Twin Research and Human Genetics

Supplementary material:

**A Genome-Wide Scan of DNA Methylation Markers for Distinguishing Monozygotic Twins**

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Supplementary Table 1: Raw Reads and Aligned Reads of MeDIP Sequencing of Eight Samples

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | Raw reads | ％of total raw reads | Aligned reads | ％of aligned reads |
| 1a | 64,946,873 | 21.54 | 56,680,610 | 87.27 |
| 1b | 13,981,764 | 4.64 | 12,037,697 | 86.10 |
| 2a | 39,890,928 | 13.23 | 34,823,240 | 87.30 |
| 2b | 32,560,160 | 10.80 | 27,414,568 | 84.20 |
| 3a | 33,542,961 | 11.12 | 29,614,140 | 88.29 |
| 3b | 36,700,864 | 12.17 | 32,591,692 | 88.80 |
| 4a | 42,104,367 | 13.96 | 35,392,795 | 84.06 |
| 4b | 37,845,357 | 12.55 | 34,191,534 | 90.35 |

Supplementary Table 2: Examples of Raw Counts Normalization

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample Name | Total Number of Mapped Reads | Total Number of Reference Reads | Raw  Read Counts | | Normalized  Read Counts | |
| Region A | Region B | Region A | Region B |
| Sample A | 13 M | 17 M | 8 | 10 | 8\*17/13=10.46 | 10\*17/13=13.08 |
| Sample B | 17 M | 17 M | 30 | 20 | 30\*17/17=30.00 | 20\*17/17=20.00 |

Supplementary Table 3: An Example of MeDIP-Score of a Specific Region Calculation

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| CGI\_name | CGI  length | Read counts (raw) | Read counts (normalized) | MeDIP score  (reads/kb) | Methylation status |
| chr1:156186237-156186647 | 410 | 108 | 269.97 | 269.97/410\*1000=658.47 | Methylated |

Supplementary Table 4: Comparison of DNA Methylation Status of Eight Samples in Different Regions of the Genome Including CGIs,   
Promoters and Gene Bodies

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample no. | CGI (27841) | | | Promoter (29402) | | | Gene body (24157) | | |
| Partially methylated | Completelymethylated | Unmethylated | Partially  methylated | Completely methylated | Unmethylated | Partially  methylated | Completely methylated | Unmethylated |
| 1a | 46.81% | 16.87% | 36.32% | 66.14% | 0.23% | 33.62% | 84.34% | 0.10% | 15.56% |
| 1b | 44.93% | 18.36% | 36.70% | 62.46% | 0.32% | 37.22% | 81.84% | 0.15% | 18.01% |
| 2a | 40.67% | 18.61% | 40.72% | 59.16% | 0.60% | 40.24% | 76.72% | 0.24% | 23.05% |
| 2b | 49.03% | 19.66% | 31.31% | 58.54% | 0.90% | 40.56% | 71.40% | 0.35% | 28.24% |
| 3a | 61.42% | 16.45% | 22.12% | 75.08% | 0.35% | 24.57% | 86.16% | 0.15% | 13.69% |
| 3b | 64.83% | 16.62% | 18.55% | 76.29% | 0.31% | 23.40% | 86.31% | 0.14% | 13.55% |
| 4a | 55.47% | 18.26% | 26.27% | 66.37% | 0.40% | 33.23% | 71.06% | 0.31% | 28.63% |
| 4b | 56.47% | 15.05% | 28.48% | 69.74% | 0.24% | 30.02% | 84.72% | 0.14% | 15.14% |

Supplementary Table 5: DNA Methylation Status of Eight Samples Across Different Categories of CGIs

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample no. | Promoter Region (13232) | | | Intergenic Region (10444) | | | Intragenic Region (4042) | | | |
| Partially methylated | Completely methylated | Unmethylated | Partially methylated | Completely methylated | Unmethylated | | Partially-  methylated | Completely methylated | Unmethylated |
| 1a | 43.84% | 1.95% | 54.21% | 50.14% | 27.42% | 22.43% | 48.02% | | 37.53% | 14.45% |
| 1b | 43.15% | 2.22% | 54.63% | 47.52% | 29.62% | 22.86% | 44.16% | | 41.17% | 14.67% |
| 2a | 35.55% | 2.24% | 62.21% | 46.59% | 30.09% | 23.31% | 42.16% | | 41.61% | 16.23% |
| 2b | 49.46% | 2.46% | 48.08% | 50.76% | 31.25% | 17.99% | 43.54% | | 44.98% | 11.48% |
| 3a | 64.62% | 1.93% | 33.45% | 60.28% | 26.55% | 13.17% | 54.40% | | 36.89% | 8.71% |
| 3b | 70.05% | 2.06% | 27.87% | 61.84% | 26.92% | 11.23% | 56.01% | | 36.71% | 7.27% |
| 4a | 58.74% | 2.34% | 38.91% | 54.32% | 29.11% | 16.57% | 48.05% | | 41.44% | 10.51% |
| 4b | 54.85% | 1.68% | 43.47% | 59.22% | 24.18% | 16.60% | 55.17% | | 34.12% | 10.71% |

Supplementary Table 6: DNA Methylation Status of Eight Samples Across the Different Categories of Promoters

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample no. | HCP (5640) | | | ICP (2347) | | | LCP (21415) | | |
| Partially  methylated | Completely methylated | Unmethylated | Partially-  methylated | Completely methylated | Unmethylated | Partially-  methylated | Completely methylated | Unmethylated |
| 1a | 62.94% | 0.14% | 36.91% | 72.94% | 0.17% | 26.89% | 66.24% | 0.27% | 33.50% |
| 1b | 59.54% | 0.14% | 40.32% | 68.04% | 0.17% | 31.79% | 62.62% | 0.38% | 37.00% |
| 2a | 57.06% | 0.39% | 42.55% | 66.21% | 1.15% | 32.64% | 58.94% | 0.60% | 40.46% |
| 2b | 59.29% | 0.41% | 40.30% | 66.21% | 1.41% | 32.38% | 57.51% | 0.98% | 41.52% |
| 3a | 76.13% | 0.41% | 23.46% | 82.36% | 0.17% | 17.47% | 74.00% | 0.36% | 25.64% |
| 3b | 79.13% | 0.21% | 20.66% | 83.68% | 0.13% | 16.19% | 74.74% | 0.35% | 24.91% |
| 4a | 69.11% | 0.21% | 30.67% | 73.75% | 1.28% | 24.97% | 64.75% | 0.67% | 34.58% |
| 4b | 70.39% | 0.14% | 29.47% | 77.46% | 1.11% | 21.43% | 68.73% | 0.17% | 31.10% |

Supplementary Table 7: DNA Methylation Status of Eight Samples Across the Categories of Gene Bodies

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample no. | HCP (5210) | | | ICP (1920) | | | LCP (17027) | | |
| Partially methylated | Completely methylated | Unmethylated | Partially methylated | Completely methylated | Unmethylated | Partially methylated | Completely methylated | Unmethylated |
| 1a | 91.96% | 0.13% | 7.91% | 88.91% | 0.10% | 10.99% | 81.49% | 0.09% | 18.42% |
| 1b | 89.69% | 0.15% | 10.15% | 86.25% | 0.10% | 13.65% | 78.94% | 0.16% | 20.90% |
| 2a | 87.37% | 0.38% | 12.25% | 82.24% | 0.89% | 16.88% | 72.84% | 0.12% | 27.05% |
| 2b | 82.34% | 0.46% | 17.20% | 76.20% | 1.51% | 22.29% | 67.52% | 0.19% | 32.30% |
| 3a | 92.74% | 0.40% | 6.85% | 90.47% | 0.10% | 9.43% | 83.64% | 0.08% | 16.27% |
| 3b | 93.22% | 0.25% | 6.53% | 91.09% | 0.10% | 8.80% | 83.66% | 0.11% | 16.23% |
| 4a | 81.09% | 0.27% | 18.64% | 76.46% | 1.88% | 21.67% | 67.36% | 0.18% | 32.47% |
| 4b | 93.17% | 0.12% | 6.72% | 90.73% | 0.83% | 8.44% | 81.46% | 0.06% | 18.47% |

Supplementary Table 8: Examples of Fold Change Calculation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| CGI name | Fold change  (2a vs. 2b) | Regulation | MeDIP-score (read-counts/kb) | |
| 2a | 2b |
| chr10:100028204-100028508 | 1 | up | 0 | 0 |
| chr10:100227438-100227832 | -100 | down | 0 | 36.7329 |
| chr10:100992156-100992687 | -1.5243 | down | 15.3264 | 23.362 |
| chr10:101089010-101090655 | 1.3182 | up | 71.2413 | 54.045 |
| chr10:101190451-101190925 | 100 | up | 6.8678 | 0 |
| chr10:101279941-101280382 | -2.5405 | down | 7.3817 | 18.7532 |
| chr10:101281181-101282116 | 1.5263 | up | 496.1335 | 325.0565 |
| chr10:101282725-101282934 | 1.0628 | up | 210.2727 | 197.8503 |
| chr10:101290025-101290338 | -1.9054 | down | 20.8008 | 39.6333 |