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Supporting Information

Analysis of Modified Nucleotide Aptamer Library Generated by Thermophilic DNA Polymerases

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Table S1. Oligonucleotide and primer sequences

| Name | Sequence (5'-3') | Primer sequences (5'-3') |
|------|-----------------|-------------------------|
| A1   | ATC CAG AGT GAC GCA GCA TGT GTT ATT TTT TCC TGT CCT TGT TTA CGC ACT TGC CTG GAC ACG GTG GCT TAG T | Forward: ATC CAG AGT GAC GCA GCA , reverse: ACT AAG CCA CCG TGT CCA |
| A2   | ATC CAG AGT GAC GCA GCA CCA GCC ACG CCA AGC CCC TTC TAA CTG CTG TGAGCT CAT CTG GAC ACG GTG GCT TAG T | Forward: ATC CAG AGT GAC GCA GCA , reverse: ACT AAG CCA CCG TGT CCA |
| A3   | CAG TGA GTG ATG GTG AGG GTG AAT CGG TGA CTA TTA AAT TAA GTT GTG GTT GTT CCC ACA CTG TCC ATA CAC G | Forward: CAG TGA GTG ATG GTG AGG G , reverse: CGT GTA TGG ACA GTG TGG G |

Table S2. Library and primer sequences

| Name | Sequence (5'-3') | Primer sequences (5'-3') |
|------|-----------------|-------------------------|
| Library | ATC CAG AGT GAC GCA GCA NNN NNN NNN NNN NNN NNN GAG ATA TCG TGC TAC CGT GA | Forward: ATC CAG AGT GAC GCA GCA , reverse: TCA CGG TAG CAC GAT ATC TC |

Figure S1. 5 µl of Therminator, Pfu, OneTaq, iProof, PWO Superyield and Q5U catalyzed reaction mixtures were separated on PAGE and visualised by GelGreen dye. The mixtures either contained only natural nucleotides (-) or dTTP was replaced by TAdUTP (+).
| Template | A1 | A2 | A3 | Aptamer library |
|----------|----|----|----|----------------|
| Enzyme   | Vent(exo-) | KOD XL | Vent(exo-) | KOD XL | Vent(exo-) | KOD XL | Vent(exo-) | KOD XL |
| PCR condition | TAdUTP | TAdUTP | TAdUTP | TAdUTP |
| Filtered reads | - | + | - | + | 263400 | 308905 | 318252 | 759835 | 322034 | 493453 | 386921 | 195415 | 335393 | 355436 | 371264 | 354417 | 403018 | 347986 | 493867 | 210661 |
| Unique reads | 4551 | 6177 | 3118 | 14186 | 18935 | 22657 | 9695 | 6061 | 6752 | 2362 | 3520 | 3043 | 398711 | 344800 | 488725 | 208546 |
| Max unique size | 118278 | 139942 | 159783 | 368350 | 90371 | 154320 | 6735 | 76416 | 137422 | 160568 | 166054 | 159134 | 17 | 8 | 21 | 17 |
| Singletons | 3467 | 4718 | 2364 | 10899 | 13254 | 15804 | 124973 | 4212 | 5050 | 1569 | 2629 | 2123 | 394442 | 341638 | 483686 | 206480 |
| Mapped to uniques (98%ID) | 260108 | 301011 | 315688 | 744850 | 296385 | 460716 | 377105 | 188327 | 327423 | 353290 | 367830 | 351445 | 8488 | 6327 | 10224 | 4312 |
| Mapped reads to uniques ratio | 98.75% | 97.44% | 99.19% | 98.03% | 92.04% | 93.37% | 97.46% | 96.37% | 97.62% | 99.40% | 99.08% | 99.16% | 2.11% | 1.82% | 2.07% | 2.05% |
| Abundance | 258487 | 300086 | 315118 | 738159 | 235431 | 436797 | 285719 | 179572 | 327375 | 353246 | 367340 | 351410 | 19 | 10 | 33 | 27 |
| Error rate (58bp) | 2.98E-04 | 3.45E-04 | 1.69E-04 | 3.22E-04 | 1.01E-03 | 7.92E-04 | 4.32E-04 | 5.35E-04 | 3.53E-04 | 1.17E-04 | 1.66E-04 | 1.51E-04 | 98.93% | 99.08% | 98.96% | 99.00% |
| Unique reads to filtered reads ratio | 98.93% | 99.08% | 98.96% | 99.00% |