

# **Supplementary figures and tables of “Evaluating preprocessing and differential expression combinations for Affymetrix GeneChip microarrays via spike-in, RT-PCR and cross-laboratory datasets”**

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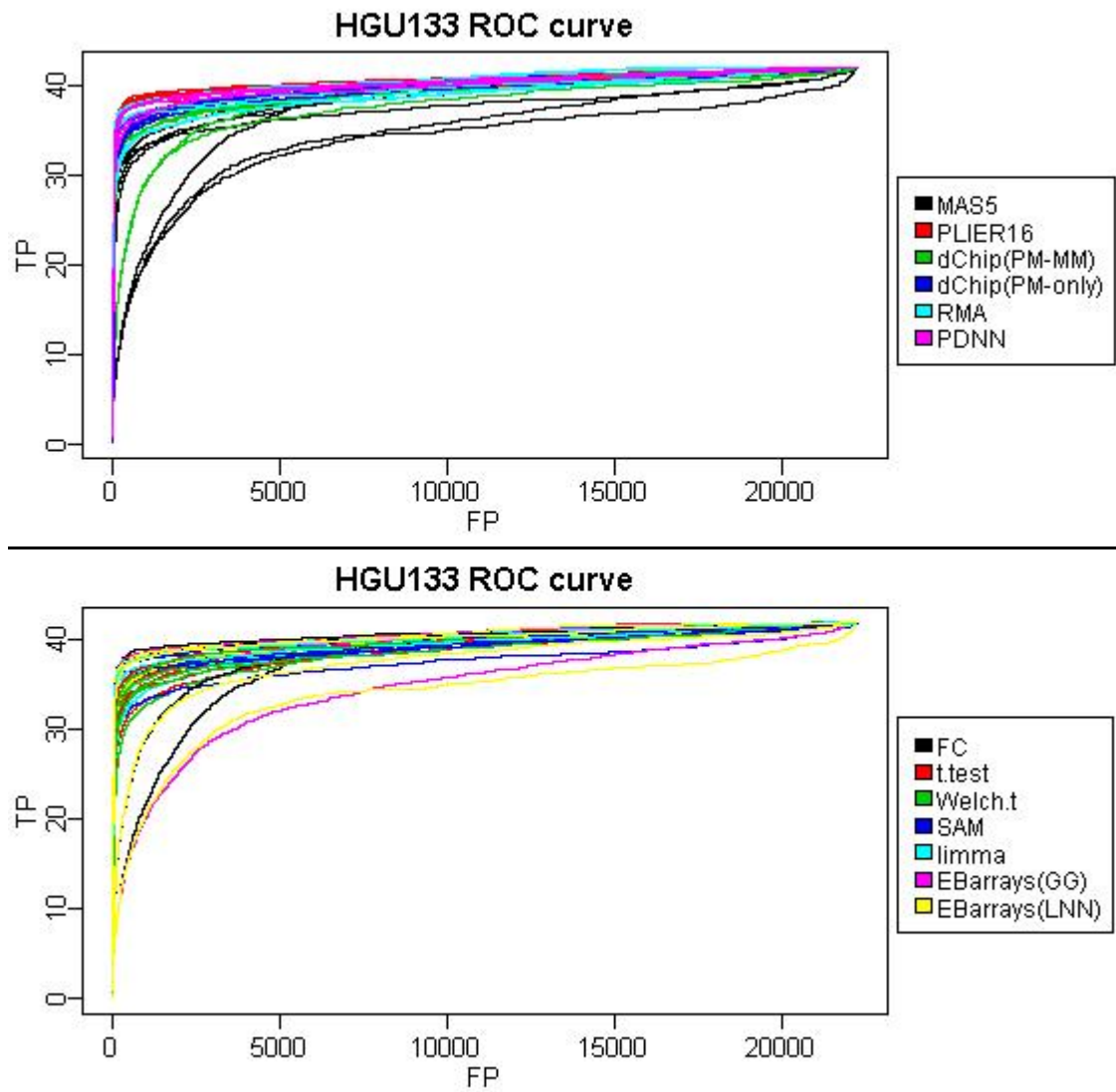
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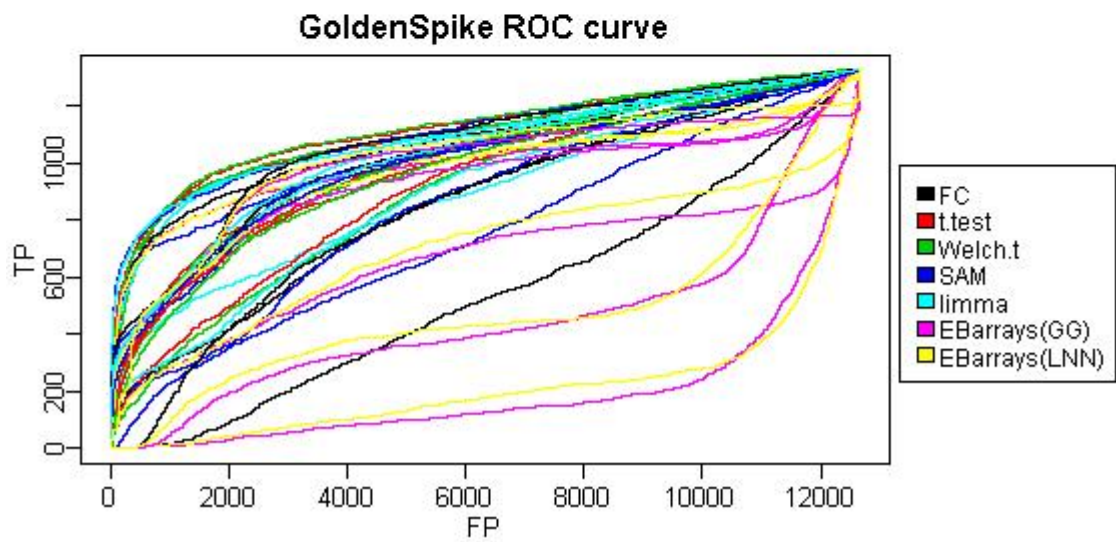
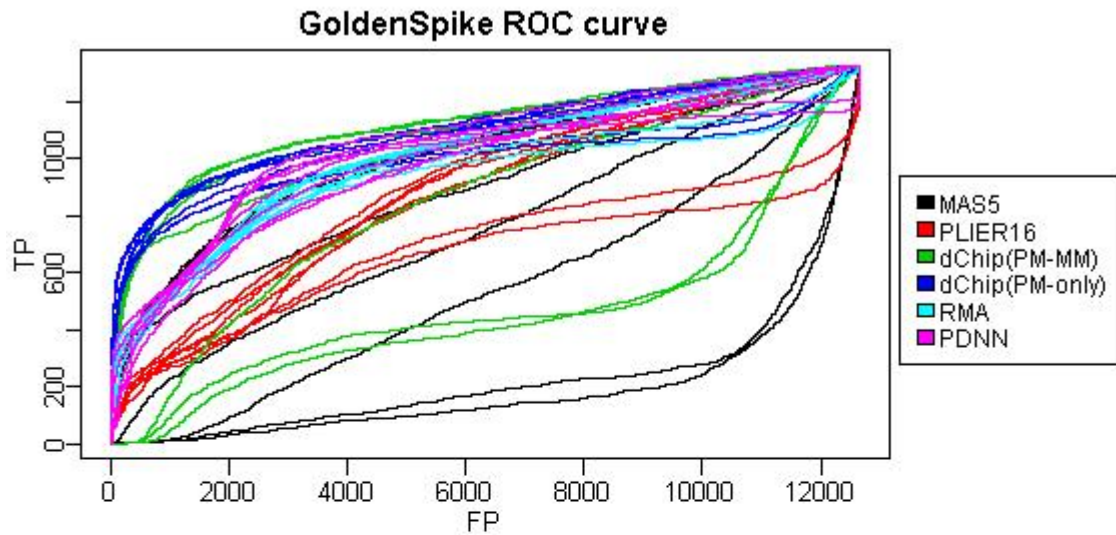
YLW: [yali0629@hotmail.com](mailto:yali0629@hotmail.com)

GHH: [ghuang@stat.nctu.edu.tw](mailto:ghuang@stat.nctu.edu.tw)



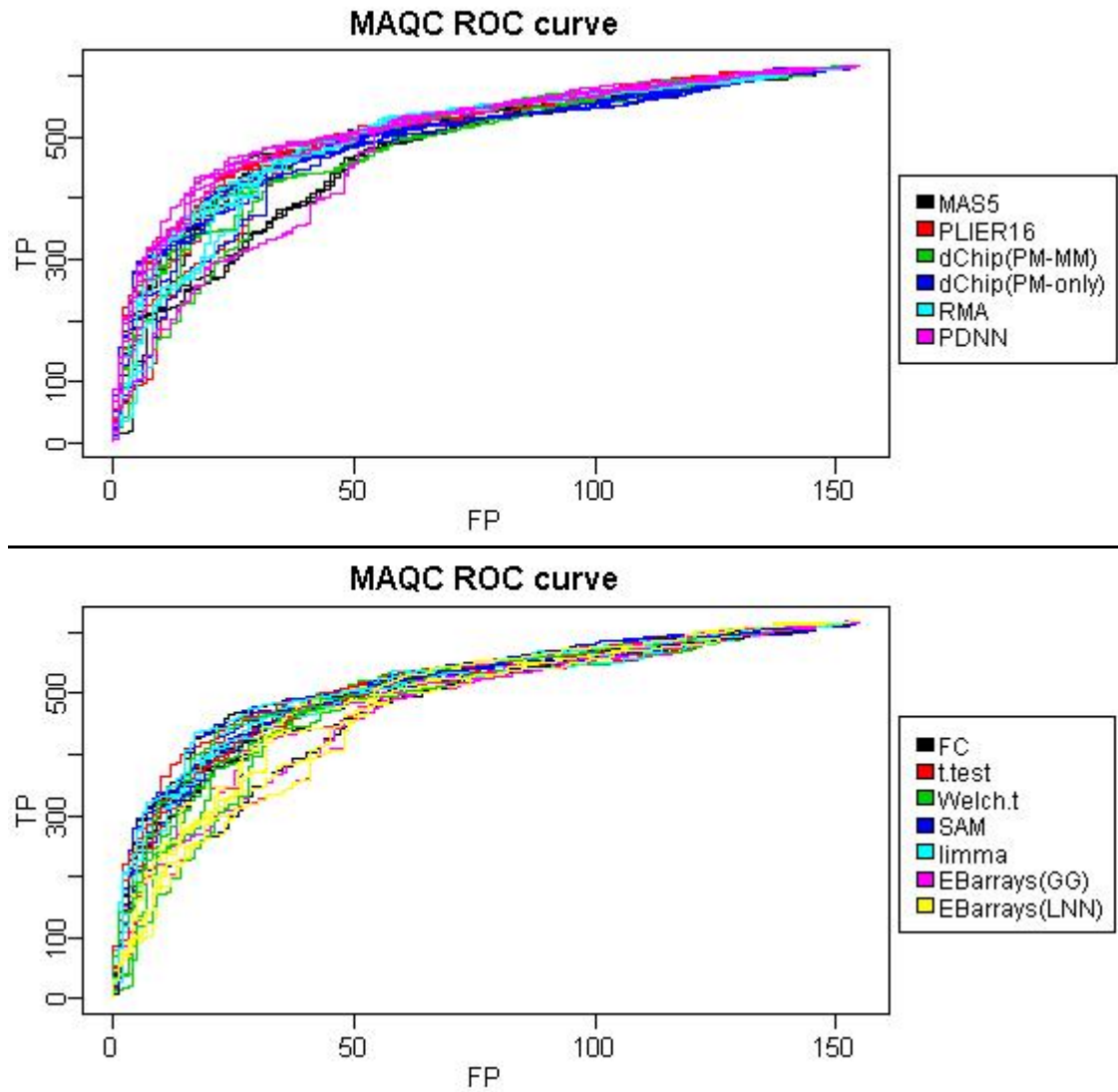
**Supplementary figure 1 - ROC curves for the HGU133 Spike-in dataset, showing all FPs.**

In the top panel, combinations using the same preprocessing method are assigned to the same color. In the bottom panel, combinations using the same differential expression method are assigned to the same color. There are 40 combinations in total.



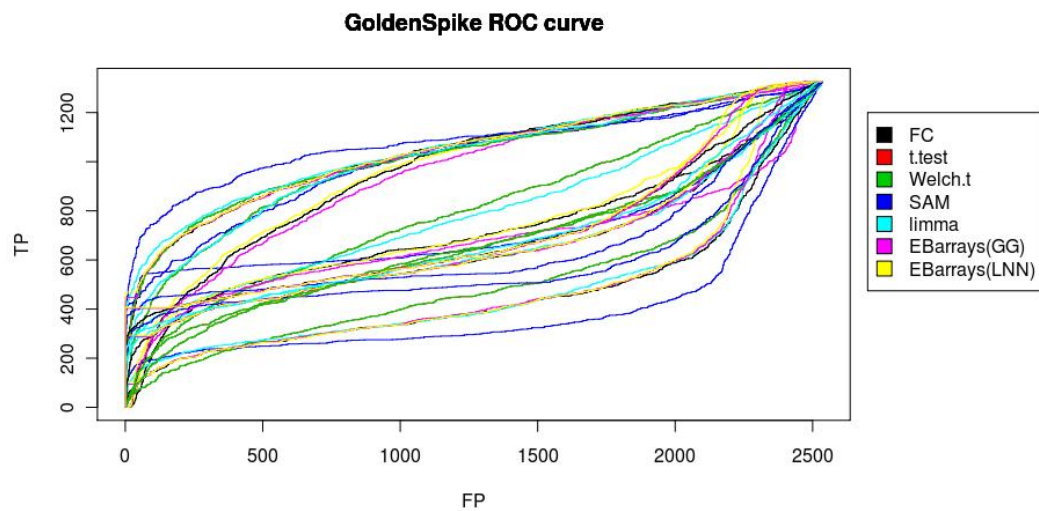
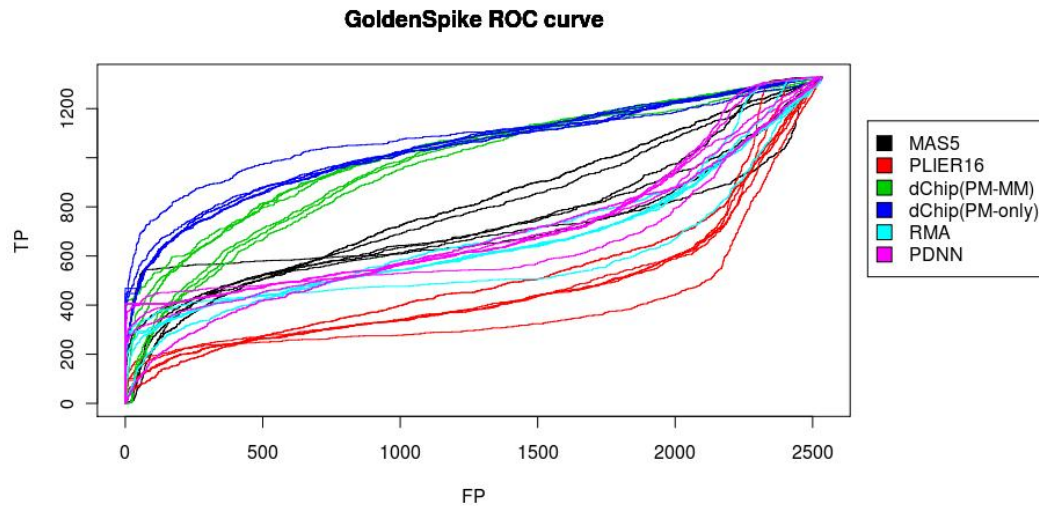
**Supplementary figure 2 - ROC curves for the Golden Spike dataset, showing all FPs.**

In the top panel, combinations using the same preprocessing method are assigned to the same color. In the bottom panel, combinations using the same differential expression method are assigned to the same color. There are 42 combinations in total.



**Supplementary figure 3 - ROC curves for the MAQC RT-PCR dataset, showing all FPs.**

In the top panel, combinations using the same preprocessing method are assigned to the same color. In the bottom panel, combinations using the same differential expression method are assigned to the same color. Genes that are significantly differentially expressed in TaqMan arrays are treated as the true positives. There are 42 combinations in total.



**Supplementary figure 4 - ROC curves for the Golden Spike dataset using genes spiked-in at equal levels as true negatives, showing all FPs.**

In the top panel, combinations using the same preprocessing method are assigned to the same color. In the bottom panel, combinations using the same differential expression method are assigned to the same color. Only genes that are spiked-in at equal levels are used as the true negatives. There are 42 combinations in total.

**Supplementary table 1 - Area under ROC curve (FP<100) for the HGU133 Spike-in dataset**

| HGU133 | Preprocessing  | Differential expression | AUC (FP<100) |
|--------|----------------|-------------------------|--------------|
| 1      | RMA            | EBarrays(LNN)           | 0.775545     |
| 2      | RMA            | EBarrays(GG)            | 0.775209     |
| 3      | RMA            | FC                      | 0.745778     |
| 4      | PLIER16        | EBarrays(GG)            | 0.737474     |
| 5      | RMA            | SAM                     | 0.735601     |
| 6      | PLIER16        | SAM                     | 0.730937     |
| 7      | PLIER16        | EBarrays(LNN)           | 0.728083     |
| 8      | RMA            | limma                   | 0.724682     |
| 9      | PLIER16        | FC                      | 0.720157     |
| 10     | PLIER16        | limma                   | 0.717157     |
| 11     | PDNN           | SAM                     | 0.715714     |
| 12     | PDNN           | limma                   | 0.710198     |
| 13     | dChip(PM-only) | SAM                     | 0.678571     |
| 14     | PDNN           | FC                      | 0.670846     |
| 15     | PDNN           | EBarrays(LNN)           | 0.670143     |
| 16     | dChip(PM-only) | limma                   | 0.666364     |
| 17     | PDNN           | t.test                  | 0.643946     |
| 18     | dChip(PM-only) | t.test                  | 0.639840     |
| 19     | dChip(PM-MM)   | SAM                     | 0.628897     |
| 20     | dChip(PM-MM)   | limma                   | 0.620192     |
| 21     | RMA            | t.test                  | 0.607460     |
| 22     | dChip(PM-MM)   | t.test                  | 0.600749     |
| 23     | PLIER16        | t.test                  | 0.593802     |
| 24     | PDNN           | Welch.t                 | 0.588472     |
| 25     | dChip(PM-only) | Welch.t                 | 0.573446     |
| 26     | RMA            | Welch.t                 | 0.556811     |
| 27     | MAS5           | SAM                     | 0.554397     |
| 28     | dChip(PM-only) | EBarrays(GG)            | 0.549018     |
| 29     | dChip(PM-MM)   | Welch.t                 | 0.542362     |
| 30     | dChip(PM-only) | FC                      | 0.537517     |
| 31     | dChip(PM-only) | EBarrays(LNN)           | 0.535917     |
| 32     | PLIER16        | Welch.t                 | 0.533251     |
| 33     | MAS5           | limma                   | 0.533016     |
| 34     | MAS5           | t.test                  | 0.516221     |
| 35     | MAS5           | Welch.t                 | 0.445383     |
| 36     | dChip(PM-MM)   | EBarrays(LNN)           | 0.217948     |
| 37     | dChip(PM-MM)   | FC                      | 0.217485     |
| 38     | MAS5           | EBarrays(GG)            | 0.155624     |
| 39     | MAS5           | FC                      | 0.154372     |
| 40     | MAS5           | EBarrays(LNN)           | 0.153766     |

**Supplementary table 2 - Area under ROC curve (FPR<0.1) for the Golden Spike dataset**

| Golden S | Preprocessing  | Differential expression | AUC (FPR<0.1) |
|----------|----------------|-------------------------|---------------|
| 1        | dChip(PM-only) | limma                   | 0.566140      |
| 2        | dChip(PM-only) | SAM                     | 0.565670      |
| 3        | dChip(PM-only) | t.test                  | 0.549932      |
| 4        | dChip(PM-only) | Welch.t                 | 0.537779      |
| 5        | dChip(PM-MM)   | t.test                  | 0.523752      |
| 6        | dChip(PM-MM)   | Welch.t                 | 0.515406      |
| 7        | dChip(PM-only) | FC                      | 0.510864      |
| 8        | dChip(PM-MM)   | limma                   | 0.504039      |
| 9        | dChip(PM-only) | EBarrays(GG)            | 0.499712      |
| 10       | dChip(PM-only) | EBarrays(LNN)           | 0.496237      |
| 11       | dChip(PM-MM)   | SAM                     | 0.484404      |
| 12       | PDNN           | FC                      | 0.366185      |
| 13       | PDNN           | limma                   | 0.346782      |
| 14       | RMA            | limma                   | 0.338811      |
| 15       | RMA            | SAM                     | 0.337653      |
| 16       | MAS5           | t.test                  | 0.335464      |
| 17       | RMA            | FC                      | 0.334335      |
| 18       | RMA            | EBarrays(GG)            | 0.329024      |
| 19       | RMA            | EBarrays(LNN)           | 0.327159      |
| 20       | PDNN           | SAM                     | 0.323364      |
| 21       | MAS5           | Welch.t                 | 0.315065      |
| 22       | PDNN           | EBarrays(LNN)           | 0.312910      |
| 23       | PDNN           | EBarrays(GG)            | 0.312688      |
| 24       | RMA            | t.test                  | 0.307614      |
| 25       | RMA            | Welch.t                 | 0.295380      |
| 26       | PDNN           | t.test                  | 0.292734      |
| 27       | MAS5           | limma                   | 0.282221      |
| 28       | PDNN           | Welch.t                 | 0.260818      |
| 29       | PLIER16        | limma                   | 0.195952      |
| 30       | PLIER16        | t.test                  | 0.192417      |
| 31       | PLIER16        | FC                      | 0.179153      |
| 32       | PLIER16        | SAM                     | 0.174857      |
| 33       | PLIER16        | EBarrays(GG)            | 0.166193      |
| 34       | PLIER16        | Welch.t                 | 0.166022      |
| 35       | PLIER16        | EBarrays(LNN)           | 0.164703      |
| 36       | MAS5           | SAM                     | 0.108998      |
| 37       | dChip(PM-MM)   | FC                      | 0.059322      |
| 38       | dChip(PM-MM)   | EBarrays(LNN)           | 0.034430      |
| 39       | dChip(PM-MM)   | EBarrays(GG)            | 0.016426      |
| 40       | MAS5           | FC                      | 0.006431      |
| 41       | MAS5           | EBarrays(LNN)           | 0.004667      |
| 42       | MAS5           | EBarrays(GG)            | 0.004140      |

**Supplementary table 3 - Area under ROC curve (FP<50) for the MAQC RT-PCR dataset**

| MAQC | Preprocessing  | Differential expression | AUC (FP<50) |
|------|----------------|-------------------------|-------------|
| 1    | PDNN           | limma                   | 0.650065    |
| 2    | PDNN           | SAM                     | 0.649417    |
| 3    | PDNN           | t.test                  | 0.648641    |
| 4    | PDNN           | FC                      | 0.648058    |
| 5    | PLIER16        | SAM                     | 0.632718    |
| 6    | PLIER16        | limma                   | 0.631974    |
| 7    | PLIER16        | t.test                  | 0.627605    |
| 8    | PDNN           | Welch.t                 | 0.619159    |
| 9    | PLIER16        | FC                      | 0.614401    |
| 10   | MAS5           | SAM                     | 0.610971    |
| 11   | dChip(PM-only) | limma                   | 0.60835     |
| 12   | MAS5           | limma                   | 0.60754     |
| 13   | dChip(PM-only) | SAM                     | 0.605922    |
| 14   | dChip(PM-only) | t.test                  | 0.605275    |
| 15   | RMA            | SAM                     | 0.60301     |
| 16   | RMA            | limma                   | 0.601327    |
| 17   | RMA            | FC                      | 0.600712    |
| 18   | MAS5           | t.test                  | 0.596408    |
| 19   | dChip(PM-only) | FC                      | 0.594563    |
| 20   | dChip(PM-MM)   | SAM                     | 0.592783    |
| 21   | dChip(PM-MM)   | limma                   | 0.588964    |
| 22   | RMA            | t.test                  | 0.587411    |
| 23   | dChip(PM-MM)   | t.test                  | 0.584045    |
| 24   | PLIER16        | Welch.t                 | 0.579061    |
| 25   | dChip(PM-only) | Welch.t                 | 0.565437    |
| 26   | dChip(PM-MM)   | FC                      | 0.555469    |
| 27   | MAS5           | Welch.t                 | 0.555275    |
| 28   | RMA            | EBarrays(LNN)           | 0.542977    |
| 29   | RMA            | EBarrays(GG)            | 0.54165     |
| 30   | RMA            | Welch.t                 | 0.536958    |
| 31   | dChip(PM-MM)   | EBarrays(GG)            | 0.534207    |
| 32   | dChip(PM-MM)   | EBarrays(LNN)           | 0.531197    |
| 33   | dChip(PM-only) | EBarrays(LNN)           | 0.509482    |
| 34   | dChip(PM-only) | EBarrays(GG)            | 0.509385    |
| 35   | PLIER16        | EBarrays(LNN)           | 0.506505    |
| 36   | PLIER16        | EBarrays(GG)            | 0.50644     |
| 37   | dChip(PM-MM)   | Welch.t                 | 0.489741    |
| 38   | MAS5           | EBarrays(GG)            | 0.479061    |
| 39   | MAS5           | EBarrays(LNN)           | 0.472006    |
| 40   | MAS5           | FC                      | 0.470259    |
| 41   | PDNN           | EBarrays(GG)            | 0.435113    |
| 42   | PDNN           | EBarrays(LNN)           | 0.433883    |



**Supplementary table 4 - Area under ROC curve (all FPs) for the Golden Spike dataset using genes spiked-in at equal levels as true negatives**

| Golden S | Preprocessing  | Differential expression | AUC      |
|----------|----------------|-------------------------|----------|
| 1        | dChip(PM-only) | SAM                     | 0.810309 |
| 2        | dChip(PM-only) | limma                   | 0.785189 |
| 3        | dChip(PM-only) | EBarrays(LNN)           | 0.784673 |
| 4        | dChip(PM-only) | EBarrays(GG)            | 0.781759 |
| 5        | dChip(PM-only) | FC                      | 0.781732 |
| 6        | dChip(PM-only) | t.test                  | 0.776872 |
| 7        | dChip(PM-only) | welch.t                 | 0.776872 |
| 8        | dChip(PM-MM)   | limma                   | 0.766347 |
| 9        | dChip(PM-MM)   | t.test                  | 0.763207 |
| 10       | dChip(PM-MM)   | welch.t                 | 0.763207 |
| 11       | dChip(PM-MM)   | SAM                     | 0.758200 |
| 12       | dChip(PM-MM)   | EBarrays(LNN)           | 0.731621 |
| 13       | dChip(PM-MM)   | FC                      | 0.730505 |
| 14       | dChip(PM-MM)   | EBarrays(GG)            | 0.716040 |
| 15       | MAS5           | t.test                  | 0.603740 |
| 16       | MAS5           | welch.t                 | 0.603740 |
| 17       | MAS5           | limma                   | 0.600205 |
| 18       | MAS5           | EBarrays(LNN)           | 0.560834 |
| 19       | MAS5           | SAM                     | 0.548898 |
| 20       | PDNN           | EBarrays(LNN)           | 0.541970 |
| 21       | MAS5           | FC                      | 0.539195 |
| 22       | PDNN           | EBarrays(GG)            | 0.538945 |
| 23       | PDNN           | FC                      | 0.528292 |
| 24       | PDNN           | limma                   | 0.515343 |
| 25       | RMA            | EBarrays(LNN)           | 0.509057 |
| 26       | MAS5           | EBarrays(GG)            | 0.507160 |
| 27       | RMA            | welch.t                 | 0.503501 |
| 28       | RMA            | t.test                  | 0.503501 |
| 29       | RMA            | EBarrays(GG)            | 0.500148 |
| 30       | PDNN           | welch.t                 | 0.499310 |
| 31       | PDNN           | t.test                  | 0.499310 |
| 32       | RMA            | limma                   | 0.494678 |
| 33       | RMA            | FC                      | 0.492670 |
| 34       | PDNN           | SAM                     | 0.490104 |
| 35       | RMA            | SAM                     | 0.439609 |
| 36       | PLIER16        | welch.t                 | 0.384444 |
| 37       | PLIER16        | t.test                  | 0.384444 |
| 38       | PLIER16        | EBarrays(LNN)           | 0.365771 |
| 39       | PLIER16        | EBarrays(GG)            | 0.360097 |
| 40       | PLIER16        | limma                   | 0.357202 |
| 41       | PLIER16        | FC                      | 0.348342 |
| 42       | PLIER16        | SAM                     | 0.300208 |

**Supplementary table 5 - Average log2-transformed signal intensity for various benchmark datasets preprocessed with different methods**

| Benchmark dataset             |                | HGU133<br>Spike-in     | Golden<br>Spike | MAQC<br>RT-PCR |       |
|-------------------------------|----------------|------------------------|-----------------|----------------|-------|
| Pre-<br>processing<br>methods | MAS5           | Positives <sup>a</sup> | 9.58            | 11.01          | 8.95  |
|                               |                | Negatives <sup>a</sup> | 7.28            | 5.34           | 7.30  |
|                               | PLIER16        | Positives              | 7.74            | 10.99          | 7.66  |
|                               |                | Negatives              | 6.35            | 7.08           | 6.61  |
|                               | dChip(PM-MM)   | Positives              | 7.72            | 10.95          | 7.57  |
|                               |                | Negatives              | 5.59            | 5.73           | 6.07  |
|                               | dChip(PM only) | Positives              | 8.60            | 11.52          | 8.36  |
|                               |                | Negatives              | 7.31            | 8.61           | 7.44  |
|                               | RMA            | Positives              | 7.94            | 11.25          | 7.71  |
|                               |                | Negatives              | 6.05            | 7.55           | 6.45  |
|                               | PDNN           | Positives              | 9.32            | 15.28          | 11.46 |
|                               |                | Negatives              | 7.94            | 12.63          | 10.62 |

<sup>a</sup> Positives=genes that were treated as truly differentially expressed; Negatives=genes that were treated as truly non-differentially expressed.