Enclosing Multiple Digital DNA Signals in a Single Analog Channel
Supplementary Materials

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Supplementary Section S1: Analysis and Simulations of Trap and Reporter System

Ordinary Differential Equation Simulations

To get the optimal $\Delta G^o_{\text{rxn}}$ to mimic step function response, and to factor in the effects of Trap and Reporter concentrations on the distribution of species in the system, we construct an ordinary differential equation (ODE) model.

Throughout this work we used toehold probes as both Trap and Reporter. Toehold probe is a dissociative probe that releases an auxiliary species upon reacting with a target.

The chemical reactions of Trap and Reporter with the same target, not including short-lived intermediates, are:

\[
\begin{align*}
T + P_{\text{Tr}}C_{\text{Tr}} & \xrightleftharpoons[k_{\text{Trap}^-}]{k_{\text{Trap}^+}} T \cdot C_{\text{Tr}} + P_{\text{Tr}} \quad \Delta G^o_{\text{Trap}} \\
T + P_{\text{R}}C_{\text{R}} & \xrightleftharpoons[k_{\text{Reporter}^-}]{k_{\text{Reporter}^+}} T \cdot C_{\text{R}} + P_{\text{R}} \quad \Delta G^o_{\text{Reporter}}
\end{align*}
\]  

wherein $k_+$ and $k_-$ represent the forward and the reverse rate constants. The values of all forward reaction rate constants $k_+$ are assumed to be $3 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$; this is estimated based on previous studies [1]. The reverse rate constants can be calculated as

\[
\begin{align*}
k_- &= k_+ e^{\Delta G^o_{\text{rxn}}/R\tau} \quad (S3)
\end{align*}
\]

where $\Delta G^o_{\text{rxn}}$ denotes the standard free energy of the relevant reaction.

For convenience, we abbreviate Target to T, Trap to Tr and Reporter to R.

The corresponding ordinary differential equations are:

\[
\begin{align*}
\frac{dT}{dt} &= -k_{\text{Trap}^+}[T][P_{\text{Tr}}C_{\text{Tr}}] + k_{\text{Trap}^-}[T \cdot C_{\text{Tr}}][P_{\text{Tr}}] - k_{\text{Reporter}^+}[T][P_{\text{R}}C_{\text{R}}] + k_{\text{Reporter}^-}[T \cdot C_{\text{R}}][P_{\text{R}}] \quad (S4) \\
\frac{d[P_{\text{Tr}}]}{dt} &= k_{\text{Trap}^+}[T][P_{\text{Tr}}C_{\text{Tr}}] - k_{\text{Trap}^-}[T \cdot C_{\text{Tr}}][P_{\text{Tr}}] \quad (S5) \\
\frac{d[P_{\text{R}}C_{\text{Tr}}]}{dt} &= -k_{\text{Trap}^+}[T][P_{\text{Tr}}C_{\text{Tr}}] + k_{\text{Trap}^-}[T \cdot C_{\text{Tr}}][P_{\text{Tr}}] \quad (S6) \\
\frac{dT \cdot C_{\text{Tr}}}{dt} &= k_{\text{Trap}^+}[T][P_{\text{Tr}}C_{\text{Tr}}] - k_{\text{Trap}^-}[T \cdot C_{\text{Tr}}][P_{\text{Tr}}] \quad (S7) \\
\frac{d[P_{\text{R}}]}{dt} &= k_{\text{Reporter}^+}[T][P_{\text{R}}C_{\text{R}}] - k_{\text{Reporter}^-}[T \cdot C_{\text{R}}][P_{\text{R}}] \quad (S8) \\
\frac{d[P_{\text{R}}C_{\text{R}}]}{dt} &= -k_{\text{Reporter}^+}[T][P_{\text{R}}C_{\text{R}}] + k_{\text{Reporter}^-}[T \cdot C_{\text{R}}][P_{\text{R}}] \quad (S9) \\
\frac{dT \cdot C_{\text{R}}}{dt} &= k_{\text{Reporter}^+}[T][P_{\text{R}}C_{\text{R}}] - k_{\text{Reporter}^-}[T \cdot C_{\text{R}}][P_{\text{R}}] \quad (S10)
\end{align*}
\]

These ordinary differential equations are simulated using MATLAB’s stiff “ode23s” solver, using relative tolerance of $10^{-6}$ and absolute tolerance of $10^{-20}$.

Simulation Results of Different Combinations of $\Delta G^o_{\text{Reporter}}$ and $\Delta G^o_{\text{Trap}}$

In Fig. 3b of main text we present simulation results of different combinations of $\Delta G^o_{\text{Reporter}}$ and $\Delta G^o_{\text{Trap}}$ indicating an optimal region (dark blue) with the smallest grayzone.

To vividly map main Fig. 3b to step function responses, here we select $\Delta G^o_{\text{Reporter}} [-4 -2.5 -1 0.5] \text{ kcal/mol}$ and $\Delta G^o_{\text{Trap}} [-11 -9.5 -8 -6.5] \text{ kcal/mol}$ with 4-by-4 combinations, and get a simulated yield with a range of initial target concentrations for each $\Delta G^o$ combination (Fig. S1-1). These results show that grayzone is a good indicator for how the target-yield response successfully mimics step function response. For example combinations of $\Delta G^o_{\text{Trap}}$ -11 to -8 kcal/mol with $\Delta G^o_{\text{Reporter}}$ -2.5 to -1 kcal/mol generate good step function responses, and also show narrow grayzone widths.

Good step function response has sharp lower and upper elbows. From Fig. S1-1 we can infer that, how sharp the lower elbow is depends on how different $\Delta G^o_{\text{Reporter}}$ and $\Delta G^o_{\text{Trap}}$ is. How sharp the upper elbow is depends on how negative $\Delta G^o_{\text{Reporter}}$ is.

1-1
FIG. S1-1: Simulation results of different combinations of $\Delta G_{\text{Reporter}}^{\circ}$ and $\Delta G_{\text{Trap}}^{\circ}$. Simulations were performed at 10 nM Reporter and 500 nM Trap reacting at 25 °C for 16 hours, with initial target concentrations ranging from 0 to 1000 nM, followed by 50× dilution (same as Fig. 3ab). Target concentrations corresponding to a 5% (left dashed line) to 95% (right dashed line) maximal yield is defined as the grayzone.

FIG. S1-2: Simulation results with one fixed Trap concentration and various Reporter concentrations. In this figure, Traps are at a concentration of 10nM. Reporters are at concentrations of 0.2 nM, 0.67 nM and 2 nM respectively. Target concentrations corresponding to a 5% (left dashed line) to 95% (right dashed line) maximal yield is defined as the grayzone. Reporter concentration is at negligible level compared to Trap concentration and can be ignored when defining the threshold. As the Reporter’s relative concentration increases, the response curve becomes less sharp and the grayzone becomes wider.

Trap and Reporter Concentration Design

Another aspect in designing the system is to assign the concentrations of Traps and Reporters.

The detection threshold of each Target equals $t$ corresponding Trap concentration. Trap concentrations are independent for different targets thus detection thresholds of different targets can be independently set. Trap concentration does not have an upper limit. The lower limit of Trap concentration correlates with Reporter concentration - Reporter concentration should be much lower than the concentration of its respective Trap.

The lowest concentration that a Reporter can go is determined by the detection limit of the instrument used to measure the signal. For example, a fluorometer’s detection limit is 10 pM, thus the Reporter concentration cannot be less than 10 pM.

The highest concentration that a Reporter can go is limited by the Trap concentration. Ideally, Reporter is negligible compared to Trap and there’s no grayzone; when Target concentration gets right above Trap, it activates all Reporters. In reality, grayzone exists, so we need to limit the Reporter concentration to limit the grayzone.
range.

Experimentally, we recommend using $[\text{Reporter}]_0 \leq \frac{[\text{Trap}]_0}{50}$. As shown in Fig. S1-2, given the fixed Trap concentration of 10 nM, when Reporter concentration increases from 0.2 nM $\rightarrow$ 0.67 nM $\rightarrow$ 2 nM, the grayzone broadens from 1.5 nM $\rightarrow$ 5.2 nM $\rightarrow$ 9.9 nM, and the response curve resembles less and less a step function.

Note that it really is a matter of grayzone width, so even if we used $[\text{Reporter}]_0 > \frac{[\text{Trap}]_0}{50}$, the combined/multiplexed output signal can still be successfully deconvoluted into each input if the target concentration is outside of the grayzone (like in main Fig. 4 and Fig. 5, CFTR’s Trap concentration is 6.25× Reporter concentration).

### Evenly Spaced Signal Levels

![Digital Step Signal (Evenly Spaced)](image)

![Digital Step Signal (Unevenly Spaced)](image)

**FIG. S1-3:** Example of (a) evenly spaced signals and (b) unevenly spaced signals. In (b), most levels are differed by $I_{\text{au}}$ from adjacent levels, while there are two $2I_{\text{au}}$ jumps marked by red ellipses.

Here we recommend using $I_H - I_L = 2^{(k-1)}I_{\text{au}}$ for the step function of the $k$th dimension, so that the signal levels are evenly spaced; $I_{\text{au}}$ is an arbitrary unit of signal that is greater than $I_{\text{Fluc}}$. For example, in a $D = 4$ system, the $I_H - I_L$ values are $I_{\text{au}}$, $2I_{\text{au}}$, $4I_{\text{au}}$, and $8I_{\text{au}}$ for each step function; the possible observed signal levels (background subtracted) are $2^D = 16$ different values evenly spaced between $0I_{\text{au}}$ and $15I_{\text{au}}$ (Fig. S1-3a).

In a system that is unevenly spaced: for example, in a $D = 4$ system, the $I_H - I_L$ values are $I_{\text{au}}$, $2I_{\text{au}}$, $5I_{\text{au}}$, and $9I_{\text{au}}$ for each step function; the possible observed signal levels (background subtracted) are $2^D = 16$ different values not unevenly spaced between $0I_{\text{au}}$ and $17I_{\text{au}}$ (Fig. S1-3b). There’re two jumps where different levels are differed by $2I_{\text{au}}$ rather than $I_{\text{au}}$.

The observed signal level should only be the result of one unique possible combination. For example, we cannot use $I_{\text{au}}$, $2I_{\text{au}}$, $3I_{\text{au}}$ for 3 different inputs, since in this case, if we observed $3I_{\text{au}}$, we would not know whether it is because of Input 1 plus Input 2, or because of solely Input 3.

The reasons we recommend the current evenly spaced setting are (1) It maximizes space utilization. In Fig. S1-3a the max signal is $15I_{\text{au}}$, while in Fig. S1-3b the max signal is $17I_{\text{au}}$. (2) It eases data interpretation. We are able to speculate and adjust data interpretation from observed signals since every two adjacent levels should have the same signal difference. If we used unevenly distributed signals, we would find it harder to distinguish between data variations due to different input combinations and data variations due to fluctuations.

[1] Zhang, J. X., Fang, J. Z., Duan, W., Wu, L., Zhang, A., Dalchau, N., & Zhang, D. (2018). Predicting DNA Hybridization Kinetics from Sequence. Nature Chemistry, 10(1), 91.
FIG. S2-1: Kinetics traces of EGFR Trap and Reporter system, measured in spectrofluorometer. Oligo sequences are the same as in main Fig. 2. 50 nM Trap and 10 nM Reporter were equilibrated at 25 °C in 5×PBS. Then 8 different concentrations of targets (20 nM, 40 nM, 50 nM, 60 nM, 65 nM, 70 nM, 80 nM, 85 nM) were added for kinetics measurements.

To mimic step function response, we design a toehold Reporter and a toehold Trap directed at one target. Fig. S2-1 shows the kinetics traces of the EGFR Trap and Reporter system with different target input concentrations.

Using 20 nM target concentration trace as an example: the toehold Reporter and the target undergo a classic strand displacement reaction with a fluorophore and a quencher on the toehold Reporter. When target reacts with the Reporter there will be increased fluorescence. The Trap is thermodynamically very favorable but without fluorophore and quencher. So the target will first react proportionally with both the Trap and the Reporter, but the Reporter reaction is reversible and re-releases the target, while the Trap permanently binds the target. At equilibrium, we will not notice any fluorescence until the Trap is used up and the Reporter starts to react with the target.

The detection threshold is set by Trap concentration: with low input (target concentration $[T]_0$ lower than Trap concentration $[Tr]_0$), Reporter is quenched at equilibrium, and baseline signal is observed; with high input ($[T]_0 > [Tr]_0$), Reporter is activated at equilibrium, and maximal signal is observed. This trend can be foreseen from the kinetics traces. In the 50 nM Trap and 10 nM Reporter system, 50 nM of target is the detection threshold, and after 50+10 = 60 nM of target, maximum signals can be reached. From Fig. S2-1, we can observe with low inputs (20 nM and 40 nM targets), traces have the trends of going to baseline signals; 50 nM and 60 nM target traces are in the grayzone; while with high inputs (65, 70, 80 and 85 nM targets), traces reach maximal signals.

From Fig. S2-1, we can see the 50 nM Trap and 10 nM Reporter system do not reach equilibrium after 2 hours. That is why in main Fig. 2 where we used 10 nM Trap and 0.2 nM Reporter, reaction was performed at 50×, incubated overnight, then diluted 50× for fluorescence measurements to ensure that we’re measuring the equilibrium signals.
Supplementary Section S3: Characterization of Multiplexed Trap and Reporter System

Fluorometer Position Correction

Fluorescence measurements were performed using a Horiba Fluoromax 4 spectrofluorometer and Hellma Semi-Micro 114F spectrofluorimeter cuvettes.

The 4 different positions each exhibited slight biases in fluorescence levels. We performed calibration experiments to correct for these systematic position biases before experimental analysis.

5 continuous data points were collected in each well after 10 min of incubation at 25 °C, 5 x PBS. To correct for position dependence, the average fluorescence of 4 positions for the same solution was used as a reference, and the fluorescence of each position for each solution was used as an independent variable. We performed linear regression between the reference fluorescences and the raw fluorescences of the four concentrations, and then applied the best-fit slope and intercept values to linearly transform the fluorescence of each well into the equivalent reference fluorescence. All experimental results in main paper Fig. 3 and Fig. 4 were corrected for position and subsequently background-subtracted.

Statistical analysis of 4-plex multiplexing characterization in a single FAM fluorescence channel

Means, standard deviations and boundaries of signals of 4-plex multiplexing characterization in main Fig. 3c is listed in Table S3-1.

A Z-score measures how many standard deviations above or below the mean a data point is. The boundary \( B_{i\sim i+1} \) values between Level \( i_{th} \) and Level \( i+1_{th} \) are calculated as:

\[
Z_{i \text{ high}} = \frac{B_{i\sim i+1} - X_i}{s_i} = Z_{i+1 \text{ low}} = \frac{X_{i+1} - B_{i\sim i+1}}{s_{i+1}}
\]

where \( X_i \) is mean of triplicated signals of Level \( i_{th} \), \( s_i \) is standard deviation of triplicated signals of Level \( i_{th} \).

| Level (i) | Mean \( (X_i) \) | STD \( (s_i) \) | Boundary \( (i_{th} \) and \( i+1_{th} \)) |
|----------|----------------|----------------|----------------------------------|
| 0        | 0.2            | 0.09           | 1.2                              |
| 1        | 2.7            | 0.15           | 3.6                              |
| 2        | 5.2            | 0.23           | 6.2                              |
| 3        | 7.5            | 0.31           | 8.4                              |
| 4        | 9.0            | 0.22           | 10.4                             |
| 5        | 12.4           | 0.31           | 13.3                             |
| 6        | 14.8           | 0.48           | 15.7                             |
| 7        | 16.7           | 0.47           | 17.3                             |
| 8        | 17.9           | 0.49           | 18.8                             |
| 9        | 19.9           | 0.53           | 21.1                             |
| 10       | 21.9           | 0.34           | 22.8                             |
| 11       | 24.0           | 0.49           | 25.3                             |
| 12       | 26.4           | 0.41           | 27.5                             |
| 13       | 28.9           | 0.48           | 30.0                             |
| 14       | 31.0           | 0.43           | 31.8                             |
| 15       | 33.0           | 0.60           |                                   |

TABLE S3-1: Summary of 4-plex multiplexing characterization in a single FAM fluorescence channel. \( X_i \) is mean of triplicate. \( s_i \) is standard deviation of triplicate. Boundary is signal boundary between Level \( i_{th} \) and Level \( i+1_{th} \).
Multiplexed detection of different DNA targets in a single fluorescence channel by Qubit

FIG. S3-1: Example of boundary calculation. $X_i$ is mean of triplicated signals of $i_{th}$ level. $s_i$ is standard deviation of triplicated signals of $i_{th}$ level. $B_{i \sim i+1}$ is signal boundary between Level $i_{th}$ and Level $i+1_{th}$.

$Z_{\text{high}} \equiv (B_{8 \sim 9} - X_8)/s_8 = Z_{\text{low}} \equiv (X_9 - B_{7 \sim 8})/s_9$

$B_{7 \sim 8} = 17.3$  $B_{8 \sim 9} = 18.8$

$s_7 = 0.47$  $s_8 = 0.49$  $s_9 = 0.53$

FIG. S3-2: Experimental demonstration of 3-plex multiplexing in a single FAM fluorescence channel using Qubit. All Trap concentrations were 50 nM. Reporter concentrations of targets CYCS, VEGFA, and CFTR were 1, 2, and 4 nM, respectively. Target concentrations for High (H) were 100 nM. Target concentrations for Low (L) were 10 nM. Signals between 8 levels were clearly distinguished.

Readout of Trap + Reporter system is valid not only on precise instruments with high sensitivity, high dynamic range and good detection limit e.g. spectrofluorometer but also on small affordable instruments such as Qubit. We tested a FAM 3-plex system on small, easy to use Qubit 3.0 Fluorometer with high reporter concentrations.
(since Qubit does not have a detection limit as good as spectrofluorometer). Signals of 8 levels were still clearly distinguished, as in spectrofluorometer.
Supplementary Section S4: 7-plex Blinded Sample Testing

To demonstrate the ability of Trap and Reporter system to nonlinearly map multiple dimensions of digital signals into analog signal space, we constructed a 7-plex system, which reports the concentration status (above or below threshold) of 7 different targets into 2 fluorescence channels, mapping 7 dimensions of digital signals into 2 analog signal spaces.

7-plex Trap and Reporter systems were used, each targeting a ≈25 nt sequence in a different gene. TFRC, GAPDH, and PPIA targets were reported by the Cy5 channel; CYCS, VEGFA, KRAS, and CFTR targets were reported by the FAM channel. Detection threshold for each target was 10 nM.

We performed a blinded experiment to determine the concentration status (above or below threshold) of 7 different targets in 5 samples; the concentrations were unknown to the experimenter YHY. The target concentration had 2 levels: above threshold or below threshold; 5 samples were prepared to have 5 different level combinations out of the $2^7 = 128$ possible combinations. All concentration status were correctly identified.

3-plex Cy5 calibration

Main Fig. 3c is the characterization/calibration of 4-plex multiplexing in a single FAM fluorescence channel. Fig. S4-1 is the characterization/calibration of 3-plex multiplexing in a single Cy5 fluorescence channel. Note that FAM and Cy5 channels were orthogonal and did not affect each other, thus we could characterize FAM system without Cy5 reporters, vice versa.

![FIG. S4-1: Experimental calibration of 3-plex multiplexing in a single Cy5 fluorescence channel. All Trap concentrations were 10 nM. Reporter concentrations of targets TFRC, GAPDH, and PPIA were 0.4, 0.8, and 1.6 nM, respectively. Final target concentrations were 2 nM for L(Low) and 20 nM for H(High). Results of triplicate experiments are shown as cross symbols. Signals of 8 levels were clearly distinguished. Boundaries of each level are indicated by gray/white zones (see boundary calculation in Supplementary Section S3).](image-url)

7-plex blinded sample complete analysis

Table S4-1 shows the 5 sample concentrations prepared by ZZ, unknown to experimenter YHY beforehand.

Each calibration was prepared and tested in triplicate, while each test was prepared and tested in quintuplicate. To interpret the data, using Sample 1 as an example:

From Fig. S4-2a, we can see Sample 1’s five FAM signals fall into Level 8, corresponding to high CFTR, low CYCS, low VEGFA, low KRAS in FAM channel calibration; From Fig. S4-2b, we can see Sample 1’s five Cy5 signals fall into Level 5, corresponding to high TFRC, high PPIA, low GAPDH in Cy5 channel calibration. Therefore with two analog signal levels (FAM Level 8 and Cy5 Level 5), we determined Sample 1’s content to be
high TFRC, low GAPDH, high PPIA, low CYCS, low VEGFA, low KRAS, high CFTR. This result is consistent with Table S4-1.

| Sample  | Target | Target Final Conc (nM) |
|---------|--------|------------------------|
| Sample 1 | TFRC   | 16                     |
|         | GAPDH  | 0                      |
|         | PPIA   | 14                     |
|         | CYCS   | 0                      |
|         | VEGFA  | 0                      |
|         | KRAS   | 0                      |
|         | CFTR   | 14                     |
| Sample 2 | TFRC   | 0                      |
|         | GAPDH  | 16                     |
|         | PPIA   | 0                      |
|         | CYCS   | 16                     |
|         | VEGFA  | 18                     |
|         | KRAS   | 20                     |
|         | CFTR   | 22                     |
| Sample 3 | TFRC   | 14                     |
|         | GAPDH  | 14                     |
|         | PPIA   | 14                     |
|         | CYCS   | 18                     |
|         | VEGFA  | 18                     |
|         | KRAS   | 18                     |
|         | CFTR   | 18                     |
| Sample 4 | TFRC   | 0                      |
|         | GAPDH  | 0                      |
|         | PPIA   | 14                     |
|         | CYCS   | 20                     |
|         | VEGFA  | 0                      |
|         | KRAS   | 0                      |
|         | CFTR   | 0                      |
| Sample 5 | TFRC   | 0                      |
|         | GAPDH  | 0                      |
|         | PPIA   | 0                      |
|         | CYCS   | 20                     |
|         | VEGFA  | 0                      |
|         | KRAS   | 0                      |
|         | CFTR   | 0                      |

TABLE S4-1: Blinded sample target concentrations prepared by ZZ. The samples were prepared by ZZ, and the tests were performed by author YHY; target concentrations were unknown to YHY until the fluorescence results were obtained. Detection threshold for each target was 10 nM.
FIG. S4-2: 7-plex complete analysis results of 5 blinded samples using distribution free statistics. (a) Analysis of FAM channel Sample 1 ∼ 5. The quintuplicate datapoints of sample fluorescence are shown as hollow red dots, and the calibration datapoints (triplicates) are shown as solid dots. The range of each level is the range from the lowest to the highest calibration datapoints. Because we did not think the measured fluorescence followed normal distribution, we used distribution free statistics in this analysis. For all 5 samples, the five datapoints clearly fell into the range of one level. For example, in sample 4, the five datapoints were far from calibration datapoints of Level 0 and Level 2, thus it could be clearly defined to be within Level 1. (b) Analysis of Cy5 channel Sample 1 ∼ 5.
Oligonucleotide sequences used for all experiments are listed here. For each probe pair (Trap and Reporter), the top oligo with sequence homologous to the target sequence is referred to as P (protector) sequence, and the bottom oligo with sequence complementary to the target sequence is referred to as C (complement) sequence. All the sequences were ordered from Integrate DNA Technologies (IDT).

/5IAbRQ/ denotes an Iowa Black RQ quencher moiety functionalized at the 5′ end of the oligo; /5IAbFQ/ denotes an Iowa Black FQ quencher moiety functionalized at the 5′ end of the oligo; /3Rox_N/ denotes the IDT entry code for the ROX fluorophore functionalized by NHS ester chemistry at the 3′ end of the oligo; /36-FAM/ denotes the IDT entry code for FAM dye (a single isomer derivative of fluorescein) at the 3′ end of the oligo; /3Cy55Sp/ denotes the IDT entry code of Cy5 dye at the 3′ end of the oligo. The fluorophore-labeled C and quencher-labeled P strands were post-synthesis HPLC purified by IDT; all other strands were ordered with standard desalting and not purified.
| Sequence Name             | Sequence                                                                 |
|--------------------------|---------------------------------------------------------------------------|
| CFTR-ReporterC           | GTACTGCTTTGACTTTCTTTATT/36-FAM/                                          |
| CFTR-ReporterP           | /5IAbkFQ/AAATAAGGAAGTCACCAAAN                                              |
| CFTR-TrapC               | GTACTGCTTTGACTTTCT                                                       |
| CFTR-TrapP               | TAGGAAGTCAACAAA                                                           |
| CFTR-Target              | AGGCAACTCAACCAAAGCAGTAC                                                              |
| CYCS-ReporterC           | GAACACATTAAGCCAAAAATCACCTGATA/36-FAM/                                      |
| CYCS-ReporterP           | /5IAbkFQ/GTATCAGGTAGTTTGCGTTTA                                              |
| CYCS-TrapC               | GAACACATTAGCCAAAAATCACC                                                     |
| CYCS-TrapP               | GTATGATTTTGCGCT                                                        |
| CYCS-Target              | GTATGATTTTGCCTTAATGTGTTCC                                                        |
| EGFR-ReporterC           | GCCGAAGGGCATGACTCTGCGTTCTC/3ROX_N/                                        |
| EGFR-ReporterP           | /5IAbRQ/GTAAACGCAGCTCAGTCGCCC                                              |
| EGFR-TrapC               | GCCGAAGGGCATGACTCTGCGT                                                    |
| EGFR-TrapP               | ACGCAGCTCAGCC                                                           |
| EGFR-Target              | AGGCAGCATAAAC                       |
| GAPDH-ReporterC          | AAAGAGGTGTAATTAAAAATTCTCCCAAAG/3Cy5Sp/                                    |
| GAPDH-ReporterP          | /5IAbRQ/CCTTTAGGGAGATAAAATTTC                                              |
| GAPDH-TrapC              | AAAGAGGTGTAATTAAAAATTCTCC                                              |
| GAPDH-TrapP              | AGGGAGATAAAATTTC                                                           |
| GAPDH-Target             | AGGGAGATAAATTCAACCTCTT                                                   |
| KRAS_FAM-ReporterC       | AATACCTTCAAAATGATTAGTTATATATCTTAATA/36-FAM/                                |
| KRAS_FAM-ReporterP       | /5IAbkFQ/TATAGAATAATAACTAATAACTTT                                      |
| KRAS_FAM-TrapC           | AATACCTTCAAAATGATTAGTTATATATCTTAATA                                      |
| KRAS_FAM-TrapP           | AATACCTTCAAAATGATTAGTTATATATCTTAATA                                      |
| KRAS_FAM-Target          | AATACCTTCAAAATGATTAGTTATATATCTTAATA                                      |
| KRAS_ROX-ReporterC       | TACTCCTCTTTACCTCGCTGTTGCATCAAAG/3ROX_N/                                   |
| KRAS_ROX-ReporterP       | /5IAbRQ/TTATAGTACACACAGCAGATGA                                           |
| KRAS_ROX-TrapC           | TACTCCTCTTTACCTCGCTGTTGCATCAAAG                                           |
| KRAS_ROX-TrapP           | TTTCGACACACAGGTA                                                        |
| KRAS_ROX-Target          | TCGACACAGCAGGTA                                                             |
| PPIA-ReporterC           | ATGTCTTTAGGGGCTCTTCTGAGTTAAG/3Cy5Sp/                                      |
| PPIA-ReporterP           | /5IAbRQ/TCTTTACTCAGAAGCCC                                               |
| PPIA-TrapC               | ATGTCTTTAGGGGCTCTGAGT                                                  |
| PPIA-TrapP               | TTACCTAGAAGGCC                                                            |
| PPIA-Target              | AACTCAGAAGGCCCTAAGCAATAGAT                                               |
| TFRC-ReporterC           | TCTCCTGCTCTCTCTCCATGAG/3Cy5Sp/                                           |
| TFRC-ReporterP           | /5IAbRQ/CTCATGTAGGAGGAGANG                                                |
| TFRC-TrapC               | TCTCCTGCTCTCTCCTCCTC                                                   |
| TFRC-TrapP               | TTGGTAGGAGGAGG                                                           |
| TFRC-Target              | GCTGAGGGAGGAGGAGGAGGAGGAG                                               |
| VCP-ReporterC            | GAGCTCACAACACTCAGGTCCGAATTTG/3Cy5Sp/                                     |
| VCP-ReporterP            | /5IAbRQ/AAACTCGAGACTCGAGTTG                                              |
| VCP-TrapC                | GAGCTCACAACACTCAGGTCC                                                  |
| VCP-TrapP                | TTGGGACCTAGTTG                                                           |
| VCP-Target               | GAGGACTGATTTGGGTGTGAGCCT                                                |
| VEGFA-ReporterC          | AGAATAATCACCCTAAAACCCCTAATAGG/36-FAM/                                    |
| VEGFA-ReporterP          | /5IAbkFQ/GTCTTATAGGGGTGTTTATTGGTG                                      |
| VEGFA-TrapC              | AGAATAATCACCCTAAAACCCCTA                                               |
| VEGFA-TrapP              | TTAGGGGTGTGTTAGG                                                        |
| VEGFA-Target             | TTAGGGGTGTGTTAGG                                                        |

**TABLE S5-1**: Oligonucleotide sequences used.