Electronic Supplementary Material (ESI) for Analytical Methods.

Supplemental Information
Derivation of Equation 3

In its basic form, the Van't Hoff equation can be expressed as follows:[19]

$$e^{-\frac{\Delta H}{RT}+\frac{\Delta S}{R}} = K \qquad (A.1)$$

In application of A.1 to DNA, ΔH and ΔS are enthalpy and entropy for hybridization, R is the universal gas constant, T is temperature, and K is the equilibrium constant for DNA hybridization. Rearranging A.1 to solve for T as a function of K yields:

$$T = \frac{\Delta H}{\Delta S - R\ln(K)} \qquad (A.2)$$

We can express K in terms of the concentration of the duplex and the limiting and excess strands:

$$K = \frac{[duplex]}{[limiting][excess]} \qquad (A.3)$$

If $[C_1]$ is the initial concentration of the excess strand, $[C_2]$ the initial concentration of the limiting strand, and f the fraction of the limiting strand in hybridization state, we substitute the following into K for any given hybridization fraction f:

$$[duplex] = f * [C_2]$$
$$[limiting] = (1-f) * [C_2]$$
$$[excess] = [C_1] - f * [C_2]$$

Substituting these into equation A.3, we find:

$$K = \frac{f * [C_2]}{[C_2] * (1-f) * ([C_1] - f * [C_2])} \qquad (A.4)$$

Multiplying through and simplifying, we arrive at:

$$K = \frac{f}{[C_1] - ([C_1] + [C_2]) * f + [C_2] * f^2} \qquad (A.5)$$

This can be substituted into equation A.2 to find T_f, or the temperature at which the fraction of the limiting DNA strand in hybridized form is equal to f:

$$T_f = \frac{\Delta H}{\Delta S - R\ln\left(\frac{f}{[C_1] - ([C_1] + [C_2]) * f + [C_2] * f^2}\right)} \qquad (A.6)$$

Note that equation A.6 is equivalent to equation 3 in the introduction of this report when the properties of logarithms are used to change K to K^{-1}:

$$T_f = \frac{\Delta H}{\Delta S + R\ln\left(\frac{[C_1] - ([C_1] + [C_2]) * f + [C_2] * f^2}{f}\right)} \qquad (A.7)$$

Simple Recursive PCR Model Used for Figure S1 and Figure 2

Amplicon copies in each cycle under primer exhaustion conditions were approximated as follows:

$$C_{N+1} = p(e-1)(C_N) + (C_N) \qquad (A.8)$$

where C_N is the number of amplicon single strands with complementarity to the primer in the current cycle, and e is a constant "efficiency" for the reaction that accounts for interfering factors—in a perfect reaction, $e = 2$. p is the probability of a single stranded amplicon attaching to a primer, taking into account competition from the complementary strand of the amplicon. The value for p at each cycle can be expressed as a function of primer copy number divided by the total number of amplicon and primer copies:

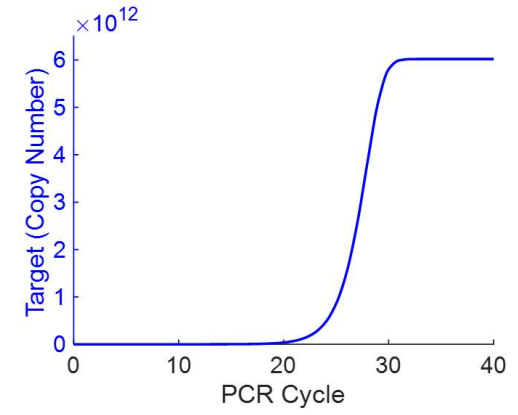

Figure S1. Theoretical model of primer limited PCR, showing typical exponential and plateau phase of amplicon copies.

$$p = \frac{primers}{amplicon + primers} \qquad (A.9)$$

For the sake of simplicity, it is assumed that the amplicon has equal binding affinity for the primer as its own reverse complement—while not necessarily true, it works for the qualitative assessment in this report, and is shown in **Figure S1**. The plot of equation A.8 has the same character as the more in-depth model used in literature,[11] and thus was deemed to be a sufficient simplification for this report.

Method for Calculating ΔH and ΔS

ΔH and ΔS were calculated from the experimental annealing point data gathered in this report by taking the reciprocal of equation 3, as is commonly done with the simplified form seen in literature:[22]

$$\frac{1}{T_f} = \frac{\Delta S}{\Delta H} + \frac{R}{\Delta H}\ln\left(\frac{[C_1] - ([C_1] + [C_2]) * f + [C_2] * f^2}{f}\right) \qquad (A.10)$$

The reciprocal of our recorded values of T_f was plotted as a function of the logarithm portion of the equation—which was known from our experimental parameters—and a linear regression was performed. The slope of the line produced was used to find ΔH, while the intercept and the calculated value for ΔH can be used to find ΔS (**Figure S2**). This method is not without its drawbacks, as the values found are intrinsically linked and should not be carried beyond the conditions in which they were derived.[22] However, it is useful for creating an example heatmap for a particular primer in PCR conditions like the ones in **Figure 6** and **Figure 8**.

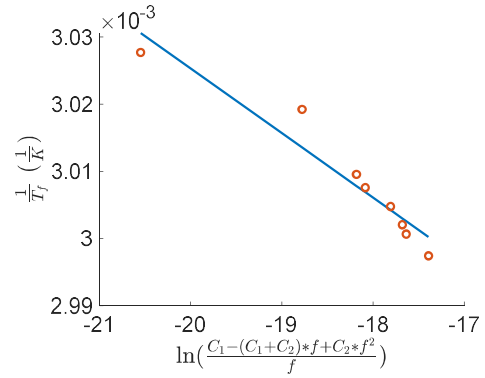

Figure S2: Example plot used to derive ΔH and ΔS, with the reciprocal of the temperature in Kelvin plotted as a function of the logarithm portion of equation A.10 (blue). Experimental data used to find the linear regression is also shown (red).

Preset Temperature PCR Results for N2 Sequence

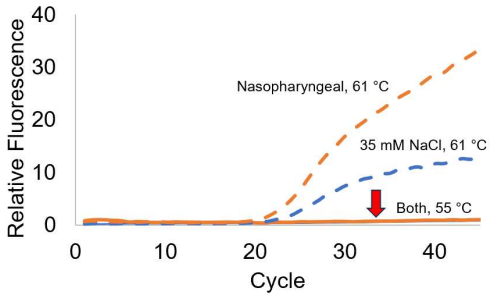

Figure S3: Average preset Rotor-Gene PCR results for the Covid N2 reaction in direct PCR nasopharyngeal and 35 mM NaCl backgrounds (averages of N=3, duplicates). False negatives are produced for the CDC's recommended annealing temperature of 55°C in both experimental groups (red arrow), while positive results are shown when setting the annealing temperature to 61°C, as seen in previous work.[16]

N1 Controls for Adaptive PCR Experiments

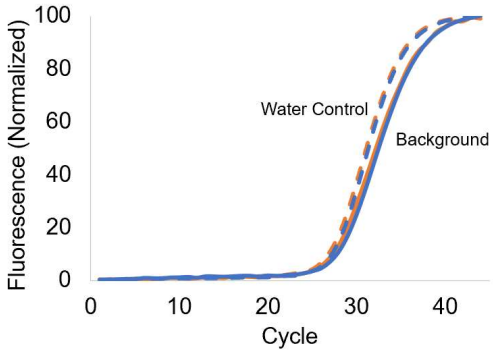

Figure S4: "Adaptive" PCR results for N1 reactions (N = 6, duplicates). Nasopharyngeal trials (solid orange) display no change from control (dashed orange). 35 mM NaCl trials (solid blue) similarly show no difference from water background control (dashed blue).