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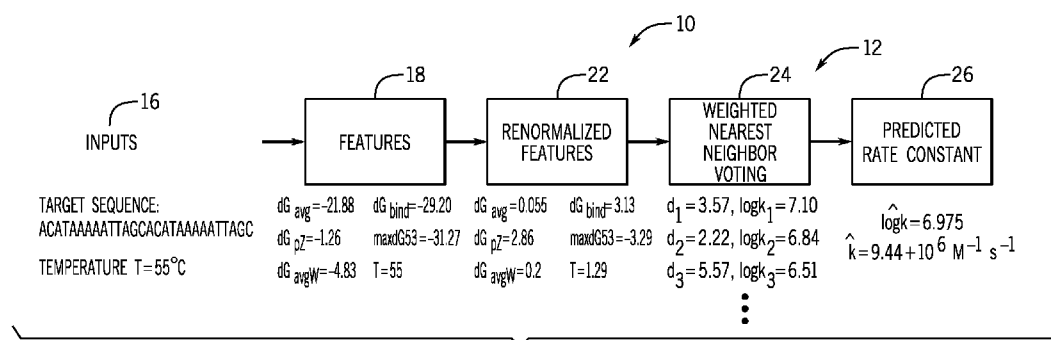


FIG. 1A

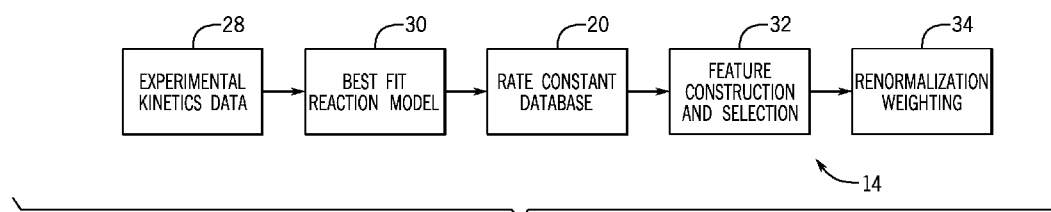


FIG. 1B

(57) **Abstract:** Embodiments of methods, systems, and tangible non-transitory computer readable medium having instructions are presented. A method includes calculating a plurality of feature values for a number of bioinformatic features of the desired hybridization reaction; and calculating distances between the plurality of feature values and corresponding database rate constant values stored in a database, the database comprising a plurality of hybridization reactions having known rate constants. The method additionally includes calculating a weighted average of a logarithm of the database rate constant values, with larger weights assigned to value instances having values lower in distance to the plurality of feature values of the desired hybridization reaction; and providing the weighted average as a predicted logarithm of the rate constant of the desired hybridization reaction.



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METHOD, APPARATUS, AND COMPUTER-READABLE MEDIUM FOR
PREDICTING A HYBRIDIZATION RATE CONSTANT OF A FIRST
SEQUENCE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from and the benefit of U.S. Provisional Application Serial No. 62/346,642, filed June 7, 2016, entitled “METHOD, APPARATUS, AND COMPUTER-READABLE MEDIUM FOR PREDICTING A HYBRIDIZATION RATE CONSTANT OF A FIRST SEQUENCE,” which is incorporated by reference in its entirety for all purposes.

STATEMENT OF GOVERNMENT INTEREST

[0002] This invention was made with U.S. government support under Grant Number ROIHG008752 awarded by the National Institutes of Health. The U.S. government has certain rights in the invention.

BACKGROUND

[0003] Oligonucleotide probes and primers play a vital role in nucleic acid analytic and diagnostic chemistry and biotechnology. Assays such as fluorescent in situ hybridization, microarray expression analysis, and hybrid-capture enrichment for next-generation sequencing (NGS) are kinetically limited by the speed of probe hybridization. Rational selection of fast-binding target subsequences and design of fast-hybridizing probes can reduce hybridization time to enable faster assays, or alternatively reduce probe concentration needed and enable more highly multiplexed assays.

[0004] To date, the effects of target and probe sequences on the hybridization kinetics have not been systematically studied, and no predictive model or algorithm exists for predicting of hybridization rate constants from sequence and experiment conditions. It is known qualitatively that secondary structure in a target or probe sequence will interfere with and slow down hybridization. Consequently, typical probe design software will apply bioinformatics to select target and probe regions that are predicted to exhibit weak or no secondary structure at equilibrium in the hybridization conditions. However, biological nucleic acids often exhibit significant secondary

structures in regions of interest, and empirical trial-and-error testing is often used to select probes to subsequences of these difficult regions.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] Fig. 1a illustrates an embodiment of a kinetics prediction workflow process suitable for deriving a predicted hybridization rate constant k_{Hyb} for a user-defined target sequence;

[0006] Fig. 1b illustrates an embodiment of a process suitable for deriving hybridization rate constant prediction model(s) based on experimental kinetics data;

[0007] Fig. 2a illustrates an embodiment of fluorescent probes with universal functionalized oligonucleotides;

[0008] Fig 2b shows embodiments of 100 different subsequences of the CYCS and VEGF genes that were selected to the target sequences;

[0009] Fig. 2c depicts examples of secondary structures encountered in target sequences;

[0010] Fig. 2d illustrates example kinetic traces (triplicate) of a hybridization reaction.

[0011] Fig. 3 illustrates correlation of individual features with hybridization rate constant k_{Hyb} ;

[0012] Fig. 4 illustrates feature renormalization. (c) Scatter plot of values for features 2 and 3 (dG_{avg} and dG_{bind});

[0013] Fig. 5 illustrates example distance calculation and rate constant prediction;

[0014] Fig. 6 illustrates an example performance of an embodiment of a WNN model, based on a particular partition of the experimental dataset into training and test sets;

[0015] Fig. 7 illustrates statistics on WNN model rate constant prediction performance, across 1000 partitions of dataset into training and test sets;

[0016] Fig. 8 illustrates statistics on multilinear regression model performance, as comparison;

[0017] Fig. 9 illustrates an exemplary computing environment that can be used to carry out the method for predicting a hybridization rate constant of a first sequence according to an exemplary embodiment;

- [0018] Fig. 10a illustrates three different reaction models considered for fitting rate constant k_{Hyb} to fluorescence kinetics data;
- [0019] Fig. 10b illustrates two examples of fit quality for 3 reaction models shown in FIG. 10a;
- [0020] Fig. 10c illustrates a summary of fit performance of the three models of FIG 10a across all 210 fluorescence kinetics experiments;
- [0021] Fig. 10d illustrates examples of observed rate constants for 96 targets at 37°C and 55°C;
- [0022] Fig. 11a illustrates rate constant prediction using a Weighted Neighbor Voting (WNV) model;
- [0023] Fig. 11b illustrates example relationship between the base-10 logarithm of the experimental hybridization rate constants k_{Hyb} (based on reaction model H3) vs. Gb values for the 210 hybridization experiments;
- [0024] Fig. 11c illustrates example feature renormalization based on 75th and 25th percentile values;
- [0025] Fig. 11d illustrates an example graph showing distances for a reaction whose rate constant k_{Hyb} is to be predicted;
- [0026] Fig. 11e illustrates an example graph showing relationship between feature space distance d and the absolute value of difference in experimental rate constants ($\log_{10}$) for two hybridization reactions;
- [0027] Fig. 11f illustrates a summary plot of $\text{Abs}(\log_{10}(k_{\text{Hyb}}(j)) - \log_{10}(k_{\text{Hyb}}(m)))$ vs. feature distance $d_{j,m}$;
- [0028] Fig. 12a illustrates example prediction accuracy of a WNV model;
- [0029] Fig. 12b illustrates rate constant prediction performance of a final WNV model;
- [0030] Fig. 12c illustrates histograms of an example WNV model using 0, 1, 3, and 6 features;
- [0031] Fig. 12d illustrates example leave-one-out (LOO) validation via graphs;

[0032] Fig. 13a illustrates example, hybridization rate constant k_{Hyb} predicted for all possible 36-mer hybridization probes to exon regions of 21 cancer-related genes;

[0033] Fig. 13b illustrates embodiments of a genomic DNA enrichment and a library preparation workflow;

[0034] Fig. 13c illustrates a Beeswarm plot embodiment of NGS reads aligned to certain probes; and

[0035] Fig. 13d illustrates an example ratio of aligned reads in a 20 minute library to a 24 hour library for certain probes.

DETAILED DESCRIPTION

[0036] While methods, apparatuses, and computer-readable media are described herein by way of examples and embodiments, those skilled in the art recognize that methods, apparatuses, and computer-readable media for predicting a hybridization rate constant of a first sequence are not limited to the embodiments or drawings described. It should be understood that the drawings and description are not intended to be limited to the particular form disclosed. Rather, the intention is to cover all modifications, equivalents and alternatives falling within the spirit and scope of the appended claims. Any headings used herein are for organizational purposes only and are not meant to limit the scope of the description or the claims. As used herein, the word "may" is used in a permissive sense (i.e., meaning having the potential to) rather than the mandatory sense (i.e., meaning must). Similarly, the words "include," "including," and "includes" mean including, but not limited to. When introducing elements of various embodiments of the present invention, the articles "a," "an," "the," and "said" are intended to mean that there are one or more of the elements. The terms "comprising," "including," and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements. Furthermore, any numerical examples in the following discussion are intended to be non-limiting, and thus additional numerical values, ranges, and percentages are within the scope of the disclosed embodiments.

[0037] The techniques described herein include methods, systems, apparatuses, and computer-readable media for predicting, for example, a hybridization rate constant k_{Hyb} of a sequence. The techniques include the steps of receiving a first sequence (such as a probe DNA sequence),

calculating a set of feature values corresponding to one or more features of a hybridization reaction, wherein the set of feature values is calculated based at least in part on a hybridization reaction between the first sequence and a second sequence (such as a target sequence), determining one or more distances (such as Euclidean distances) between the set of feature values and one or more other sets of feature values corresponding to one or more other hybridization reactions, and calculating a predicted hybridization rate constant for the hybridization reaction between the first sequence and the second sequence based at least in part on the one or more distances and one or more other hybridization rate constants corresponding to the one or more other hybridization reactions.

[0038] The one or more features can include a temperature of the hybridization reaction. The one or more features can also be based on one or more of a calculated ensemble standard free energy of the first sequence, a calculated ensemble standard free energy of the second sequence, a calculated ensemble standard free energy of a duplex formed through hybridization of the first sequence and second sequence at predetermined temperature and buffer conditions, a calculated standard free energy of a minimum free energy structure (mfe) of the first sequence, a calculated standard free energy of a mfe of the second sequence, a calculated standard free energy of a mfe of a duplex formed through hybridization of the first sequence and second sequence at predetermined temperature and buffer conditions, a difference between a calculated ensemble standard free energy and a calculated standard free energy of a mfe of a duplex formed through hybridization of the first sequence and second sequence at predetermined temperature and buffer conditions, a calculated standard free energy of strongest-binding N nucleotide subsequence of the first sequence at predetermined temperature and buffer conditions, a calculated maximum probability of a N nucleotide subsequence of the first sequence being all in unpaired states at equilibrium, and/or a calculated maximum probability-weighted standard free energy of binding of a N nucleotide subsequence of the first sequence being in all in unpaired states at equilibrium.

[0039] Calculating a predicted hybridization rate constant for the hybridization reaction between the first sequence and the second sequence may be based at least in part on the one or more distances and one or more other hybridization rate constants corresponding to the one or more other hybridization reactions can include weighting each of the one or more other hybridization rate constants based at least in part on the one or more distances between the set of feature values and one or more other sets of feature values corresponding to one or more other hybridization reactions, calculating a weighted average of a logarithm of the one or more other hybridization rate constants,

and transmitting the weighted average as a predicted logarithm of the hybridization rate constant for the hybridization reaction between the first sequence and the second sequence.

[0040] Additionally, the method can include normalizing the set of feature values prior to determining one or more distances. In this case, determining one or more distances between the set of feature values and one or more other sets of feature values corresponding to one or more other hybridization reactions can include determining one or more distances between the normalized set of feature values and one or more other sets of normalized feature values corresponding to one or more other hybridization reactions.

[0041] Turning now to FIGS. 1a and 1b, the techniques described herein include a novel Weighted Nearest Neighbor (WNN) process 10 suitable for predicting hybridization kinetics based on target sequence and experimental conditions. The WNN process 10 may include a hybridization rate constant prediction process 12 shown in FIG. 1a, and a model building process 14 suitable for constructing a hybridization rate constant model. In one embodiment of the WNN process 10, each hybridization reaction, comprising a target sequence 16, a probe sequence, and a set of hybridization conditions (e.g., temperature, buffer), may be represented by a set of bioinformatic features 18 that are constructed. Two hybridization reactions with similar feature values (e.g., having a smaller distance in feature space) are expected to be more likely to exhibit similar hybridization kinetics. A desired hybridization reaction's features 18 is compared with a database of known hybridization reactions 20, and each instance of the database 20 makes a "vote" for the predicted rate constant, with smaller vote weights for more dissimilar instances.

[0042] To quantify the similarity or dissimilarity between two hybridization reactions, each reaction can be abstracted into a number of bioinformatic features 18. The value of each feature 18 for a particular hybridization reaction is computable based at least in part on one or more of the sequence of the target 16, the sequence of the probe, the reaction temperature, and/or buffer conditions. Each hybridization reaction is thus a point in feature space. With an optimally designed set of features 18, the two points close in feature space (small Euclidean distance) should exhibit similar values of k_{Hyb} , the second order rate constant of hybridization. The converse is not necessarily true, two hybridization reactions with coincidentally similar k_{Hyb} values may possess very different feature values.

[0043] Mapping the hybridization reactions into feature space may be important because targets that are similar in sequence space may not be similar in hybridization kinetics, and vice versa, due

to the sensitivity of secondary structure to small changes in DNA sequence in certain regions, but not in others.

[0044] For example, oligonucleotide (2) with sequence "ACACACACAAAAAAGTGTGTGT" has higher Hamming distance to oligonucleotide (1) with sequence "ACACACACTTTTTTTTGTGTGTGT" than oligonucleotide (3) with sequence "AGTCAGACTTTTTTTTGTGTGTGT", but is expected to exhibit much more similar kinetics in hybridization to each's respective complement. In this case, one possible feature 18 can be the number of base pairs formed in the stem of any hairpins in the minimum free energy structure of the oligonucleotide: oligonucleotide (1) and (2) would have feature value 8, while oligonucleotide (3) has feature value 6.

[0045] As shown in FIG. 1a, the hybridization rate constant prediction process 12 may take us the target sequence 16 as input. In the depicted embodiment, the features 18 include six bioinformatics features such as Temperature (T), dGavg, dGbind, dGpZ, maxdG53, and dGavgW. One set of example values is shown in the figure for each of the six features 18, which may be computed as described in more detail below, for example, with respect to FIGS. 2-3. The hybridization rate constant prediction process 12 may then renormalize the features 18 resulting in renormalized features 22 as described in more detail below, for example, with respect to FIG. 4. The process 12 may then apply a weighed neighbor voting 24, with weight decreasing based on distance to the target 16 in feature 18 space. The weighed neighbor voting 24 then may result in the derivation of a predicted rate constant, such as k_{Hyb} . It is to be understood that while the techniques described herein are described with respect to DNA and/or RNA, the techniques may alternatively or additionally be used for protein structures, such as amino acid sequences, ensembles, and the like.

[0046] The process 14 suitable for creating hybridization rate constant prediction model(s) may apply experimental kinetics data 28, such as reaction data, to one or more best fit reaction model(s) 30. The model(s) 30 may access the rate constant database 20 for feature construction and selection 32. As mentioned above, the features 18 as constructed and selected (block 32) via the model 30 and database 20 may then be renormalized and weighed (block 34) to derive the predicted rate constant 26. In this manner, hybridization rate constants from sequence and reaction conditions (e.g., temperature, buffer, salinity) may be provided, useful for a variety of biomedical applications.

[0047] Experimental Data:

[0048] Turning now to FIGS. 2a-d, the figures shows an example experimental setup and data 28 used for training a WNN model, such as model 30. 210 different hybridization experiments, such as an experiment labeled 200, were performed on 100 different pairs of target 16 and probe sequences 210 (e.g., fluorescent probes), as shown in FIG. 2a. . Fluorescence is initially high, and decreases as the hybridization reaction proceeds because the fluorophore (star) becomes localized to the quencher (dot). Each hybridization reaction was monitored in real time using fluorescence, and the rate constant k_{Hyb} was fitted to the kinetics traces. The target sequences 16 were selected as subsequences of the VEGF and CYCS genes, 212, 214, respectively, as shown in FIG. 2b. For this study, all target and probe sequences are 36 nt long (excluding universal regions). 25 targets for each gene were chosen randomly with uniform distribution across the entire intron and exon region, and the other 25 targets were selected as close overlapping frames to systematically test the position effects of secondary structures.

[0049] FIG. 2c illustrates example secondary structures encountered in target sequences, including systematic structures 216 and random structures 218. Shown are predicted minimum free energy (mfe) structures predicted for the target sequences at 37 °C. FIG. 2d depicts example kinetic traces 220 for a hybridization reaction test, showing decay over time at 37 °C. All reactions proceeded in 5x PBS buffer.

[0050] Bioinformatic Features:

[0051] From an initial candidate pool of over 40 bioinformatic features, the feature list was pruned to remove features that did not contribute significantly to the prediction of hybridization rate constant k_{Hyb} , either because they did not significantly impact k_{Hyb} or because their effects were redundantly captured by other features. The final WNN modeling uses 6 features 18: Temperature, dGavg, dGbind, dGpZ, maxdG53, and dGavgW. Temperature is simply the temperature of the hybridization reaction, in Celsius.

[0052] dGbind is the standard free energy of hybridization of the target subsequence and the probe, at the hybridization reaction conditions. Its value can be calculated from nucleic acid thermodynamics prediction parameters, by summing the standard free energies of each base stack. Its value can also be calculated via thermodynamics prediction tools such as Nupack and mFold, e.g. via the "mfe" function.

[0053] $dG_{avg} = \sum Pr_{ij} * dG_{ij}$, over all $1 \leq i < j \leq N$, where N is the length of the target subsequence, corresponds to the standard free energy of hybridization a subsequence from nucleotides i through j to its exact complement (dG_{ij}), weighted by the probability of all nucleotides from i through j being in an unbound state in single-stranded form (Pr_{ij}). Pr_{ij} can be calculated as the product of Pr_k , with k ranging from i through j . Pr_k can be calculated using nucleic acid thermodynamics prediction tools such as Nupack, e.g. via the "pairs" function. dG_{ij} can be calculated using nucleic acid thermodynamics prediction parameters as in dG_{bind} , counting only the base stacks from nucleotides i through j .

[0054] $dG_{avgW} = (\sum Pr_{ij} * dG_{ij}) / (\sum Pr_{ij})$ is calculated similarly to dG_{avg} , but is weighted by the total sum of the probabilities of all subsequences being in unbound states.

[0055] dG_{pZ} is the partition function energy of folding of the single-stranded probe oligonucleotide, and can be calculated using nucleic acid thermodynamics prediction tools such as Nupack, e.g. via the "pfunc" function.

[0056] $maxdG_{53}$ is calculated as the stronger (more negative) standard free energy of binding of the 5' -most and 3' -most unpaired nucleotides of the target in its single-stranded minimum free energy state. In standard dot-parenthesis notation, the standard free energy of binding of the first i nucleotides that are unpaired (dots) and the last j nucleotides that are unpaired are both evaluated, and $maxdG_{53}$ is set as the more negative value.

[0057] FIG. 3 shows the rate constants k_{Hyb} plotted against each of six feature 18 example values. More specifically, six graphs 302, 304, 306, 308, 310, and 312 are shown, based on Temperature, dG_{avg} , dG_{bind} , dG_{pZ} , $maxdG_{53}$, and dG_{avgW} , respectively. Each of the graphs 302-312 includes an x-axis having feature values and a y-axis having an observed rate constant. Each of the graphs 302-312 also includes a feature weight for each of the six features 18. A correlation indicates that these feature values may be effective for rate constant prediction. The calculated feature values for all hybridization reactions (temperature and sequence) are listed later in this document under the heading "Sequences, Feature Values, and Observed Rate Constants".

[0058] Feature Renormalization:

[0059] The constructed features can have different units and different ranges of values. In order to accurately calculate a Euclidean distance between two hybridization reactions, the different

features can be normalized. Because the distributions of most feature values may be distinctively non-Gaussian, normalization can be performed based on the interquartile range. Turning now to FIG. 4, the figure depicts graphs 400 and 402 suitable for illustrating before and after normalization and weighing of features 18. More specifically, graph 400 depicts dGavg in the x-axis and dGbind in the y-axis before normalization and weighing.

[0060] In normalized and weighed graph 402, the 75th percentile feature value is mapped to a score of $+w/2$, and the 25th percentile value is mapped to $-w/2$. Different features can be assigned different weights w , to indicate their importance in prediction of the rate constant k_{Hyb} . A feature with larger weight w allows a larger range of scores, and can contribute more to the overall distance. The data set was divided into a training set (80% of experiments, dots) and a test set (20%, X's); the depicted box indicates the 25th to 75th percentile ranges of the training set for each feature. Renormalization of feature values. The 75th percentile value of feature j is renormalized to $+w_j/2$ and the 25th percentile value is renormalized to $-w_j/2$. All other feature values are linearly transformed based on these reference values. Optimal weights w_j were determined through numerical optimization.

[0061] Distance Calculation and Rate Constant Prediction:

[0062] From a database of hybridization experiments that pairs normalized feature values with k_{Hyb} , such as database 20, a prediction for k_{Hyb} of a new hybridization reaction can be made as shown in FIG. 5. The Euclidean distance between a target sequence's features (X) and each training instance's features is computed and used to determine the weight of the training instance's vote. Displayed numbers are the log (base 10) of the observed experimental rate constants for the training instances, which are integrated to predict the log of the hybridization rate constant of the target sequence. Farther training instances contribute exponentially less to the overall prediction. The formula used for prediction can be:

$$\mathbf{[0063]} \quad \log \hat{k} = \frac{1}{Z} \sum_i \log k(i) e^{-d_i/D}$$

[0064] where $\log k(i)$ is the logarithm (base 10) of the i th database entry, d_i is the distance, e.g., $d_i = \sqrt{(f_j(target) - f_j(i))^2}$, D is a distance constant (arbitrarily set as 5), and $Z = \sum_i e^{-d_i/D}$ is the "partition function" of the distance. Thus, in this model, the weight of a database entry i drops off exponentially with distance. The value of distance constant D does not have significant impact on

the performance of the WNN model, as the same k_{Hyb} predictions can be achieved by scaling the feature weights w with D .

[0065] The six features described earlier (Temperature, dGavg, dGbind, dGpZ, maxdG53, and dGavgW) have final weights of 2.58, 2.42, 3.82, 4.12, 2.89, and 2.31, respectively, under the assumption of $D = 5$. Using this set of feature weights maximizes the prediction accuracy of the model on our current dataset.

[0066] Cross-validation and Prediction Accuracy:

[0067] FIG. 6 illustrates prediction accuracy of an embodiment of the final WNN model for one particular partition of the dataset into training and test sets. For example, graph 600 of FIG. 6 shows real versus predicted data in axes y and x , respectively. A darker shaded region 602 indicates correctness to within a factor of 2, and a lighter shaded region 604 indicates correctness to within a factor of 3. FIG. 7 shows the statistics for 1000 different partitions of the data into training and test sets. The median performance of the WNN model was 73.8% accuracy to within a factor of 2 (F2accuracy) shown in graph 700, 85.7% to within a factor of 3 (F3accuracy) shown in graph 702, and 0.371 root mean square error (RMSE) shown in graph 704. These represent marked improvement over the naive model in which all training data were weighted equally. Different training and test set partitions result in variation in prediction performance, because some partitions result in regions in feature space that are sparsely represented, resulting in larger distances and less accurate prediction.

[0068] Another approach to rate constant prediction is a multi-linear regression based on the constructed features as shown in FIG. 8. Using the same approach of testing on 1000 different partitions of the dataset, the best-performing 6-feature 18 multilinear regression provided median F2accuracy = 69.0% shown in graph 800, F3accuracy = 81.0% shown in graph 802, and RMSE = 0.408 shown in graph 804. It is believed that the WNN model performs better than the multilinear regression approach because the latter approach over-weighs the information from hybridization experiments highly dissimilar to the reaction of interest. Indeed, performance is inferior to WNN model on all three metrics.

[0069] Additional Enhancements:

[0070] Based on biophysical knowledge of the hybridization process, over 30 features were constructed that are believed to be correlated to the hybridization rate constant k_{Hyb} ; these were pruned down to 6 final features without reduction of prediction accuracy. The high cross-validation accuracy of the WNN model indicates that these features capture a significant, if not majority, portion of the complexity of the hybridization process. Simultaneously, there remain pairs of experiments in our database with similar feature values (distances ≤ 1) but with a 10-fold difference in k_{Hyb} . This implies that there are features that distinguish these experiments and these additional features can also be incorporated into the WNN model.

[0071] The WNN model is highly scalable to the addition of new experimental data, as the underlying weights and features are not changed. This is an advantage over multilinear regression-based approaches, in which new data necessitates new regression coefficients. With every additional hybridization experiment and its accompanying fitted k_{Hyb} value, the 6dimensional feature space becomes denser, ensuring that on average a new hybridization experiment will be closer to an existing instance.

[0072] Sequences, Feature Values, and Observed Rate Constants:

[0073] A listing of 100 sequences is presented in the table below:

1	AAGATGGTGAGTGCCATCTTAAACTTACTGGAGAT
2	TTTTCACAAAGATGGTGAGTGCCATCTTAAACTTA
3	TGTTCAACTTTTTCACAAAGATGGTGAGTGCCATCTT
4	TTCCCTCCTGGAAAGCCGAAGCTTAGAGCTTCACGT
5	ACTTCCCTCCTGGAAAGCCGAAGCTTAGAGCTTCAC
6	AGACTTCCCTCCTGGAAAGCCGAAGCTTAGAGCTTC
7	TGGGATGTCCCCGGGGGACCGTGCAGCCTGCCCCTG
8	GTTGGGATGTCCCCGGGGGACCGTGCAGCCTGCCCC
9	GGAGTTGGGATGTCCCCGGGGGACCGTGCAGCCTGC
10	CAGGCGTGAGCCACCACGCCTGGCCAATTATGTAAT
11	GGGATTACAGGCGTGAGCCACCACGCCTGGCCAATT
12	AAGTGCTGGGATTACAGGCGTGAGCCACCACGCCTG
13	ACATAAAAATTAGCCAGGTGTGGTGGTGGGCACCTG
14	AATTAGCCAGGTGTGGTGGTGGGCACCTGTAATCTC
15	CAGGTGTGGTGGTGGGCACCTGTAATCTCAGCTACT
16	TTGGGAGGCCAAGGCAGGCAGATCACCTGAGGTCAG
17	ACTTTGGGAGGCCAAGGCAGGCAGATCACCTGAGGT
18	AGCACTTTGGGAGGCCAAGGCAGGCAGATCACCTGA
19	ACATTTAGAGTAGTCCTTGGAGATTTTATGGAGATG
20	AAGTTGCGGTTGTGGTGATTTTGGCTTAATGTGTTC
21	TCACAAGACTAAAGATAATTAAGAAAGAAAACCACAG
22	GAAACCCCATCTCTACCAAAAATATAAAAAGTAGCT

23	CTTAGTTGGAGTTTGGGGTATTTGAAAACGTCATGC
24	TCTGGTGGGGAATTTAAAAATGCATCCTGGAAATCC
25	CTTGGAGATTTTATGGAGATGGTGAGCACAAGGTAA
26	GCACTTCTCTTGAATTCCTTTATAGATGTACAGTTT
27	ACAATAGTGAAACTCCGTCTCAAAAAGAAAAAAGT
28	AAGATTAAATGGTTAGGTCTTTTTAAAAGTTGCGGT
29	AAATATTCATTCATGAGCTCTTTTGGCAATCCGTCA
30	TTTTATTTTTATTTTTTTTGAGATAATTTCACTCTTG
31	GGTCGCCCCAGGAGATCACAGGTAGGGGAGTTGGGA
32	CTCCAATTCAGTAAATGGTATCACTGTTTACCCCTT
33	ATCCGTCCACTTGCCTTGGCTCCCCAAAGTGCTGGG
34	AGGTTATCTTAGTTGGAGTTTGGGGTATTTGAAAAC
35	GCTATCATTTCCCTCAGAAAGCTAAGTAAATTTACT
36	AAATGTTTTTGGTATTAAAGAATATTTGGTATAAAG
37	ATTCATTTCTCAAAGAGTAAAAGTGCAGGTTGTATG
38	CCAGGTTATCTTAGTTGGAGTTTGGGGTATTTGAAA
39	TATTCAGGGACAGTGTAGCAAGTAGCTTACAAGGGG
40	AATTTTACCATAAGTTTTACCTATTCGTAAGTTGGC
41	TGTCTCTTCTGAAACTGGAGTTTGAATTAGGTTCCC
42	TATAATTACATACTGAATTATTTTCATGCATAGTCTG
43	GCTCTTTTGGCAATCCGTCATCAGTATATTCTGAAA
44	TACATTATATTGCCCTTCAGAATAGATTCCAGTTCC
45	TGGAGTTTGGGGTATTTGAAAACGTCATGCCTTCAG
46	GCCCAGCTTATTTTGTGTTTTTAGTAGAGACAGGGT
47	CCAAGCGGGGAGCATTTCGAGTGGAGCCCGCGCTGGG
48	AGGAGGACTGCTTGTGCCCAGAAGTTCGAGGCTGCA
49	AACTTTGTCTCCACATAAGTCTCTTCTAGGCACTG
50	TTTTAAAAGGACATTTCTATCAGGGATATATACCT
51	CTGGGGCTGTTCTCATACTGGGGCTTTCTGCCCCAG
52	GTTCTCATACTGGGGCTTTCTGCCCCAGGACCACAC
53	CTGGGGCTTTCTGCCCCAGGACCACACCTTCCTGTC
54	GCTCCAGTGCACCCCAGGCTTCGTGGCCAGCCTGGG
55	GTGCACCCCAGGCTTCGTGGCCAGCCTGGGAAACTG
56	CCCAGGCTTCGTGGCCAGCCTGGGAAACTGTCTCTA
57	CTGTGAACTTCCCTCCCAGGCCAGCAGAGGGGCTGGC
58	CCCTCCCAGGCCAGCAGAGGGCTGGCTGTAGCTCCC
59	GCCAGCAGAGGGCTGGCTGTAGCTCCCAGGCGCCCC
60	GTGTCAGGAGCCCCCTCTCTCCCTCTCTTGGAGAGAG
61	GAGCCCCCTCTCTCCCTCTCTTGGAGAGAGTCCTGAG
62	CTCTCTCCCTCTCTTGGAGAGAGTCCTGAGTGCCCC
63	CCCTGTCACCCCGCTTATTTTCATTTCTCTCTGCGG
64	TCACCCCGCTTATTTTCATTTCTCTCTGCGGAGAAG
65	CCGCTTATTTTCATTTCTCTCTGCGGAGAAGATCCA
66	GCCATCCAATCGAGACCCTGGTGGACATCTTCCAGG
67	ATCGAGACCCTGGTGGACATCTTCCAGGAGTACCCT
68	CCTGGTGGACATCTTCCAGGAGTACCCTGATGAGAT
69	TTTATATATATATATATTATATATATATAAAAATAA

70	TTCCATACCTTCACAACACTTGTGCCTCCCCCAGGG
71	TACCTTCACAACACTTGTGCCTCCCCCAGGGCCTCT
72	CACAACACTTGTGCCTCCCCCAGGGCCTCTTTCTCA
73	CCCTGTACTTTCCACTGCCCTACCTAGATGTCCCTG
74	GAGATTTTGTCCCTTCATCCACCGGCTTCTAGATTA
75	GGACTTGACATTTTAGGGTTTTAGGTGATTATTCT
76	ACACACTGAAGGAGCTGTAGCATCCAAGAATACTAG
77	TGTCAACAAAGCACAGATGCTCTCGCTGGGGCCTTG
78	TCTGTCCTCAGTGGTCCCAGGCTGCACCCATGGCAG
79	ACCCAGTCTCGGCTTCCCACCAAAGCCTTGTCAGGG
80	AGCTGCCTCCCCCTTTGGGTTTTGCCAGACTCCACA
81	GCTCCGATGGGGGCAACAGCAGTTGGGTCCCTGTGG
82	AATGTGACTTGGGTCCATTTGAATCCAAAGTCCCTG
83	GGCCGCTGGTCCCGGACGAACTGGAAGTCTGAGCAG
84	AGGTGAGCATGCCTGGGGGTGTTGGGGAGATGCAAT
85	TAAGTGAAGTCAAGTTGTTTCAGGGGGCTAAGCCCAT
86	GAGGGCAGGGCTGGGGCTGTTCTCATACTGGGGCTT
87	AGCCCCCTATTCCGGCCCAACCCATGGCACCCACAG
88	GCCAGCCTTGACACACTTTGTCCTGGTGAAAGGCA
89	CTATAAATCCATGAGCAGAAAAATACATAAAATGTG
90	TCCCTGTACCTCCTATAAAATCAGCATGGAGCCTGG
91	CCAGGCAGTGGAGGCCAGCCCTCCTTGGAGGGGCGG
92	CCTGCATTTTCGAGCTCCCCAGCCCCAACATCTGGT
93	TACCTTTGTGAGCCCCGGGCATCTGTACCTCTTTCC
94	TCCGTTTCGGGGCTCCCCAGAAGGGTAGGGCCTGTT
95	GAGCAAACCTCCCCCACCCTTTCCAAAGCCCATT
96	AGTTTGCCCTCTTGGGCGGGGTATCAGTGGCTGGC
97	ACCCCTTGCCCAGGCCAGACCTTCCTGCTATCCCCT
98	GCCTGATACACAGCCCTCCCTCCCCTCCTGCTCCC
99	CTTATGGCAGCCTCTCCCTGCACTCTCTGCCCCTCT
100	TCTCCCTCCTCAGACTGGGGCTCTGAGGGCAAGGGG

[0074] The calculated values of each of the six previously identified features for these 100 sequences is listed in the table below:

Sequence	k_Hyb	T	dG_average	binding Dg	pfprobe	max(5'/3' free)	Dg_average_2
1	6.493599	37	-16.69946109	-41.772	-5.6635	-17.632	-4.46734967
1	6.645121	55	-13.16411774	-27.998	-3.1656	-11.813	-4.104588151

1	6.404107	28	-18.09615307	-48.658	-7.1336	-1.0247	-4.634540636
1	6.65908	46	-15.16937238	-34.885	-4.3347	-14.723	-4.359451342
2	5.758972	37	-18.91803374	-40.617	-5.8439	-8.4381	-5.697503272
2	5.879055	55	-13.1730106	-26.782	-3.41	-5.6545	-4.08237026
3	5.921924	37	-19.32477581	-43.128	-5.8176	-18.589	-6.493503982
3	6.316482	55	-14.89303642	-29.207	-3.4284	-12.622	-5.347620864
4	4.926437	37	-19.19118253	-47.612	-6.8235	-4.9707	-4.428226929
4	6.251435	55	-16.26375592	-33.626	-3.8974	-3.669	-3.424338266
5	4.700764	37	-16.75932916	-46.735	-7.1581	-1.4198	-3.330225727
5	6.235281	55	-15.17467572	-32.86	-3.9622	-18.829	-2.882208655
6	4.758499	37	-17.64374545	-46.424	-7.0057	-3.9557	-4.151263153
6	6.112713	55	-15.67362999	-32.584	-4.0829	-22.223	-3.374865045
7	5.412774	37	-18.84403557	-55.293	-7.027	-6.2711	-3.668593584
7	6.288939	55	-18.14106706	-41.451	-4.0291	-17.703	-3.363972993
8	5.524343	37	-19.44492457	-54.993	-6.8304	-3.5904	-3.568772209
8	6.401901	55	-18.45326235	-41.151	-3.8827	-15.812	-3.349735215
9	5.698041	37	-20.4938987	-53.938	-5.9418	-6.7118	-4.277208547
9	6.483438	55	-19.17868955	-40.035	-3.2413	-15.799	-3.789398319
10	6.152152	37	-19.27520474	-49.099	-9.9333	-14.793	-6.002278899
10	6.104575	55	-15.63221011	-35.019	-6.6098	-9.732	-5.705765446
11	5.716302	37	-21.77455825	-51.143	-9.916	-8.1835	-6.403441244
11	6.046932	55	-17.25577763	-37.107	-6.5616	-5.8321	-5.378867074

12	6.541378	37	-24.52809539	-51.107	-9.3771	-17.176	-10.80521603
12	6.751653	55	-18.25979401	-36.969	-6.3782	-12.102	-8.057295998
12	6.334811	28	-27.52736985	-58.175	-10.983	-19.713	-11.94168999
12	6.745367	46	-21.43026192	-44.038	-7.8387	-14.639	-9.503891602
13	6.354415	37	-23.18120623	-46.299	-5.0826	-13.907	-6.720039287
13	6.585083	55	-17.26571175	-32.504	-2.848	-8.8351	-5.061446508
14	5.492402	37	-21.55748754	-46.768	-5.6554	-6.7765	-5.325758114
14	6.172355	55	-16.68349847	-32.971	-3.0592	-4.4305	-4.062337838
15	6.364534	37	-25.41418931	-47.566	-5.2578	-15.549	-8.610489803
15	6.52458	55	-18.75625553	-33.693	-2.9089	-10.444	-5.877435942
16	6.086519	37	-22.8819649	-49.768	-5.0281	-8.4316	-4.865360384
16	6.717518	55	-19.97321831	-35.971	-2.6673	-14.746	-3.84722431
17	5.517892	37	-19.00207327	-49.441	-5.1463	-4.469	-4.153507101
17	6.661886	55	-17.86082733	-35.665	-2.6814	-17.211	-3.40622034
18	5.947284	37	-18.09801207	-49.852	-5.2373	-7.5541	-4.212835345
18	6.71578	55	-18.00377806	-35.99	-2.7112	-20.859	-3.561363471
19	6.806991	37	-28.29172329	-39.408	-1.3134	-41.376	-6.405634406

19	6.96155	55	-22.16433101	-25.736	-0.87917	-27.806	-6.2915949
20	7.083721	37	-34.8733754	-44.416	-0.76711	-46.383	-8.688773585
20	7.13876	55	-27.25067955	-30.401	-0.47992	-32.471	-8.737169947
21	6.939231	37	-33.23915941	-37.506	-0.82059	-39.474	-10.23622608
21	6.927577	55	23.28217285-	-23.757	-0.441	-25.828	-9.127799158
22	7.112589	37	-37.5616697	-39.015	-0.99673	-40.983	-15.67717349
22	7.102685	55	-25.93534419	-25.389	-0.6085	-27.459	-12.45488968
23	6.372783	37	-26.45689597	-42.835	-1.3538	-10.915	-5.450793737
23	6.944557	55	-23.69447266	-28.96	-0.58986	-31.031	-5.816061771
24	6.215896	37	-25.9709614	-42.839	-1.9715	-10.4	-5.207686436
24	6.995671	55	-23.32301401	-29.22	-1.0313	-31.29	-5.701615276
25	6.714481	37	-31.23487577	-42.32	-1.0855	-23.685	-7.099491319
25	7.027868	55	-24.91471368	-28.56	-0.5824	-30.631	-7.013092143
26	6.38593	37	-27.3905512	-39.237	-2.3169	-41.205	-5.415354031
26	6.806777	55	-22.92353276	-25.438	-1.2373	-27.509	-6.540902946
27	6.579883	37	-27.15977403	-39.911	-2.602	-25.41	-5.727915586
27	6.734515	55	-20.95157137	-26.029	-1.664	-17.14	-5.317950766
28	6.860599	37	-26.80159943	-39.716	-2.4901	-19.254	-5.494430375

28	7.174687	55	-21.44914453	-25.934	-1.2424	-28.004	-5.043411424
29	6.195249	37	-25.87191145	-42.035	-2.2299	-15.891	-5.238222299
29	6.942867	55	-21.21304647	-28.109	-1.2381	-30.179	-4.993485145
30	6.811387	37	-24.87789255	-34.001	-1.5688	-15.295	-5.44329072
31	6.643434	37	-29.63771149	-49.819	-3.2141	-9.9337	-6.985684744
31	7.065788	55	-22.54070203	-36.112	-1.7156	-7.2312	-5.092906075
32	6.084429	37	-20.69936309	-41.032	-3.4047	-11.228	-5.086586389
32	6.925882	55	-18.82320277	-27.367	-1.7422	-29.437	-3.82343689
33	5.780442	37	-17.74581148	-50.538	-4.9237	-15.607	-3.390510457
33	6.71647	55	-20.36527301	-36.687	-2.4407	-11.237	-3.886928135
34	6.864782	37	-29.16611682	-40.222	-1.0342	-19.027	-7.355423553
34	7.194446	55	-23.52963699	-26.59	-0.49414	-28.661	-7.145071361
35	6.30882	37	-18.1699364	-39.033	-3.2304	-7.5632	-3.958629007
35	6.804477	55	-18.26038676	-25.315	-1.4168	-27.386	-3.976988447
36	6.508046	37	-22.07167661	-34.411	-1.8244	-16.456	-3.900994314
37	6.481893	37	-27.13964671	-39.955	-2.2413	-41.923	-5.475190865
37	6.853413	55	-22.21475651	-26.117	-1.1892	-28.187	-5.661913282
37	6.132725	28	-27.74783493	-46.874	-3.0903	-25.825	-5.268975608
37	6.827725	46	-25.35248021	-33.036	-1.6252	-35.055	-5.657714226

38	7.098691	37	-29.1618495	-41.063	-0.00005	-20.781	-7.647254967
38	7.298904	55	-22.67756282	-27.451	-0.54774	-29.522	-6.489019546
39	6.448974	37	-27.27795942	-43.91	-2.8254	-19.564	-5.67255902
39	7.075777	55	-22.30750708	-30.132	-1.4803	-13.694	-5.287102365
39	6.361354	28	-28.38429871	-50.798	-3.8022	-12.531	-5.729724035
39	6.872587	46	-25.28380899	-37.021	-2.0639	-16.629	-5.598896555
40	6.572649	37	-26.01382104	-38.641	-2.2487	-40.608	-5.23926118
40	7.038522	55	-21.14643517	-24.865	-1.2275	-26.936	-5.509088764
41	6.619415	37	-24.89904202	-42.185	-3.478	-14.907	-4.976884579
41	7.119339	55	-21.17484379	-28.453	-1.8266	-10.328	-4.622083254

42	6.234078	37	-21.13565236	-35.672	-2.9729	-17.6	-4.678284585
43	6.164563	37	-22.92308117	-42.042	-2.6259	-0.96097	-4.962054782
43	6.828428	55	-20.30724322	-28.11	-1.2416	-30.181	-4.656494392
44	6.540634	37	-29.69458582	-39.594	-1.9572	-21.112	-6.654721325
44	6.951088	55	-23.54882189	-25.932	-1.0028	-28.003	-6.901939765
45	6.182305	37	-24.13632028	-44.397	-1.9102	-16.568	-4.291761074
45	7.127481	55	-23.26219179	-30.526	-0.94558	-32.596	-5.026341836
46	6.506334	37	-24.49240493	-42.716	-1.6745	-44.684	-5.469966637
46	6.891185	55	-24.52321065	-28.934	-0.62352	-31.004	-6.979206408
47	6.684362	37	-14.03276311	-54.997	-5.9115	0	-4.32595933
47	5.331332	55	-13.85548219	-40.789	-3.2339	-2.8701	-3.744739899
48	6.081154	37	-25.29976863	-50.081	-3.4615	-20.024	-4.5581571 0
48	6.866657	55	-23.77897548	-36.041	-1.8933	-38.112	-4.628040149
49	6.420859	37	-21.3806966	-43.058	-2.9457	0	-4.22532639
49	6.969484	55	-20.99919607	-29.243	-1.5576	-31.314	-4.382877842
50	6.377586	37	-20.16876181	-36.704	-2.2026	-22.446	-4.191932856
50	6.836831	55	-15.61179067	-23.164	-1.2289	-25.234	-3.41337009
51	6.388603	37	-19.69215094	-49.095	-8.8813	-21.973	-5.410169781
51	6.704311	55	-15.55700297	-35.381	-5.9143	-15.732	-4.912134016
52	5.701102	37	-18.19188013	-48.134	-9.0318	-10.19	-8.442818444
52	6.303601	55	-15.2514429	-34.363	-6.0454	-7.392	-4.870095249
53	6.516417	37	-20.63824092	-50.157	-9.5625	-22.66	-6.144991218

53	6.750861	55	-16.36516155	-36.446	-6.3348	16.378	5.280465471
54	5.734849	37	-23.78206744	-54.408	-9.081	-17.104	-8.188236698
54	6.175647	55	-20.47240221	-40.451	-5.683	-12.659	-8.270196835
55	4.437089	37	-25.32774414	-52.501	-9.2594	-7.8926	-7.872486261
55	5.450797	55	-18.86066495	-38.549	-5.9313	-5.8203	-5.912725069
56	6.523657	37	-24.31994347	-49.866	-8.8287	-13.075	-10.51289077
56	6.542725	55	-17.45275741	-35.993	-5.7153	-8.7556	-7.483788261
57	5.737655	37	-25.26096992	-51.468	-8.151	-25.084	-7.729212877
57	6.56944	55	-19.71512553	-37.671	-5.0515	-18.107	-6.842819887
58	5.869908	37	-26.93831784	-53.023	-7.9007	-12.987	-7.391718053
58	6.428207	55	-20.84849405	-39.287	-4.9493	-9.4637	-5.860494227
59	5.124995	37	-23.18458	-55.222	-8.3928	-29.379	-5.869685748
59	6.336184	55	-21.04092601	-41.254	-5.0525	-22.251	-6.138808531
60	5.783375	37	-20.7797813 6	-47.491	-7.1478	-18.35	-5.821081755
60	6.637639	55	-16.41606066	-33.806	-4.1634	-13.634	-4.072282545
61	5.658536	37	-22.38310507	-47.181	-7.5475	-8.3257	-6.408472659
61	6.158557	55	-17.28229897	-33.529	-4.3383	-6.383	-4.454339153
62	6.693126	37	-24.31924855	-47.491	-7.6917	-18.725	-9.077606969
62	7.014858	55	-18.91920666	-33.806	-4.4053	-14.055	-6.033626161
63	6.03039	37	-31.84289221	-46.697	-3.0695	-13.615	-14.56183675
63	6.830978	55	-23.99358341	-32.774	-1.5356	-10.122	-9.579511807
64	6.366036	37	-26.07740681	-45.039	-4.0672	-5.9236	-6.618810546

64	6.853962	55	-18.80396058	-31.084	-2.2064	-4.3464	-4.510062059
65	6.418173	37	-25.28400363	-43.946	-3.5233	-11.377	-6.214304624
65	6.758708	55	-19.22132148	-29.985	-1.9069	-7.8872	-4.711443428
65	6.323671	28	-28.44631582	-50.926	-4.4953	-13.121	-6.870811953
65	6.693575	46	-22.10342767	-36.966	-2.6499	-9.6319	-5.441748081
66	6.254255	37	-24.43824541	-47.975	-4.5915	-21.217	-6.512154008
66	6.76502	55	-19.64445666	-34.173	-2.3839	-15.228	-4.874223962
67	5.82361	37	-24.18865054	-47.03	-4.9168	-9.499	-6.215419941
67	6.626818	55	-18.2151185	-33.289	-2.6315	-6.8234	-4.455359547
68	5.601396	37	-19.44394209	-45.377	-5.1255	-19.132	-4.146021699
68	6.581059	55	-16.22596297	-31.703	-2.8537	-13.313	-3.642266878
70	6.159312	37	-24.71995753	-47.581	-5.4923	-12.296	-6.253905672
70	6.947577	55	-20.81145857	-33.878	-2.9372	-13.69	-5.018051995
70	5.897735	28	-27.35539161	-54.433	-6.9047	-14.217	-7.008665359
70	6.499526	46	-22.50863877	-40.729	-4.1486	-10.375	-5.573090768
71	6.123368	37	-24.77989108	-49.061	-5.9752	-5.9909	-5.121741445
71	6.886395	55	-20.88130794	-35.321	-3.1935	-19.512	-4.280191329
72	6.506021	37	-25.77205652	-48.954	-5.211	-12.751	-6.362779345
72	6.938456	55	-21.31104008	-35.168	-2.7314	-24.315	-4.812842442
73	6.928302	37	-38.82914005	-45.894	-1.8656	-47.862	-10.10717616
73	7.310583	55	-30.84704866	-32.232	-1.0033	-34.303	-11.63776512
74	6.422231	37	-33.48181004	-43.11	-1.9843	-45.077	-7.865090386
74	7.027814	55	-26.12883894	-29.332	-1.1539	-31.403	-8.081875887

75	6.979617	37	-29.06302649	-39.129	-0.92277	-41.097	-6.499169819
75	7.137104	55	-21.78479239	-25.492	-0.59567	-27.562	-5.821464987
76	6.291089	37	-22.71969812	-43.087	-2.8092	-12.037	-4.12979974
76	6.686476	55	-21.8839244	-29.198	-1.2608	-31.268	-4.825534358
76	5.963582	28	-22.49897977	-50.031	-4.0407	-12.276	-4.047519533
76	6.509928	46	-22.91340966	-36.142	-1.8937	-13.99	-4.428300729
77	5.874221	37	-25.53986414	-49.151	-4.2985	-10.324	-5.026372156
77	6.699569	55	-22.81659211	-35.057	-2.5185	-17.008	-5.149002384
78	5.081317	37	-23.24868627	-51.004	-5.0915	0	-4.983275299
78	6.243148	55	-23.80418165	-37.127	-2.6035	-21.302	-4.893177948
79	4.220745	37	-20.09979462	-50.027	-6.2036	0	-4.974575965
79	6.221251	55	-21.46606362	-36.21	-2.8928	-9.4247	-4.843784904
80	5.699717	37	-30.63521783	-49.782	-4.6835	-22.291	-6.393951817
80	6.28254	55	-26.03811048	-36.026	-2.4188	-15.959	-6.217263781
81	5.435628	37	-17.47892435	-52.004	-5.4445	-10.784	-3.581000028
81	6.141796	55	-15.89058724	-38.127	-2.9029	-7.9388	-3.127012976
82	4.490768	37	-17.77322497	-43.888	-4.9679	-4.6476	-3.968110667
82	6.213266	55	-16.37079568	-30.179	-2.3663	-3.3254	-3.286039565
82	5.156059	46	-16.67898195	-37.034	-3.5201	-3.9007	-3.577181097
83	5.920113	37	-22.39496242	-52.113	-3.0251	-7.921	-4.012456619
83	6.685215	55	-22.64017573	-37.971	-1.5963	-7.2936	-4.177382083
84	6.409595	37	-23.79565597	-49.765	-2.3335	-7.1776	-4.841827211
84	7.118607	55	-21.77068055	-35.996	-1.0055	-38.067	-4.184045577

85	6.67084	37	-21.50033636	-45.113	-4.4006	-27.409	-3.935994363
85	6.722208	55	-18.00071051	-31.359	-2.3388	-18.75	-4.095268836
86	7.394846	37	-24.9156486	-50.702	-2.3031	-15.302	-6.392169095
86	7.209255	55	-22.01861156	-36.982	-1.1562	-11.065	-4.99275664
87	6.611552	37	-31.5877987	-52.126	-3.5505	-19.458	-7.121974874
87	7.110619	55	-27.3995776	-38.414	-1.7197	-14.424	-6.54765261
88	4.884468	37	-16.30169957	-49.385	-5.8439	-5.4214	-5.446779655
88	5.869976	55	-13.8171601	-35.368	-2.708	-4.2097	-4.248217977
89	6.582693	37	-26.3389174	-36.968	-2.233	-26.131	-7.878803782
89	6.810758	55	-19.15464541	-23.223	-1.2064	-25.293	-5.828278454
90	6.039519	37	-27.31367875	-44.708	-2.8753	-10.954	-5.944350006
90	6.836369	55	-24.92455266	-31.036	-1.3855	-33.106	-6.528264466
91	5.509794	37	-19.68058646	-54.437	-6.7833	-6.1258	-3.778210659
91	6.201765	55	-17.50082363	-40.69	-3.7937	-4.8331	-3.43536854
92	6.272034	37	-28.70146551	-50.279	-3.9966	-15.559	-6.205580195
92	7.101642	55	-26.72103301	-36.448	-1.8625	-38.518	-6.466232255
93	6.565171	37	-30.97831029	-47.513	-3.0403	-18.787	-7.151679424
93	6.82859	55	-25.30969944	-33.707	-1.593	-12.977	-6.600306619
94	5.815779	37	-15.73269044	-51.151	-6.0932	-8.5498	-3.098197985
94	6.314285	55	-14.54829421	-37.393	-3.5147	-6.121	-2.68477169
95	6.693808	37	-36.26341953	-48.948	-2.0714	-6.8182	-9.199995825
95	7.412106	55	-32.24715674	-35.4	-0.95865	-37.47	-11.08256153

96	6.874767	37	-24.51210503	-50.59	-5.6869	-4.5958	-4.752012687
96	7.134715	55	-18.06210693	-36.724	-3.1578	-19.621	-3.876489151
97	6.501341	37	-33.40407013	-50.666	-3.521	-14.442	-7.037981446
97	6.9055	55	-28.28637204	-37.078	-1.9548	-13.545	-6.761792623
98	6.817971	37	-38.45842011	-50.564	-3.4668	-34.027	-10.07664379
98	7.302731	55	-32.25886797	-36.848	-1.4805	-38.918	-9.86858742
99	6.026689	37	-30.3137576	-49.763	-4.5807	-6.0867	-7.786580229
99	6.690746	55	-23.16568785	-35.815	-2.3491	-4.4087	-5.580669209
100	4.431251	37	-16.7137184	-50.525	-7.9263	-2.5359	-5.259399149
100	5.130717	55	-13.84844443	-36.917	-4.2136	-1.7544	-3.851673493

[0078] One or more of the techniques described herein for all figures can be implemented in or involve one or more computer systems. Fig. 9 illustrates a generalized example of a computing environment 900. The computing environment 900 is not intended to suggest any limitation as to scope of use or functionality of a described embodiment.

[0079] The computing environment 900 includes at least one processing unit 910 and memory 920. The processing unit 910 executes computer-executable instructions and can be a real or a virtual processor. In a multi-processing system, multiple processing units execute computer-executable instructions to increase processing power. The memory 920 can be volatile memory (e.g., registers, cache, RAM), non-volatile memory (e.g., ROM, EEPROM, flash memory, etc.), or some combination of the two. The memory 920 can store software 980 implementing described techniques.

[0080] A computing environment can have additional features. For example, the computing environment 900 includes storage 940, one or more input devices 950, one or more output devices 960, and one or more communication connections 990. An interconnection mechanism 970, such as a bus, controller, or network interconnects the components of the computing environment 900. Typically, operating system software or firmware (not shown) provides an operating environment for other software executing in the computing environment 900, and coordinates activities of the components of the computing environment 900.

[0081] The storage 940 can be removable or non-removable, and includes magnetic disks, magnetic tapes or cassettes, CD-ROMs, CD-RWs, DVDs, or any other medium which can be used to store information and which can be accessed within the computing environment 900. The storage 940 can store instructions for the software 980.

[0082] The input device(s) 950 can be a touch input device such as a keyboard, mouse, pen, trackball, touch screen, or game controller, a voice input device, a scanning device, a digital camera, remote control, or another device that provides input to the computing environment 900. The output device(s) 960 can be a display, television, monitor, printer, speaker, or another device that provides output from the computing environment 900.

[0083] The communication connection(s) 990 enable communication over a communication medium to another computing entity. The communication medium conveys information such as computer-executable instructions, audio or video information, or other data in a modulated data signal. A modulated data signal is a signal that has one or more of its characteristics set or changed in such a manner as to encode information in the signal. By way of example, and not limitation, communication media include wired or wireless techniques implemented with an electrical, optical, RF, infrared, acoustic, or other carrier.

[0084] Implementations can be described in the general context of computer-readable media. Computer-readable media are any available media that can be accessed within a computing environment. By way of example, and not limitation, within the computing environment 900, computer-readable media include memory 920, storage 940, communication media, and combinations of any of the above.

[0085] Of course, Fig. 9 illustrates computing environment 900, display device 960, and input device 950 as separate devices for ease of identification only. Computing environment 900, display device 960, and input device 950 can be separate devices (e.g., a personal computer connected by wires to a monitor and mouse), can be integrated in a single device (e.g., a mobile device with a touch-display, such as a smartphone or a tablet), or any combination of devices (e.g., a computing device

operatively coupled to a touch-screen display device, a plurality of computing devices attached to a single display device and input device, etc.). Computing environment 900 can be a set-top box, personal computer, or one or more servers, for example a farm of networked servers, a clustered server environment, or a cloud network of computing devices.

[0086] Having described and illustrated the principles of our invention with reference to the described embodiments, it will be recognized that the described embodiments can be modified in arrangement and detail without departing from such principles. It should be understood that the programs, processes, or methods described herein are not related or limited to any particular type of computing environment, unless indicated otherwise. Various types of general purpose or specialized computing environments can be used with or perform operations in accordance with the teachings described herein. Elements of the described embodiments shown in software can be implemented in hardware and vice versa.

[0087] Discussion below is based on model construction based on using dGavg, Temperature, Pap, nGp, Gb, and Pm as the features 18.

[0088] Turning now to FIG. 10a, the figure illustrates three different reaction models 1000, 1002, 1004, considered for fitting rate constant k_{Hyb} to fluorescence kinetics data. Model H1 (e.g., model 1000) is the simplest model and has 2 free parameters: k_{Hyb} and $[P_{good}]$. $[P_{good}]$ denotes the concentration of properly synthesized probes that are capable of hybridization, with the remainder of the $[P]$ assumed to be unhybridizable and constitutively fluorescent. Model H2 (e.g., model 1002) has 3 free parameters: k_{Hyb} , k_1 , and k_2 . Model H3 (e.g., model 1004) has 4 parameters: k_{Hyb} , $[P_{good}]$, k_1 , and k_2 . An even simpler model H0 with only k_{Hyb} as a parameter appears to fail to reasonably fit the observed fluorescence data.

[0089] FIG. 10b illustrates two examples 1006, 1008 of fit quality for the 3 reaction models 1000, 1002, and 1004. The y-axis of FIG. 10b plots the relative error RE as a function of time for each model 1000, 1002, and 1004 using best-fit parameters. For Target 12 (e.g., target number 12 of all targets 16), all three models 1000, 1002, and

1004 show low relative error across all time points, but model 1000 is significantly worse than 1002 and 1004 for Target 37 (e.g., target 37 of all targets 16).

[0090] FIG. 10c illustrates a summary of fit performance for the three models 1000, 1002, 1004, across all 210 fluorescence kinetics experiments. Each point in graph 1010 corresponds to the root mean square relative error (RMSRE) of all time points for a particular fluorescence experiment. Based on this result, we chose to proceed with H3 and its best-fit k_{Hyb} values for all subsequent studies. FIG. 10d illustrates a graph 1012 of observed rate constants for 96 targets at 37°C and 55°C. 4 of the targets did not stably bind to their corresponding probes at 55°C due to being A/T rich and were not included in the graph 1012.

[0091] FIG. 11a illustrates rate constant prediction using a Weighted Neighbor Voting (WNV) model. More specifically, the figure illustrates values 1100 of a number of rationally designed features are computed based on the sequences of the target 16 and probe 210, as well as the reaction conditions (temperature, salinity). Shown in the figure is an example calculation for feature Gb, the weighted average ΔG° of the hybridized complex. The ΔG° of formation for a number of likely states S_a are computed using Nupack, and subsequently used to compute their probability of existence at equilibrium $\text{Pr}(S_a)$.

[0092] FIG. 11b illustrates a graph 1102 having an example relationship between the base-10 logarithm of the experimental hybridization rate constants k_{Hyb} (based on reaction model 1004) vs. Gb values for the 210 hybridization experiments. There is moderate correlation between k_{Hyb} and Gb, indicating that Gb may be an effective feature for rate constant prediction. FIG. 11c illustrates feature renormalization based on 75th and 25th percentile values. Plotted on graph 1104 are the raw values of the Gb (e.g., weighted average ΔG° of the hybridized complex, such as TP complex) and dGavg features 18 for all 210 experiments. These feature 18 values are linearly transformed (re-normalized), as shown on graph 1106, based on a set of feature weights $w(i)$: The 75th percentile value of a feature i is renormalized to $+\frac{w(i)}{2}$ and the 25th percentile value is renormalized to $-\frac{w(i)}{2}$.

[0093] FIG. 11d illustrates a graph 1108 showing that given a reaction whose rate constant k_{Hyb} is to be predicted (middle dot), the reaction's renormalized feature values are first calculated and compared to the feature values of reactions with known k_{Hyb} values (dots surrounding middle dot). The feature space distance $d_{j,m}$ between the unknown reaction j and each known reaction m is used to determine the prediction weight of reaction m . Prediction weight drops exponentially with distance and is calculated as $2^{-d_{j,m}}$; Z_j is the sum of all prediction weights involving j . Both the predicted rate constant k_{Hyb} and the known rate constants are expressed in logarithm base 10.

[0094] FIG. 11e illustrates a graph 1110 showing relationship between feature space distance d and the absolute value of difference in experimental rate constants ($\log_{10}$) for two hybridization reactions. Here, feature space distances are calculated using the final 6-feature model (see FIGS. 12), for one reaction (arbitrarily assigned $j = 1$) vs. all 209 other reactions. Pairs of reactions with small d generally have similar rate constants. The converse statement is not true because two very different reactions may coincidentally have similar rate constants.

[0095] FIG. 11f illustrates a summary plot 1112 of $\text{Abs}(\log_{10}(k_{\text{Hyb}}(j)) - \log_{10}(k_{\text{Hyb}}(m)))$ vs. feature distance $d_{j,m}$ for all $\frac{210}{2}$ pairs of experiments. A black line 1114 shows the mean and a region 1116 shows ± 1 standard deviation on the mean.

[0096] FIG. 12a illustrates prediction accuracy of a WNV model. For example, a graph 1200 is shown, with curves 1202 and 1204. The dots of curve 1204 show the results of a greedy optimization algorithm in which individual weight-optimized features are sequentially added to the WNV model. The Badness metric (y-axis) is calculated based on the fraction accurate to within a factor of 2 (F2acc), fraction accurate to within a factor of 3 (F3acc), and the root-mean-square error (RMSE). The final 6-feature WNV model (labeled 'X') comprise the 6 features from the 8-feature model with the largest weights. Performance of a multilinear regression (MLR) model is also shown in curve 1202 for comparison.

[0097] FIG. 12b includes a graph 1206 illustrating rate constant prediction performance of the final WNV model, using feature weights that were optimized on all 210 experimental reactions. Each dot corresponds to the prediction of each reaction's rate constant based on the data from the other 209 reactions' rate constants. A shaded region 1208 indicates correctness to within a factor of 2, and a shaded region 1210 indicates correctness to within a factor of 3.

[0098] FIG. 12c illustrates embodiments of histograms 1212, 1214, 1216, and 1218 of experimental minus predicted $\log_{10}(k_{Hyb})$ values for WNV model using 0, 1, 3, and 6 features. The 0-feature model represents a naive model predicting the same $\log_{10}(k_{Hyb})$ value for all sequences, and the 1-feature model represents a simple model that considers only secondary structure.

[0099] FIG. 12d illustrates leave-one-out (LOO) validation via graphs 1220 and 1222. More specifically, to estimate the accuracy of the final WNV model on prediction of rate constants for new sequences, LOO validation was performed. In graph 1220, feature weights were optimized on the 209 data points, and this model was used to predict the rate constant of a single "unknown" data point 1224. The bottom panel summarizes the prediction performance of the 210 distinct models (with weights optimized on different sets of 209 points).

[00100] FIGS. 13a-d illustrates comparison of probes predicted to possess median vs. fast hybridization kinetics for enrichment from human genomic DNA. For example, FIG. 13a illustrates hybridization rate constant k_{Hyb} 1300 predicted for all possible 36-mer hybridization probes to exon regions of 21 cancer-related genes 1302. Middle graph 1304 and lower graph 1306 express the idea behind probe selection and library design, but are for example only and may not accurately reflect kinetics distributions or trajectories of any particular gene or probe.

[00101] FIG. 13b illustrates a genomic DNA enrichment and library preparation workflow 1308. As shown, the workflow includes a shearing stage (block 1310), an end repair and adaptor ligation stage (block 1312), a heat denature stage (block 1314), a hybridizing of all probes stage (block 1316), a magnetic bead capture stage (block 1318),

and a PCR to append index and normalize library stage (block 1320). All hybridization probes were present at 50 pM concentration.

[00102] FIG. 13c illustrates a Beeswarm plot 1322 of NGS reads aligned to each probe, excluding 15 fast and 15 median probes to 4 genes with low read depth. In the library in which probes were hybridized to the fragmented gDNA for 24 hours (top panel 1324), there is no significant difference in the read count distribution between the median and fast probes. In the 20 minute hybridization library shown in bottom panel 1326, the fast probes showed significantly higher reads than the median probes, indicating that the probes the algorithm predicted to be faster did in fact provide a higher degree of hybridization within 20 minutes.

[00103] FIG. 13d illustrates a ratio of aligned reads in the 20 minute library to the 24 hour library for each probe. A high ratio indicates fast hybridization kinetics; ratio can exceed 1 because libraries were normalized, so that fast probes are more dominant and occupy more reads in the 20 minute library.

[00104] Details of Model Construction - Hybridization rate constant (kHyb) fitting.

[00105] Turning back to FIG. 10a, from the experimental kinetics traces (e.g., traces 220 shown in FIG. 2d), we wish to determine a single rate constant kHyb that describes the dominant hybridization kinetics pathway. However, a simple two-state $T + P \rightarrow TP$ reaction model fails to reasonably capture a significant portion of the kinetic behavior. Most notably, over 40% of the observed reactions appeared to asymptote to a final reaction yield of less than 85%, based on positive and negative control fluorescence values. Consequently, we considered 3 slightly more complex reaction models 1000, 1002, 1004 of hybridization, in order to evaluate which best fits the observed data, as shown in FIG. 10a.

[00106] Model H1 1000 assumes that the $T + P \rightarrow TP$ reaction is correct, but that a fraction of the probes P are poorly synthesized, or otherwise incapable of proper hybridization with target T or the accompanying fluorescence quenching. Thus, in addition to kHyb, H1 has one extra fitting parameter: [Pgood]₀, the initial concentration (or fraction) of viable probe P.

[00107] Model H2 1002, in contrast, assumes that all probe P is correctly synthesized, but that some fraction of the T + P reaction undergoes an alternative pathway with rate constant k_1 to result in a state TP_{bad} with high fluorescence. This frustrated state TP_{bad} may represent states in which T and P are co-localized by misaligned base pairs. Model H2 1002 assumes that TP_{bad} undergoes first-order rearrangement with rate constant k_2 to form the correct product TP. Model H2 1002 has a total of 3 fitting parameters: k_{Hyb} , k_1 , and k_2 . Model H3 1004 is a simple combination of models H1 1000 and H2 1002, wherein there exists both a fraction of poorly synthesized P as well as the alternative pathway involving TP_{bad} , and has a total of 4 fitting parameters: k_{Hyb} , $[P_{\text{good}}]_0$, k_1 , and k_2 .

[00108] For each of our 210 fluorescence kinetics experiments, we used a custom stochastic fitting function to determine the best-fit values of each rate constant parameter for each model. Here, best-fit is determined as the minimal sum-of-square relative error RE, where $RE = \left(\frac{\text{Data} - \text{Simulation}}{\text{Data}} \right)$. Minimum and maximum fluorescence values corresponding to 0% and 100% yields were determined through separate control experiments. Fig. 10b shows RE values of best-fit parameters for two hybridization reactions. While all three models describe the observed fluorescence data well for some reactions, other reactions show a significant difference among the three models.

[00109] For each hybridization reaction, we have between 60 and 180 RE values, each corresponding to a time point at which fluorescence was measured. The RE values of each hybridization experiment are summarized as a single root mean square relative error (RMSRE) value, defined as

$$[00110] \quad RMSRE = \sqrt{\frac{1}{\alpha} \sum_t RE(t)^2}$$

[00111] where α is the total number of time points t during which fluorescence was measured for the reaction. Fig. 10c shows the distribution of RMSRE values for each hybridization model; model H3 1004 appears to give the best overall fit to the data. More complex reaction models were also evaluated, with additional fitting parameters, but these did not significantly improve RMSRE over model H3 1004 (data not shown).

Consequently, model H3's best-fit k_{Hyb} rate constants were used for all subsequent work.

[00112] Fig. 10d summarizes Model H3's best-fit k_{Hyb} values for paired hybridization experiments at 37 °C and 55 °C. The values of k_{Hyb} ranged roughly 3 orders of magnitude at both temperatures. Even among relatively fast reactions corresponding to target/probe sequences with relatively low secondary structure, there is still significant variation in hybridization kinetics. The large diversity of k_{Hyb} values for different sequences and the imperfect correlation between rate constants for the same sequence at different temperatures emphasizes the difficulty and need for a predictive kinetics model, such as the models described herein.

[00113] Weighted Neighbor Voting (WNV) Model.

[00114] To predict the rate constant of a new hybridization reaction, a WNV model checks the reaction for similarity against labeled instances (hybridization reactions with known rate constants) in an existing database (e.g., database 20), and allow each instance in the database to make a weighted "vote." Instances that are more similar to the new reaction are weighted more heavily.

[00115] To quantitate the similarity or dissimilarity between two hybridization reactions, we abstract each reaction into a number of features. The value of each feature for a particular hybridization reaction is computable based on the sequences of the target and probe, and the reaction temperature and buffer conditions. Each hybridization reaction is thus a point in feature space. With an optimally designed and weighted set of features, the two points close in feature space should exhibit similar k_{Hyb} values. The converse is not necessarily true: two hybridization reactions with coincidentally similar k_{Hyb} values may possess very different feature values.

[00116] As mentioned previously, mapping the hybridization reactions into feature space is important because targets that are similar in sequence space may not have similar hybridization kinetics, and vice versa, due to the sensitivity of secondary structure to small changes in DNA sequence in certain regions, but not in others. For example,

oligonucleotide (2) with

sequence “ACACACACTTAAAATTGTGTGTGTCCC” has higher Hamming distance to oligo (1) with sequence “ACACACACTTTTTTTTGTGTGTGTCCC” than oligo (3) with sequence “ACTCAGACTTTTTTTTGTGTGTGTCCC”, but is expected to exhibit much more similar kinetics in hybridization to each’s respective complement. In this case, one possible feature could be the number of base pairs formed in the hairpin stem of the minimum free energy structure: oligos (1) and (2) would have feature value 8, while oligo (3) would have feature value 6.

[00117] There are many potential approaches to the prediction of an analog desired parameter (k_{Hyb} in this application) based on a set of features, the simplest of which is multilinear regression (MLR). WNV was selected because WNV may significantly outperform MLR when the relationships between the desired parameter and the features are nonlinear. Simultaneously, WNV is a highly scalable framework, in the sense that additional labeled instances can easily be incorporated for improved prediction accuracy without requiring reoptimization of model parameters (feature weights).

[00118] Feature Construction and Normalization.

[00119] Starting by rationally designing 38 potential features, each based on some aspect of DNA biophysics that it is believed may influence kinetics. Fig. 11a shows Gb, one of the 6 features used in a final model; Gb can be thought of as the weighted average of the #G# of formation of the TP complex, based on probabilities of state existence/occupancy at equilibrium. Fig. 11b shows the relationship between the observed k_{Hyb} (according to model H3 1004) and the value of Gb. There is significant correlation between k_{Hyb} and Gb; simultaneously, the relationship is not clearly linear. There may not be good physical interpretations of all effective features—in these cases, the feature in question is likely correlated with a yet-undiscovered complex feature with a firm physical basis.

[00120] The features constructed had different units and different ranges of values. In order to calculate the distance between two hybridization reactions, it may be necessary to normalize the different features into a consistent scale. Because the distributions of most feature values were distinctively non-Gaussian for the 210

reactions, normalization was performed based on the interquartile range: the 75th percentile feature value is mapped to a score of $+\frac{w(i)}{2}$, and the 25th percentile value is mapped to $-\frac{w(i)}{2}$ (Fig. 11c), where $w(i)$ is the weight of feature i . The feature space distance $d_{j,m}$ between an unknown reaction j and a known reaction m is calculated as a Euclidean distance:

$$[00121] \quad d_{i,j} = \sqrt{\sum_i (f_i(j) - f_i(m))^2}$$

[00122] where $f_i(j)$ is the value of renormalized feature i for reaction j (Fig. 4d). Because a feature i with larger weight $w(i)$ allows a larger range of scores, it can contribute more to the distance between two hybridization reactions. Fig. 11e and f confirm that the difference in k_{Hyb} values for a pair of reactions increases with feature space distance d .

[00123] Rate Constant Prediction.

[00124] From a database of hybridization experiments m with known $k_{Hyb}(m)$ and renormalized feature values, the WNV model makes the following prediction for $k_{Hyb}(j)$ of an unknown hybridization reaction j :

$$[00125] \quad \log_{10}(k_{Hyb}(j)) = \frac{1}{Z_j} \sum_m 2^{-d_{j,m}} \cdot \log_{10}(k_{Hyb}(m))$$

[00126] where $\sum_m 2^{-d_{j,m}}$ is the “partition function” of the distances involving reaction j .

[00127] To quantitate the overall performance of a particular WNV model (defined by its set of features and corresponding feature weights $w(i)$), the following “Badness” metric may be constructed:

$$[00128] \quad \text{Badness} = 3 \cdot (1 - \text{F2acc}) + 3 \cdot (1 - \text{F3acc}) + 4 \cdot \text{RMSE}$$

[00129] where F2acc is the fraction all predicted reactions j in which predicted $k_{Hyb}(j)$ and the experimental $k_{Hyb}(j)$ agrees to within a factor of 2, F3acc the fraction that agrees to within a factor of 3, and

$$[00130] \quad RMSE = \sqrt{\frac{1}{N} \sum_j (\log_{10}(k_{Hyb}(j)) - \log_{10}(k_{Hyb}(j)))^2}$$

[00131] is the root mean square error of the logarithm of the hybridization rate constant (where N = 210 is the number of experiments).

[00132] The Badness metric was chosen rather than RMSE only (i.e. a least-squares fit) because it may be more relevant for many applications involving the design of DNA oligonucleotide probes and primers: Rather than marginally improving the predictions of outlier sequences that are off by more than an order of magnitude, the Badness metric as described above emphasizes instead improving the fraction of predictions that are correct to within a factor of 3, or better yet within a factor of 2. Simultaneously, to allow efficient computational optimization of feature weights, the Badness metric to be minimized may not be locally flat, so RMSE is included as a component of Badness. Use of different Badness metrics may result in optimized feature weights that exhibit a different tradeoff between the magnitude and frequency of large prediction errors.

[00133] Feature Selection and Weighting.

[00134] All 38 potential features constructed showed significant correlation with kHyb, but it may be inappropriate to include all of these in the WNV prediction model both because several features may consider redundant information, and because large sets of feature weights are computationally difficult to optimize. It may be useful to first manually prune the list of potential features down to 17 most promising features, based on single-feature WNV performance (using each feature's optimized feature weight). Due to the complexity and nonlinearity of the Badness landscape over the feature weight parameter space, it may not be feasible to determine an analytic solution of optimal weights. Instead, it may be useful to use a stochastic numerical optimization algorithm to find weight values that achieve Badness minima.

[00135] Next, a greedy algorithm may be implemented in which individual features that best improve the Badness at each round are iteratively added to an initially empty feature set. Fig. 12a shows that the Badness decreases as the number of features included increases up to 8; at 9 features, the WNV model shows no additional Badness

improvement. Also plotted in Fig. 12a are the Badness of for MLR model using various numbers of features. Up to 4 features, the WNV and MLR models provide similar prediction accuracies; however, WNV continues improving with additional features whereas MLR performance plateaus.

[00136] The optimized feature weights for the 8-feature WNV model includes two features very small weights ($w < 0.1$); these may be removed, and the resulting WNV model consist of the following 6 features: dGavg, Pap, Gb, T, nGp, and Pm, with weights of 12.30, 11.89, 10.72, 6.88, 6.54, and 0.94, respectively. A brief text description of these each feature follows. dGavg corresponds to the sum of the ΔG° of binding for all subsequences of the target weighted by the probability of all nucleotides of the subsequence being unpaired. Pap corresponds to the sum of the probability-weighted ΔG° of the strongest continuous subsequence that is expected to be unpaired. Gb was described with respect to Fig. 11a and corresponds to the sum of the probability-weighted ΔG° of formation of the target-probe complex. T is the reaction temperature in Celsius. nGp corresponds to the partition function energy of the probe secondary structure, as calculated by Nupack. Pm is similar to Pap, but is calculated for misaligned target-probe complexes.

[00137] Fig. 12b shows the accuracy of the final 6-feature WNV model. Each blue dot plots the predicted $\log_{10}(K_{Hyb})$ value vs. the experimentally observed $\log_{10}(K_{Hyb})$ value for a single hybridization experiment. Each prediction was performed using 209 labeled instances (all reactions except the one to be predicted), using the feature weights trained on all 210 data points. Predictions were accurate to within a factor of 2 for 80% of the reactions, and within a factor of 3 for 94.8% of the reactions. For comparison, Fig. 12c shows histograms of the distribution of prediction errors in $\log_{10}(K_{Hyb})$ for WNV models using 0, 1, 3, and 6 features.

[00138] Leave-one-out Validation of Final WNV Model.

[00139] The fact that the final model's feature weights were fitted to all 210 experiments raises potential concern regarding whether the WNV model's prediction accuracy would generalize to new hybridization reactions, because the latter's

(unknown) rate constant may not be used for training feature weights. It may be beneficial to perform leave-one-out (LOO) validation on the model to study the generalizability of the WNV model.

[00140] Accordingly, in LOO studies, 210 separate feature weight optimizations were performed, each using a different set of 209 hybridization experiments. Thus, each of the 210 models possessed different feature weights, and each model was used to predict the hybridization rate constants of the single hybridization experiment not included for its feature weight optimization (dot 1224 in top panel 1220 of Fig. 12d). The aggregate performance of these 210 LOO models are shown in the bottom panel 1222 of Fig. 12d. F2acc and F3acc are marginally lower than in Fig. 5b at 73.8% and 92.4%, respectively.

[00141] Applicants in order to help the research community predict hybridization rate constants for DNA oligo probes and primers, have constructed a web-based software tool, available at <http://nablab.rice.edu/nabtools/kinetics>. The software typically completes predicting kHyb within 30 seconds, with the bulk of the computing time devoted to computation of the Pap and Pm feature values. It is currently seeded with the 210 hybridization experiment results performed in this paper, but will be updated with additional hybridization experiment results in the future, which should further improve prediction accuracy.

[00142] Enrichment from Human Genomic DNA.

[00143] The human genome is over 3 billion nucleotides long, but the coding regions that form the exome collectively only span 30 million nucleotides, or 1% of the genome. Within the 20,000 genes of the exome, typically there are only between 10-400 that are relevant to any particular disease. Consequently, solid-phase enrichment of relevant gene regions using highly multiplexed hybridization of synthetic DNA oligonucleotide probes may be a preferred approach for targeted sequencing.

[00144] Current commercial multiplex hybrid-capture panels generally use a very large number of synthetic probe oligonucleotides to fully tile or overlap-tile the genomic regions of interest; for example, the whole exome requires more than 200,000

distinct oligonucleotide probe species. Due to the large number of oligo species involved, the concentration of each species is thus necessarily quite low (tens of picomolar), resulting in hybrid-capture protocols that typically span at least 4 hours, and more frequently more than 16 hours. Because of the varying hybridization kinetics of different probes (Fig. 10d), it is likely that many probes do not contribute significantly to hybridization yield, and in fact slow down the hybrid-capture process by forcing lower concentrations of the fast-hybridizing probes.

[00145] To experimentally test this possibility, we first applied our hybridization rate constant prediction algorithm to all possible 36 nt probes to exon regions of 21 genes. Because the exon regions are typically 3000 nt long, this corresponds to roughly 3000 possible probes. Predicted rate constants typically range about 2 orders of magnitude, with the fast ($\geq 95^{\text{th}}$ percentile) probes being typically a factor of 3 faster than median probes ($\sim 50^{\text{th}}$ percentile). NGS hybrid-capture enrichment typically uses probes longer than 36 nt (e.g. Agilent SureSelect uses 120 nt probes), but there is likely a similar if not greater range of hybridization kinetics rate constants for longer probes due to the greater possibility of secondary structure and nonspecific interactions.

[00146] Subsequently, a total of 65 fast probes and 65 median probes may be picked across the exon regions of 21 different cancer-related genes. The expectation is that after a 24 hour hybridization protocol, the fast and median probes would produce similar reads, but with a short 20 minute hybridization protocol, the fast probes would exhibit significantly greater reads than median probes (Fig. 13a). A library preparation protocol is summarized in Fig. 13b; all 130 probes are hybridized to the adaptor-ligated DNA simultaneously. However, the number of reads aligned to a particular probe is not directly proportional to its hybridization yield, due to well-documented sequencing bias. For example, some adaptor-ligated amplicons exhibit significant secondary structure and is less efficiently PCR amplified during normalization, or less efficiently sequenced due to lower flow cell binding efficiency. For this reason, 15 fast and 15 median probes targeting 4 genes resulted in less than 100x sequencing depth, and were excluded from subsequent analysis. It is not believed this to affect the conclusions from the genomic DNA enrichment study performed.

[00147] Comparison of reads for the 20 minute hybridization library and for the 24 hour hybridization library indicates that the probes predicted to be fast on average exhibited both a 2-fold increase in reads in the 20 minute library, and a 2-fold increase in the ratio of reads at 20 min vs. 24 hours. This is slightly worse than the algorithm's predicted 3-fold difference between median and fast probes, but understandable given that the rate constant prediction algorithm was trained on single-plex hybridization rather than on multiplex hybridization. Subsequent calibration experiments indicate that the correlation constant between single-plex and multiplex k_{Hyb} values are roughly $r^2 = 0.6$.

[00148] Results thus suggest that sparse hybrid-capture enrichment panels would produce faster kinetics at a significantly lower cost. Rather than fully tiling or overlap-tiling the genetic regions of interest, it would be better to use a higher concentration of a few probes with fastest hybridization kinetics. Multiple probes appear to only be needed insofar as biological genomic DNA may be fragmented, and a different probe is needed to capture each fragment. With the notable exception of cell-free DNA, most genomic DNA from clinical samples are longer than 500 nucleotides.

[00149] The concentrations of the probes used for this application was 50 pM per probe, and was intentionally selected so as to be similar to the concentrations of probes used by commercial enrichment kit providers. At 50 pM concentrations, up to 200,000 probes can be used and the total oligo concentration would still be at a reasonable 10 μ M. At the significantly (e.g. 10x) higher individual probe concentrations that become feasible with a sparse coverage of target genetic regions, even the 20 minutes allotted here for hybridization could be further reduced, greatly speeding up the NGS library preparation workflow from current practice of 4-24 hours.

[00150] Summary Discussion and Technical Effects

[00151] In the instant application, we combined the rational design of features and the WNV framework with computational optimization of feature selection and feature weights, resulting in a final model that is capable of accurately predicting hybridization kinetics rate constants based on sequence and temperature information. The final WNV

rate constant prediction model is highly scalable and easily incorporates new experimental data to provide improved predictions, without requiring model retraining. With every additional hybridization experiment and its accompanying fitted k_{Hyb} value, the 6-dimensional feature space becomes denser, ensuring that on average a new hybridization experiment will be closer to an existing labeled instance. Thus, prediction accuracy will further increase and as additional hybridization kinetics data is further collected.

[00152] To seed the model with a reliable initial database of labeled instances that is representative of the diversity of genomic DNA sequences, applicants experimentally characterized the kinetics of 210 hybridization experiments across 100 biological target sequences using fluorescence. The X-probe architecture allowed more economically study kinetics for a reasonably large number of target sequences, but extra nucleotides of the universal arms may cause hybridization kinetics to differ slightly from that of a standard single-stranded probe. For example, there may be a systematic bias towards lower rate constants because of the reduced diffusion constants. Nonetheless, because all targets/probes use the same universal arm sequences, it is likely that the relative ordering of rate constants is preserved.

[00153] Research was started with 38 rationally designed features that were eventually pruned down to 6 in the final model. The high LOO validation accuracy of the WNV model indicates that these features capture a significant, if not majority, portion of the complexity of the hybridization process. Simultaneously, there remain pairs of experiments in our database with similar feature values (feature space distance $d \leq 3$) but with 3-fold differences in k_{Hyb} .

[00154] The hybridization reactions experimentally characterized in the work were all performed in 5x PBS buffer, and all target and probe sequences were 36 nt long. These experiment constraints were designed to reduce the diversity of hybridization reactions, in order to ease the training of the WNV model. Additionally, with genomic DNA targets, the long-range secondary structure and the fragmentation pattern of genomic DNA targets should also be considered. An expanded model to accommodate

varying length targets and probes (including targets overhangs) and other buffer conditions will require the construction of new features.

[00155] Multiplex hybrid-capture panels for enriching target regions from genomic DNA is commonly used in targeted sequencing for scientific and clinical studies. In the absence of reliable kinetics prediction software, researchers and companies have taken a brute-force probe design approach, using fully tiled or overlapping-tiled probes to cover genetic loci of interest. While this approach ensures the presence of at least some fast-binding probes, it is both expensive (in terms of synthesis and QC of thousands of probes) and results in slower workflows. Accurately predicting multiplexed hybridization kinetics will enable precision design of sparse, high-performance probe panels for target enrichment.

[00156] It may be beneficial to list some equations as they may improve understanding of the claimed subject as follows:

[00157] $F = a \cdot \Delta G_{pf}^{\circ} + b$ may apply to bioinformatic features that comprise one or more features based on a first calculated ensemble standard free energy of the first nucleic acid molecule, a second calculated ensemble standard free energy of the second nucleic acid molecule, or a third calculated ensemble standard free energy of a duplex formed through hybridization of the first and second nucleic acid molecules at the reaction temperature and the reaction buffer conditions.

[00158] $F = a \cdot \Delta G_{mfe}^{\circ} + b$ may apply to bioinformatic features that comprise one or more features based on a first calculated standard free energy of a minimum free energy structure (mfe) of the first nucleic acid molecule, a second calculated standard free energy of the mfe of the second nucleic acid molecule, or a third calculated standard free energy of the mfe of a duplex formed through hybridization of the first and second nucleic acid molecules at the reaction temperature and the reaction buffer conditions.

[00159] $F = a \cdot (\Delta G_{pf}^{\circ} - \Delta G_{mfe}^{\circ}) + b$ may apply to bioinformatic features that comprise one or more features based on a difference between a calculated ensemble standard free energy and a calculated standard free energy of the mfe of a duplex formed

through hybridization of the first and second nucleic acid molecules at the reaction temperature and the reaction buffer conditions.

[00160] $F = a \cdot \min\{\Delta G^\circ(i:i+N-1)\} + b$ may apply to bioinformatic features comprise one or more features based on a calculated standard free energy of strongest-binding N nucleotide subsequence of the first nucleic acid molecule at the reaction temperature and the reaction buffer conditions.

[00161] $F = a \cdot \max\{P_{open}(i:i+N-1)\} + b$ may apply to bioinformatic features that comprise one or more features based on a calculated maximum probability of a N nucleotide subsequence of the first nucleic acid molecule being all in unpaired states at equilibrium.

[00162] $F = a \cdot \max\{P_{open}(i:i+N-1) \cdot \Delta G^\circ(i:i+N-1)\} + b$ may apply to bioinformatic features that comprise one or more features based on a calculated maximum probability-weighted standard free energy of binding of a N nucleotide subsequence of the first nucleic acid molecule being all in unpaired states at equilibrium.

[00163] In view of the many possible embodiments to which the principles of our invention can be applied, we claim as our invention all such embodiments as can come within the scope and spirit of the following claims and equivalents thereto.

CLAIMS:

1. A method for predicting a rate constant of a desired hybridization reaction given sequences of a first nucleic acid molecule and a second nucleic acid molecule, a reaction temperature, and a reaction buffer, the method comprising:

calculating a plurality of feature values for a number of bioinformatic features of the desired hybridization reaction;

calculating distances between the plurality of feature values and corresponding database rate constant values stored in a database, the database comprising a plurality of hybridization reactions having known rate constants;

calculating a weighted average of a logarithm of the database rate constant values, with larger weights assigned to value instances having values lower in distance to the plurality of feature values of the desired hybridization reaction; and

providing the weighted average as a predicted logarithm of the rate constant of the desired hybridization reaction.

2. The method of claim 1, wherein the bioinformatic features comprise one or more features based on a first calculated ensemble standard free energy of the first nucleic acid molecule, a second calculated ensemble standard free energy of the second nucleic acid molecule, or a third calculated ensemble standard free energy of a duplex formed through hybridization of the first and second nucleic acid molecules at the reaction temperature and the reaction buffer conditions.

3. The method of claim 1, wherein the bioinformatic features comprise one or more features based on a first calculated standard free energy of a minimum free energy structure (mfe) of the first nucleic acid molecule, a second calculated standard free energy of the mfe of the second nucleic acid molecule, or a third calculated standard free energy of the mfe of a duplex formed through hybridization of the first and second nucleic acid molecules at the reaction temperature and the reaction buffer conditions.

4. The method of claim 1, wherein the bioinformatic features comprise one or more features based on a difference between a calculated ensemble standard free

energy and a calculated standard free energy of the mfe of a duplex formed through hybridization of the first and second nucleic acid molecules at the reaction temperature and the reaction buffer conditions.

5. The method of claim 1, wherein the bioinformatic features comprise one or more features based on a calculated standard free energy of strongest-binding N nucleotide subsequence of the first nucleic acid molecule at the reaction temperature and the reaction buffer conditions.

6. The method of claim 1, wherein the bioinformatic features comprise one or more features based on a calculated maximum probability of a N nucleotide subsequence of the first nucleic acid molecule being all in unpaired states at equilibrium.

7. The method of claim 1, wherein the bioinformatic features comprise one or more features based on a calculated maximum probability-weighted standard free energy of binding of a N nucleotide subsequence of the first nucleic acid molecule being all in unpaired states at equilibrium.

8. The method of claim 5, 6, or 7, wherein N has a value of 3, 4, 5, 6, 7, or 8.

9. The method of any of the preceding claims, wherein feature weight coefficients a and b of a linear transformation are calculated on Xth and Yth percentile values of untransformed feature values in the database.

10. The method of claim 9, wherein the Xth percentile value is between 5 and 40, and the Yth percentile value is between 60 and 95.

11. The method of any of claims 1-10, wherein the distance to the plurality of feature values of the desired hybridization reaction comprises an Euclidean distance.

12. The method of any of claims 1-10, wherein the distance to the plurality of feature values of the desired hybridization reaction comprises a Hamming distance.

13. The method of any of claims 1-12, wherein weights of value instances are calculated as $\text{InstanceWeight} = c \cdot \exp(-\text{Distance} / D)$, where c and D comprise constants identical in value for all value instances.

14. The method of claims 1-13, wherein the weighted average is calculated as $\text{WeightedAverage} = \sum \text{InstanceWeight} \cdot \log(\text{RateConstant})$

15. A computing system, comprising a processor, the processor configured to predict a rate constant for a hybridization reaction between a first sequence and a second sequence by:

calculating a plurality of feature values for a number of bioinformatic features of the hybridization reaction;

calculating distances between the plurality of feature values and corresponding database rate constant values stored in a database, the database comprising a plurality of hybridization reactions having known rate constants;

calculating a weighted average of a logarithm of the database rate constant values, with larger weights assigned to value instances having values lower in distance to the plurality of feature values of the hybridization reaction; and

providing the weighted average as a predicted logarithm of the rate constant of the hybridization reaction.

16. The system of claim 15, wherein the processor is configured to generate a set of candidate probe sequences that are complementary to a target nucleic acid sequence, wherein the hybridization reaction comprises the target nucleic acid sequence, and wherein the target nucleic acid sequence comprises the first sequence.

17. The system of claim 16, wherein the processor is configured to predict a predicted hybridization rate constant for each of the candidate probe sequences and

to select one or more of the candidate probe sequences if the predicted hybridization rate constant is in a top Z%.

18. The system of claim 17, wherein the top Z% comprises 1, 2, 5, 10, 20, or a combination thereof.

19. The system of any of claims 15-18, wherein the first sequence comprises a target sequence and the second sequence comprises a probe sequence.

20. The system of any of claim 15-19, wherein the bioinformatics features of the hybridization reaction are based on one or more of:

- a calculated ensemble standard free energy of the first sequence;

- a calculated ensemble standard free energy of the second sequence;

- a calculated ensemble standard free energy of a duplex formed through hybridization of the first sequence and second sequence at predetermined temperature and buffer conditions;

- a calculated standard free energy of a minimum free energy structure (mfe) of the first sequence;

- a calculated standard free energy of a mfe of the second sequence;

- a calculated standard free energy of a mfe of a duplex formed through hybridization of the first sequence and second sequence at predetermined temperature and buffer conditions;

- a difference between a calculated ensemble standard free energy and a calculated standard free energy of a mfe of a duplex formed through hybridization of the first sequence and second sequence at predetermined temperature and buffer conditions;

- a calculated standard free energy of strongest-binding N nucleotide subsequence of the first sequence at predetermined temperature and buffer conditions;

- a calculated maximum probability of a N nucleotide subsequence of the first sequence being all in unpaired states at equilibrium;

a calculated maximum probability-weighted standard free energy of binding of a N nucleotide subsequence of the first sequence being in all in unpaired states at equilibrium, or a combination thereof.

20. A tangible, non-transitory computer readable medium comprising executable instructions, the instructions configured to:

calculate a plurality of feature values for a number of bioinformatic features of a desired hybridization reaction between a first sequence and a second sequence;

calculate distances between the plurality of feature values and corresponding database rate constant values stored in a database, the database comprising a plurality of hybridization reactions having known rate constants;

calculate a weighted average of a logarithm of the database rate constant values, with larger weights assigned to value instances having values lower in distance to the plurality of feature values of the desired hybridization reaction; and

provide the weighted average as a predicted logarithm of the rate constant of the desired hybridization reaction.

21. The tangible, non-transitory computer readable medium of claim 21, wherein weights of value instances are calculated as $\text{InstanceWeight} = c \cdot \exp(-\text{Distance} / D)$, where c and D comprise constants identical in value for all value instances.

22. The tangible, non-transitory computer readable medium of any of claims 20-21, wherein the bioinformatics features of the hybridization reaction are based on one or more of:

a calculated ensemble standard free energy of the first sequence;

a calculated ensemble standard free energy of the second sequence;

a calculated ensemble standard free energy of a duplex formed through hybridization of the first sequence and second sequence at predetermined temperature and buffer conditions;

a calculated standard free energy of a minimum free energy structure (mfe) of the first sequence;

a calculated standard free energy of a mfe of the second sequence;

a calculated standard free energy of a mfe of a duplex formed through hybridization of the first sequence and second sequence at predetermined temperature and buffer conditions;

a difference between a calculated ensemble standard free energy and a calculated standard free energy of a mfe of a duplex formed through hybridization of the first sequence and second sequence at predetermined temperature and buffer conditions;

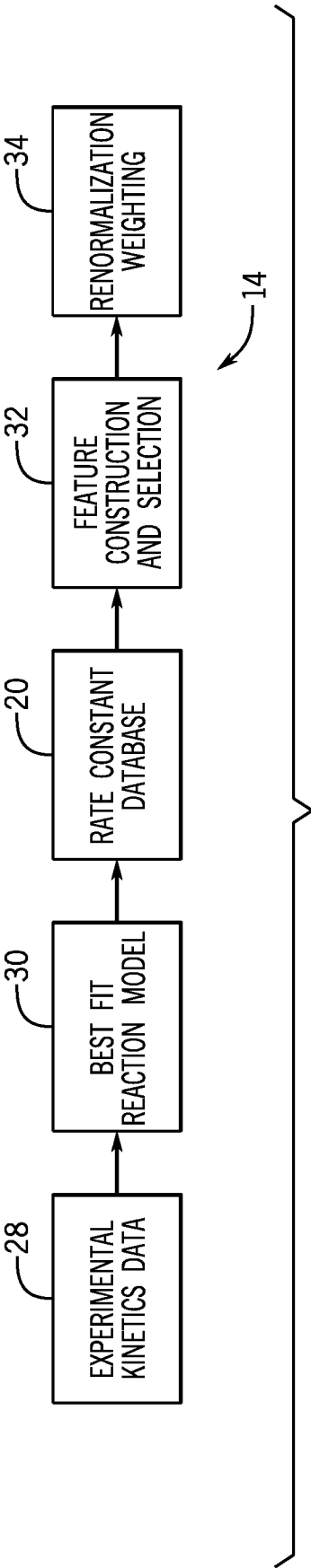
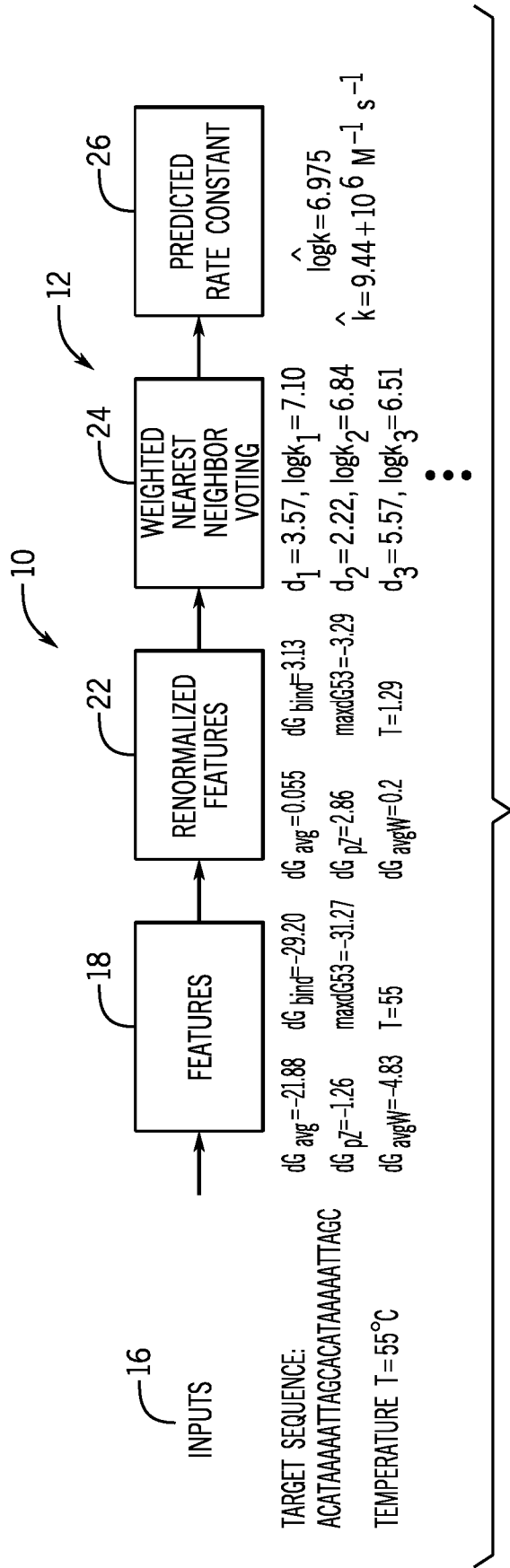
a calculated standard free energy of strongest-binding N nucleotide subsequence of the first sequence at predetermined temperature and buffer conditions;

a calculated maximum probability of a N nucleotide subsequence of the first sequence being all in unpaired states at equilibrium;

a calculated maximum probability-weighted standard free energy of binding of a N nucleotide subsequence of the first sequence being in all in unpaired states at equilibrium, or a combination thereof.

23. The tangible, non-transitory computer readable medium of any of claims 20-22, wherein the instructions are configured to generate a set of candidate probe sequences that are complementary to a target nucleic acid sequence, wherein the hybridization reaction comprises the target nucleic acid sequence, and wherein the target nucleic acid sequence comprises the first sequence.

24. The tangible, non-transitory computer readable medium of any of claims 20-23, wherein the instructions configured to predict a predicted hybridization rate constant for each of the candidate probe sequences and to select one or more of the candidate probe sequences if the predicted hybridization rate constant is in a top Z%.



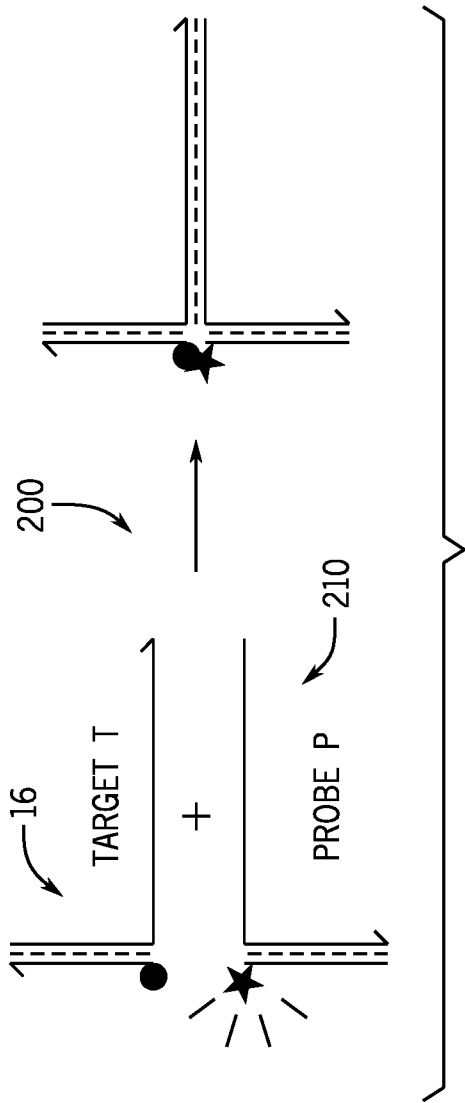


FIG. 2A

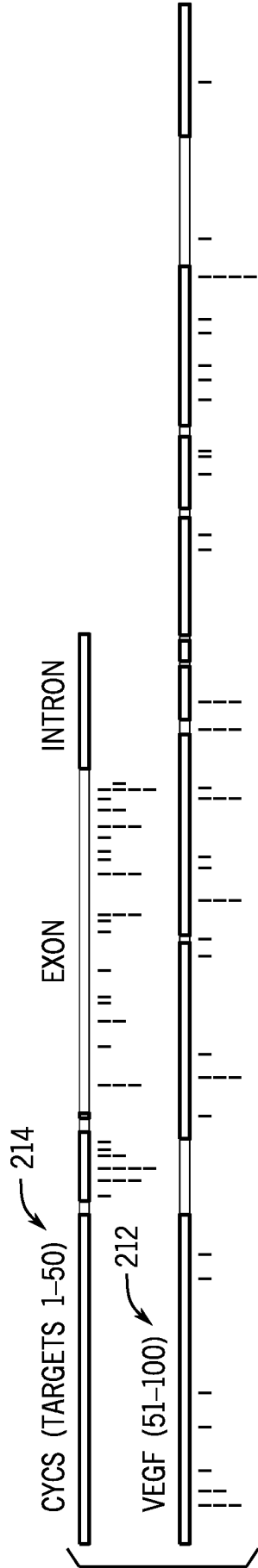
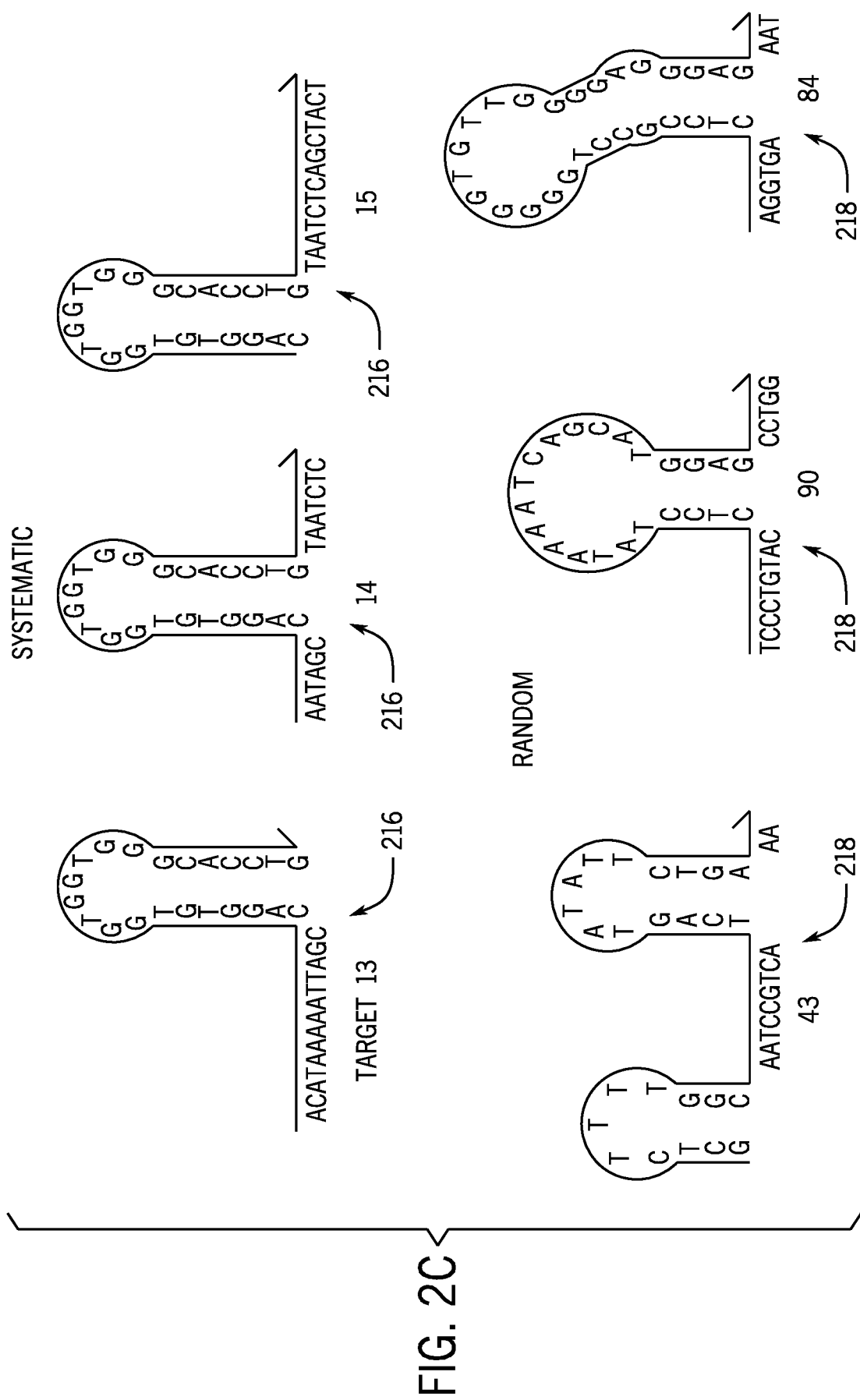


FIG. 2B



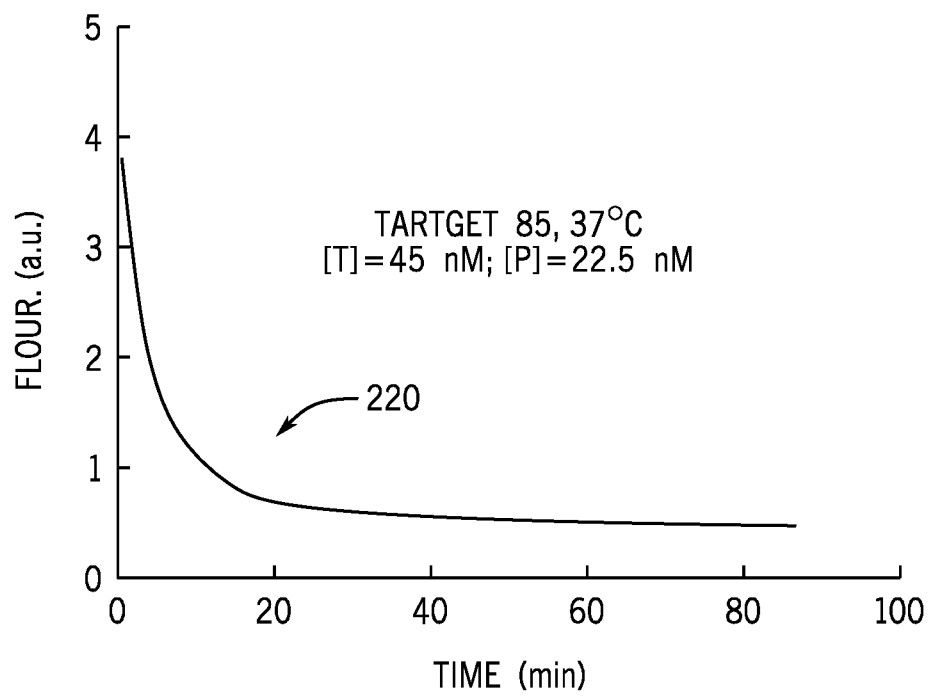


FIG. 2D

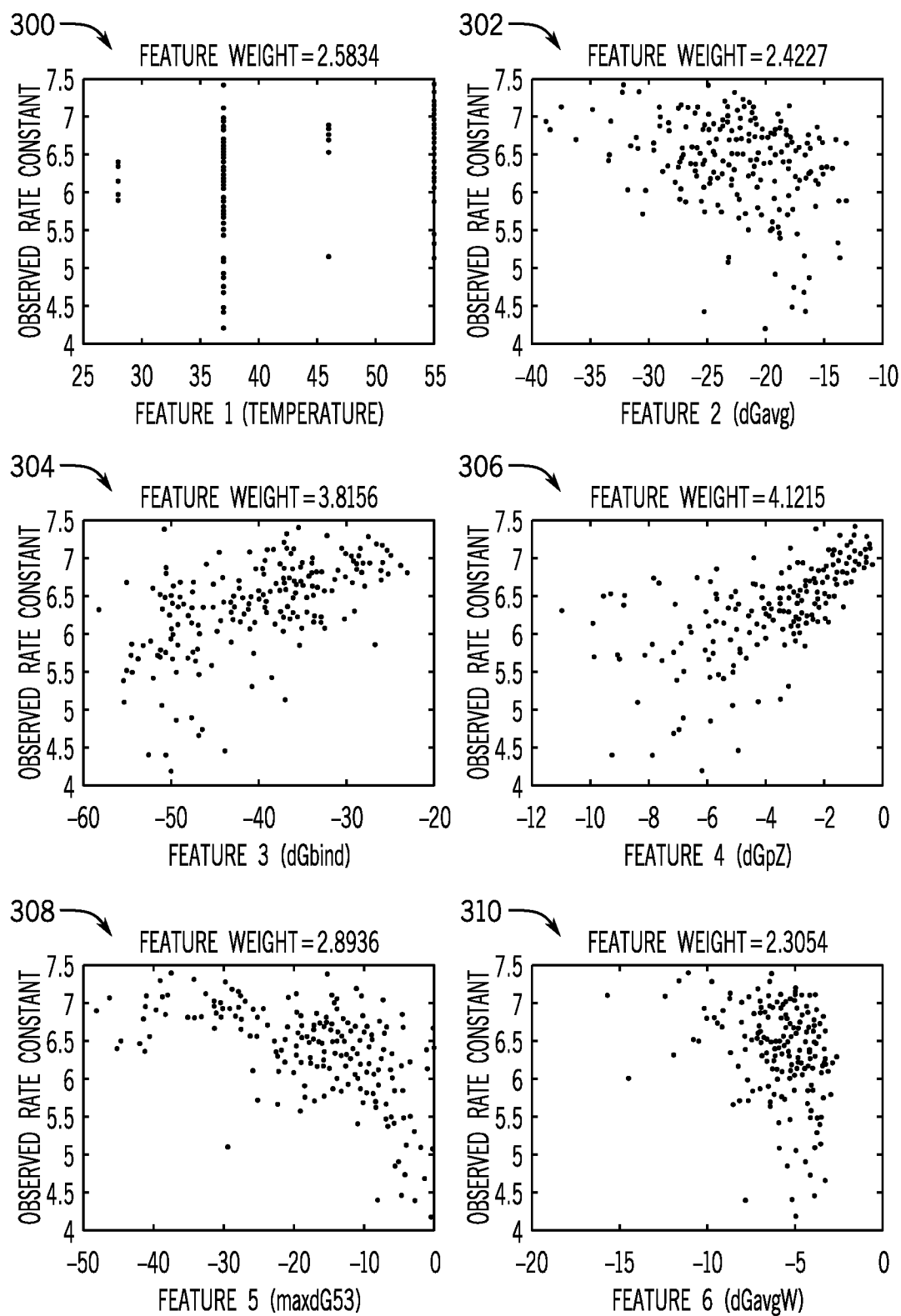


FIG. 3

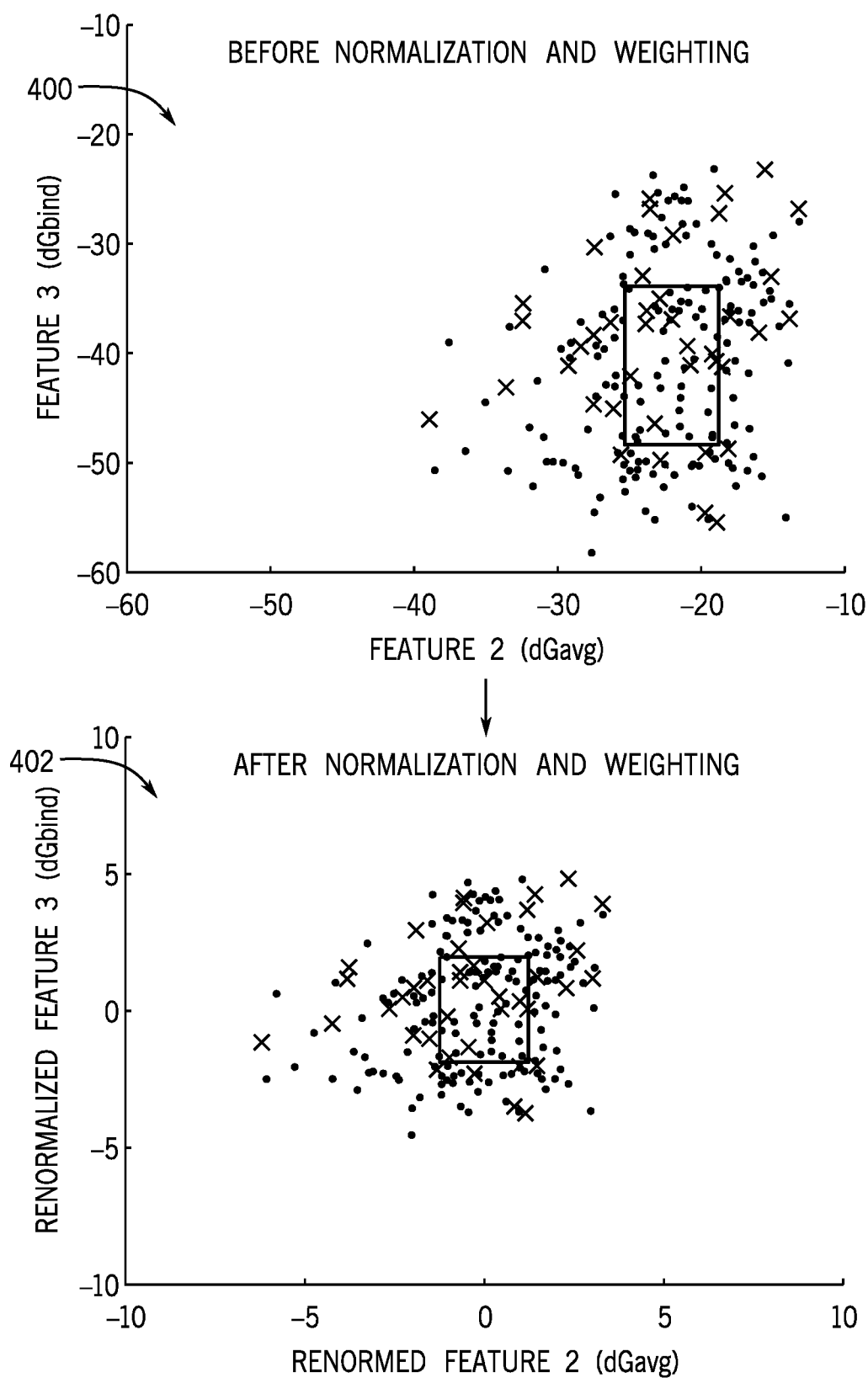


FIG. 4

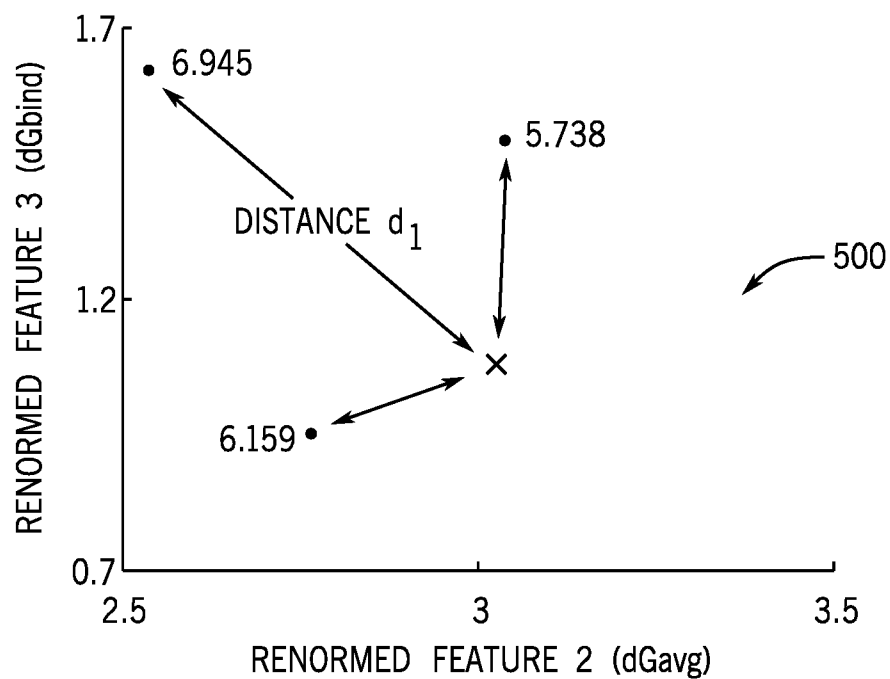


FIG. 5

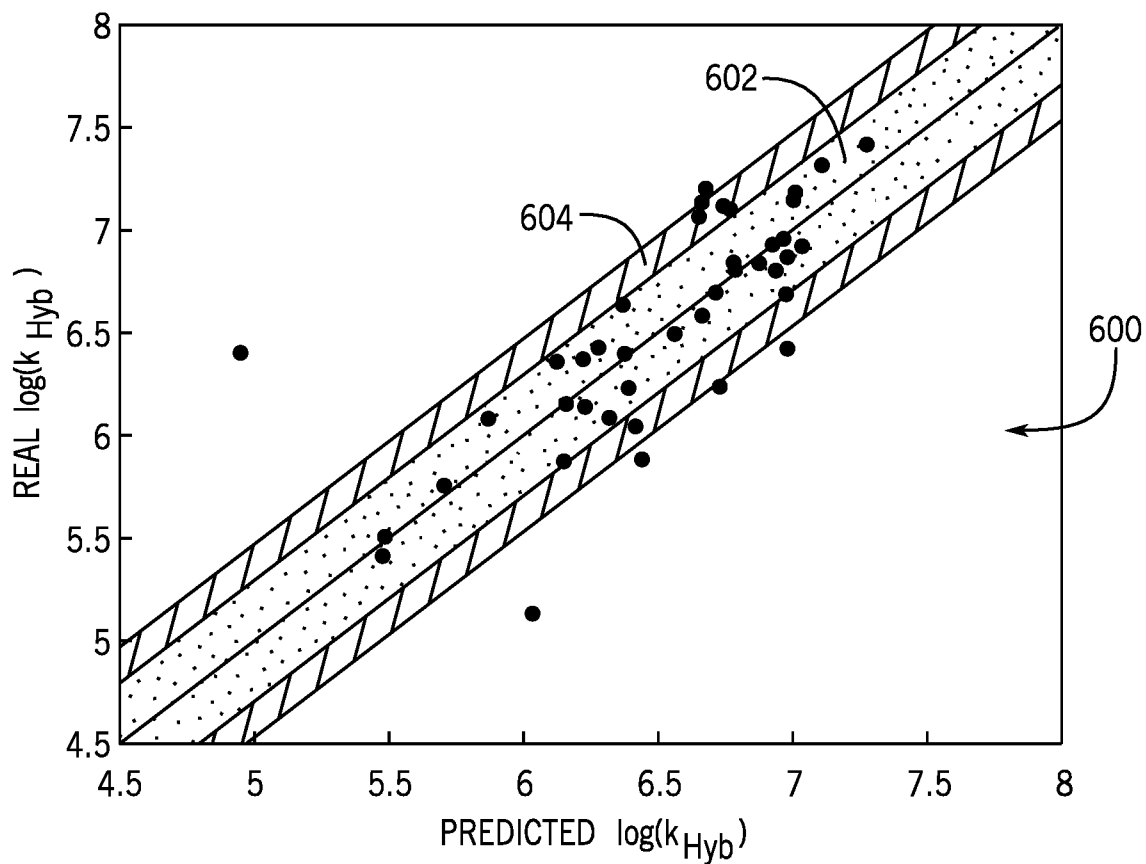


FIG. 6

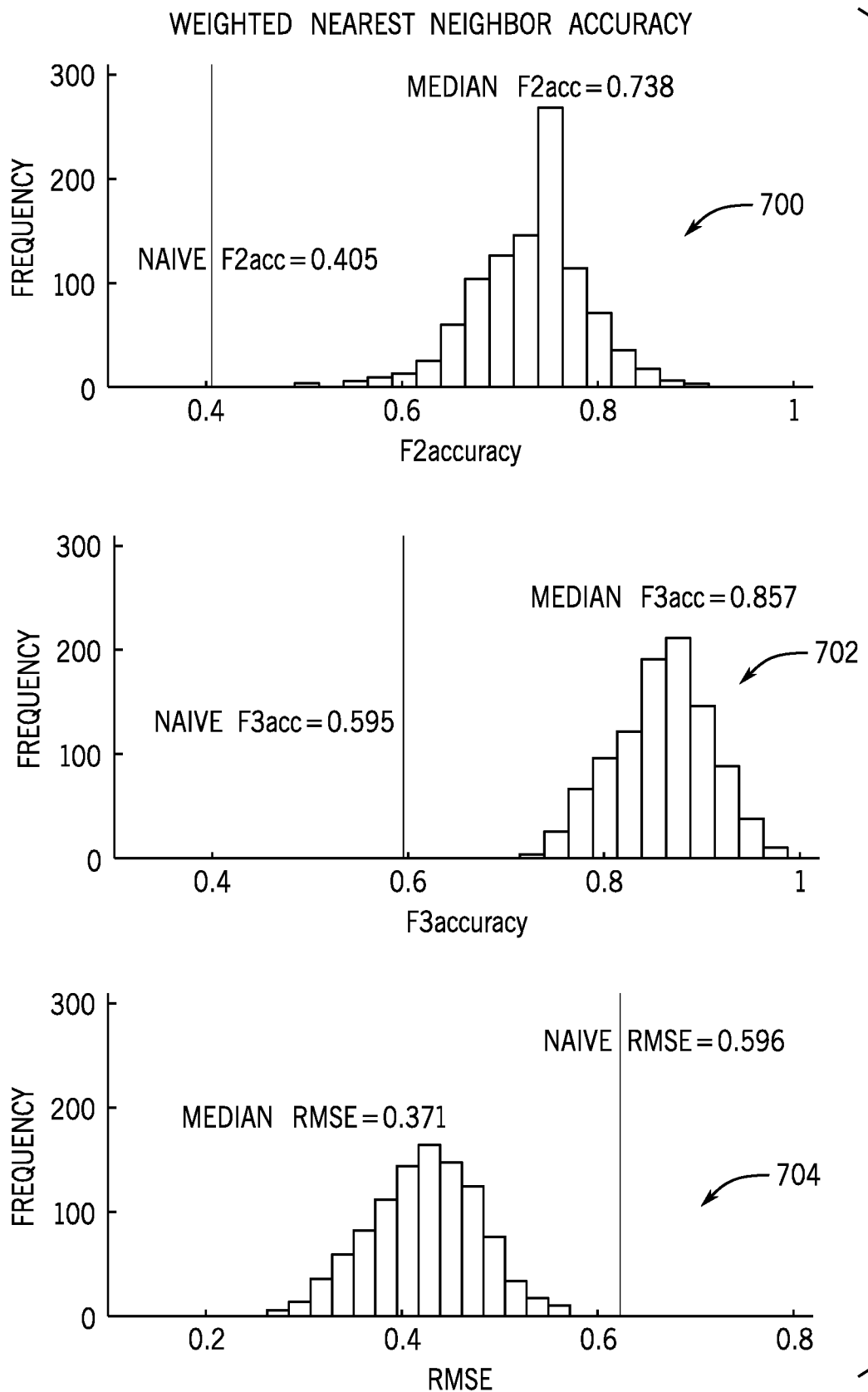


FIG. 7

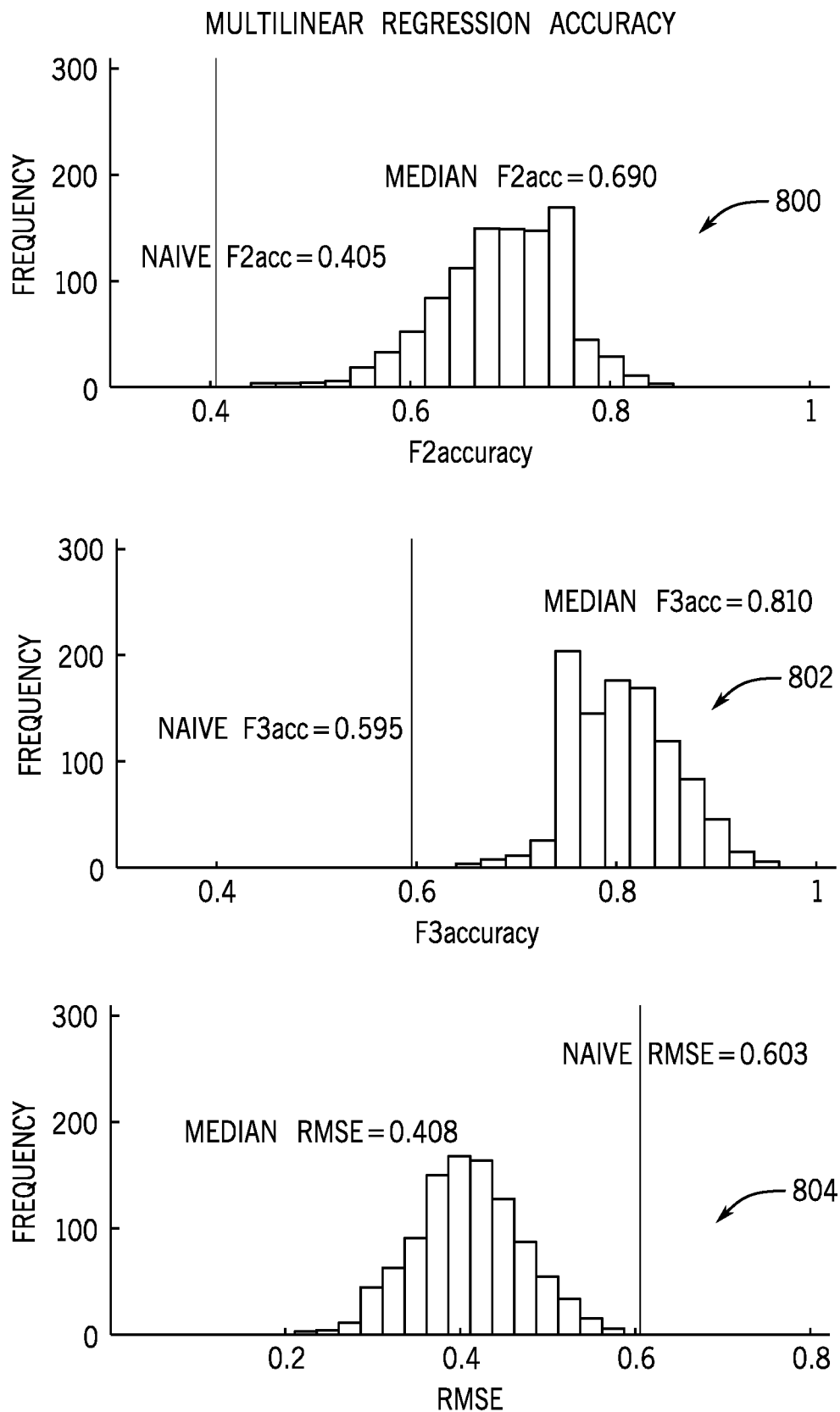


FIG. 8

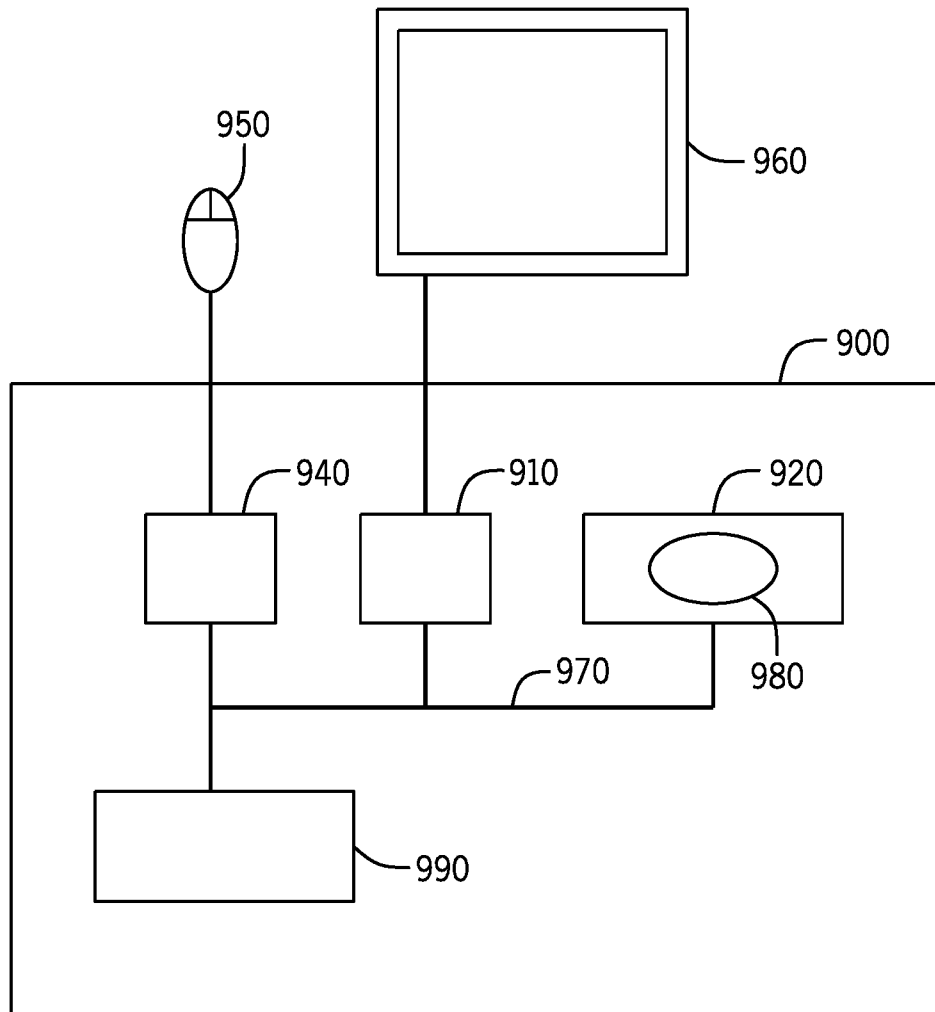
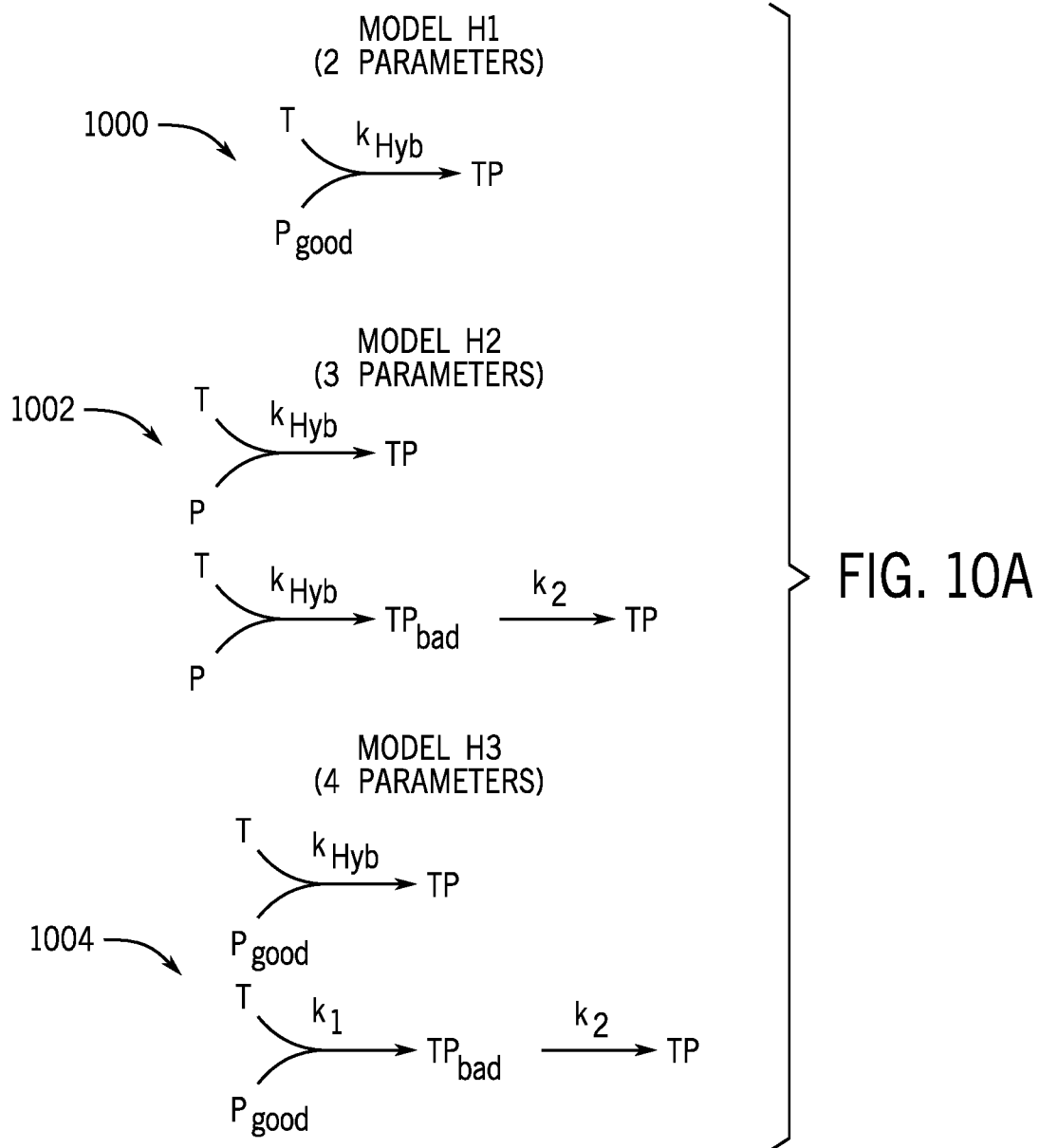
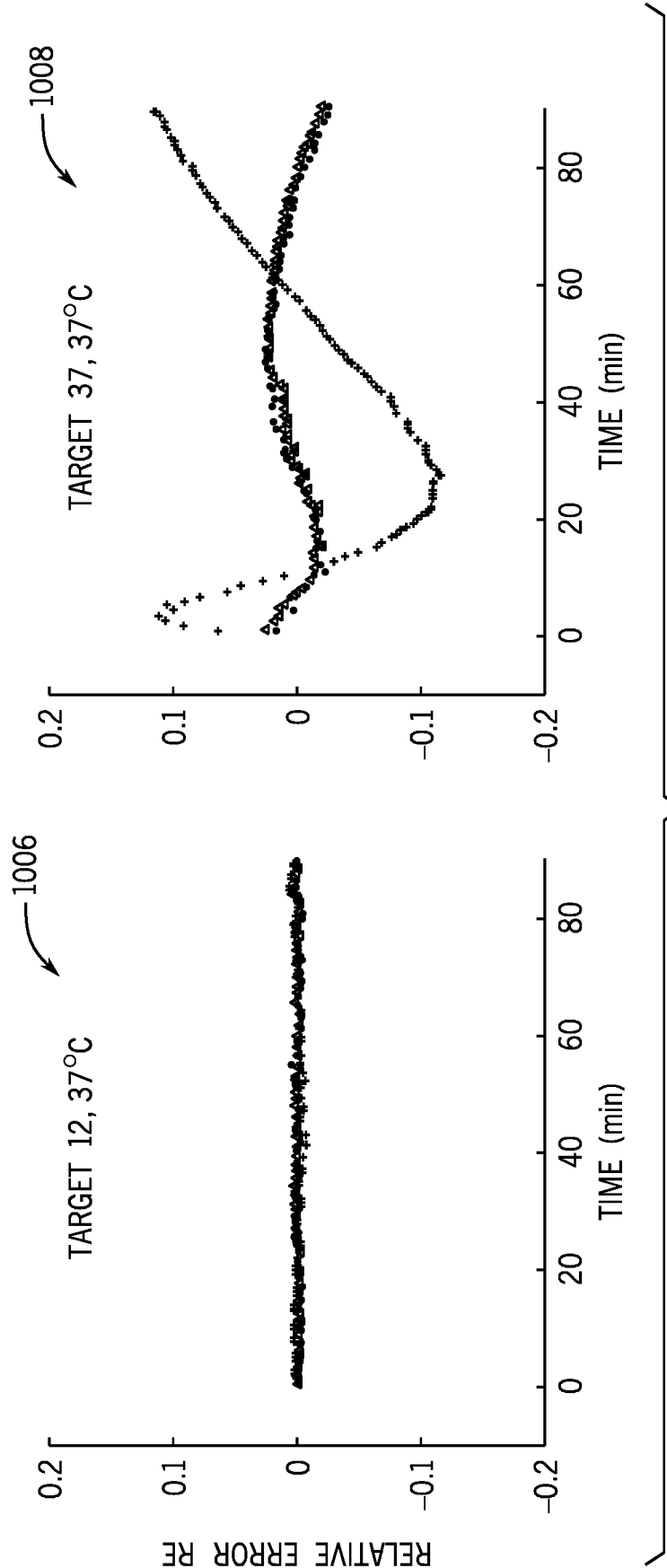
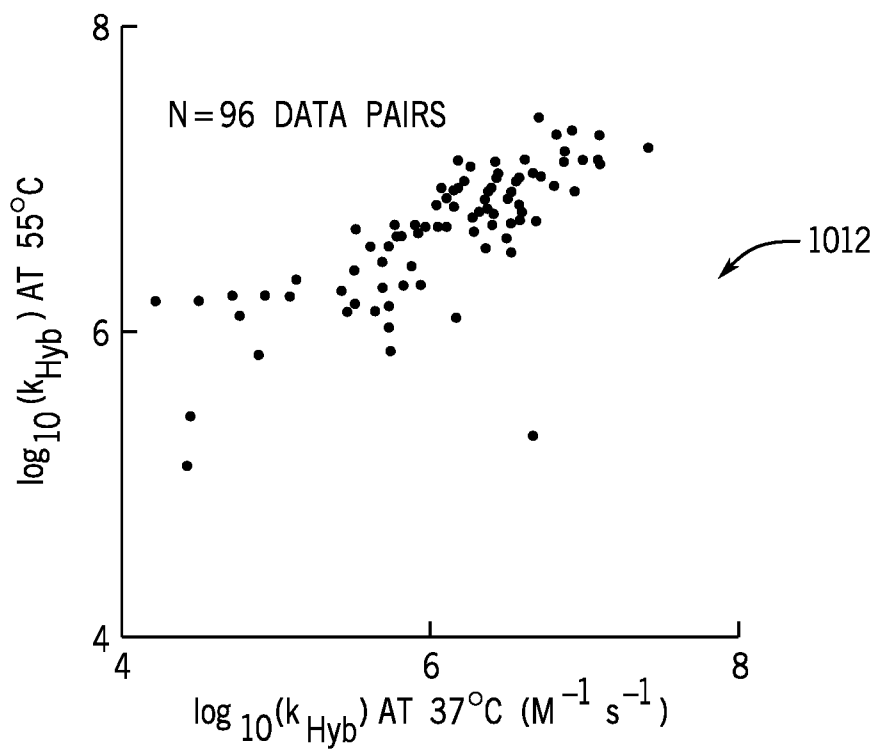
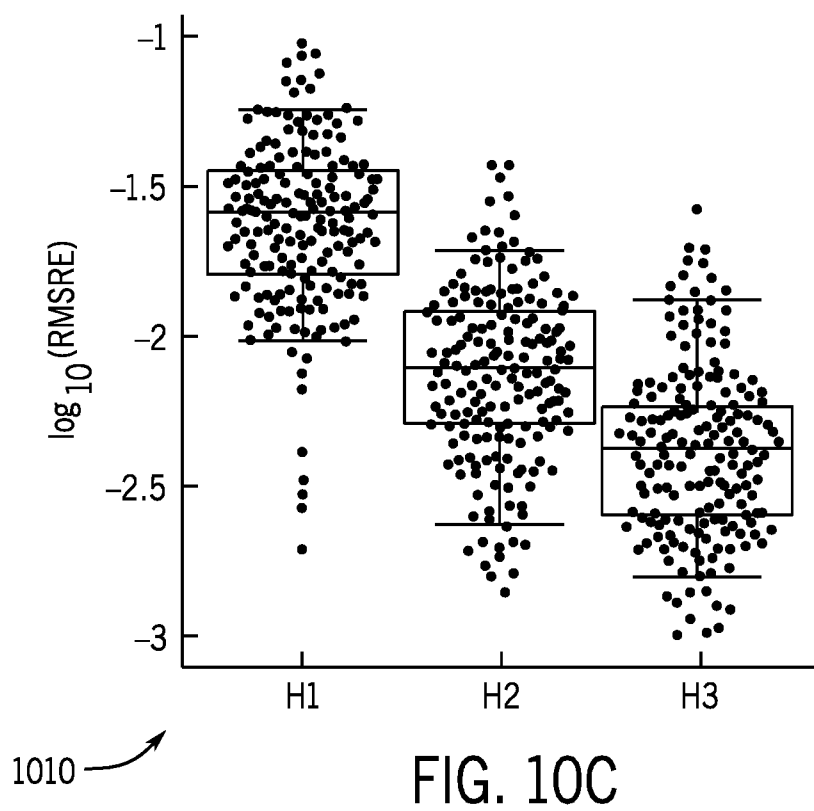


FIG. 9







1100

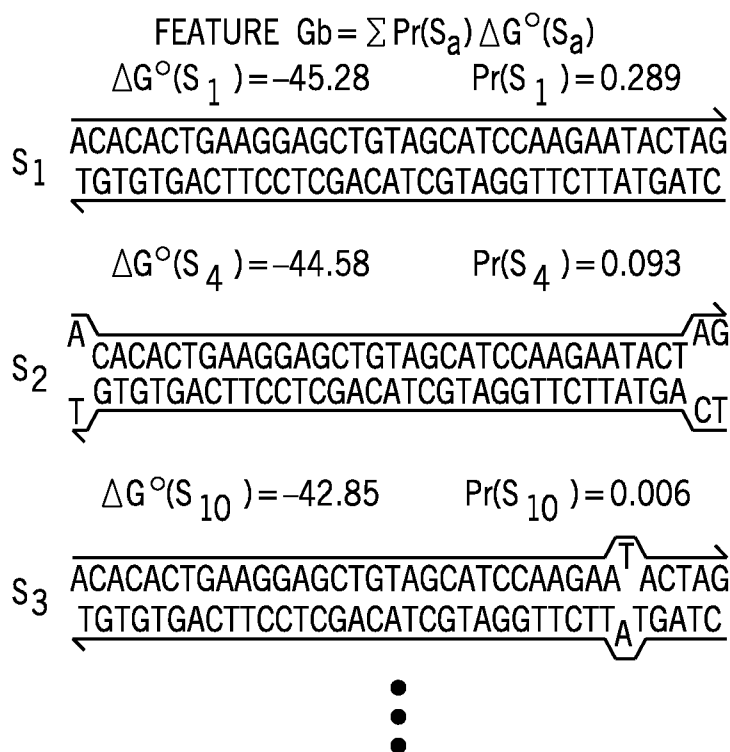


FIG. 11A

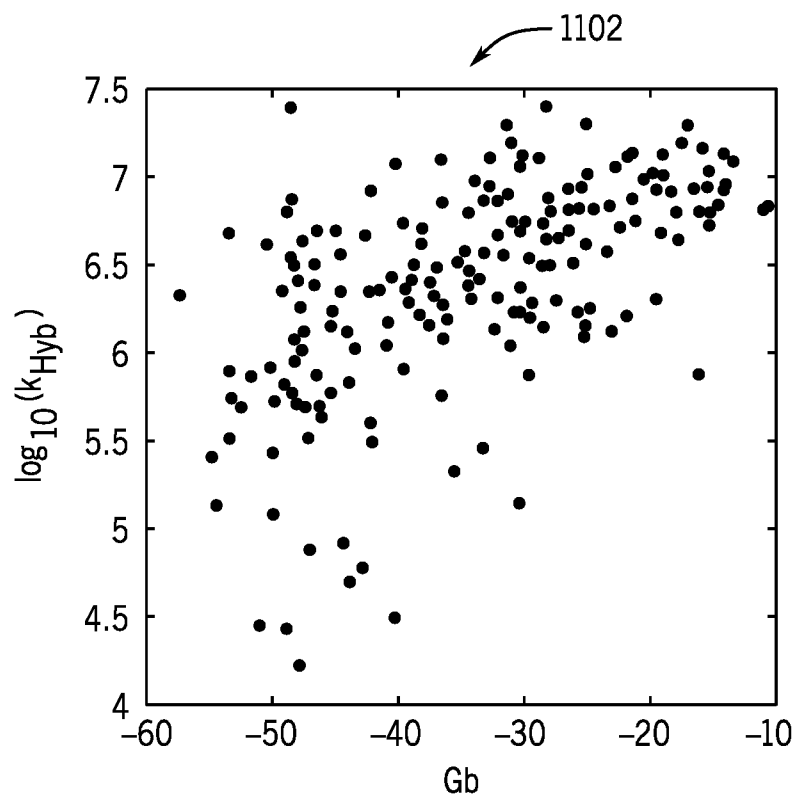


FIG. 11B

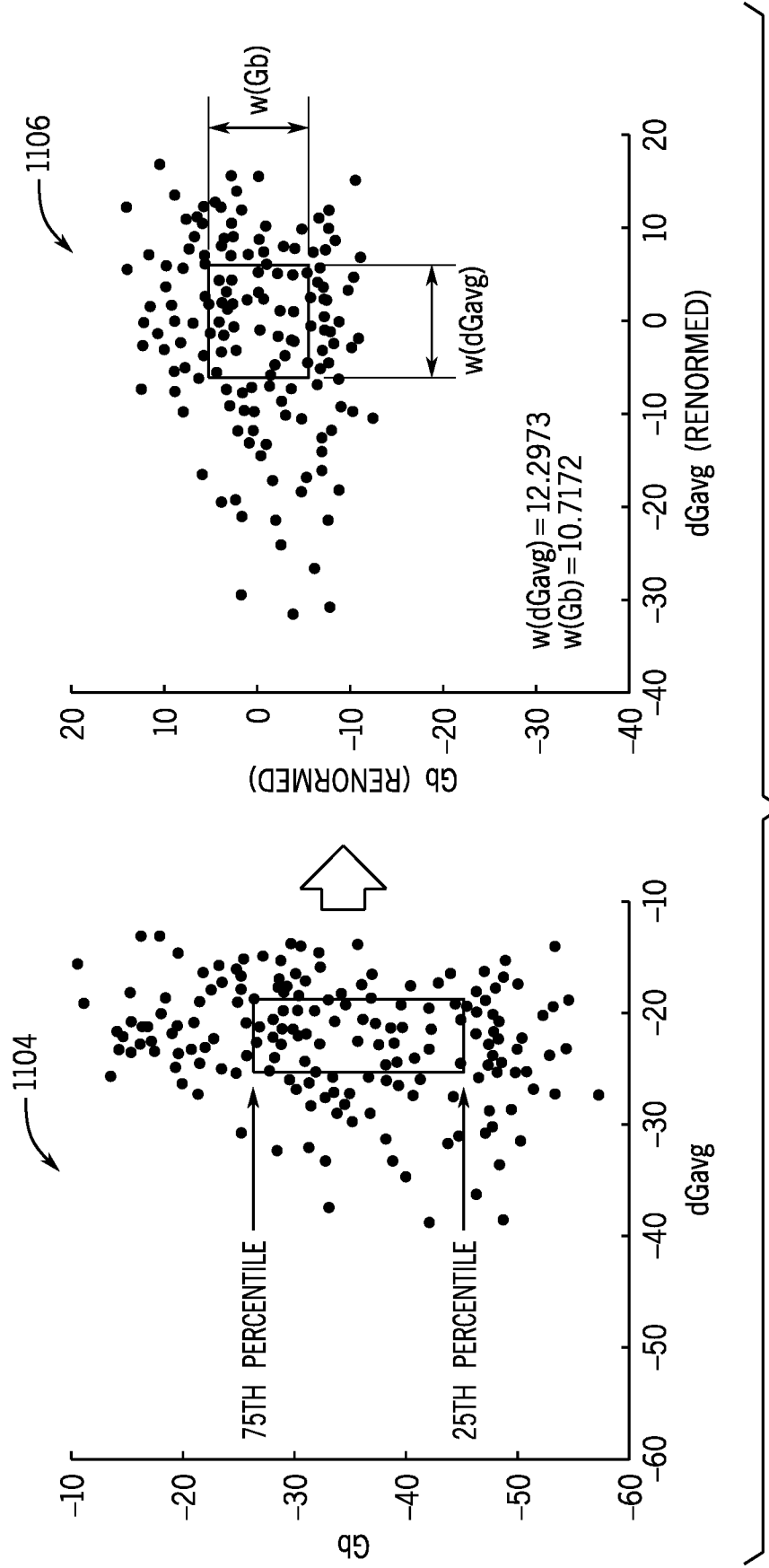


FIG. 11C

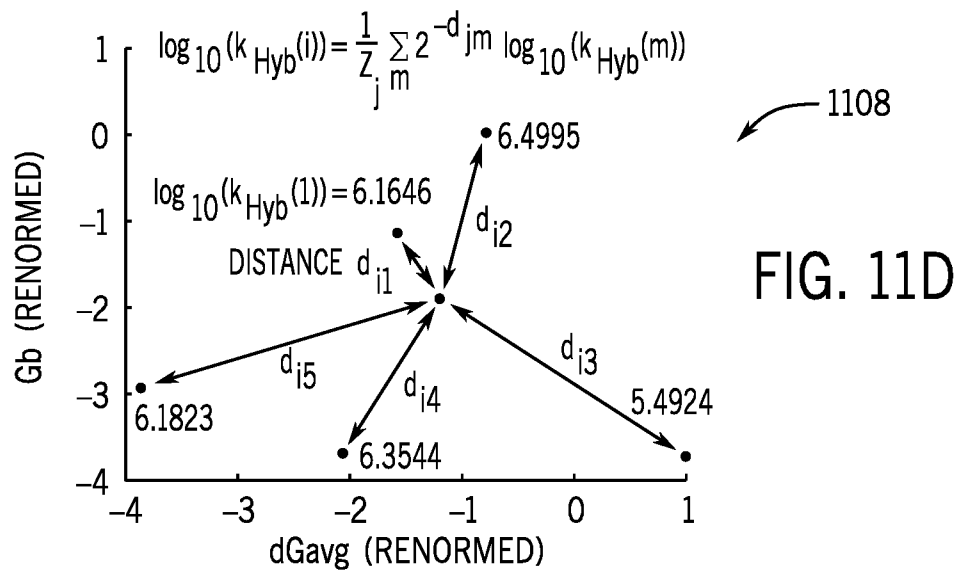


FIG. 11E

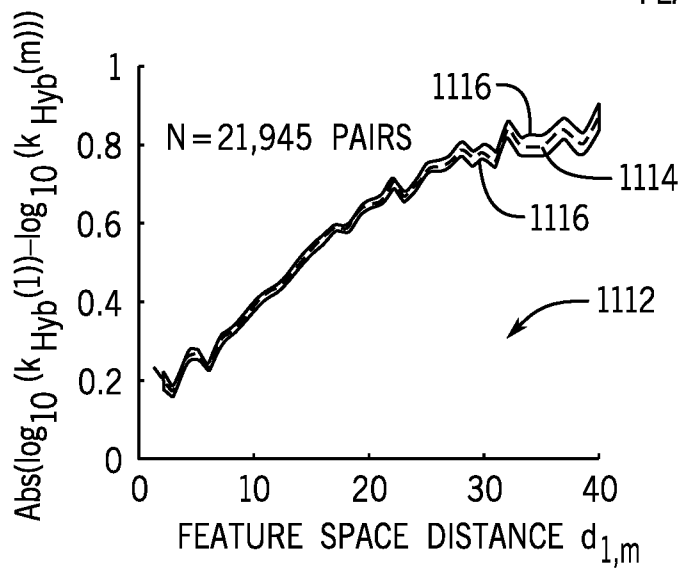
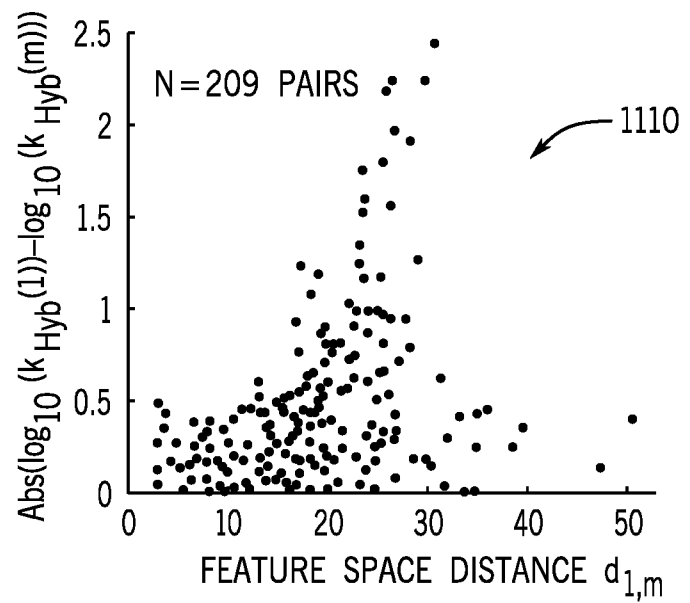


FIG. 11F

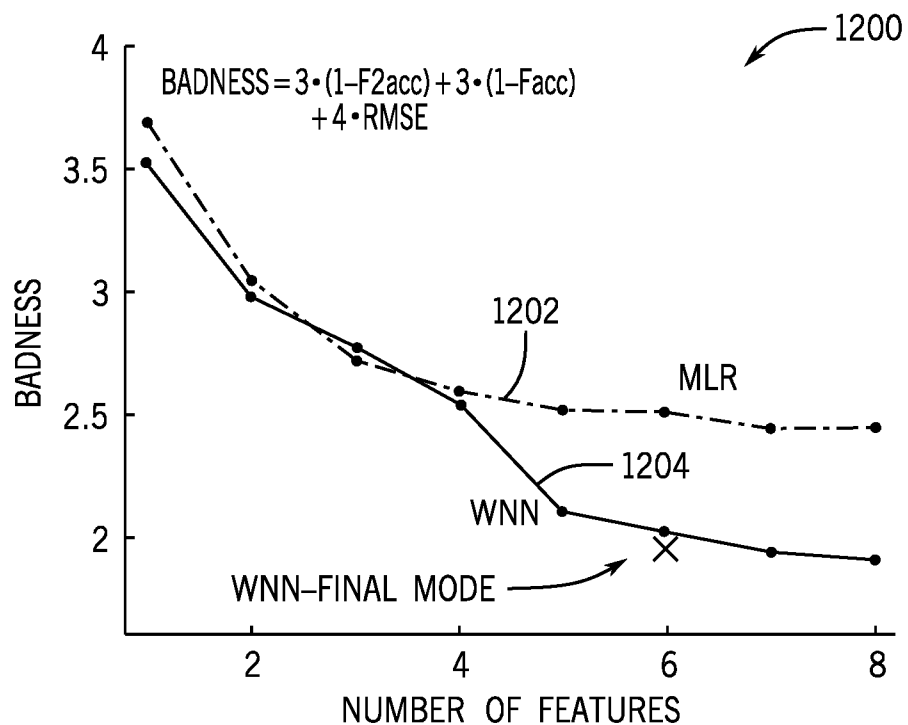


FIG. 12A

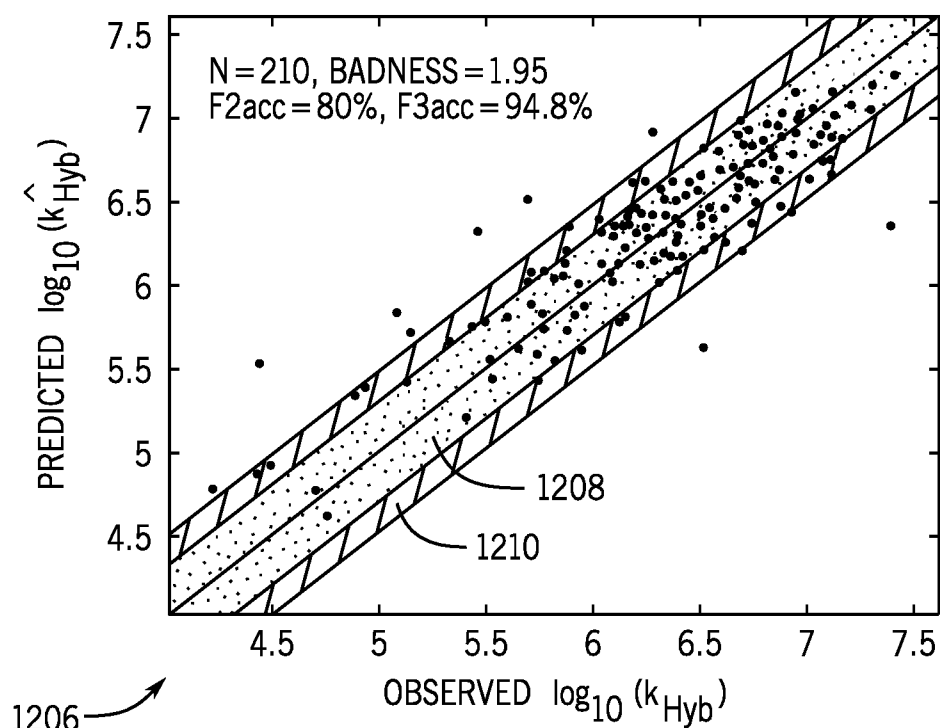


FIG. 12B

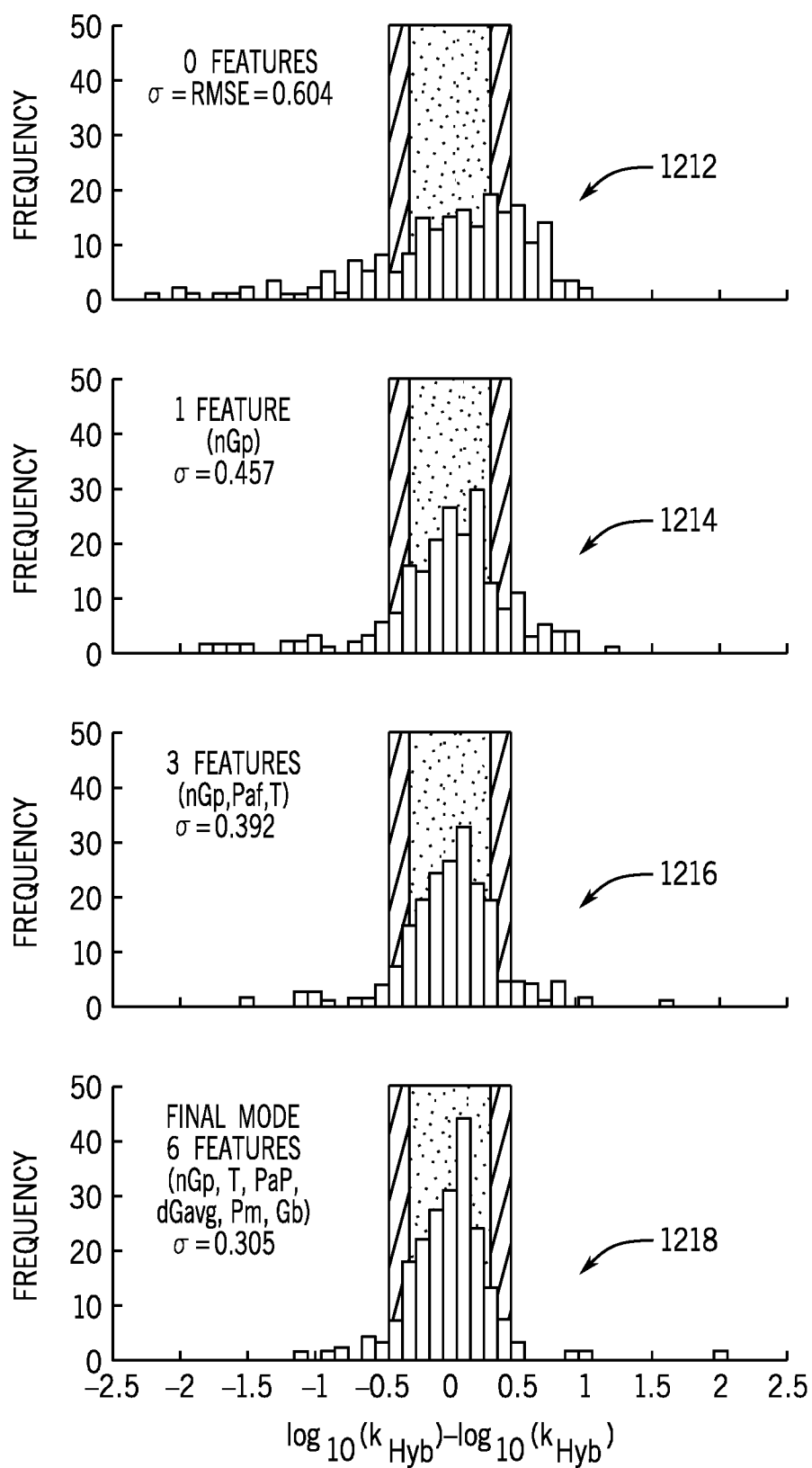


FIG. 12C

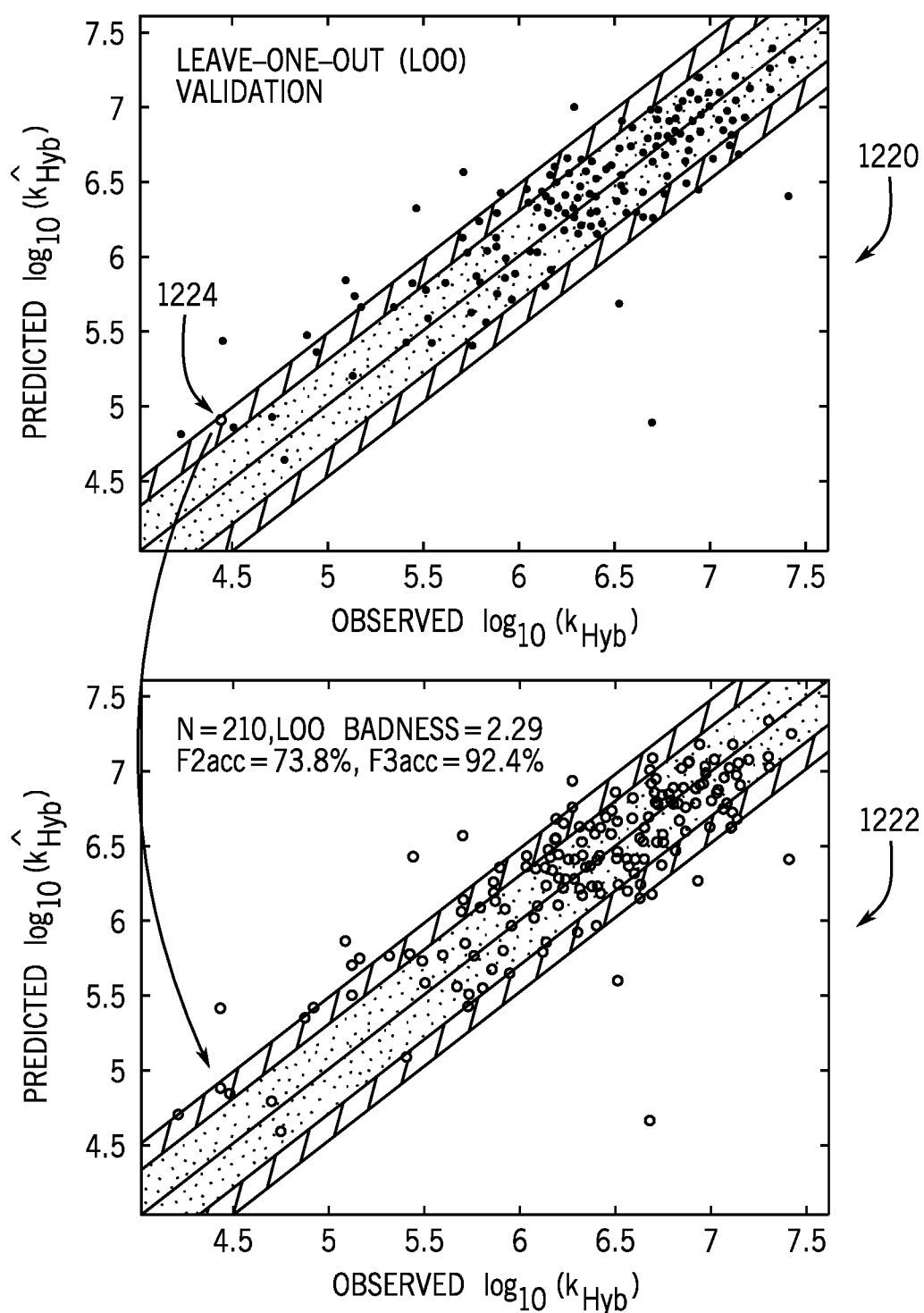


FIG. 12D

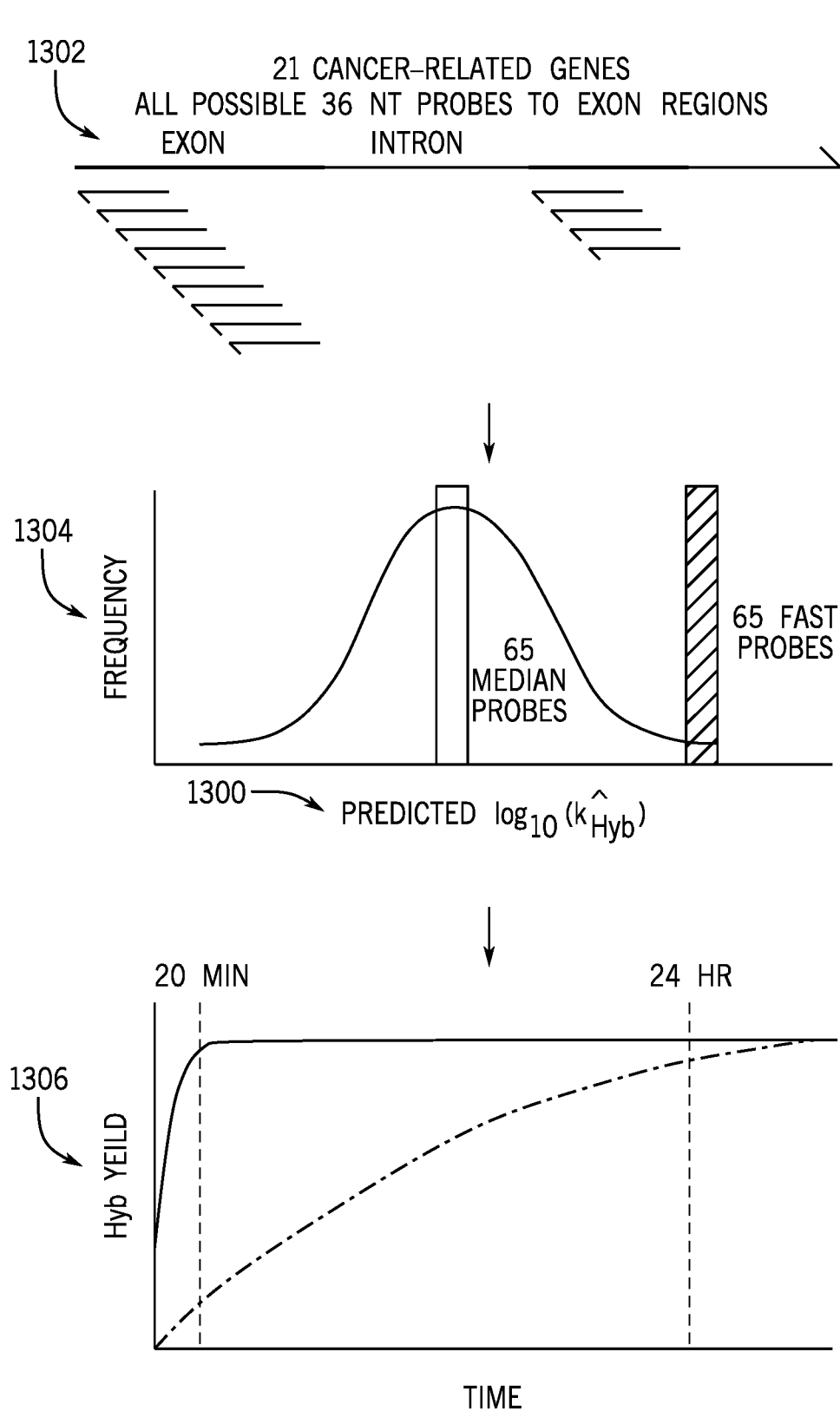


FIG. 13A

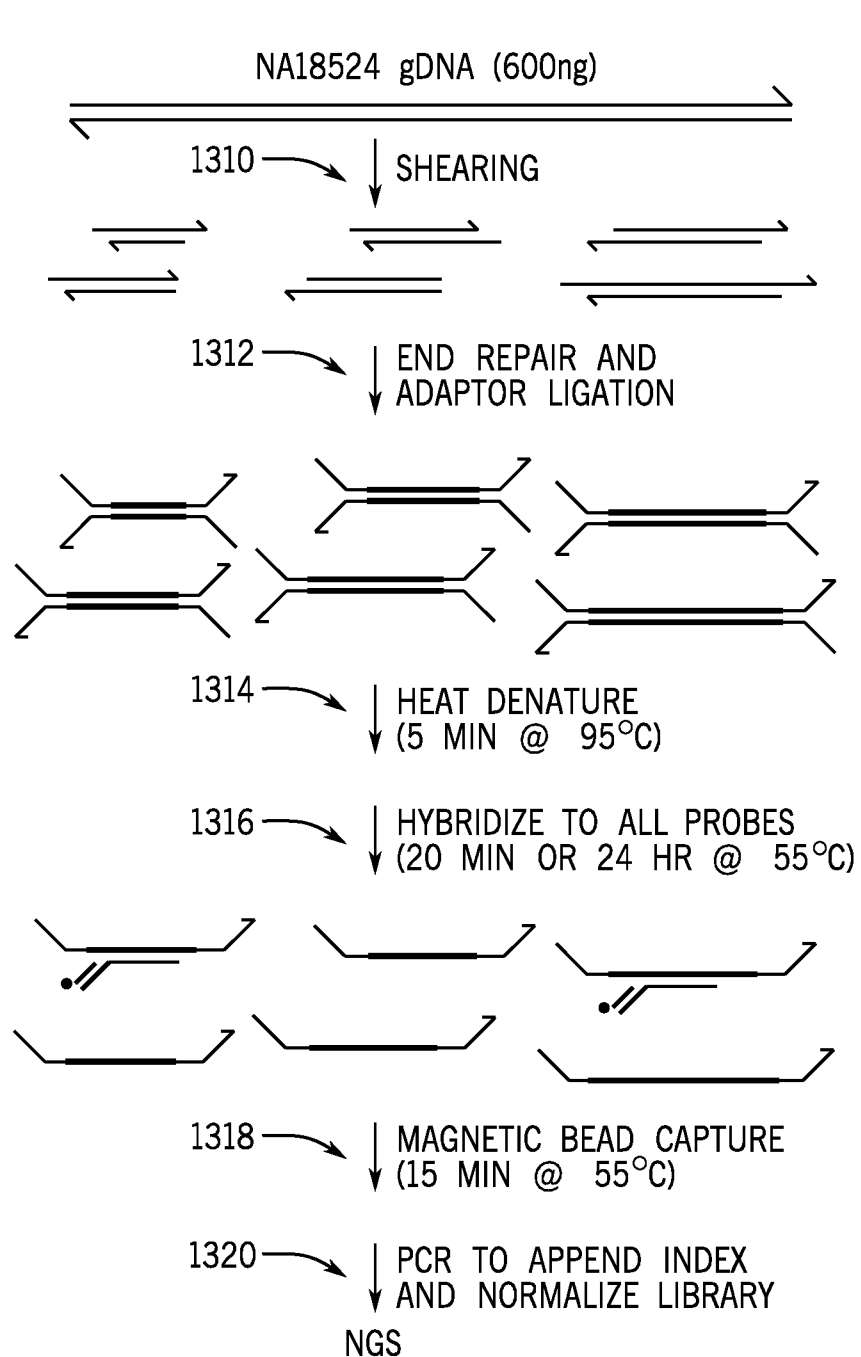


FIG. 13B

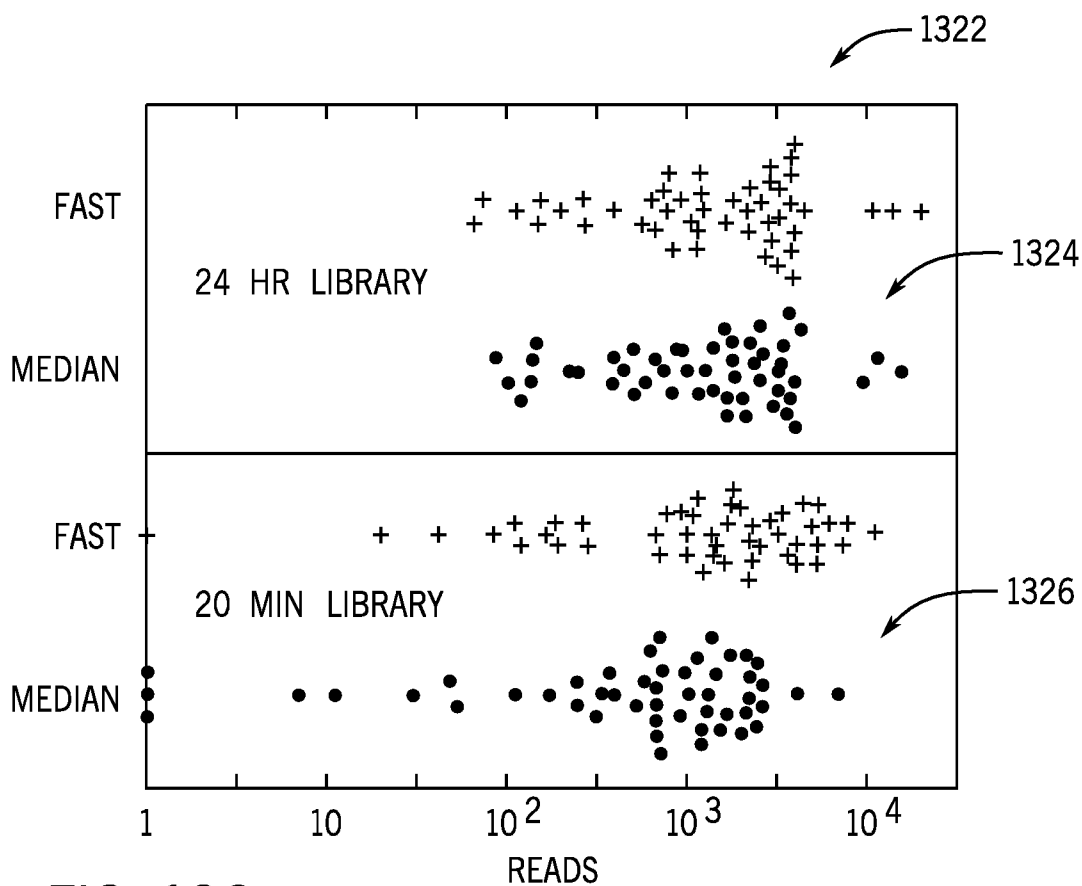


FIG. 13C

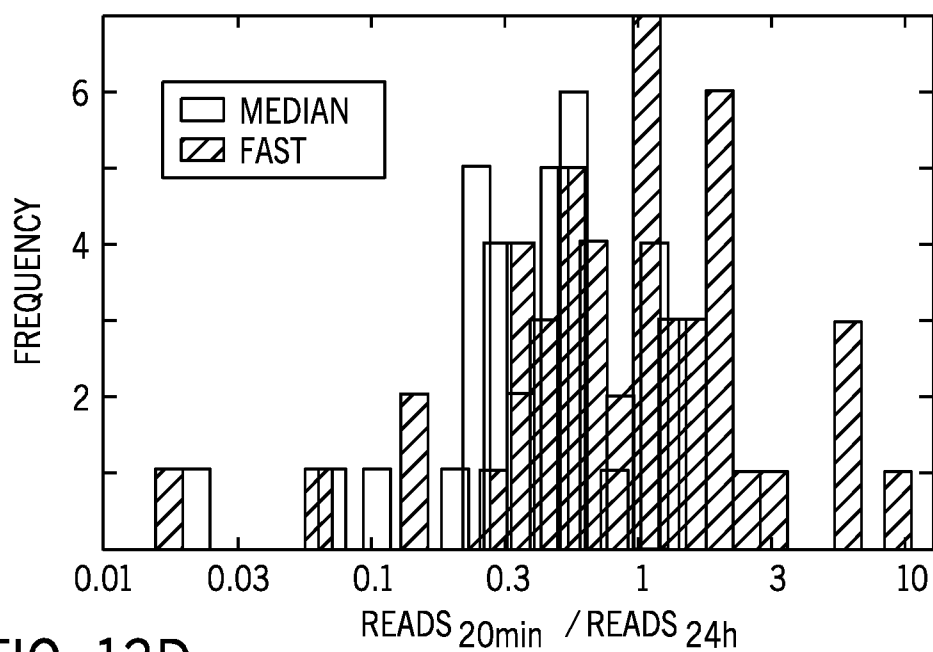


FIG. 13D

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/036410

A. CLASSIFICATION OF SUBJECT MATTER
INV. G06F19/20
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G06F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MICHAEL F. HAGAN ET AL: "Hybridization dynamics of surface immobilized DNA", JOURNAL OF CHEMICAL PHYSICS, vol. 120, no. 10, 8 March 2004 (2004-03-08), pages 4958-4968, XP055404311, US	1,2,11, 12, 14-20, 22-24
A	ISSN: 0021-9606, DOI: 10.1063/1.1645786 page 4958 left col 1st par, page 4959 right col 3rd par, page 4965 left col. last par. - right col. 1st par, fig. 6 and 10 ----- -/--	3-5,13, 21



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

28 September 2017

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2017/036410

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Anonymous: "Inverse distance weighting - Wikipedia", 19 December 2015 (2015-12-19), XP055404431, Retrieved from the Internet: URL:https://en.wikipedia.org/w/index.php?title=Inverse_distance_weighting&oldid=695845427 [retrieved on 2017-09-06] page 1 1st par, page 2 second half of the page - page 3 1st par, figure on page 2 -----	1-5, 11-24
Y	US 2007/003938 A1 (FU GLENN [US] ET AL) 4 January 2007 (2007-01-04) -----	1,2,11, 12, 14-20, 22-24
A	abstract -----	3-5,13, 21
A	US 2002/133301 A1 (HUBBELL EARL [US]) 19 September 2002 (2002-09-19) par. 56, 59, 126; abstract -----	1-5, 11-24
A	Ian H. Moal ET AL: "Kinetic Rate Constant Prediction Supports the Conformational Selection Mechanism of Protein Binding", 12 January 2012 (2012-01-12), XP055404089, Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3257286/ [retrieved on 2017-09-05] abstract, page 4 2nd and 3rd par, -----	1-5, 11-24

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2017/036410

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2007003938	A1	04-01-2007	NONE

US 2002133301	A1	19-09-2002	NONE
