A_23_P100469     427.00000
A_23_P100478     785.50000
A_23_P100486    11739.00000
```

A_23_P100499	152.00000
A_23_P100501	3271.00000
A_23_P100517	586.00000
A_23_P100522	4774.00000
A_23_P100539	387.00000
A_23_P100556	649.00000
A_23_P100576	5485.00000
A_23_P100583	103.00000
A_23_P100595	94.50000
A_23_P10060	79.00000
A_23_P100602	687.00000
A_23_P10062	91.00000
A_23_P100632	19335.00000
A_23_P100642	366.00000
A_23_P100654	1244.00000
A_23_P100660	3949.00000
A_23_P100676	375.00000
A_23_P100693	670.50000
A_23_P100704	592.50000
A_23_P100711	3972.50000
A_23_P100730	436.00000
A_23_P100754	231.50000
A_23_P100764	2644.00000
A_23_P10077	270.50000
A_23_P100779	367.50000
A_23_P100788	211.00000
A_23_P100795	408.00000
A_23_P100802	7216.00000
A_23_P10081	178.00000
A_23_P100868	2487.00000
A_23_P100872	97.00000
A_23_P100883	2943.00000
A_23_P10091	163.50000
A_23_P100912	190.00000
A_23_P100931	80.00000
A_23_P100935	82.00000
A_23_P100959	99.00000
A_23_P100963	223.00000
A_23_P100990	101.00000
A_23_P100991	81.00000
A_23_P101007	387.00000
A_23_P101013	1405.00000
A_23_P101025	364.00000
A_23_P101038	83.00000
A_23_P101048	98.50000
A_23_P101054	180.00000
A_23_P101073	106.00000
A_23_P101084	98.00000
A_23_P101093	827.00000
A_23_P101111	857.00000
A_23_P101121	511.00000
A_23_P101131	93.00000
A_23_P101141	196.00000
A_23_P101185	4124.00000
A_23_P101193	563.00000
A_23_P101208	3651.00000
A_23_P10121	3057.00000
A_23_P101237	1711.00000
A_23_P101240	15390.50000
A_23_P101246	379.00000

A_23_P101253	381.50000
A_23_P101258	220.00000
A_23_P10127	7540.00000
A_23_P101272	1224.50000
A_23_P101281	497.50000
A_23_P101297	295.00000
A_23_P101303	1100.00000
A_23_P101308	263.50000
A_23_P101319	130.00000
A_23_P101321	159.50000
A_23_P101332	1987.00000
A_23_P101342	316.00000
A_23_P10135	546.50000
A_23_P101351	740.00000
A_23_P101374	203.00000
A_23_P101380	834.00000
A_23_P101392	320.00000
A_23_P1014	1205.50000
A_23_P101407	7430.50000
A_23_P101427	166.50000
A_23_P101434	106.00000
A_23_P101461	6686.00000
A_23_P101476	161.50000
A_23_P101480	356.00000
A_23_P101505	794.50000
A_23_P101516	175.00000
A_23_P101521	78.00000
A_23_P101532	64534.20000
A_23_P101551	2714.50000
A_23_P10156	394.00000
A_23_P101564	91.00000
A_23_P101573	83.00000
A_23_P101615	330.00000
A_23_P101623	135.00000
A_23_P101636	84.00000
A_23_P101642	805.00000
A_23_P101655	1207.00000
A_23_P101671	231.00000
A_23_P101683	81.50000
A_23_P101699	566.00000
A_23_P101707	1906.50000
A_23_P10172	92.00000
A_23_P101737	339.00000
A_23_P101742	1413.00000
A_23_P101759	205.50000
A_23_P101761	910.00000
A_23_P101774	89.00000
A_23_P101783	115.00000
A_23_P101796	392.00000
A_23_P101806	698.00000
A_23_P101811	223.00000
A_23_P10182	603.00000
A_23_P101829	215.00000
A_23_P101871	326.00000
A_23_P101893	163.00000
A_23_P101905	269.00000
A_23_P101908	192.50000
A_23_P10194	5600.00000
A_23_P101943	1189.00000
A_23_P101950	9901.00000

A_23_P101960	7311.00000
A_23_P101972	156.00000
A_23_P101992	169.00000
A_23_P102000	3021.10000
A_23_P102017	95.00000
A_23_P102037	282.00000
A_23_P102058	352.00000
A_23_P10206	297.00000
A_23_P102060	1751.00000
A_23_P102071	975.50000
A_23_P102083	68.00000
A_23_P102109	1917.00000
A_23_P10211	379.00000
A_23_P102113	279.00000
A_23_P102117	133.00000
A_23_P102122	6253.50000
A_23_P102160	205.00000
A_23_P102172	79.00000
A_23_P102183	2426.00000
A_23_P102192	355.00000
A_23_P102202	11726.00000
A_23_P102235	5885.00000
A_23_P102242	683.00000
A_23_P102258	5873.50000
A_23_P102262	7163.00000
A_23_P102278	87.00000
A_23_P102286	85.50000
A_23_P10232	99.00000
A_23_P102320	1915.00000
A_23_P102331	81.00000
A_23_P102351	184.00000
A_23_P102364	2503.50000
A_23_P102391	386.00000
A_23_P102404	21629.90000
A_23_P102412	747.00000
A_23_P102420	49593.30000
A_23_P102439	119.00000
A_23_P102454	2043.00000
A_23_P102462	117.00000
A_23_P102471	3809.15000
A_23_P102481	89.00000
A_23_P102494	149.00000
A_23_P102508	1182.50000
A_23_P102517	1165.00000
A_23_P102523	288.50000
A_23_P102542	931.50000
A_23_P102551	95.50000
A_23_P102571	15099.00000
A_23_P102575	3246.50000
A_23_P102582	9381.00000
A_23_P102607	105.50000
A_23_P102611	8720.50000
A_23_P102622	285.00000
A_23_P102634	79.00000
A_23_P102681	135.00000
A_23_P102694	83.50000
A_23_P102706	247.00000
A_23_P102731	781.00000
A_23_P102743	287.00000
A_23_P102759	138.00000

A_23_P102769	498.00000
A_23_P102770	1588.00000
A_23_P102816	216.00000
A_23_P102832	2042.00000
A_23_P102842	2041.50000
A_23_P102864	242.50000
A_23_P102876	12896.00000
A_23_P102884	129.00000
A_23_P102890	7136.00000
A_23_P1029	467.00000
A_23_P10291	114.00000
A_23_P102917	91.50000
A_23_P102919	87.00000
A_23_P102925	1436.00000
A_23_P102937	1490.00000
A_23_P102950	180.00000
A_23_P102965	600.00000
A_23_P102973	726.00000
A_23_P102988	173.00000
A_23_P102994	3032.00000
A_23_P103011	537.00000
A_23_P103034	90.00000
A_23_P103041	106.00000
A_23_P10305	257.00000
A_23_P103062	281.00000
A_23_P103070	2849.00000
A_23_P103084	177.00000
A_23_P103099	472.00000
A_23_P103104	488.00000
A_23_P103110	717.00000
A_23_P103120	3092.00000
A_23_P103131	3394.00000
A_23_P103149	5649.50000
A_23_P103172	2215.00000
A_23_P103180	680.00000
A_23_P103201	2201.00000
A_23_P103226	98.00000
A_23_P103232	6305.00000
A_23_P103248	533.00000
A_23_P103256	197.50000
A_23_P103269	114.50000
A_23_P103276	299.00000
A_23_P103282	9325.00000
A_23_P103310	107.50000
A_23_P103315	11200.50000
A_23_P103328	128.00000
A_23_P103334	192.00000
A_23_P103349	83.50000
A_23_P103361	1188.75000
A_23_P103371	135.00000
A_23_P103398	2641.00000
A_23_P103405	178.00000
A_23_P103414	4946.00000
A_23_P103424	81.00000
A_23_P103433	534.50000
A_23_P103442	2955.00000
A_23_P103454	11044.00000
A_23_P103465	118.50000
A_23_P103476	698.00000
A_23_P103486	214.00000

A_23_P103496	108.00000
A_23_P103503	554.00000
A_23_P103511	712.50000
A_23_P103522	82.00000
A_23_P103532	217.00000
A_23_P10356	102.00000
A_23_P103561	274.00000
A_23_P103571	121.00000
A_23_P103588	96.00000
A_23_P103601	1778.50000
A_23_P103617	224.00000
A_23_P103628	2768.00000
A_23_P103631	16923.50000
A_23_P103661	3438.00000
A_23_P103672	2898.50000
A_23_P103690	1005.00000
A_23_P103698	3372.00000
A_23_P103703	166.50000
A_23_P103720	1096.00000
A_23_P10374	3247.00000
A_23_P103743	87.00000
A_23_P103756	156.00000
A_23_P103765	81.50000
A_23_P103775	160.00000
A_23_P103787	1023.00000
A_23_P103795	617.00000
A_23_P1038	196.00000
A_23_P103803	81.00000
A_23_P103812	144.50000
A_23_P103824	1076.00000
A_23_P103837	819.00000
A_23_P10385	1040.40000
A_23_P103864	485.00000
A_23_P103877	914.00000
A_23_P103885	8531.00000
A_23_P103897	104.00000
A_23_P103905	1208.50000
A_23_P10391	430.00000
A_23_P103919	1757.00000
A_23_P103923	131.00000
A_23_P103932	1080.00000
A_23_P103942	1387.50000
A_23_P103951	5832.00000
A_23_P103968	1337.00000
A_23_P103971	97.00000
A_23_P103981	19732.00000
A_23_P103996	629.00000
A_23_P10401	251.50000
A_23_P104012	92.00000
A_23_P104022	2387.00000
A_23_P104025	569.00000
A_23_P104046	501.00000
A_23_P104054	1738.50000
A_23_P104065	911.00000
A_23_P104073	181.00000
A_23_P104086	173.00000
A_23_P104098	100.00000
A_23_P104109	457.00000
A_23_P104116	21624.70000
A_23_P104138	219.00000

A_23_P104146	728.00000
A_23_P104151	424.00000
A_23_P104188	540.00000
A_23_P104193	2763.00000
A_23_P104199	1128.55000
A_23_P104201	5256.00000
A_23_P104224	229.00000
A_23_P104237	98.00000
A_23_P104252	226.00000
A_23_P104275	90.00000
A_23_P104282	254.00000
A_23_P104295	297.00000
A_23_P1043	539.00000
A_23_P104318	21087.70000
A_23_P104323	586.00000
A_23_P104335	80.00000
A_23_P104346	457.00000
A_23_P104351	2140.00000
A_23_P104362	6140.00000
A_23_P104372	6645.00000
A_23_P104387	4674.00000
A_23_P104413	9091.00000
A_23_P10442	876.00000
A_23_P104438	196.50000
A_23_P104445	177.50000
A_23_P104451	5345.00000
A_23_P104464	181.00000
A_23_P104471	1338.00000
A_23_P104484	80.00000
A_23_P104493	321.00000
A_23_P104509	121.00000
A_23_P104522	166.00000
A_23_P104555	91.00000
A_23_P104563	224.00000
A_23_P104579	569.00000
A_23_P10458	91.00000
A_23_P104583	443.50000
A_23_P104594	102.00000
A_23_P104607	2069.00000
A_23_P104617	244.50000
A_23_P104624	1572.00000
A_23_P10463	16057.00000
A_23_P104641	2339.00000
A_23_P104651	5964.00000
A_23_P104676	3952.00000
A_23_P104689	525.45000
A_23_P104692	694.00000
A_23_P104705	179.00000
A_23_P104734	4757.50000
A_23_P104741	250.00000
A_23_P104762	545.00000
A_23_P104798	186.10000
A_23_P104804	277.50000
A_23_P104819	98.00000
A_23_P104845	89.00000
A_23_P104853	94.50000
A_23_P104865	79.00000
A_23_P104876	1045.00000
A_23_P10488	160.00000
A_23_P104881	117.50000

A_23_P104892	13696.00000
A_23_P104942	3675.00000
A_23_P10497	499.00000
A_23_P104972	90.00000
A_23_P104996	125.00000
A_23_P10500	85.00000
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A_23_P111171	15640.0000
A_23_P111188	1657.0000
A_23_P11119	122.0000
A_23_P111194	929.0000
A_23_P111206	1437.0000
A_23_P111228	1647.0000
A_23_P111240	8219.5000
A_23_P111260	531.0000
A_23_P111267	254.0000
A_23_P111273	18023.0000
A_23_P111288	111420.0000
A_23_P111297	1531.0000
A_23_P111303	5959.0000
A_23_P111306	2925.0000
A_23_P111311	244.0000
A_23_P111321	895.5000
A_23_P111343	12614.0000
A_23_P111360	673.5000
A_23_P111361	267.0000
A_23_P111373	15759.0000
A_23_P111381	12513.0000
A_23_P111395	201.0000
A_23_P111402	110.5000
A_23_P111413	1390.0000
A_23_P11144	659.0000
A_23_P111452	87240.2000
A_23_P111480	322.5000
A_23_P111481	75866.6000
A_23_P111487	2107.0000
A_23_P111492	1238.5000
A_23_P111506	589906.0000
A_23_P111517	288.0000
A_23_P111525	3045.5000
A_23_P111531	1006.0000
A_23_P111571	601132.0000
A_23_P111583	257553.0000
A_23_P111593	1028.0000
A_23_P11160	589.0000
A_23_P111604	133.0000
A_23_P111621	12597.5000
A_23_P111635	10722.0000
A_23_P111657	256.0000
A_23_P111662	180.0000
A_23_P111672	3361.0000
A_23_P111698	453.0000
A_23_P1117	667.0000
A_23_P111701	362.5000
A_23_P111712	1396.5000
A_23_P111724	129.0000
A_23_P111737	235.0000

A_23_P111745	14369.0000
A_23_P111753	29929.4000
A_23_P111766	1319.0000
A_23_P111797	8004.0000
A_23_P111804	15423.0000
A_23_P111811	3979.5000
A_23_P111826	172.0000
A_23_P111835	51332.3000
A_23_P111843	74128.6000
A_23_P111860	262.0000
A_23_P111865	1140.0000
A_23_P111888	321.0000
A_23_P111919	133.5000
A_23_P11192	32297.6000
A_23_P111929	422.0000
A_23_P111947	116.0000
A_23_P111961	4956.0000
A_23_P111978	526.0000
A_23_P111981	3947.5000
A_23_P111995	1500.0000
A_23_P112004	2790.5000
A_23_P11201	5492.0000
A_23_P112016	290.0000
A_23_P112026	387.0000
A_23_P112061	42474.7000
A_23_P112078	4556.0000
A_23_P112086	202.0000
A_23_P112100	127.5000
A_23_P112103	2445.0000
A_23_P112113	25867.4000
A_23_P112135	85984.6000
A_23_P11214	3756.0000
A_23_P112159	17420.5000
A_23_P112162	16918.0000
A_23_P112173	194.0000
A_23_P112187	325.0000
A_23_P112201	3832.0000
A_23_P112220	164.5000
A_23_P11224	39850.4000
A_23_P112241	47629.7000
A_23_P112251	94985.0000
A_23_P112260	29112.0000
A_23_P112275	164.0000
A_23_P112289	216.0000
A_23_P112296	148.5000
A_23_P112311	2030.5000
A_23_P112327	215.0000

```
> dim(dataout)
```

```
[1] 999 4
```

To remove these files again and empty the output directory use :

```
> unlink(file.path(system.file(package="agilp"), "output", "", "*. *" ,
+ fsep = .Platform$file.sep), recursive=FALSE)
```

For further detail on function variables see individual help files.

- **AAloess.** Normalises a set of gene expression data files using LOESS against a reference data set. An important aspect of analysing sets of microarray expression data is to be able to compare different experiments carried

out at different times. This requires being able to normalize data sets as far as possible correcting for global differences between experiments. *AALoess* function normalises data sets which share a common set of identifiers (typically probe names if all the experiments are carried out on a single platform) by comparison to a baseline file which typically contains an average of many datasets (for example as produced by *Baseline*). If the files to be analysed contain different numbers of identifiers, the output will contain only the data for the IDs common to the whole set. The underlying assumption is that the overall expression distribution over all the probes is the same in all samples, and that most probes are unchanged when comparing any two samples. The normalisation rationale is discussed in more detail in Chain et al. (see Bibliography) . The function also outputs a frequency histogram of the distances between each sample and the baseline which has proved useful in identifying technical outliers (e.g. if there is a problem in hybridization, for example).

Examples. This example takes the files in `agilp/extdata/raw`, *LOess* normalises them and saves them in `agilp/output` folder.

```
> inputdir<-file.path(system.file(package="agilp"),"extdata","raw/", "", fsep = .Platform$file.sep)
> outputdir<-file.path(system.file(package="agilp"),"output/", "", fsep = .Platform$file.sep)
> baselinedir<-file.path(system.file(package="agilp"),"extdata","testbase.txt", fsep = .Platform$file.sep)
> AALoess(input=inputdir, output=outputdir, baseline = baselinedir, LOG="TRUE")
```

After running this function review the SSE plot and file to identify outliers - note these in template file.

Alternatively the following example does not log transform the data.

```
> AALoess(input=inputdir, output=outputdir, baseline = baselinedir, LOG="FALSE")
```

To remove these files again and empty the output directory use :

```
> unlink(file.path(system.file(package="agilp"),"output", "", "*. *" ,
+ fsep = .Platform$file.sep), recursive=FALSE)
```

- **IDswop. Maps expression data across different bioinformatic identifiers.** *IDswop* replaces one set of identifiers associated with an expression data set (for example platform specific probe IDs) by another set (for example ENSEMBL transcript IDs). This allows expression data sets from different platforms to be combined for analysis . Any appropriate mapping spreadsheet file which contains the source identifiers in one column and the equivalent target IDs in another column can be used. We have typically used files produced by biomaRt, but suitable annotation files can also be made using the `select` function, in the AnnotationDbi package. If multiple source IDs(e.g. probe IDs) map to the same target ID the function returns the mean or the maximum value within the set.

Examples. This example maps expression data from Agilent ProbeIDs (found in column one of input files) to corresponding EnsemblID. Each output file has the name of the original file with the suffix "_EnsemblID". If multiple probes map to the same ID and vary by more than 20% they are discarded.

```
> inputdir<-file.path(system.file(package="agilp"),"extdata","raw/", "", fsep = .Platform$file.sep)
> outputdir<-file.path(system.file(package="agilp"),"output/", "", fsep = .Platform$file.sep)
> annotation<-file.path(system.file(package="agilp"),"extdata","annotations_sample.txt", fsep = .Platform$file.sep)
> IDswop(input=inputdir,output=outputdir,annotation=annotation,source_ID="ProbeID",
+ target_ID="EnsemblID", ERR=0.2)
```

Alternatively the following keeps all output IDs, even when multiple probes map to a single annotation.

```
> IDswop(input=inputdir,output=outputdir,annotation=annotation,source_ID="ProbeID",
+ target_ID="EnsemblID", ERR=1)
```

More details of how to use this function are given in the *IDSwop* individual help file. *IDSwop* and *Equaliser* (see below) should be used BEFORE *Baseline* and *AALoess*. To remove these files again and empty the output directory use :

```
> unlink(file.path(system.file(package="agilp"),"output", "", "*. *" ,
+ fsep = .Platform$file.sep), recursive=FALSE)
```

- **Equaliser. Trims a set of gene expression data files to include only the set of identifiers common to all files.** Different platforms will typically generate files with different numbers of distinct identifiers (for example compare the Agilent 44K and Agilent 60K whole genome expression arrays. Different numbers of identifiers will also often result from use of *IDSwop*. *Equaliser* is a utility which takes a set of gene expression data files and trims each file so as to include only the set of IDs which are common to all files in the set. More details of how to use this function are given in the *IDSwop* individual help file. *Equaliser* and *IDSwop* (see above) should be used BEFORE *Baseline* and *AALoess*.

Examples Takes four files in folder agilp/dataset/raw, selects only those entries common to all four, and outputs the shortened files to the folder agilp/output.

```
> inputdir<-file.path(system.file(package="agilp"),"extdata","raw/", "", fsep = .Platform$file.sep)
> outputdir<-file.path(system.file(package="agilp"),"output/", "", fsep = .Platform$file.sep)
> Equaliser(input = inputdir, output = outputdir)
```

To remove these files again and empty the output directory use :

```
> unlink(file.path(system.file(package="agilp"),"output", "", "*. *" ,
+ fsep = .Platform$file.sep), recursive=FALSE)
```

Examples

All the examples given above and in the individual help files use data files contained within the /extdata folder within the installed agilp folder. The output is sent to the agilp/output folder. To locate the path of the agilp folder on your computer type

```
> file.path(system.file(package="agilp"))
```

```
[1] "/private/var/folders/v1/y6dg5h4n163dzmrfl6t_r5480000gp/T/RtmpBjkTKh/Rinstbaa81f30b1d9/agilp"
```

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