Specifically, we calculated the FPKM for parental genes and retrocopy using these biological replicates separately. We first noticed that either parental genes or retrocopies were positively correlated between brain or heart replicates suggesting that the FPKM values are largely reproducible across replicates.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tissue | Pearson coefficient of parental gene | P value | Pearson coefficient of retrocopy | P value |
| Brain | 0.93 | 1.46E-23 | 0.85 | 1.53E-15 |
| Heart | 0.92 | 4.8E-22 | 0.82 | 6.67E-14 |

Second, we compared the expression level of parental genes and retrocopies: we divided FPKM of each retrocopy by FPKM of its parental gene in and tissue, which was defined as i. 52 gene pairs were included, except the ones with FPKM=0 in parental genes. If i <1 in a certain tissue, the expression level was higher in parental gene. Alternatively, if i >1, the expression level was higher in retrocopy. We carried out a two-sample test for equality of proportions with continuity correction. The proportion that the retrocopies showed lower expression level than corresponding parental gene was significant higher.

As shown below, the result of biological replicates was roughly consistent with each other in brain and heart, respectively.

|  |  |  |  |
| --- | --- | --- | --- |
| Tissue | i<1 | i>1 | Adjusted P value |
| SRR891511(Brain) | 34 | 15 | 2.99E-04 |
| ERR35545(Brain) | 35 | 15 | 1.45E-04 |
| SRR891495(Heart) | 37 | 13 | 5.50E-06 |
| ERR35546(Heart) | 33 | 12 | 2.48E-05 |

Third, the tissue specificity was also recalculated and compared:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Retrocopies | | Parental Gene | |
| Tissue | No. of unexpressed genes | No. of expressed genes | No. of unexpressed genes | No. of expressed genes |
| ERR35545(Brain) | 10 | 42 | 5 | 47 |
| SRR891511(Brain) | 18 | 34 | 8 | 44 |
| ERR35546(Heart) | 18 | 34 | 11 | 41 |
| SRR891495(Heart) | 23 | 29 | 9 | 43 |

We found that in each condition, mean number of retrocopies that were not expressed in each tissue was significantly greater than the number of parental genes (*P*=1.01E-4, *P*=1.09E-5, respectively, Mann-Whitney U test). Besides, the mean of the Shannon entropy (another measure of specificity ) were 0.42 and 0.37 (*P=*0.03)for retrocopies and parental genes (SRR891511, SRR891495), respectively. The value were 0.39 and 0.27 (*P* = 0.011， Mann-Whitney U test) for retrocopies and parental genes (ERR35545, ERR35546), respectively. These analyses were consistent with the previous conclusion where the retrocopies showed higher tissue specificity than parental genes.