**Supplementary file 1. Parameters for directed evolution of polymerase ribozymes.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Round | Selection method |  +NTPs | Time (h) |  [Mg2+] (mM) | Mutagenesis |
|  1 | gel-shift |  20 |  24 |  200 |  |
|  2 |  " |  " |  " |  " |  |
|  3 |  " |  " |  " |  " |  |
|  4 |  " |  " |  " |  " |  + |
|  5 | vitamin B12 aptamer |  12 |  4 |  " |  |
|  6 |  " |  " |  " |  " |  |
|  7 |  " |  " |  " |  " |  |
|  8 | gel-shift |  18 |  24 |  " |  |
|  9 |  " |  " |  " |  " |  |
|  10 |  " |  " |  " |  " |  |
|  11 |  " |  " |  " |  " |  |
|  12 |  " |  " |  " |  " |  + |
|  13 |  " |  30 |  2 |  " |  |
|  14 |  " |  " |  " |  " |  |
|  15 |  " |  " |  " |  " |  |
|  16 |  " |  " |  " |  " |  |
|  17 | gel-shift & GTP aptamer |  " |  " |  " |  + |
|  18 | gel-shift |  32 |  6 |  " |  |
|  19 |  " |  " |  1 |  " |  |
|  20 |  " |  " |  0.25 |  " |  |
|  21 |  " |  40 |  " |  " |  |
|  22 | gel-shift & GTP aptamer |  30 |  " |  " |  + |
|  23 |  " |  " |  " |  " |  |
|  24 |  " |  " |  " |  " |  |
|  25 | hammerhead |  33 |  2 |  " |  |
|  26 |  " |  " |  0.33 |  " |  |
|  27 |  " |  " |  " |  " |  |
|  28 |  " |  " |  " |  " |  |
|  29 |  " |  " |  " |  " |  |
|  30 |  " |  " |  " |  " |  |
|  31 | gel-shift & hammerhead |  " |  " |  " |  |
|  32 |  " |  " |  " |  " |  |
|  33 |  " |  " |  " |  " |  |
|  34 |  " |  " |  0.08 |  " |  |
|  35 |  " |  " |  " |  " |  |
|  36 |  " |  " |  " |  " |  |
|  37 |  " |  " |  " |  " |  |
|  38 |  " |  " |  " |  " |  10% |
|  39 | urea wash |  28 |  15 |  " |  |
|  40 |  " |  " |  2.5 |  " |  |
|  41 | gel-shift & hammerhead |  33 |  20 |  " |  |
|  42 |  " |  " |  2 |  " |  |
|  43 |  " |  " |  " |  " |  + |
|  44 |  " |  " |  1 |  " |  + |
|  45 |  " |  " |  0.4 |  50 |  + |
|  46 |  " |  " |  4 |  " |  + |
|  47 |  " |  " |  1 |  " |  + |
|  48 |  " |  " |  0.25 |  " |  + |
|  49 |  " |  " |  " |  " |  + |
|  50 |  " |  " |  " |  " |  + |
|  51 |  " |  " |  1 |  25 |  |
|  52 |  " |  " |  24 |  50 |  |

The third column indicates the number of NTPs to be added in order to meet the selection criterion. “+” indicates PCR mutagenesis; “10%” indicates random mutagenesis of the 38-6 polymerase at 10% degeneracy per position.