Optimal Control of DNA Amplification

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1. INTRODUCTION

In "Dynamics and Control of DNA Sequence Amplification", we have formulated the PCR optimal control problem and discussed the strategies for solving it. In this chapter, we implement those strategies and solve a simple PCR optimal control problem. The foundational concepts of Optimal Control were developed as early as the 1750s in classical mechanics as the principle of least action, a variational approach that minimizes the action (the time integral of a Lagrange cost) of a moving particle to derive the equations of motion in Hamilton's form. A more general and formal version of the optimal control problem, especially from a control engineering perspective, was developed by Pontryagin, who also derived the conditions for the optimality which now comprise Pontryagin Maximum Principle (PMP) ([1, 2]). Optimal control is used in diverse applications ranging from satellite control to chemical plant control, from quantum control to biological process control, etc. As a result of this, there are several classes of optimal control problems such as constrained optimal control, time optimal control, etc. The complexity of optimal control problems pose challenging issues in developing efficient numerical schemes. There are tremendous research efforts directed towards developing various numerical schemes to solve various optimal control problems. Though the primary objective of our work is to solve a PCR optimal control problem, in this chapter we also briefly review several numerical approaches that are available to solve a large scale dynamic optimization problem so that it will be useful in future development of an efficient software platform for solving PCR optimal control problems. Development of an efficient numerical scheme for the optimal control of PCR is beyond the scope of this work. The primary goal of this work is the development of a foundation for the derivation of optimal control laws for amplification of DNA sequences through application of the PMP to sequence-dependent models of DNA amplification. When applied to a given DNA sequence, these laws prescribe the optimally controlled dynamics of amplification of that sequence.

2. SOLUTION OF THE OPTIMAL CONTROL PROBLEM

In order to solve the optimal control problems that we have formulated in "Dynamics and Control of DNA Sequence Amplification"we keep temperature (T) as control variable. Even though the upper and lower limits for the temperature are specified as inequality constraints there, the optimum lies in the interior of the constraint set; hence, if the algorithm does not sample manipulated variables that are outside the constraint set, the constraints can be ignored. Hence, we solve an unconstrained optimization without any bounds on the temperatures. Moreover, by appropriately applying the equality constraints $h(x)$ to eliminate variables, we can reduce the number of state variables by 5 and solve the control problem on a reduced dimensional state manifold. The conditions for optimality for the above optimal control problem are given below as per the PMP.

2.1. Solution of the Optimal Control Problem

Necessary conditions for optimal solutions to the above optimal control problem are derived using Pontryagin's Maximum principle, and they are given as

$$H\left[x(t),\phi(t),T(t)\right] = \phi^{Tr}(t)\sum_{i=1}^{10} k_{i,seq}(T(t))g_i(x(t)) \tag{1}$$

$$\frac{d\phi}{dt} = -\frac{\partial H}{\partial x(t)} = -\sum_{i=1}^{10} k_{i,seq}(T(t))\frac{\partial g_i(x(t))^{Tr}}{\partial x(t)}\phi(t) \tag{2}$$

$$\phi(t_f) = \left[\frac{\partial F(x(t))}{\partial x(t)}\right]_{t=t_f} = \left[\frac{\partial}{\partial x(t)}\sum_{i=1}^{4n+4} x_i(t)\right]_{t=t_f} \tag{3}$$

$$\frac{\partial H}{\partial T(t)}\left[x^*(t), \phi^*(t), T^*(t)\right] = \phi^{*,Tr}(t) \sum_{i=1}^{10} \left.\frac{\partial k_{i,seq}(T(t))}{\partial T(t)}\right|_{T^*(t)} g_i(x^*(t)) = 0, \quad \forall t \in [0, t_f] \tag{4}$$

$H[x(t), \phi(t), T(t)]$ is the PMP-Hamiltonian function. ϕ represents the co-state (a function of time similar to a Lagrange multiplier and analogous to momentum in Hamiltonian mechanics). Equations **??**,2 and 4 represent a system of differential algebraic equations (DAE) with the initial and terminal boundary conditions for state and co-state variables. Eq. (4) specifies the first order condition for optimality that must be satisfied by the solution $T^*(t)$. An optimal control $T^*(t)$ must also satisfy the second order (Legendre-Clebsch) conditions for optimality, $\frac{\partial^2 H}{\partial T(t) \partial T(t')} \geq 0$, i.e., that the Hessian of the Hamiltonian must be positive semidefinite at T^*. For Mayer cost functionals, the Hessian is generally semidefinite at T^* due to the existence of a level set of solutions. This DAE should be solved simultaneously to obtain the optimal temperature profile T. If the target length is n, the number of state equations is $4n + 4$ and typically n will be on the order of 10^3. Thus, the PCR optimal control problem is a large scale optimization problem which requires an efficient numerical algorithm to be solved.

2.2. Classification of Optimal Control Problem Solution Strategies

While we developed the necessary conditions for the optimality in the previous section, in this section several numerical strategies to solve an optimal control problem are briefly discussed. Numerical schemes that were developed to solve optimal control problems are broadly classified as *Optimize then Discretize* and *Discretize then Optimize* ([3]). These two methods can be further classified into many types. In the first type, the optimal conditions for the optimal control problem is derived using PMP principle using the variational approach and then the resulting differential algebraic system of equations is solved using an appropriate numerical solver. In the second type, the given optimization problem is discretized to convert it into a Nonlinear Linear Programming Problem (NLP) which is then solved using efficient solvers.

One variational approach to solving optimal control problems is the so-called shooting approach. In shooting algorithms one solves for the optimal control $u^*(t)$ in terms of $x(t)$, $\phi(t)$ using the algebraic constraint from the PMP optimality condition and then integrates the x, ϕ ODEs simultaneously in terms of known $x(0)$ and unknown $\phi(0)$ initial conditions. Although $\phi(0)$ is unknown, terminal boundary conditions are available in terms of either $x(t_f) = x_f$ (Lagrange functionals) or $\phi(t_f) = \nabla_x F(x(t_f))$ (Bolza or Mayer functionals). This is known as a system of differential equations with split boundary conditions or a two-point boundary value problem. For nonlinear problems, $\frac{\partial H}{\partial u(t)}$ is an explicit function of $u(t)$ and hence one can solve for $u(t)$ in terms of $x(t)$,$\phi(t)$, even for Mayer functionals. There are multiple solutions to this problem and one can find an approximate solution via line search. Shooting algorithms converge iteratively upon the target $x(t_f) = x_f$or $\phi(t_f)$ vector by making successive changes in the initial conditions $\phi(0)$; i.e., they shoot from x(0),$\phi(0)$, trying to hit the terminal boundary conditions. Numerical algorithms for this problem are typically based on a combination of Newton-Raphson and Runge-Kutta (RK) ODE integration; RK is used to integrate the state/costate ODEs at each step, given $x(0)$ and a guess for $\phi(0)$; NR is used to solve for roots of the boundary condition equations, i.e., $\phi(t_f) - \phi_f = 0$ or $x(t_f) - x_f = 0$. Denoting these by f_i and the initial costate parameters by $\phi_i(0) = c_i$, the NR step is $\delta c = \alpha J^{-1} F(c)$, where the elements of the Jacobian J are $J_{ij} = \frac{\partial f_i}{\partial c_j}$. Shooting algorithms are particularly convenient for Lagrange functionals for which $u(t)$ can be expressed analytically in terms of $x(t), \phi(t)$. Shooting algorithms applied to nonlinear problems wherein $u(t)$ cannot be expressed analytically in terms of $x(t)$, $\phi(t)$ require integration of the state,co-state equations simultaneously with nonlinear root finding to obtain u at each time step. Hence we did not apply them here.

In this work we used the variational control vector iteration (CVI) method to solve a fixed time simplex PCR optimal control problem. The software implementation of this algorithm was developed fully by the authors. CVI is commonly used for Bolza or Mayer functionals since $\phi(t_f) = \nabla F(x(t_f))$ is available from solution of the state equations and an expression for $u = g(x(t), \phi(t))$ is not required.

2.3. Solution to Fixed time optimal control problem for PCR

For stage 1, we solve a fixed time optimal control problem for a short reaction time (45 seconds of annealing time and 30 seconds of extension time). In stage 1, pseudo first order kinetics for the primer annealing and enzyme binding reaction is applicable. In addition to this, nucleotide concentration can also be assumed to be a constant throughout the cycle. Hence, the algebraic mass balance equations $h(x)$ for these variables need not be solved along with the minimum number of state equations Since the concentrations of primers, enzyme and nucleotides are constants, they

can be multiplied with the second order rate constant. Note that the minimum number of state equations is $4n+4$. Under these conditions, the costate and first order optimality conditions in the PMP become:

$$\frac{d\phi}{dt} = -\frac{\partial H}{\partial x(t)} = \sum_{i=1}^{10} k_{i,seq}(T(t)) B_i^{Tr} \phi(t) \tag{5}$$

$$\frac{\partial H}{\partial T(t)} = \sum_{i=1}^{10} \frac{\partial k_{i,seq}(T(t))}{\partial T(t)}\bigg|_{T^*(t)} B_i x^*(t) = 0, \ \forall t \in [0, t_f]. \tag{6}$$

We use the methods of optimize and discretize (Variational approach) which gives a set of Differential Algebraic Equations (DAE) that provide a solution for the optimal control problem. Due to the reasons mentioned above, we use Control Vector Iteration (CVI) ([1, 3]), which is also called the self consistent iterative method ([4]) to solve the above DAE. This iterative process starts with the specification of the initial conditions for the state variables and an assumed manipulated variable profile. The state equations are now integrated for the fixed reaction time from t_0 to t_f. The boundary condition for the co-state variables are obtained as per Eq. (2) and the co-state equations are integrated backwards.With the available state and co-state variables, the manipulated variable is updated based on the steepest descent method such that the nominal control profile satisfies Eq. (4). The following equation describes the steepest descent method.

$$T_{i+1}(t) = T_i(t) - \alpha \frac{\partial H}{\partial T(t)} \tag{7}$$

Where α is a steepest descent parameter which is generally assumed to be a constant in a conventional CVI ([1]), In order to speed up the iterative process, α can be estimated through line search via the following minimization.

$$\min_{\alpha} \ F(\alpha(t)) = H\left[x(t), \phi(t), T(t) - \alpha \frac{dH}{dt}(t)\right] \tag{8}$$

We used a golden section search algorithm for the above minimization.
The following steps and Fig. 1 explains the implementation of CVI.

1. For a given initial trajectory of $T_0(t)$ and initial condition x_0, the state equations are integrated from t_0 to t_f and $x(t)$ is obtained.
2. Boundary condition for the co-state vector is obtained using Eq. (4).
3. Co-state equations are integrated backwards from t_f to t_0 and $\phi(t)$
4. Using $x(t)$, $\phi(t)$ and $T_0(t)$, at each t, $\frac{\partial H}{\partial T(t)}$ was found.
5. Using $x(t_f)$, the objective function value J was found.
6. If $J \leq J_{desired}$ and $\|\frac{\partial H}{\partial T(t)}\| \leq \epsilon$, the iterative process was stopped at this point, otherwise the iteration was continued to the next step.
7. Golden section line search method was followed to estimate the optimal $\alpha(t)$.
8. Control profile is updated as per Eq. (7)
9. With the updated control profile, step 1 is started again and other steps are followed till the iteration satisfied the convergence criteria, $J \leq 0.05$ and $\|\frac{\partial H}{\partial T(t)}\| \leq 10^{-4}$

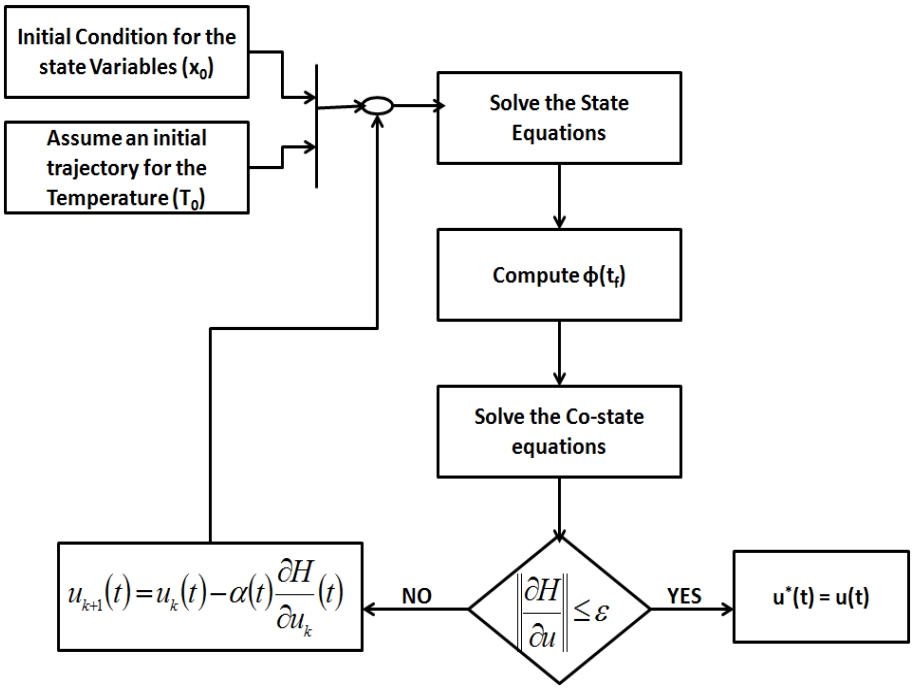

FIG. 1: Control Vector Iteration to solve optimal control problem

3. FIXED TIME OPTIMAL CONTROL PROFILE

A fixed time PCR optimal control problem with the primer sequences mentioned in "Dynamics and Control of DNA Sequence Amplification" and the same reaction conditions (length of target = 500 base pairs) has been solved using the CVI. The reaction time is fixed to be 75 seconds. Figs. 2 and 3 shows the optimal temperature profile and the corresponding optimal DNA concentration profile. The efficiency at the optimal operating condition is 96%. As discussed in "Dynamics and Control of DNA Sequence Amplification"the optimal control solutions $T^*(t)$ for stage 1 are periodic. Hence the $T^*(t)$ from Fig. 3 can be applied repeatedly for the first 14 PCR cycles. These are optimal PCR cycling strategies for fixed cyclic time. The same algorithm can be applied to multi-step PCR cycles by appropriate choice of t_f in Eq. (**??**). Since the experimental parameter estimation for the denaturation model in "Dynamics and Control of DNA Sequence Amplification" has not yet been completed, we don't solve the multi-step fixed time problem here. Note that since the cycle time was arbitrarily fixed in the analysis in that paper, greater efficiency could be achieved in a specified reaction time. In the next section we consider how to obtain the optimal reaction time t_f.

4. TIME OPTIMAL CONTROL PROBLEM

In order to obtain the optimal cycle time for PCR, a multiple cycle time optimal problem of the form introduced in "Dynamics and Control of DNA Sequence Amplification" can be solved. In particular, this provides the optimal switching time between cycles. In stage 1, for the reasons discussed in that paper, solution of the time optimal problem for $m = 2$ provides the optimal cycling strategy for all cycles in the stage.

The PMP-Hamiltonian for time optimal control problem (**??**), expressed using temperature as the control variable, is:

$$H\left[x(t), T(t), \phi(t)\right] = L\left[x(t), T(t)\right] + \phi^{Tr}(t)\sum_{i=1}^{10} k_{i,seq}(T(t))g_i(x(t)) = 1 + \phi^{Tr}(t)\sum_{i=1}^{10} k_{i,seq}(T(t))g_i(x(t)) \qquad (9)$$

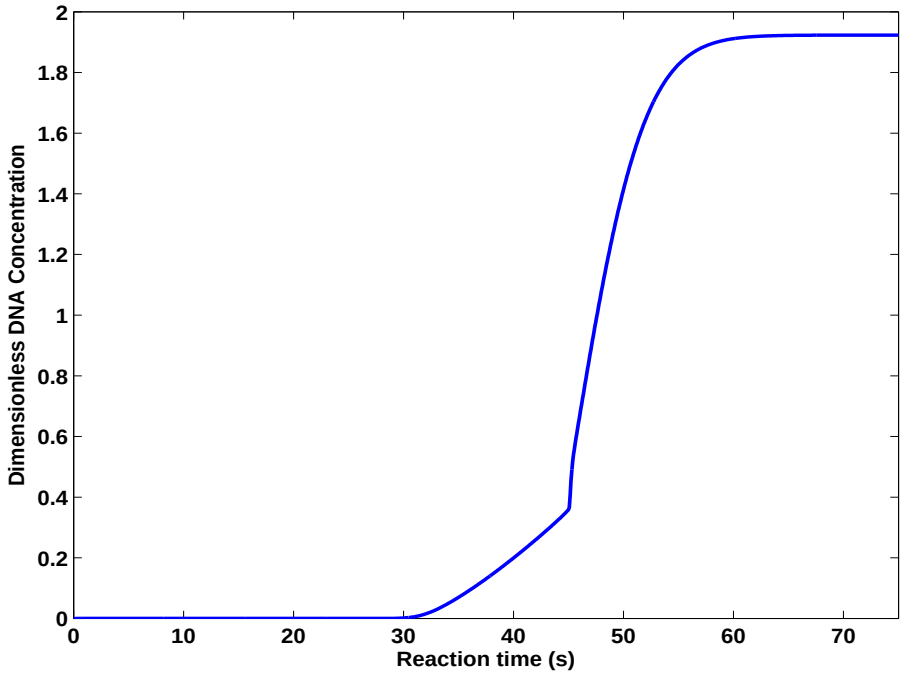

FIG. 2: Optimal DNA concentration profile that was obtained by solving PCR optimal control problem using control vector iteration.

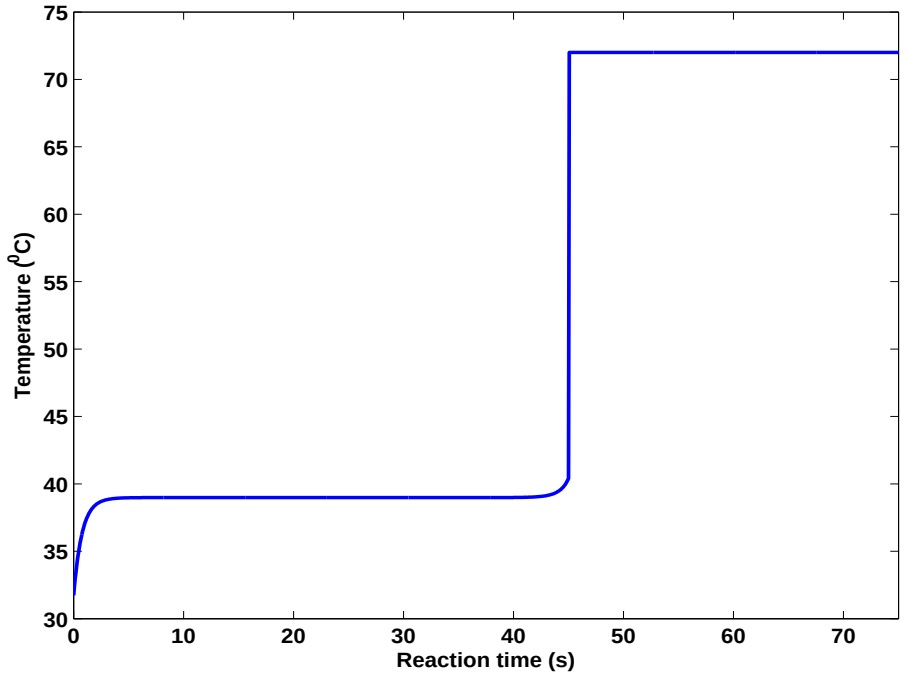

FIG. 3: Optimal temperature profile that was obtained by solving PCR optimal control problem using control vector iteration.

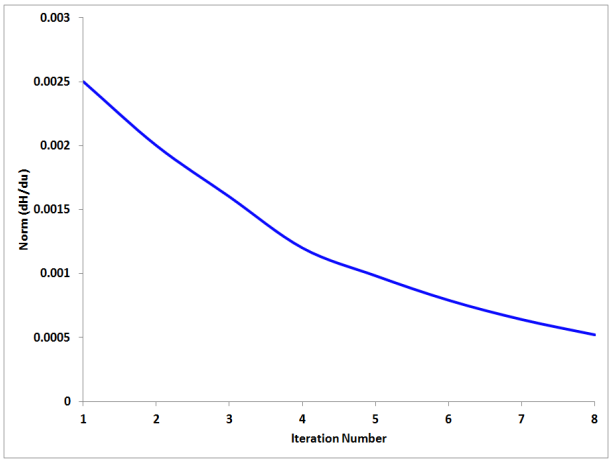

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FIG. 4: Variation of the $\| \frac{dH}{du}(t) \|$ with respect to number of iterations of control vector iteration

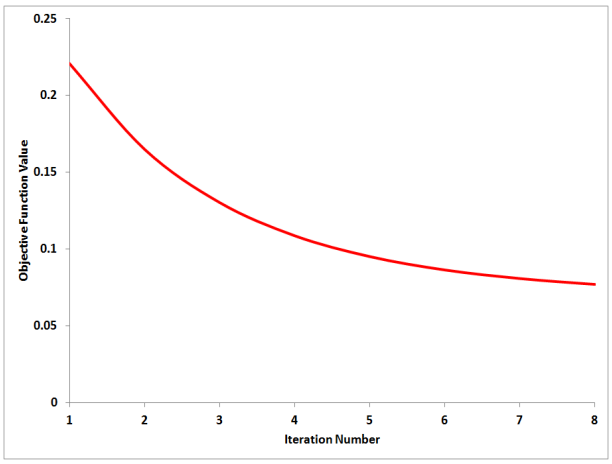

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FIG. 5: Variation of the Objective function value with respect to number of iterations of control vector iteration

FIG. 6: Variation of $\| \frac{dH}{du}(t) \|$ and J value indicates the convergence of control vector iteration. Note that (2 - objective function value)/ 2 expresses the efficiency of the PCR

The first-order conditions for the minimal time problem are:

$$\frac{d\phi}{dt} = -\frac{\partial H}{\partial x(t)} = -\sum_{i=1}^{10} k_{i,seq}(T(t)) \frac{\partial g_i(x(t))^{Tr}}{\partial x(t)} \phi(t) \tag{10}$$

$$\frac{\partial H}{\partial T(t)} \left[x^*(t), \phi^*(t), T^*(t) \right] = \phi^{*,Tr}(t) \sum_{i=1}^{10} \frac{\partial k_{i,seq}(T(t))}{\partial T(t)} \Big|_{T^*(t)} g_i(x^*(t)) = 0, \quad \forall t \in [0, t_f] \tag{11}$$

In addition to the $4n+4$ initial conditions, the following $4n+4$ terminal boundary conditions are applied to the co-state and state to provide the two-point boundary value problem:

$$\phi_i(t_f) = 0, \quad i = 1, ..., 4n+4 \tag{12}$$

$$\sum_{i=1}^{4n+4} x_i(t_f) = S_{10} + S_{20} - x_{4n+9,f}, \quad x_{4n+9,f} > 2x_{4n+9}(0). \tag{13}$$

We choose $x_{4n+9,f} = 2$ to obtain the time optimal stage 1 cycle protocol; the resulting t_f corresponds to less than 2 cycles unless the time optimal cyclic efficiency is $> \sqrt{2}$. The PMP first-order optimality conditions for time optimal control include an additional so-called algebraic transversality condition for the final time t_f:

$$H\Big|_{t_f} = 0 \implies \phi^{Tr}(t_f) \sum_{i=1}^{10} k_{i,seq}(T(t_f)) g_i(x(t_f)) = -1. \tag{14}$$

For stage 1, Eqs. 10 and 14 are replaced by

$$\frac{d\phi}{dt} = -\sum_{i=1}^{10} k_{i,seq}(T(t)) B_i^T \phi(t)$$
$$\phi^{Tr}(t_f) \sum_{i=1}^{10} k_{i,seq}(T(t_f)) B_i x(t_f) = -1.$$

Various optimization algorithms are available for solving the DAE corresponding to this two-point boundary value problem subject to the algebraic and transversality constraints Eqs. 11 and 14. The transversality constraint can be associated with a new Lagrange multiplier. The solution provides both the optimal control function $T^*(t)$ and the minimal time t_f, which is greater than the period of the function in stage 1. The minimal time cycling strategy for stage 1 is the period of the function. A property of the solution to this problem is that it will satisfy the time optimal cycle switching conditions for geometric growth discussed in "Dynamics and Control of DNA Sequence Amplification". Recall from that analysis that the following bound applies:

$$\min_{\eta} \; t_{total}(\eta) \geq \min_{\eta} \; t\left(\eta, T^*\right) \frac{log\left(y\right)}{log\left(1+\eta\right)} \tag{15}$$

Since experimental data for estimation of DNA denaturation model parameters are not yet available, we do not solve the multi-step time optimal problem here. In the absence of a denaturation model, assuming a fixed denaturation time, solution of the stage 1 time optimal problem requires tracking of possible cycle efficiencies and computation of $t^*\left(\eta\right)$ to identify the optimal cyclic efficiency. Due to the computational complexity of this problem, here we use an approximate approach to the optimal time synthesis for a single cycle for each possible η.

4.1. Approximate approach to time optimal control: high processivity assumption

In Section 3 we have obtained the optimal control profile for a fixed reaction time. In this section we seek the temperature profile that minimizes the overall reaction time for a target level of amplification. In "Dynamics and Control of DNA Sequence Amplification" the time optimal cycle switching analysis was introduced for a TIM model (equality constrained controls). Here, we consider the true time optimal cycle switching analysis for the fully time-varying PCR control system. Generation of the full cycle switching curve requires computation of the function $t(\eta)$ for $0 \leq \eta \leq 1$, an interval over which $\eta(t)$ is injective. In an approximate approach to generation of this curve, the efficiency of PCR is defined based on the disappearance of single strand molecules (S_1 and S_2) and single strand primer duplexes (S_1P_1 and S_2P_2). Under the assumption of very high polymerase processivity (where enzyme does not dissociate from partially extended primer-template duplexes), disappearance of SP molecules is the rate limiting step. Indeed, the traditional 3-step PCR cycling protocol can be justified based on the assumption of high polymerase processivity. Since nucleotide addition at the optimal extension temperature is faster than enzyme binding, we define the efficiency of a PCR cycle as

$$\eta = 1 - \frac{S_1P_1 + S_1}{S_{10}} - \frac{S_2P_2 + S_2}{S_{20}} \tag{16}$$

We repeated the minimum time optimal control analysis (in "Dynamics and Control of DNA Sequence Amplification") with the above defined efficiency to generate the cycle time switching curve and estimate the optimal cycle efficiency - and hence the overall optimal reaction time. Fig. 7 shows existence of an optimal cyclic efficiency (92.3%) and the corresponding optimal reaction time is 94.1 seconds. According to this, the total number of PCR cycles needed to multiply the initial concentration of the DNA into 10^4 time is 14 and the corresponding overall reaction time is 1325 seconds. This is 100 seconds less than what has been estimated to be the optimal reaction time using the grid-based sampling approach. Note that in this analysis we have fixed the extension time based on our minimum

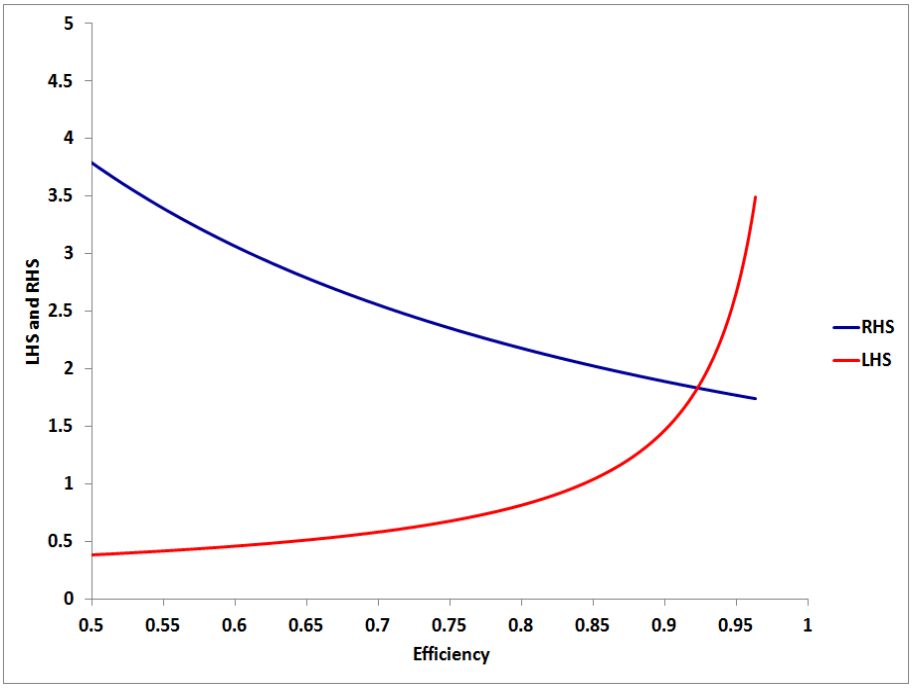

FIG. 7: Optimal PCR cycle efficiency that minimizes the overall reaction time. Y-axis represents the LHS and RHS of the time optimal cycle switching condition introduced in "Dynamics and Control of DNA Sequence Amplification" and the point of intersection is the solution of the Eq. $t(\eta)$ used to calculate the LHS and RHS was computed at each η via the approximation method described in the text. The intersection point specifies the optimal switching time between cycles.

extension time analysis in "Dynamics and Control of DNA Sequence Amplification". Fig. 8 shows the optimal temperature profile that minimizes the reaction time. Though the performance of the grid-based sampling approach is comparable to that of the above minimal time strategy, the former is computationally very expensive. Furthermore, these kind of optimal temperature profiles can be used as initial guesses for the multi-step PCR fixed time and time optimal control problem. Note that bound (15) still applies to the $t(\eta)$ generated by this approach.

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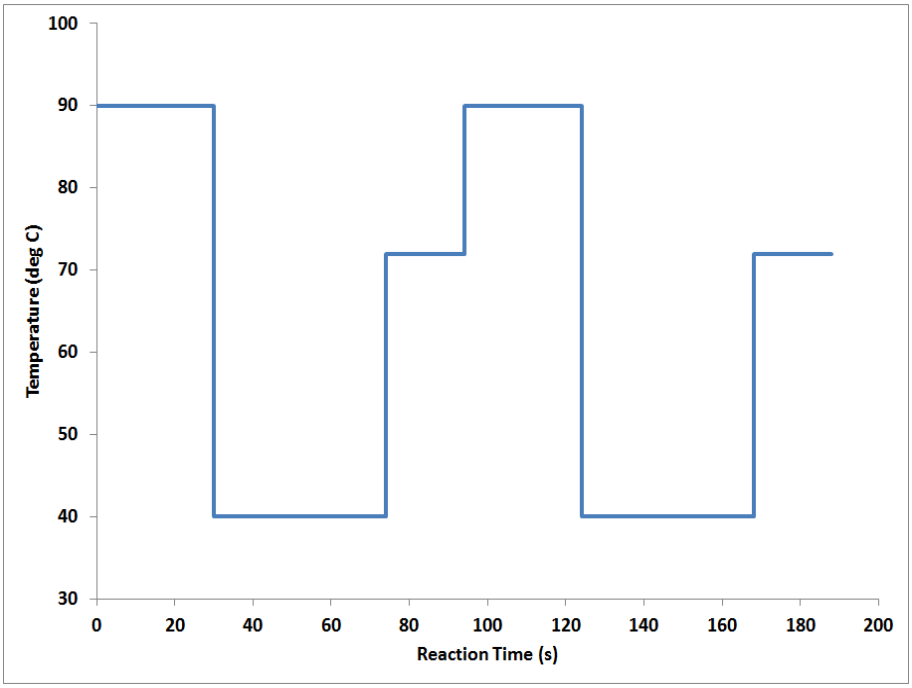

FIG. 8: Optimal temperature profile that minimizes the overall reaction time by cycling switching at the intersection point in Fig. 2.6.