Supplementary Materials

1. Material and Methods

1.1 Standard PCR

Primers used in standard PCR were identical to ones used in aPCR. Phusion polymerase for the PCR reaction was purchased from NEB. Each PCR reaction mixture was carried out in 50 ul final volume, composed of 1x HF buffer (NEB), 200 nM dNTP mix (NEB), 500 nM sense primer (GFP exo-sense = undesired strand), 500 nM antisense primer (GFP exo-anti = desired strand), 10ng/ul plasmid template (pEGFP-N1; Addgene), 0.5 ul Phusion DNA polymerase, and nuclease-free water to volume. Each PCR was performed using the following thermocycler steps: 30s at 98°C, 30s at 58°C, and 1 min at 72°C for 30 cycles. For evaluation of the PCR reactions, each 50 ul PCR product was mixed with 10 ul 6x loading dye purchased from NEB, and then loaded onto 1% agarose cast pre-stained with 1x SYBR Safe. The gel was electrophoresed and visualized as above.

1.2 Verification through restriction digestion

MluI was purchased from NEB. Single-stranded GFP (ssGFP) digestion mixtures was prepared in 20 ul, composed of 1x NEBuffer r3.1 (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 ug/ml Recombinant Albumin, pH 7.9 at 25°C), 60-80 ng ssGFP from each ssDNA production method (lambda specific degradation, formamide separation, and aPCR), 1ul MluI and nuclease-free water to final volume. Each reaction mixture was incubated at 37 °C for 15 min. Reaction products were mixed with 6x loading dye (NEB) and loaded onto 1% agarose gel pre-stained with SYBR Safe (Thermofischer). Electrophoresis was carried out at 110V for 1 hour. The SYBR Safe-stained DNA was visualized using a 490nm wavelength (blue) transilluminator and an amber filter.

1.3 Verification through Single-Stranded Binding Protein (SSB)

T4 Gene 32 Protein (G32P) (SSB) was purchased from NEB. Binding mixtures were prepared in 20 ul, composed of 1x NEBuffer 4.1 (50 mM Potassium acetate, 20 mM Tris-acetate, 10 mM Magnesium acetate, 1 mM DTT, pH 7.9 at 25 °C) (NEB), 60-80 ng ssGFP from each ssDNA production method (lambda specific degradation, formamide separation, and aPCR), 1 ul 10 ug/ul G32P, nuclease-free water to fill volume up to 20 ul. Mixtures were incubated at 37 °C for 30 min. The products were mixed with 6x loading dye (NEB) and loaded onto 1% agarose gel pre-stained with SYBR Safe (Thermofischer). The gel was electrophoresed and visualized as above.

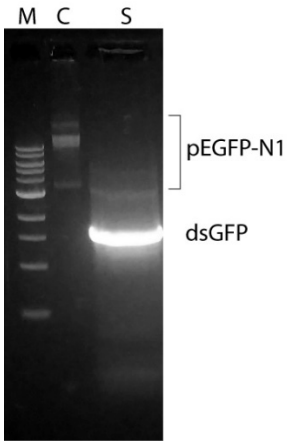

Figure S1. AGE result of standard PCR. M: marker; C: pEGFP-N1; S: standard PCR product.

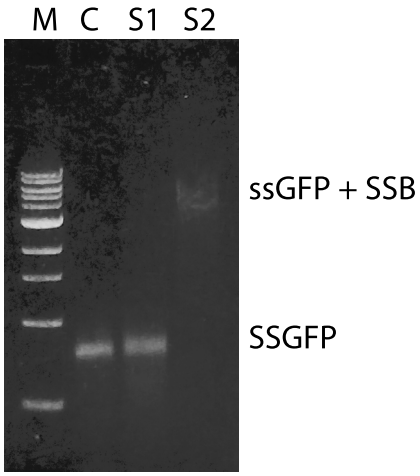

Figure S2. AGE result of ssGFP (from strand-specific Lambda exonuclease degradation) verification through digestion and SSB treatment. M: marker; C: ssGFP; S1: ssGFP digested with MluI; S2: ssGFP treated with SSB.

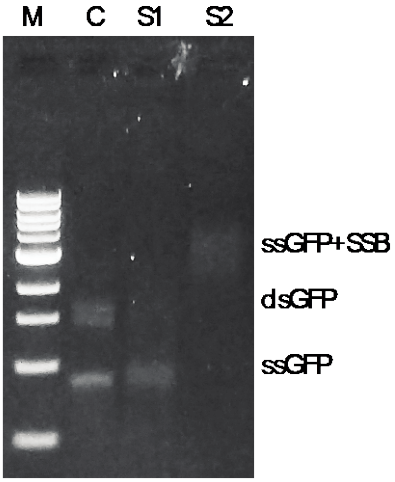

Figure S3. AGE result of ssGFP (from formamide separation) verification through digestion and SSB treatment. M: marker; C: ssGFP; S1: ssGFP digested with MluI; S2: ssGFP treated with SSB.

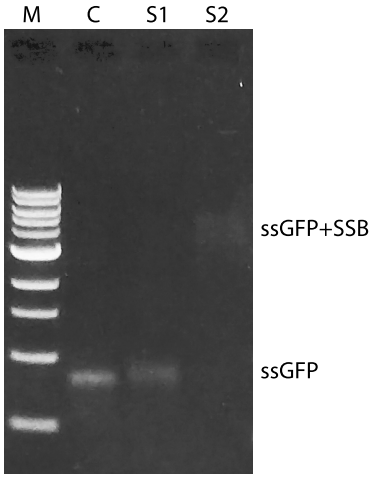

Figure S4. AGE result of ssGFP (from aPCR) verification through digestion and SSB treatment. M: marker; C: ssGFP; S1: ssGFP digested with MluI; S2: ssGFP treated with SSB