

exons vs. decoy regions

selection for single-strandedness in exons:

| | | | |
|-----------------------------|-----------------|-----------------------|-------------------|
| 773 of 2268 neutral motifs | 34.08% | | |
| 450 of 867 ESE motifs | 51.90% | differential fraction | 17.82% |
| 149 of 457 ESS motifs | 32.60% | differential fraction | -1.48% |
| <u>Fisher's exact test:</u> | ESE vs. ESS | P-value: <0.0001 | (odds ratio 2.23) |
| | ESE vs. neutral | P-value: <0.0001 | (odds ratio 2.09) |
| | ESS vs. neutral | P-value: 0.5517 | (odds ratio 1.07) |

selection for single-strandedness in decoy regions:

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|-----------------------------|-----------------|-----------------------|--------------------|
| 211 of 353 neutral motifs | 59.77% | | |
| 58 of 112 ESE motifs | 51.79% | differential fraction | -7.99% |
| 36 of 39 ESS motifs | 92.31% | differential fraction | 32.53% |
| <u>Fisher's exact test:</u> | ESE vs. ESS | P-value: <0.0001 | (odds ratio 11.17) |
| | ESE vs. neutral | P-value: 0.1536 | (odds ratio 1.38) |
| | ESS vs. neutral | P-value: <0.0001 | (odds ratio 8.08) |

exons vs. pseudo exons

selection for single-strandedness in exons:

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|-----------------------------|-----------------|-----------------------|-------------------|
| 684 of 2618 neutral motifs | 26.13% | | |
| 426 of 979 ESE motifs | 43.51% | differential fraction | 17.39% |
| 133 of 496 ESS motifs | 26.81% | differential fraction | 0.69% |
| <u>Fisher's exact test:</u> | ESE vs. ESS | P-value: <0.0001 | (odds ratio 2.10) |
| | ESE vs. neutral | P-value: <0.0001 | (odds ratio 2.18) |
| | ESS vs. neutral | P-value: 0.7388 | (odds ratio 0.97) |

selection for single-strandedness in pseudo exons:

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|-----------------------|--------|-----------------------|---|
| 2 of 3 neutral motifs | 66.67% | | |
| 0 of 0 ESE motifs | 0.00% | differential fraction | - |
| 0 of 0 ESS motifs | 0.00% | differential fraction | - |

exons vs. intron flanks

selection for single-strandedness in exons:

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|-----------------------------|-----------------|-----------------------|-------------------|
| 515 of 1820 neutral motifs | 28.30% | | |
| 343 of 675 ESE motifs | 50.81% | differential fraction | 22.52% |
| 145 of 439 ESS motifs | 33.03% | differential fraction | 4.73% |
| <u>Fisher's exact test:</u> | ESE vs. ESS | P-value: <0.0001 | (odds ratio 2.09) |
| | ESE vs. neutral | P-value: <0.0001 | (odds ratio 2.62) |
| | ESS vs. neutral | P-value: 0.0537 | (odds ratio 0.80) |

selection for single-strandedness in intron flanks:

| | | | |
|-----------------------------|-----------------|-----------------------|-------------------|
| 583 of 801 neutral motifs | 72.78% | | |
| 173 of 304 ESE motifs | 56.91% | differential fraction | -15.88% |
| 52 of 57 ESS motifs | 91.23% | differential fraction | 18.44% |
| <u>Fisher's exact test:</u> | ESE vs. ESS | P-value: <0.0001 | (odds ratio 7.88) |
| | ESE vs. neutral | P-value: <0.0001 | (odds ratio 2.03) |

Table S4: Number of ESEs, ESSs and neutral motifs that are selected for single-strandedness comparing exons with decoy regions, pseudo exons and intron flanks.