An energy-flexible mechanism for qPCR thermal cycling using shape memory alloys

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Abstract

We present a mechanism for thermal cycling that does not require electricity; instead, the device functions as a heat engine and requires only a generic heat source and a shape memory alloy (SMA) spring. The SMA spring mechanically translates to a low-temperature reservoir when heated, and the subsequent cooling of the spring causes translation back to a high-temperature reservoir. The usefulness of the mechanism is displayed by performing the quantitative polymerase chain reaction (qPCR), an important biological assay that requires thermal cycling for amplification of nucleic acids. The ability to perform qPCR with a generic heat source enables a variety of significant health diagnostic tests to be performed in resource limited settings, where electricity access may not be available or reliable. We demonstrate robust thermal cycling using a direct flame, sunlight, and electricity as heat sources, with maximum heating and cooling rates of 4.4 °C s⁻¹ and −2.7 °C s⁻¹, respectively.

Supplementary material for this article is available online

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(Some figures may appear in colour only in the online journal)

1. Introduction

Temperature modulation is an important process across many scientific disciplines. Thermal cycling is used for testing the stability of phase change materials [1–3], for the study of fatigue [4–6] and phase transformation temperatures [7–9] of shape memory alloys (SMAs), and for nucleic acid tests such as the polymerase chain reaction (PCR) [10] and the ligase chain reaction [11, 12]. Such processes are easily accomplished with the equipment of modern laboratories, which use electricity to perform some combination of Joule heating, forced convection, and the Peltier effect for temperature change. However, what capability for thermal cycling exists when electricity is unavailable? Although we are not aware of any current technological solution, the ability to perform quantitative PCR (qPCR) in locations without electricity would have large impact for the field of health diagnostics in resource limited settings. For example, in sub-Saharan Africa there is a large need for diagnosis of human immunodeficiency virus (HIV) in infants [13], but there is a lack of reliable electricity in healthcare facilities [14] to perform the necessary nucleic acid quantification.

We built an energy-flexible thermal cycler using a shape memory alloy spring. SMAs exhibit the shape memory effect, where a temperature-dependent crystal structure transformation between martensite and austenite can be observed as a change in material stiffness. Nitinol (NiTi) is perhaps the most common SMA today, and has seen broad use in medical implants [15], smart material systems [16], and actuators [17, 18], including one driven by sunlight [19]. NiTi has also been used in elastocaloric refrigerators and heat pumps...

[Note: The text continues with further details on the mechanism, its design, and results of the experiments.]
NiTi spring is used to construct a heat engine that also functions as a thermal cycler. A NiTi spring is heated on a high-temperature reservoir, increasing its stiffness and causing it to mechanically translate towards a low-temperature reservoir. There its stiffness is subsequently lowered and an antagonistic spring causes translation back to the high-temperature reservoir, starting the cycle again.

This manuscript begins with the operating principles of our energy-flexible mechanism, including a force balance describing the motion of the NiTi spring. We then show that the thermal cycler can use a generic source of heat to operate, describing the motion of the NiTi spring. We then show that two different objects between high and low heat reservoirs, or positions x, are translated as one component between the reservoirs. The location of the carriage is determined by its temperature reservoir. The spring inside the carriage heats, gradually increasing its stiffness and causing it to mechanically translate towards a low-temperature reservoir. There its stiffness is subsequently lowered and an antagonistic spring causes translation back to the high-temperature reservoir, starting the cycle again.

The design of the carriage enables the spring and target to reach two different temperatures during cycling even though they translate as one component between the reservoirs. The carriage is made from two pieces of aluminum that are connected via thin (0.4 mm wall thickness) stainless steel spacers. Due to the geometry of the spacers and the relative material thermal conductivities (k_{NiTi}/k_{Al} ≈ 10), heat transfer between spring-hold and target is relatively slow compared to heat transfer between the carriage components and the heat reservoirs. The faces of the carriage and heat reservoirs are machined smooth and are forced parallel to one another for optimal heat transfer. A portion of the aluminum where the spring-hold would contact the T_{high} reservoir was machined out (figure 1(b)) so that the temperature of the spring (T_{NiTi}) lags behind the temperature of the target (T_{target}) during heating. This ensures that the spring continues to experience some phase transformation as the target approaches its high-temperature goal (about 94 °C), as a change in stiffness is required to cause translation back to the low-temperature reservoirs. Our measurements (figure 1(d)) confirm that T_{target} is usually about 10 °C higher than T_{NiTi}.

2. Results

2.1. Mechanism operating principles

Figure 1(a) shows an image of the mechanism, with a schematic in figure 1(b). The mechanism consists of heat reservoirs at three different temperatures (T_{high}, T_{low}, and T_{ambient}), and a carriage (figure 1(c)) that translates between the reservoirs in a step-like manner (Movie S1 is available at stacks.iop.org/SMS/29/045038/mmedia). The reservoirs and carriage are machined from aluminum for rapid heat transfer. The location of the carriage is determined by its temperature because inside it is placed a NiTi spring in tension. When the spring is heated, its stiffness increases to a point where the carriage is pulled towards the cooler reservoirs (T_{low} and T_{ambient}), and when the spring is cooled its stiffness is lowered to a point where the carriage is pulled back towards the T_{high} reservoir due to the force from an antagonistic spring. This process is cyclical and the carriage repeatedly translates between high and low heat reservoirs, or positions x = x_{high} and x = x_{low}.

The mechanism contains three heat reservoirs (as opposed to two) so that the thermal cycling of a target object can occur at temperatures distinct from those the spring must be cycled between. While the transition temperature of the NiTi spring is fixed (near 80 °C), thermal cycling of the target object is relatively flexible between T_{low} and T_{ambient}, as long as T_{high} is above the transition temperature of the spring and T_{low} below. In effect, the mechanism performs thermal cycling of two different objects (the NiTi spring and the target) between two sets of temperature limits (table 1). Four heat reservoirs would allow for complete disentanglement of the thermal cycling limits. The motivation of our chosen target temperatures is discussed later in the manuscript.

The carriage is fixed to a low-friction linear slide that restricts its movement to be perpendicular to the faces responsible for heat transfer (figure 1(b)). The NiTi spring itself is also oriented perpendicular to the direction of movement. This is an intentional and crucial design feature. The mechanism cycles based on a change in tension in the NiTi spring, and that change can be larger the longer the spring is (assuming the same change in stiffness). Orienting the spring perpendicular to the movement allowed us to maximize the spring length while keeping translation of the carriage to a minimum. The NiTi spring is attached to a stainless steel wire rope that is wrapped around a pulley, ensuring that the tensile force has a component in the direction of the carriage movement (we call this force F_{NiTi}). The linear slide is of the low-friction type so that the force on the pulley in the direction perpendicular to movement does not adversely affect translation.

A constant-force spring with force F_{cfs} is attached to the linear slide and always pulls the carriage towards the T_{high} reservoir (antagonistic to F_{NiTi}). In addition to the two springs there are also two magnets that affect the carriage’s movement (figure 1(b)). The magnet near the high-temperature reservoir provides force F_{m,high} and the magnet near the low-temperature reservoir provides force F_{m,low}. These magnetic forces allow the spring to increase or decrease in stiffness by variable amounts depending upon the position of the magnets, which is easily adjusted.

A simple force balance decides the movement of the carriage. Consider the case where the carriage is on the T_{high} reservoir. The spring inside the carriage heats, gradually transforms to austenite, and increases in stiffness (its length is fixed and is greater than its free-length). F_{NiTi} subsequently increases. F_{cfs} and F_{m,high} prevent the carriage from moving as they oppose F_{NiTi}. Eventually, F_{NiTi} increases enough such
that the carriage can translate to the $T_{\text{low}}$ reservoir:

$$F_{\text{NiTi}}(T, x = x_{\text{high}}) > -[F_{\text{cfs}} + F_{\text{m,high}}(x = x_{\text{high}})].$$  \hspace{1cm} (1)

The above equation can be equivalently stated as: the carriage accelerates in the $+x$ direction (see figure 1(b)) when the sum of the forces are greater than zero. In equation (1) we assume that the magnetic force from the opposite reservoir ($F_{\text{m,low}}$) is zero as it is relatively far away. We also emphasize which forces are dependent upon position, $x$, and/or temperature, $T$. 

Figure 1. Mechanism overview and schematic. (a) An image of the mechanism. Heat reservoirs made from aluminum are at three different temperatures. A carriage mounted on a linear slide translates between the reservoirs to achieve thermal cycling. (b) A schematic of the same mechanism. The carriage is built from two parts, one housing the NiTi spring (called the spring-hold) is cycled between $T_{\text{high}}$ and $T_{\text{ambient}}$, and the other (called the target) is thermal cycled between $T_{\text{high}}$ and $T_{\text{low}}$. The force balance determining the carriage’s position is also shown. A constant-force spring provides a force ($F_{\text{cfs}}$) antagonistic to the force from the NiTi spring ($F_{\text{NiTi}}$). Two magnets provide forces $F_{\text{m,high}}$ and $F_{\text{m,low}}$. (c) An image from computer aided design emphasizes the geometry of the carriage. (d) Four representative cycles of the mechanism, when heated by electricity. (Top) The two components of the carriage have offset temperatures because of a difference in contact area and because they cycle between different heat reservoirs. (Bottom) $F_{\text{m,high}}$ and $F_{\text{m,low}}$ can be deduced from $F_{\text{NiTi}}$, which was measured with a small load cell.

Table 1. Approximate temperature limits of the cycled components. See figure 1(c) for visualization of the two components.

| Component of carriage | Position | Component exchanges heat with | Component final temperature |
|-----------------------|----------|-------------------------------|-----------------------------|
| Spring-hold           | $x = x_{\text{high}}$ | $T_{\text{high}}$ (105 °C) | 85 °C                      |
|                        | $x = x_{\text{low}}$ | $T_{\text{ambient}}$ (25 °C) | 45 °C                      |
| Thermal cycling target| $x = x_{\text{high}}$ | $T_{\text{high}}$ (105 °C) | 94 °C                      |
|                        | $x = x_{\text{low}}$ | $T_{\text{low}}$ (45 °C) | 60 °C                      |
We exclude noting these parameters in other parts of this text for the purpose of brevity.

Once movement begins, $F_{m,high}$ drops significantly as the carriage moves away from it. Note that the magnets are vital to the mechanism’s operation. Without the magnetic force, at $x = x_{hot}$ $F_{NiTi}$ would only rise to the value of $F_{cfs}$. Then the carriage would slightly lift off the hot reservoir, but with not enough stiffness to continue to overcome $F_{cfs}$ over a significant distance ($F_{NiTi}$ drops as the spring length increases). The carriage would indefinitely hover very near the $T_{high}$ reservoir.

Now consider that the carriage is on $T_{low}$ and $T_{ambient}$. $F_{NiTi}$ has dropped significantly because of the spring’s increase in length, and continues to drop as the spring transforms back to martensite and decreases in stiffness. $F_{m,low}$ and $F_{NiTi}$ hold the carriage in position until the constant-force spring can overcome these forces and pull the carriage back to the $T_{high}$ reservoir:

$$-F_{cfs} > F_{NiTi}(T, x = x_{low}) + F_{m,low}(x = x_{low})$$

The above equation can be equivalently stated as: the carriage accelerates in the $-x$ direction (see figure 1(b)) when the sum of the forces are less than zero.

### 2.3. Tuning the thermal cycling parameters

Although most thermal cyclers adjust their operating parameters (i.e. temperature limits and the time spent at those limits) via electronic control, the operating parameters of our mechanism result solely from mechanical inputs. $T_{high}$, $T_{low}$, and the phase transformation temperature of the spring define the absolute temperature limits of our system, but tuning within those limits can be controlled via the magnets near the heat reservoirs.

The magnets are mounted on fine screws so that their position in $x$ can be precisely set. Bringing a magnet closer to the steel post on the carriage will increase $F_{m,low}$ or $F_{m,high}$ and tend to bring $T_{target}$ closer to the temperature of the reservoir it is in contact with, as long as both equations (1) and (2) can eventually be satisfied. Figure 2(a) shows that as the distance between the steel post and the $T_{high}$ magnet is decreased, the final temperature of the target and the cycle time increases. As equation (1) suggests, $F_{NiTi}$ must be greater to overcome the increase in $F_{m,high}$, requiring a greater portion of the spring to transform from martensite to austenite. We indirectly measured the change in $F_{m,high}$ using $F_{NiTi}$ (figure 2(a)). Figure 2(b) similarly shows that the low-temperature of the target can be altered by changing $F_{m,low}$. Increasing the distance between magnet and steel post caused the carriage to spend less time on the $T_{low}$ reservoir. Equation (2) helps to illustrate that as $F_{m,low}$ decreases, $F_{cfs}$ overcomes the opposing forces more quickly.

Fine-tuning of the cycle may also be achieved by changing heat transfer parameters. As mentioned, we intentionally slowed heat exchange from the $T_{high}$ reservoir to the spring-hold by machining aluminum from the reservoir (figure 1(b)). If the same were performed on the $T_{ambient}$ reservoir then heat transfer between $T_{low}$ and the target would be relatively fast compared to heat transfer between $T_{ambient}$ and spring-hold. This could be used to increase the amount of time the target spends on the $T_{low}$ reservoir, useful for producing more of a steady-temperature dwell.

**Figure 2.** Mechanical modulation of cycle parameters. (a) Movement of the magnet near the $T_{high}$ reservoir causes a change in $F_{m,high}$, subsequently modulating the high target temperature. Also note that the cycle time is affected. The background shading shows the time when the magnet was at different distances from the steel post on the carriage, where $a$ is an arbitrary starting distance. (Top) Measurement of the force on the NiTi spring and (bottom) temperature of the target. (b) Modulation of the cold target temperature when the magnet near the $T_{low}$ reservoir is moved. $b$ is an arbitrary starting distance.

### 2.4. Mechanism heated by three different heat sources

The mechanism functions as a heat engine and can therefore operate on a generic heat source; thermal cycling will proceed as long as the heat reservoirs are at appropriate temperatures. We performed a series of experiments to show that sunlight, direct flame, and electricity are three such possible sources of heat.
A single cartridge heater (figure 1(a)) and standard PID controller were used to heat the mechanism using electricity. The $T_{\text{low}}$ reservoir is heated via a thermal bridge with the $T_{\text{high}}$ reservoir that provides a variable amount of heat transfer depending upon how tightly it is fastened to the reservoirs. If cycling is rapid the thermal bridge is unnecessary as the $T_{\text{low}}$ reservoir is intermittently heated by heat exchange with the target. The $T_{\text{ambient}}$ reservoir rejects the heat it receives from the spring-hold to the environment by an array of aluminum heat sinks.

We also heated the mechanism using a standard butane torch with adjustable flame (figure 3(a)), where the flame was applied directly to the $T_{\text{high}}$ reservoir. When heated via sunlight (figure 3(b)), a 225 cm$^2$ black aluminum plate was fastened to the $T_{\text{high}}$ reservoir and was used to absorb light concentrated from a large (1960 cm$^2$) Fresnel lens.

The resulting cycling from the three heat sources is shown in figure 3(c). The maximum heating rate was measured at 4.4 $^\circ$C s$^{-1}$ and the maximum cooling rate was measured at $-2.7$ $^\circ$C s$^{-1}$ (figure 3(d)). Using the mass of the carriage (12 g spring-hold and 5 g target), we estimate that the average heating and cooling power of the mechanism is on the order of 10 W (but is dependent upon $F_{m,\text{high}}$ and $F_{m,\text{low}}$). Electricity and torch-heated cycling was extremely repeatable. For example, the variation in the maximum temperature of the cycle when heated via torch or electricity was at most 0.8 $^\circ$C.
over nine cycles (Figure 3(e)). Furthermore, the time period of the cycling was very stable: when heated via either heat source the maximum variation in the half-period was 2.3 s over nine cycles (Figure 3(e)).

When heated via sunlight, cycling was successful but repeatability diminished. Over nine cycles, the maximum variation in peak temperature was about 2.5 °C (high-temperature) and 4.2 °C (low-temperature). The maximum variation in half-period was about 65 s \((T_{\text{high}} \rightarrow T_{\text{low}})\) and about 11 s \((T_{\text{low}} \rightarrow T_{\text{high}})\). While we did not perform enough experiments in the sunlight to verify as much, we suspect that these large variations were caused by \(T_{\text{ambient}}\) rising above 25 °C, making it difficult for \(F_{\text{NiTi}}\) to decrease enough to fulfill equation (2). The heat sinks used to reject heat to the environment on the \(T_{\text{ambient}}\) reservoir are anodized black and were likely heating the reservoir by absorbed sunlight.

Heating via any of the three methods was sufficiently rapid. Heating via electricity or torch to about 105 °C was achieved in less than 9 min, and heating via sunlight was achieved in about 15 min (Figure 3(f)).

2.5. Thermal cycling used for qPCR

The temperature limits of the target (Table 1) were chosen so that the mechanism would be useful to perform the PCR. PCR is a ubiquitously-used biological assay that can identify the presence or absence of specific nucleic acids by repeated heating and cooling of a sample. PCR was traditionally performed by cycling a sample between three distinct temperatures, but a two-step version of the assay is also common today. Quantification of nucleic acid is possible by measuring fluorescence in real-time and finding the cycle that the signal starts to dramatically increase: the earlier the threshold cycle, the greater the concentration of nucleic acid (this methodology is called qPCR).

We attached optical components to the target of the carriage so that the fluorescence of a qPCR assay could be tracked during thermal cycling (Figure 4(a)). We inserted a standard 200 µl PCR tube into the target component of the carriage and heated it between roughly 94 °C and 60 °C for 40 cycles. The full methodology of our assay can be found in Methods, along with details of the target sequence. We performed three trials of qPCR using a sample positive for our target nucleic acid, and for all three samples fluorescence began to increase rapidly near the 25th cycle (Figure 4(b)). When the same assay was performed on a commercial qPCR machine, similar results were found.
3. Conclusions

We have presented a mechanism for thermal cycling that can be powered by electricity, flame, or sunlight. We are not aware of other thermal cyclers which share this energy flexibility. When heat is input to the system at temperatures below and above the transition temperature of a shape memory alloy spring, thermal cycling autonomously commences due to the change in stiffness of that spring. A simple force balance was presented that dictates the motion of the mechanism; these equations may be used to expedite the design of similar systems in the future. Subsequent work might consider spring fatigue to assess device lifetime, as the applicability of this technique will be limited by the number of cycles to failure for the NiTi spring. We encountered no signs of material failure during our testing (at least 10^3 cycles), and other work shows that ordinary NiTi springs can last more than 10^5 cycles [29].

Although energy-flexible thermal cycling may be of use to many fields, we demonstrated the utility of our device by performing a gold-standard nucleic acid assay: quantitative PCR. The heating and cooling rates we have demonstrated (up to 4.4 °C s^{-1} and -2.7 °C s^{-1}) are comparable to those in machines built exclusively for the purpose of qPCR, but are achieved by heat conduction with heat reservoirs instead of by Peltier elements, which require electricity. The capability to perform qPCR with heat sources such as torch and sunlight might be especially important for health diagnostics in resource limited settings, where access to electricity is sometimes available but cannot be guaranteed. Future iterations of the mechanism might be made smaller to improve portability and to decrease the amount of energy required for operation, bolstering their practicality as diagnostic tools.

4. Methods

4.1. Mechanism construction and temperature measurement

All heat reservoirs and carriage components were machined from 6061 aluminum. The \(T_{\text{low}}\) and \(T_{\text{ambient}}\) reservoirs were initially one piece, but later cut into two, ensuring that the faces contacting the carriage would be parallel to one another. The temperatures of the \(T_{\text{high}}\) and \(T_{\text{low}}\) reservoirs were monitored via shielded K-type thermocouples with wire diameter 0.13 mm. MAX31855 thermocouple amplifiers (0.25 °C resolution, ±2 °C accuracy) were used to sample temperature at 1 Hz. To measure \(T_{\text{target}}\) we placed the same type of thermocouple at the bottom of an empty 200 μl plastic PCR tube. \(T_{\text{NiTi}}\) was estimated by measuring the temperature of the spring-hold.

The carriage was fixed onto a M-2 linear ball slide from Del-Tron Precision, Inc. A constant-force spring with 1.9 N load pulled on the slide at all times. Magnets on the low and low-temperature reservoirs were mounted to 10–32 UNF screws (1 turn ≈ 0.8 mm), and were attracted to an alloy steel shoulder screw on the carriage, which also served to fix a 12.7 mm diameter pulley with bearing. A 0.6 mm diameter, braided, stainless steel wire rope was wrapped around the pulley and was used to attach the NiTi spring to the \(T_{\text{ambient}}\) reservoir, or to a load cell mounted on the \(T_{\text{low}}\) reservoir (\(F_{\text{NiTi}}\) was not measured during all experiments, which is why the load cell cannot be seen in Movie S1). The tensile force on the NiTi spring was measured by a full bridge load cell from Omega Engineering (part LCL-816G).

4.2. NiTi spring specifications

The nitinol spring was manufactured by Kellogg’s Research Labs with the following specifications: 3.2 mm inner diameter, 0.5 mm wire diameter, 2 mm pitch, and 80 °C transition temperature. The spring was manufactured longer than necessary so that its free-length could be adjusted. Extra windings exited a hole in the spring-hold opposite the load cell, and the free-length was set via set screws.

4.3. Heating methods

A 54 W cartridge heater from Comstat Inc. (part MCH1-240W-004) and standard PID controller were used to heat and regulate the mechanism when using electricity. When heating via flame, a standard butane torch was used, where \(T_{\text{high}}\) was controlled via a mechanical regulator on the torch. A 0.5 m diameter acrylic Fresnel lens from Knight Optical (part LFQ250500) was used to concentrate sunlight onto an absorption plate that was mounted to the \(T_{\text{high}}\) reservoir. When \(T_{\text{high}}\) reached above about 105 °C, the amount of incident sunlight was attenuated by partially occluding the Fresnel lens with an opaque object. The absorption plate was manufactured from 6061 aluminum and painted flat black. To slow heat loss to the environment, the plate was covered with glass on the side facing the Sun, and with 12.7 mm thick melamine insulation on the opposite side. Insulation of the heat reservoirs was not necessary using any of the heating methods described here.

4.4. qPCR assay and method

A sample mixture was prepared and consisted of 1X Fast Evagreen qPCR Master Mix (Biotium product #31003), 0.67 μM forward and reverse primer, and target sample. The target sample consisted of about 38 000 copies of a plasmid made from a region of the human herpesvirus 8 (Kaposi’s sarcoma herpesvirus) open reading frame (ORF) 26 gene (see sequence NC_009333 from the National Center for Biotechnology Information). Kaposi’s sarcoma is a cancer common today in parts of East Africa. Please refer to our group’s past work on nucleic acid diagnosis of Kaposi’s sarcoma for more information [26–28]. Primer sequences were (5' to 3') AGCCGAAAAGATTCCACCA (forward) and GCTGCGCAGCACCAT (reverse).

When qPCR was performed in the commercial machine (ViiA 7 Real-Time PCR System, manufactured by Applied Biosystems), 10 μl of the sample mixture was amplified. The following thermal cycling parameters were used: a 2 min hold at 50 °C followed by a 10 min hold at 95 °C, and then 40
cycles of 15 s at 95°C and 1 min at 60°C, where the heating and cooling rates were \( \pm 1.6 \text{ °C}s^{-1} \).

To perform qPCR in the mechanism, 50 \( \mu l \) of the same sample mixture was topped with 50 \( \mu l \) of paraffin oil in a standard 200 \( \mu l \) PCR tube and placed in the target component of the carriage. To track the temperature of the sample a small thermocouple (shielded K-type with wire diameter 0.13 mm) was inserted into the tube such that it rested in the paraffin oil but did not touch the sample, and then the lid of the tube was closed. We first performed a 2 min denaturation step (necessary for hot-start polymerases) near 95°C by greatly increasing \( F_{n,\text{high}} \) to indefinitely keep the carriage on the \( T_{\text{high}} \) reservoir. We then decreased \( F_{n,\text{high}} \) to proceed with thermal cycling, where we aimed to keep the sample between 55°C and 60°C for at least a few seconds for the annealing/extension step and between 91°C and 96°C for a few seconds for the denaturation step. Although it is typical in qPCR to have a longer annealing/extension step, much shorter steps have shown to be possible [30–32]. Thermal cycling was performed for at least 40 cycles. Because of the initial denaturation step, manual adjustment of \( F_{n,\text{high}} \) and \( F_{n,\text{low}} \) was necessary for the first 5–10 cycles of qPCR but generally was not needed in later cycles.

The sample was illuminated by a blue light emitting diode (LED) and fluorescence was recorded using a high-sensitivity photodiode (device: TSL237, manufacturer: ams AG, sensitivity: 2.3 kHz cm\(^{-2}\) \( \mu \text{W}^{-1} \)) which was placed underneath an optical filter (Omega Optical 530/660DB) to block the illumination source. The LED was only turned on near the annealing/extension step to prevent photo-bleaching. Because the current version of the mechanism is not optically isolating the mechanism from the environment, qPCR experiments were performed in a dark room; signal quality could be improved by optically isolating the mechanism from the environment. Fluorescence was measured as the photodiode value when \( T_{\text{target}} \) was near 62°C in an effort to eliminate temperature-dependent changes in the LED or photodiode. Moving median and moving mean filters were applied to the fluorescence signals to help eliminate signal noise.

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**Author contributions**

RS wrote the manuscript. DE, DM, and PB reviewed and edited the manuscript. RS, PB, and DE conceptualized the principles of the mechanism. RS, PB, and DM performed experiments. RS completed all design and construction of the mechanism. RS analyzed the data and generated the figures.

**Competing interests**

The authors declare no competing interests. Product manufacturers are noted only for completeness of description and does not constitute recommendation or endorsement.

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