

# Single-Channel Array Normalization (SCAN)

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## 1 Background

This vignette describes how to normalize samples with the *Single-Channel Array Normalization (SCAN)* method. This new approach has been described in detail in a recent paper (Piccolo et al., 2012). Individuals interested in understanding the motivations and methodology behind this method can read this paper for extensive details.

Briefly, SCAN is a single-sample approach for gene-expression microarrays. This means that the output for a given microarray sample will be the same whether that sample is processed in isolation or jointly with other samples. The probe-sequence content within each microarray is used to correct for binding-affinity biases that can arise during processing and to standardize variances across the probes. Because samples can be processed individually, the user can process extremely large batches of files with a modest computer-memory footprint. Unlike many normalization methods, SCAN can be applied to any Affymetrix microarray for which an annotation package (that has been constructed using the pdInfoBuilder package) exists in Bioconductor. In the paper mentioned above, we demonstrate that SCAN performs as well as or better than several popular normalization methods on simulated and “real-world” data sets.

## 2 How to use SCAN

This section demonstrates how to normalize an Affymetrix microarray file. In the examples below, an example CEL file is downloaded from Gene Expression Omnibus, saved to a temporary file, and then normalized using SCAN. Various optional parameters are also demonstrated.

The first step is to download an example CEL file (which was obtained via Gene Expression Omnibus).

```
> celFilePath = "Vignette_Example.CEL.gz"
> download.file("http://www.ncbi.nlm.nih.gov/geo/download/?acc=GSM555237&format=file&
```

To normalize the file, the `SCAN` function must be invoked. This function requires one mandatory parameter: a path specifying the location of the file to be normalized.

```
> library(SCAN.UPC)
> normalized = SCAN(celFilePath)
```

```
Reading in : ./Vignette_Example.CEL.gz
```

The `SCAN` function returns an *ExpressionSet* object containing a row for each probeset (transcript/gene) value. Detailed status information, including the number of iterations required for mathematical convergence of the mixture models, are printed to the console.

Multiple input files can also be specified using wildcard characters (e.g., "\*.CEL"). In this case, the `SCAN` function returns an *ExpressionSet* object with a row for each probeset and a column for each input file.

Using the optional `outFilePath` parameter, the normalized values also can be saved to a text file. The example below demonstrates this option. (The optional `verbose` parameter can also be used. When set to `FALSE`, `SCAN` outputs only minimal status information while processing.)

```
> normalized = SCAN(celFilePath, outFilePath="output_file.txt")
```

```
Reading in : ./Vignette_Example.CEL.gz
```

By default, `SCAN` uses the default mappings between probes and genes that have been provided by the manufacturer. However, these mappings may be outdated or may include

problematic probes (for example, those that cross hybridize). The default mappings also may produce multiple summary values per gene. Alternative mappings, such as those provided by the BrainArray resource (see [http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF/genomic\\_curated\\_CDF.asp](http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF/genomic_curated_CDF.asp)), allow SCAN to produce a single value per gene and to use updated gene definitions. Users can specify alternative mappings using the `probeSummaryPackage` parameter. If specified, this package must conform to the standards of the AnnotationDbi package. The BrainArray packages can be downloaded from [http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF/CDF\\_download.asp](http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF/CDF_download.asp). When using BrainArray, be sure to download the R source package for probe-level mappings (example below).

← → ↻ brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF/15.0.0/entrez.asp

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Home > Service > Custom CDF > Download

Download (Version 15.0.0, ENTREZG)

Species	Chip	Original Probe Count	Custom CDF Name	Statistics of Current Version		Statistics of Previous Version		% of Common Probes in Version		% of Common Probesets in Version		% of Identical Probesets in Version		R Packages		CDF Seq Map Desc
				Probe %	Probeset #	Probe %	Probeset #	Current	Previous	Current	Previous	Current	Previous	Source	Win32	
Anopheles gambiae	PlasmodiumAnopheles	250758	PlasmodiumAnopheles_Ag_ENTREZG	1.7	408	1.7	408	100.00	100.00	100.00	100.00	100.00	100.00	CP	CP	O
Arabidopsis thaliana	AG	131822	AG_At_ENTREZG	83.2	7359	83.1	7352	99.78	99.89	99.71	99.81	99.06	99.16	CP	CP	O
Arabidopsis thaliana	AGRONOMICS1	6046951	AGRONOMICS1_At_ENTREZG	20.0	30851	19.7	30749	98.69	99.79	99.45	99.78	92.63	92.93	CP	CP	O
Arabidopsis thaliana	ATH1121501	251078	ATH1121501_At_ENTREZG	84.9	21236	84.9	21225	99.77	99.84	99.69	99.74	99.16	99.21	CP	CP	O
Arabidopsis thaliana	aragene10st	628424	aragene10st_At_ENTREZG	93.3	27602									CP	CP	O
Arabidopsis thaliana	aragene11st	628424	aragene11st_At_ENTREZG	93.3	27602									CP	CP	O
Bos taurus	Bovine	265627	Bovine_Bt_ENTREZG	43.1	9409	43.0	9403	99.83	100.00	99.94	100.00	98.35	98.42	CP	CPA	O
Caenorhabditis elegans	Celegans	249165	Celegans_Ce_ENTREZG	78.8	17165	78.9	17198	99.83	99.68	99.84	99.65	97.08	96.89	CPA	CPA	O
Canis familiaris	Canine2	473162	Canine2_Cf_ENTREZG	53.1	16755	50.1	15664	88.21	93.44	89.33	95.56	70.34	75.24	CPA	CPA	O
Canis familiaris	Canine2PM	486081	Canine2PM_Cf_ENTREZG	51.7	16755	48.8	15664	88.21	93.44	89.33	95.56	70.34	75.24	CPA	CPA	O
Canis familiaris	cangene11st	621953	cangene11st_Cf_ENTREZG	66.6	17768									CPA	CPA	O
Danio rerio	Zebrafish	249752	Zebrafish_Dr_ENTREZG	53.1	8548	47.0	7696	84.62	95.52	84.45	93.80	75.96	84.37	CPA	CPA	O
Danio rerio	zebgene10st	1245559	zebgene10st_Dr_ENTREZG	41.8	23877									CPA	CPA	O
Danio rerio	zebgene11st	1245558	zebgene11st_Dr_ENTREZG	41.8	23877									CPA	CPA	O
Drosophila melanogaster	DrosGenome1	195994	DrosGenome1_Dm_ENTREZG	91.0	11719	91.2	11788	99.78	99.54	99.69	99.11	98.99	98.41	CPA	CPA	O
Drosophila melanogaster	Drosophila2	265400	Drosophila2_Dm_ENTREZG	70.6	12746	71.0	12847	99.55	99.00	99.36	98.58	97.97	97.20	CPA	CPA	O
Equus caballus	equgene10st	537520	equgene10st_EQca_ENTREZG	69.1	17624									CP	CP	O
Gallus gallus	Chicken	424097	Chicken_Gg_ENTREZG	43.3	12492	43.3	12492	100.00	100.00	100.00	100.00	100.00	100.00	CPA	CPA	O
Gallus gallus	chigene10st	464100	chigene10st_Gg_ENTREZG	69.6	13310									CPA	CPA	O
Gallus gallus	chigene11st	464100	chigene11st_Gg_ENTREZG	69.6	13310									CPA	CPA	O
Homo sapiens	HCG110	30313	HCG110_Hs_ENTREZG	72.2	1292	72.3	1295	99.70	99.62	99.85	99.61	98.84	98.61	CPA	CPA	O
Homo sapiens	HGFocus	98149	HGFocus_Hs_ENTREZG	78.9	7820	79.2	7856	99.59	99.17	99.65	99.20	98.82	98.37	CPA	CPA	O
Homo sapiens	HGU133A	247965	HGU133A_Hs_ENTREZG	69.0	12012	69.2	12078	99.28	99.07	99.55	99.01	98.04	97.50	CPA	CPA	O
Homo sapiens	HGU133A2	247899	HGU133A2_Hs_ENTREZG	69.0	12012	69.2	12078	99.28	99.07	99.55	99.01	98.04	97.50	CPA	CPA	O

Once such a probe-summary has been downloaded, it must be installed in R using code such as the following.

```
> download.file("http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF/15.0.0/entrezg.tar.gz", repos=NULL, type="source")
> install.packages("hgu95ahsentrezgprobe_15.0.0.tar.gz", repos=NULL, type="source")
> library(hgu95ahsentrezgprobe)
```

Then the mappings can be applied to a CEL file using code such as the following.

```
> normalized = SCAN(CELFilePath, probeSummaryPackage=hgu95ahsentrezgprobe)
```

Finally, we clean up files that were created in this demo.

```
> unlink(c(celFilePath, "output_file.txt", "hgu95ahsentrezgprobe_15.0.0.tar.gz"))
```

## References

Stephen R. Piccolo, Ying Sun, Joshua D. Campbell, Marc E. Lenburg, Andrea H. Bild, and W. Evan Johnson. A single-sample microarray normalization method to facilitate personalized-medicine workflows. *Genomics*, 100(6):337–344, 2012. doi: <http://dx.doi.org/10.1016/j.ygeno.2012.08.003>.