Supporting Information

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Non-Enzymatic Assembly of a Minimized RNA Polymerase Ribozyme

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Materials & Methods

1. Chemicals
2-Methylimidazole, N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC) and reaction buffers Tris(hydroxymethyl)aminomethan (TRIS), N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), 3-[4-(2-Hydroxyethyl)piperazin-1-yl]propane-1-sulfonic acid (EPPS) and 2-(Cyclohexylamino)ethanesulfonic acid (CHES) were from Sigma Aldrich. NTP’s for RPR catalysed primer extensions were ordered as lithium salts from Roche.

2. Oligonucleotides
Table S1 specifies the DNA and RNA oligonucleotides used in this study. All DNA and RNA oligonucleotides were purchased from Integrated DNA Technologies (IDT). DNA templates for RNA in-vitro transcription were generated by fill-in of two DNA oligonucleotides (in reverse template sequence and primer P T7) using GoTaq (Promega) followed by DNA purification using QiaQuick (Qiagen). RNAs were transcribed using the MegaShortScript high-yield transcription kit (Ambion) and purified by RNeasy (Qiagen). Oligonucleotides were purified, if necessary, by denaturing PAGE on 10%, 15%, or 20% denaturing polyacrylamide gels containing 8 M urea. After visualisation by UV shadowing, oligonucleotides were excised from the gel, extracted overnight by crush and soak into 0.3 M NaOAc, followed by precipitation with absolute ethanol on dry ice, two washing steps with 70% ethanol and dried by speed vac. The oligonucleotide concentration was determined by Nanodrop (Thermo Fisher) at a wavelength of 260 nm.

3. Compartmentalized bead-tagging (CBT)
Compartmentalized bead-tagging (CBT) selection procedure with the primer extension step performed in ice. The CBT selection strategy is described in detail in previous papers. In short, biotinylated ribozyme genes are bound to streptavidin-coated magnetic beads (MyOne Streptavidin C1 Dynabeads, Invitrogen) and clonal ribozyme populations are produced by coupled in-emulsion transcription (Megashortscript High Yield transcription kit, Ambion), followed by decoration of the beads with primer/template duplexes. After in emulsion RPR catalysed primer extensions in the eutectic-phase of water-ice at -7°C with 200 mM MgCl2, 50 mM TRIS pH 8.3, extended primers are selectively amplified by rolling circle amplification (RCA) based on predefined DNA minicircles (DC +7 RP, DC 0 RP, DC -3 RP and DC +6 RP, DC +4 RP, DC 0 RP, DC -4 RP). After staining with SYBR® Gold active RNA sequences are isolated by FACS sorting (Sony Synergy) applying two different sorting gates (high gate: top 1-5% of single beads, low gate: top 5-10% of single beads, than mixed in a 1:1 ratio), and amplified by PCR (30-38 cycles, depending on number of sorted single beads, 95°C for 30 s, 50°C for 30 s, 72°C for 60 s). Every second selection round an additional error prone PCR step (25 cycles, 95°C for 30 s, 50°C for 30 s, 72°C for 60 s) using the GeneMorph II Random Mutagenesis Kit (Agilent) was introduced.
4. Synthesis of 5’phosphorimidazole RNA oligonucleotides

5’-phosphorylated RNA oligonucleotides (IDT) were suspended in Millipore water to a concentration of 100 µM. 20 µl were added to 20 µl of 1 M 2-Methylimidazole (pH 6) and 5.7 mg of EDC and the mixture was incubated for 2h at 25°C. The activated RNA oligonucleotides were purified and concentrated using Vivaspin® 500 concentrators, 3000MWCO (Sartorius) and, after concentration measurement by Nanodrop, directly used in the templated ligation reaction.

5. Templated RNA ligation

Templated RNA ligation reactions were performed in 10 µl volumes with 5’ fluorescently labelled primer (0.5 µM), 5’ 2-Methylimidazole activated RNA oligonucleotides (1.5 µM each), RNA or DNA template strands (50 µM each), 10 mM MgCl₂ and 50 mM CHES pH 9. The reactions were incubated for 24h at 37°C, stopped by the addition of equal volumes of 10 mM EDTA in 8 M urea, 0.05% bromophenol blue and full-length ligation products were isolated on 20% polyacrylamide/ 8 M urea gels, analysed using a Typhoon Trio scanner (GE Healthcare) and quantified using Image Quant software (Molecular Dynamics).

6. Functional activity and sequencing of full-length ligation products

Full-length ligation products were excised from the gel, extracted as described above and reverse transcribed and amplified by PCR using primers (P F and P F rev) and the SuperScript® III One-Step RT-PCR System with Platinum® Taq DNA Polymerase (Invitrogen). After in-vitro transcription RNA sequences were analysed for their primer extension capability (S6, S7). For sequencing, DNA products were inserted into pGEM®-T (Promega) vectors and sequenced (Sanger sequencing) by Genewiz.

6.1 Sequencing result of full-length ligation product F 1-4, see Fig. S6, red marked nucleotides indicate the DNA sequence of the F ribozyme.

NNNNNNNNNNNGGGCCCCTCCCCNCCGCGCCGACCCTGCAGGCGGCCGCACTACTGATTGATCGATCTAGAGGAGGCCAGCCTCGTGCGTGCGGAAATACGACTCACTATA
GGACAACCAAAAAGACAAATCTGCCCTCAGAGCTTG
AGAACATCTTCGGATGTAGAGGAGGCAGCCTTCGGTGCAATGCGCCAACGTTCTCAACAGAA
TACTTGACCTGACGAAAGGCGCTTGTAAGACAGCGCCAGTCATCATCAAATCCGCGGCCATGG
CGGCCGGAAGCATGCGACGTGCGGCCAATTCGCCCCTATAGTGAGTCGATTAAATCTCGGCC
GTCGTTTTACACGTCTGCGATGGAACCCGCTCGGTTACCCAACTTAATCGCCTCTGACGACATCC
CCCCCTTCGCCACGTGGGAATAGGAAGGGCCGACCAGCTCGCCCTCCCAAACGTTGCGCA
GCGTGAAAGGCGGAATAGGCGCCGCCGCTCGTGGCGCATTAGCGCGCGGTTGTTGTTGTTGTTACGC
GCAAGCTGACACCACTACCTTGCCAGCCGCTCGCCCTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT
GCCACCGTCGCCGGCTCTCCCGCTCAAGCTCCTCAATCGCGGGGCTCTCCCTAGGGCTCCCTCCATCAC
CGCTNTACGGCACCTGACCCACCACAACTGNTACGGTGTTACGTTACGTTACGCTGTACGTGACATCCCTCC
CGCTACGCTTTTTCGCGCTCCGCTCGACCTCCCTCTCTCCTCCTCTTTTTTNGACTCTATTTACGACTTTTTA
6.2 Sequencing result of full-length ligation product F 1-7, see Fig. S7, red marked nucleotides indicate the DNA sequence of the F ribozyme.

NNCATATGGTCGACCTGCAGGCGGCCGCACTAGTGATTGATGATCTTGGAATTAATACGATCTCACTATAAGACAACAAAAAGACACAAATCTGCCCCCTGAGAGACTCTTTCCGAGATGTAGAG
GAGGCAGCCCTGCTGGCCGAATAGGGCCCAACGTTGCTCAAGATGCTGAATAGCTCTCACTATA
GGACAACCAAAAAGACAAATCTGCCCTCAGAGCTTGAGAACATCTTCGGATGTAGAG
TAGCGAAGAGGGCGGACCCGATGCGACGTCGCGCCGAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTGCTGACT
GGGAAAACCTGGCGTTACCAAATCTGAGCAGCAGACTCCCTCCCCCTGCCGCACTGCTGGCCGCAATAGCGCCAACGTTCTCAACAGATACTTGACCTGACGAAAAGGC
GGCTGGAAGGCGGAGGGCCAGCTGGCCATGGCGGCCGGGAGCATGCGACGTCGCCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTGCTGACT
GGGAAAACCTGGCGTTACCAAATCTGAGCAGCAGACTCCCTCCCCCTGCCGCACTGCTGGCCGCAATAGCGCCAACGTTCTCAACAGATACTTGACCTGACGAAAAGGC
GGCTGGAAGGCGGAGGGCCAGCTGGCCATGGCGGCCGGGAGCATGCGACGTC

7. RPR catalysed primer extensions

Primer extension reactions were performed in 10 µl volumes with 5' fluorescently labelled primer (0.5 µM), template (0.6 µM) and ribozyme (0.7 µM). Primer/template and ribozyme were separately annealed (80°C, 2 min, 0.1°C/s to 17°C, 17°C for 10 min). After the addition of reaction buffer (final conc. 10 mM MgCl₂, 0.8 mM NTP's, 50 mM CHES pH 9 or other buffer/pH) or (final conc. 200 mM MgCl₂, 4 mM NTPs, 50 mM TRIS pH 8.3), the reaction mixture was flash-frozen on dry ice and transferred to a cooling bath (Grant) at -7°C or directly put to 17°C. The reactions were quenched by the addition of equal volumes of 10 mM EDTA in 8 M urea, 0.05% bromophenol blue, 3 µM competing RNA oligonucleotide or 50 µM competing DNA oligonucleotide respectively. Reactions containing 200 mM MgCl₂ were stopped using quenching buffer containing 200 mM EDTA. Primer extensions were separated on polyacrylamide/8M urea gels and analysed using a Typhoon Trio scanner (GE Healthcare).
Supporting figures:

| time (d) | MgCl₂ (mM) | Buffer pH |
|----------|------------|-----------|
| 7        | 200        | CHES 8.5  |
| 14       | 200        | TRIS 8.3  |
| 21       | 10         | CHES 8.5  |
| 30       | 10         | TRIS 8.3  |
Figure S1. Optimisation of in-ice RNA polymerase ribozyme activity. a) Full reaction time course (30 d) of the RPR (Z) catalysed primer (P HP) extension along template (T HP) in the eutectic phase of water ice (-7°C) at 10 mM and 200 mM MgCl₂ applying CHES 8.5 or TRIS 8.3. b) PH dependence (pH 8.3-11) of the RPR (Z) catalysed extension on primer/template (P HP/T HP) in ice at -7°C (10d) using different MgCl₂ conc. (2 mM, 5 mM, 10 mM, 20 mM, 30 mM, 200 mM) (above) and c) at 17°C (4d) applying two different MgCl₂ concentrations (20 mM, 200 mM).
Figure S2. Optimisation of in-ice RNA polymerase ribozyme activity II. RPR (Z) catalysed primer (P HP) extension on template (T HP) in ice (-7°C) applying a) different Mg salts (Cl⁻: Chloride, SO₄²⁻: Sulfate, NO₃⁻: Nitrate, CH₃COO⁻: Acetate, HCOO⁻: Formiate) with a concentration of 10 mM, at CHES pH 9 for 14 d, or b) different buffer systems (TRIS, BES, CHES) in the pH range 7-9 using a constant MgCl₂ concentration of 10 mM for 7d.
Figure S3. Selection library. The partly randomised starting library for selection was generated from three previously selected RPR variants (Z, W, Y) with a distribution of 50% for Z and 25% for W and Y and each comprising a randomised linker region of 12, 18 and 24 nt. The diversity of the library was increased by 2 rounds of error prone PCR (25 cycles, 95°C for 30 s, 50°C for 30 s, 72°C for 60 s) using the GeneMorph II Random Mutagenesis Kit (Agilent). Red marked parts in the starting library indicate fully or partly randomised nucleotides/regions.
Figure S4. Selected polymerase ribozyme clones. a) Secondary structure of three different clones (C1, C2, K) identified by CBT selection, showing b) improved primer extensions on the selected primer/template combination (P HP/T HP) in comparison to the RPR variant Z; reaction conditions: ice (-7°C), 10 mM MgCl₂, CHES pH 9, 5d.
Figure S5. In-ice ribozyme activity. RPR concentration dependence of reactions in ice. PAGE gels of a) Z and b) F catalysed primer (P RP) extensions in ice (7d), on three difficult templates (F2a, F2b, F3a) encoding parts of the RPR structure applying different final ribozyme concentrations (1x: 0.7µM, 2x: 1.4µM, 10x: 7µM) and c) Z (Z1: 0.7µM) and F (F1: 0.7µM, F10: 7µM) primer (P RP) extension on an established template sequence (T RP).
**Figure S6. Non-enzymatic RNA polymerase ribozyme assembly.** Templated non-enzymatic ligation of the full-length F RNA polymerase ribozyme (150 nt) from 4 pieces. a) Secondary structure of the F ribozyme; each ligation fragment (F1-F4) is indicated by a different color. b) PAGE gel of the non-enzymatic ligation of the full-length F ribozyme (F1+F2+F3+F4) after 24h. Quantification of band intensities yielded: F1: 71.4%, F12 (F1 + F2): 22.7%, F123 (F1 + F2 + F3): 4.2%, F1234 (F1 + F2 + F3 + F4): 1.7%. c) F catalysed primer (P RP) extension on template (T RP) in ice for 7d of 1) control F ribozyme and 2) full length-ligation variant (F1234).
Figure S7. Activity of assembled ribozyme. a) PAGE gel of the templated non-enzymatic assembly of the F ribozyme from 7 fragments applying the following reaction conditions: 50 µl volumes (10 mM MgCl₂, 50 mM CHES pH 9) with 0.5 µM 5' fluorescently (FITC) labelled primer, 5' 2-Methylimidazole activated RNA oligonucleotides (1.5 µM each), 50 µM each RNA template strand at 37°C for 24h.b) Quantification of band intensities, the values (% of combined intensities in one line) are the mean of three individual experiments. c) Primer (P RP) extension on template (T RP) in ice for 7d of 1) control F ribozyme and 2) ligated (F_{1234567}) variant.
Supporting Tables

Table S1. Table of DNA and RNA sequences. DNA sequences are coloured in grey and RNA sequences in black. IVT denotes RNA sequences generated by in vitro transcription. Primer binding sites in template sequences are underlined and 5' modifications (FITC: Fluorescein isothiocyanate, Bio: biotinylation, C18: 18-atom hexaethylene-glycol spacer, p: phosphorylation) are marked in red. Standard oligonucleotides for the CBT selection procedure are as described previously.[1]

| Application | Name | Description | Sequence 5'       | 3'       | Note |
|-------------|------|-------------|------------------|----------|------|
| P HP        | Primer HP |                | FITCGAAGGGCA      |          |      |
| T HP        | Template HP |                | UAUUGUUCUCUUUGACUUCUCUGCCUUC |          |      |
| T HP C      | Complement T HP for PAGE |                | GAAGGGCAGAGAAGUCAAAGAGAG |          |      |
| P RP        | Primer RP |                | FITCCUGCCAACCG    |          |      |
| T RP        | Template RP |                | CAAUGAAUCCACGCUUCGCACGGUUGGCAG |          |      |
| T RP comple ment | Complement T RP for PAGE |                | CUGCCAACCGUGCGAAGCGUG |          |      |
| P F         | Primer for F ribozyme |                | GGACAACAAAAAGACAATCTGCC |          |      |
| P F rev     | Reverse primer for F ribozyme |                | GGATTATGACCTGGGGCTGTC |          |      |
| P T7        | Primer for fill-in |                | GATCGAGATCTCGATCCCGCAGAAAATACGACTCACTATA |          |      |
| F1a         | Template 1 encoding the catalytic core |                | UUGGUUGCCCGGUUGGCAG |          |      |
| F1b         | Template 2 encoding the catalytic core |                | AUUUGUCCUGGUUGGCAG |          |      |
| F2a         | Template 3 encoding the catalytic core |                | CUGAGGGCAGCGGUUGGCAG |          |      |
| F2b         | Template 4 encoding the catalytic core |                | UUCUCAGCUCCGUUGGCAG |          |      |
| F3a         | Template 5 encoding the catalytic core |                | UCCGAAGAUGCGGUUGGCAG |          |      |
| CBT selection | Primer for poly U extension | Template for poly U extension, IVT |
|---------------|-----------------------------|----------------------------------|
| P pU          | FITCUUUUGACGUCUGCCAACCG     | GGAAAAAAACGGUUGGCAGACGUC         |
| T pU          | BioUUUUUUUUUCUGCCAACCG      | GUCAAUGACACCUUCGACGGUUGCCAGAAAAAAA |
| P RP CBT      | BioUUUUUUUUUCUGCCAACCG      | GUCAAUGACACCUUCGACGGUUGCCAGAAAAAAA |
| T RP CBT      | CUCUUUGACUUCUCUGCCAACCG     | GUCAAUGACACCUUCGACGGUUGCCAGAAAAAAA |
| P HP CBT      | BioUUUUUUUUUCUGCCAACCG      | GUCAAUGACACCUUCGACGGUUGCCAGAAAAAAA |
| T HP CBT      | CUCUUUGACUUCUCUGCCAACCG     | GUCAAUGACACCUUCGACGGUUGCCAGAAAAAAA |
| DC -3 RP DNA minicircle -3 for T RP CBT | GTTACTTTTTCAATGAAATCCACGGTTTCTGTAACGACTTTTCGGA | GATTTCTAGGATCTCAAGTATGTTCTAAAGTC |
| DC 0 RP DNA minicircle 0 for T RP CBT | GTTACTTTTTCAATGAAATCCACGGTTTCTGTAACGACTTTTCGGA | GATTTCTAGGATCTCAAGTATGTTCTAAAGTC |
| DC +7 RP DNA minicircle +7 for T RP CBT | GTTACTTTTTCAATGAAATCCACGGTTTCTGTAACGACTTTTCGGA | GATTTCTAGGATCTCAAGTATGTTCTAAAGTC |
| DC -4 HP DNA minicircle -4 for T HP CBT | GTTACTTTTTCAATGAAATCCACGGTTTCTGTAACGACTTTTCGGA | GATTTCTAGGATCTCAAGTATGTTCTAAAGTC |
| DC 0 HP DNA minicircle 0 for T HP CBT | GTTACTTTTTCAATGAAATCCACGGTTTCTGTAACGACTTTTCGGA | GATTTCTAGGATCTCAAGTATGTTCTAAAGTC |
| DC +4 HP DNA minicircle +4 for T HP CBT | GTTACTTTTTCAATGAAATCCACGGTTTCTGTAACGACTTTTCGGA | GATTTCTAGGATCTCAAGTATGTTCTAAAGTC |
| DC +6 HP DNA minicircle +6 for T HP CBT | GTTACTTTTTCAATGAAATCCACGGTTTCTGTAACGACTTTTCGGA | GATTTCTAGGATCTCAAGTATGTTCTAAAGTC |
| F I           | pGAACACUGCGGAUGAGAGGAGGAGGCCUGCUUCGUGGC | pGAACACUGCGGAUGAGAGGAGGAGGCCUGCUUCGUGGC |
| F II          | pGAACACUGCGGAUGAGAGGAGGAGGCCUGCUUCGUGGC | pGAACACUGCGGAUGAGAGGAGGAGGCCUGCUUCGUGGC |
| F III         | pGAACACUGCGGAUGAGAGGAGGAGGCCUGCUUCGUGGC | pGAACACUGCGGAUGAGAGGAGGAGGCCUGCUUCGUGGC |
|   | Piece 4 for templated ligation (4 pieces) | pGAAAAGGCGCUGUUAGACAGCAGGCAUAAUCC |
|---|-----------------------------------------|-----------------------------------|
| Sp I | Splint 1 (F I+F II) for templated ligation (4 pieces) | CCGAAGATGTCTCAAGCCTGAG |
| Sp II | Splint 2 (F II+F III) for templated ligation (4 pieces) | TGGCGCTATTGCGCCACCGAAGGC |
| Sp III | Splint 3 (F III+F IV) for templated ligation (4 pieces) | CAGCGCCTTCTCGTCAAGT |
| F 1 | Piece 1 for templated ligation (7 pieces) | FITCC18 C18GGACAACCAAAAAGACAAU |
| F 2 | Piece 2 for templated ligation (7 pieces) | pCUGCCUCAGAGCUUGAGAA |
| F 3 | Piece 3 for templated ligation (7 pieces) | pCAUCUUCGGAUAGAGGAG |
| F 4 | Piece 4 for templated ligation (7 pieces) | pGCAGCUCCGUGGCGCAUU |
| F 5 | Piece 5 for templated ligation (7 pieces) | pAGCgccACGUCUCAACAG |
| F 6 | Piece 6 for templated ligation (7 pieces) | pAUACUUGACCGAGCAAAAG |
| F 7 | Piece 7 for templated ligation (7 pieces) | pGCGCUGUUAGACAGCAGCCAGGCAUAAUCC |
| Sp 1 | Splint 1 (F1+F2) for templated ligation (7 pieces) | CUGAGGCGAGAUUGUCUUUGGUGU |
| Sp 2 | Splint 2 (F2+F3) for templated ligation (7 pieces) | UCCGAAGUUGUCUCAAGCU |
| Sp 3 | Splint 3 (F3+F4) for templated ligation (7 pieces) | CGAAGGCUCUCUCUACA |
| Sp 4 | Splint 4 (F4+F5) for templated ligation (7 pieces) | CGUGGCGCUAUUGGCAC |
| Component | Description | Sequence |
|-----------|-------------|----------|
| Sp 5      | Splint 5 (F5+F6) for templated ligation (7 pieces) | GGUCAAGUAUCUGUUGAGAA |
| Sp 6      | Splint 6 (F6+F7) for templated ligation (7 pieces) | CUAACAGCGCCUUUUUGCUA |
| Z/Y/W library | IVT | GGAACAACAAAAAGACAAAUUCUGCCCUCAGAGCUUGAGAAC AUCUUCGGAUGYAGAGGGGACGCCCUCUGUGGCGGCGAUA GCGCCAACGGUCUCAACAGYRCAAA (N)12, 18, 24 GACCUAG CGAAAAGGGCMUGUAGACACGCMACCAGGUACUAUCCCG GAGCUUCGGCUC |
| Z         | IVT | GGAACAACAAAAAGACAAAUUCUGCCCUCAGAGCUUGAGAAC AUCUUCGGAUGYAGAGGGGACGCCCUCUGUGGCGGCGAUA GCGCCAACGGUCUCAACAGYRCAAA (N)12, 18, 24 GACCUAG CGAAAAGGGCMUGUAGACACGCMACCAGGUACUAUCCCG GAGCUUCGGCUC |
| K         | IVT | GGAACAACAAAAAGACAAAUUCUGCCCUCAGAGCUUGAGAAC AUCUUCGGAUGYAGAGGGGACGCCCUCUGUGGCGGCGAUA GCGCCAACGGUCUCAACAGYRCAAA (N)12, 18, 24 GACCUAG CGAAAAGGGCMUGUAGACACGCMACCAGGUACUAUCCCG GAGCUUCGGCUC |
| C1        | IVT | GGAACAACAAAAAGACAAAUUCUGCCCUCAGAGCUUGAGAAC AUCUUCGGAUGYAGAGGGGACGCCCUCUGUGGCGGCGAUA GCGCCAACGGUCUCAACAGYRCAAA (N)12, 18, 24 GACCUAG CGAAAAGGGCMUGUAGACACGCMACCAGGUACUAUCCCG GAGCUUCGGCUC |
| C2        | IVT | GGAACAACAAAAAGACAAAUUCUGCCCUCAGAGCUUGAGAAC AUCUUCGGAUGYAGAGGGGACGCCCUCUGUGGCGGCGAUA GCGCCAACGGUCUCAACAGYRCAAA (N)12, 18, 24 GACCUAG CGAAAAGGGCMUGUAGACACGCMACCAGGUACUAUCCCG GAGCUUCGGCUC |
| F         | IVT | GGAACAACAAAAAGACAAAUUCUGCCCUCAGAGCUUGAGAAC AUCUUCGGAUGYAGAGGGGACGCCCUCUGUGGCGGCGAUA GCGCCAACGGUCUCAACAGYRCAAA (N)12, 18, 24 GACCUAG CGAAAAGGGCMUGUAGACACGCMACCAGGUACUAUCCCG GAGCUUCGGCUC |
| CL        | cloning linker | pCTGTAGGACCACCATCAAT/3ddC/ |
| P CL rev  | Rev Primer for cloning linker | ATTGATGGTGCCTACAG |
| P CL RP   | RP Primer for cloning linker | GTACTGCTGCCAACC |

Ribozymes

Illumina sequencing
| Primer | Sequence | Primer | Sequence |
|--------|----------|--------|----------|
| P Seq  | AATGATACGCGACCCGAGATCTACACTCTTTCCCTACACGACGCTCTTCGATCTNNNGAGTGGGTACTGCTGCCAACCG | P Seqrev | CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGTTGAACCGCTCTTCGATCTATTGATGGTGCCTACAG |

**Supplemental References**

[1] A. Wochner, J. Attwater, A. Coulson, P. Holliger, *Science* **2011**, *332*, 209-212.
[2] J. Attwater, A. Wochner, P. Holliger, *Nat Chem* **2013**, *5*, 1011-1018.