Regression modeling on DNA encoded libraries

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Abstract

DNA encoded libraries (DELs) are pooled, combinatorial compound collections where each member is tagged with its own unique DNA barcode. DELs are used in drug discovery for early hit finding against protein targets. Recently, several groups have proposed building machine learning models with quantities derived from DEL datasets. However, DEL datasets have a low signal-to-noise ratio which makes modeling them challenging. To that end, we propose a novel graph neural network (GNN) based regression model that directly predicts enrichment scores from raw sequencing counts while accounting for multiple sources of technical variation and intrinsic assay noise. We show that our GNN regression model quantitatively outperforms standard classification approaches and can be used to find diverse sets of molecules in external virtual libraries.

Introduction

Small molecule drug discovery begins with the identification of putative chemical matter that binds to protein targets of interest. This can be be achieved with experimental techniques such as high-throughput screening or in silico methodologies such as docking and generative modeling. DNA encoded library (DEL)[11, 6, 2, 5, 4, 7] screening is a high throughput experimental technique used to identify diverse sets of chemical matter against targets of interest.

DELs are DNA barcode labeled pooled compound collections (Figure 1.A) that are incubated with an immobilized protein target in a process referred to as panning. The mixture is then washed to remove non-binders, and the remaining bound compounds are eluted, amplified, and sequenced to identify putative binders. DELs provide a quantitative readout for hundreds of millions of compounds. This readout can contain substantial experimental noise and biases caused by sources such as DEL members binding the protein immobilization media or differences in starting population (load). This noise is often controlled for by computing an enrichment metric[6, 11, 7, 5] that compares the compound population to its starting population and control experiments. Often, multiple replicates and off-target counter pans are used to improve the signal-to-noise ratio.

Recently, several groups [14, 12] have demonstrated powerful applications of ML to DELs. McCloskey et al. [14] trained classification models on labels generated with aggregated DEL counts and used the models to perform inference on large virtual libraries (VLs). This inference yielded diverse hits for several protein targets. However, their approach required determining a label boundary, which has no standard process and may bias the dataset. Moreover, classification models struggle to differentiate between weak and strong binders. This becomes important when prioritizing compounds for testing due to the multi-billion scale of VLs. Lim et al. [12] improved on the classification approach by directly modeling an enrichment metric (the ratio between counts from a target and an off-target pan). Their model was trained using a custom negative-log-likelihood loss function derived from a Poisson ratio test. However, it is not obvious that their method can be expanded to multiple off-target experiments or extended to include additional covariates.

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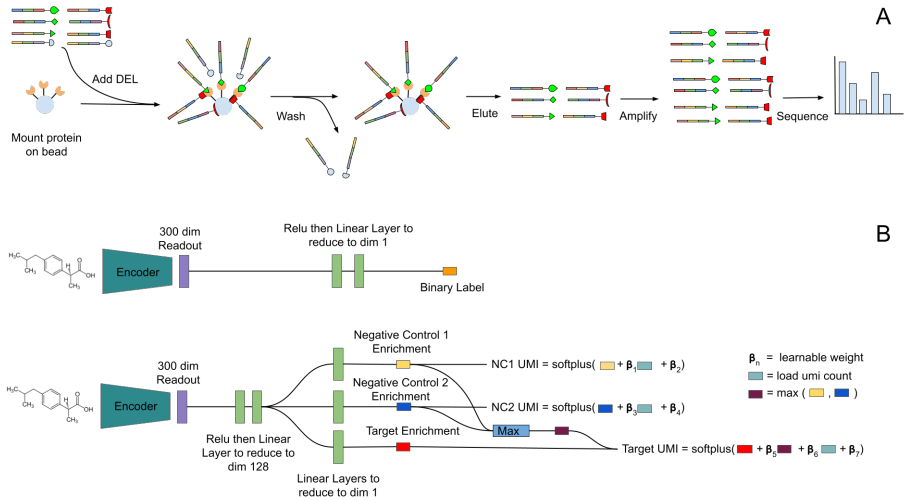

Figure 1: A. Diagram of DEL panning experiment. The sequencing counts can be attributed to both target specific binding (green) and non-target binding modes (red). B. Model schematics. Both classification and regression use a GIN-E encoder. The classification network maps the encoder output to a single class prediction. The regression network has multiple heads, each predicting an enrichment value from a reduced embedding of the encoder output. A linear sum of the enrichments and covariates is used to predict observed counts.

By contrast, we introduce a general framework for quantitative modeling of DELs that incorporates multiple sources of experimental noise. We show that GNNs trained using our framework and regularized negative binomial loss outperform classification models in replicating experimental compound rankings generated with proxy measurements of binding affinity on a held out DEL. We end by examining key metrics from a virtual screen of the Enamine REAL and Wuxi VLs.

Methods

We obtained multiple proprietary DEL panning datasets screened against a challenging protein target. These datasets include control and off-target pans. Here, we present results for a diversity screening library of 100M compounds (Lib1) that we used for training and a separate expansion library of 2.5M compounds used for validation (Lib2).

Classification Model

To provide a competitive baseline, we built and optimized a classification model using the same GNN architecture as the regression model (GIN-E network with virtual node [15]). We assigned binary labels for binders (positives) and non-binders (negatives) using a two-step thresholding process. First, we discarded compounds with on-target unique molecular identifier (UMI) counts below a noise threshold. Second, we normalized compound UMIs in each pan by the sum of all UMIs in the pan to yield molecular frequencies (MFs). We then calculated the ratio between the on-target and max control or off-target MF. If a compound's MF ratio exceeded a positive cutoff or fell below a negative cutoff, we assigned it a positive or negative label, respectively. Compounds with ratios falling between the cutoffs were discarded. This yielded ~74K positives and ~5.6M negatives. We experimented with combinations of sampling schemes and losses to address the class imbalance, and found that Focal Loss [13] without balanced sampling performed best. Additionally, we regularized the model with dropout in the layers after graph readout and with input augmentations.

Regression Model

We used negative binomial regression to model the UMI from each panning experiment. This approach for denoising counts has previously been shown by [8] who used a regularized negative binomial regression model to de-noise UMI readouts from single cell RNA sequencing. They defined

enrichment as the Pearson residual after regressing the count on sequencing depth. Similarly, we modeled the enrichment for each compound as the residual after accounting for various covariates such as binding to beads. As a generalization of Poisson regression, negative binomial regression incorporates a dispersion parameter α in addition to a mean variable μ. For one target pan and two no-target control pans, $C_{i,target} \sim NB(\mu_{i,target}, \alpha_{target})$, $C_{i,control_1} \sim NB(\mu_{i,control_1}, \alpha_{control_1})$, and $C_{i,control_2} \sim NB(\mu_{i,control_2}, \alpha_{control_2})$ represent the UMI counts of ith compound in the respective panning experiments. We modeled μ_i as the combination of enrichment from binding to the target ($R_{i,target}$), enrichment from binding to the non-target media ($R_{i,control_1}$, $R_{i,control_2}$), and observed count of the compound in the original starting population load (S_i).

$$\mu_{i,control_1} = \sigma(R_{i,control_1} + \beta_1 S_i + \beta_2) \tag{1}$$

$$\mu_{i,control_2} = \sigma(R_{i,control_2} + \beta_3 S_i + \beta_4) \tag{2}$$

$$\mu_{i,target} = \sigma(R_{i,target} + \beta_5 max(R_{i,control_1}, R_{i,control_2}) + \beta_6 S_i + \beta_7) \tag{3}$$

β_i are learned from the data and σ represents the softplus function, which we found to be more stable during training than the typical exponential function. The dispersion parameter, α, of the negative binomial is a single scalar, learned for each experiment. We related $R_{i,target}$ and $R_{i,control}$ to each compound's structure by deriving their values with a GNN operating on the compound's molecular graph. A shared encoding network generates a 128 dimensional embedding vector from atom and bond features. This embedding vector is then transformed into $R_{i,target}$, $R_{i,control_1}$, and $R_{i,control_2}$ by separate feed forward networks (Fig 1.B). For our experiments, we used a GIN-E network with virtual node [15, 9, 10] for the initial encoding and two layers in each of our feed forward networks. During training, we summed the negative log likelihood of the observed counts for the target and control pans. Furthermore, we L2 regularized the enrichment values, which empirically prevented over-fitting. For a single example with count c_i for each panning experiment , the loss can be written:

$$P(c_i|\mu_i, \alpha) = \frac{\Gamma(c_i + \alpha^{-1})}{\Gamma(c_i + 1)\Gamma(\alpha^{-1})} \left(\frac{1}{1 + \alpha\mu_i}\right)^{\alpha^{-1}} \left(\frac{\alpha\mu_i}{1 + \alpha\mu_i}\right)^{c_i} \tag{4}$$

$$L_{i,target} = -\log P(c_{i,target}|\mu_{i,target}, \alpha_{target}) \tag{5}$$

$$L_{i,control_1} = -\log P(c_{i,control_1}|\mu_{i,control_1}, \alpha_{control_1}) \tag{6}$$

$$L_{i,control_2} = -\log P(c_{i,control_2}|\mu_{i,control_2}, \alpha_{control_2}) \tag{7}$$

$$L_i = L_{i,target} + L_{i,control_1} + L_{i,control_2} + \gamma R^2_{i,target} + \gamma R^2_{i,control_1} + \gamma R^2_{i,control_2} \tag{8}$$

where $\Gamma(x)$ is the gamma function and γ is the L2 regularization rate. This negative binomial regression can be further extended with other covariates such as enrichment in other negative control pans, other target pans, compound synthesis yield, and reaction type. For our experiments, we used 13 negative control pans. During validation and inference for virtual screening, we used our de-noised enrichment value $R_{i,target}$ to rank compounds.

Cross Library Validation

After training on Lib1, we validated our models on Lib2 which had proxy binding affinity measurements. Binding affinity of a compound to a target can be measured by the equilibrium disassociation constant Kd and corresponding negative log value pKd. Lib2 was used in a set of target titration panning experiments[3] to produce titration-based pKds (t-pKds). A small portion of these t-pKds were validated with off-DNA pKd measurements (R^2 = 0.84). We measured model performance by calculating the Spearman correlation coefficient between model predictions and the t-pKds. This metric aligned with our intended use of the models to rank VLs for candidate selection.

The R_{target} predicted by our regression model had a 0.41 (95% CI [0.40, 0.43]) Spearman correlation with t-pKds (Fig 2.A). This exceeds both a Random Forest classification baseline (0.28) and our GNN classification model (0.35 (95% CI [.34,.37])) (Fig 2.B). Furthermore, both the GNN regression and GNN classification models trained from Lib1 showed better correlation with t-pKds from Lib2 than UMI counts from a single pan of Lib2. This illustrates both the high noise in the raw UMI output from a single panning experiment and our models' ability to generalize. Finally, we compared the two GNN models' retrieval rates for strong binders in their top prediction results. The regression model had more binders in its top prediction results than the classification model (Fig 2.C).

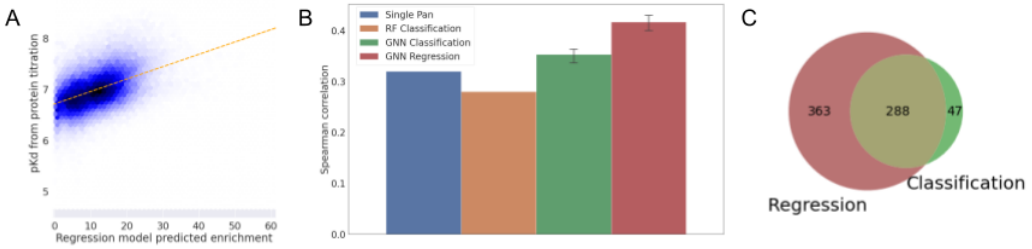

Figure 2: A. Bivariate histogram showing correlation between predicted enrichment (R_{target}) from GNN regression model and t-pKds derived from protein titration. Regression line is plotted in orange. B. Spearman correlation between model predictions and t-pKds. For RF Classification and GNN Classification, predicted probability of the binder class is used for ranking. "Single Pan" represents the UMI counts from a single panning experiment done with Lib2 at the same target concentration as experiments with Lib1. Error bars on GNN Regression and Classification represents the 95% confidence interval as estimated by three model replicates. C. Venn diagram showing number of compounds with t-pKds >= 8 (n=1327) retrieved in the top 10K of the GNN Regression versus the GNN Classification model.

Virtual Screening

We used our regression model to perform a virtual screen of 3.7 billion compounds in Enamine and WuXi's VLs. We then thresholded the regression model's output to select a limited number of top scoring compounds for analysis. We established the threshold using a quantitative analysis of the enrichment distribution in both the validation and inference sets (Fig 3.A). We clustered this top set using Taylor-Butina clustering[1] with a similarity cut-off of 0.6. Structural similarity was calculated via Jaccard similarity of Morgan fingerprints. This clustering yielded 26 clusters, and we selected top scoring compounds from each cluster for testing. These selected compounds are structurally diverse (Fig 3.C).

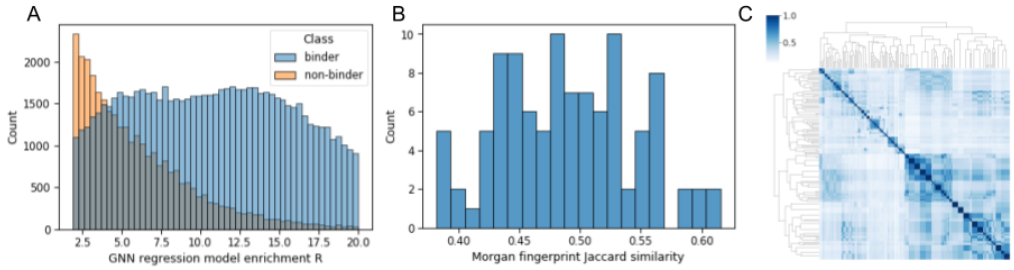

Figure 3: Regression models pick diverse compounds from the VLs. A. Histogram of predicted enrichment for the overlapping region between binders and non-binders on the validation set (Lib2). B. Histogram of Jaccard similarities for inference compounds with predicted enrichment >10 to their closest neighbor in the training set (Lib1). C. Heatmap of pairwise Jaccard similarities between compounds with predicted enrichment >10.

Conclusion

DEL experiments yield datasets with low signal-to-noise ratio. In our work, we show a novel regression technique for modeling DEL sequencing counts that accounts for various sources of variation, such as media binding and differences in initial load. Our model's predicted enrichment values have better correlation with proxy binding affinities than those of baseline classification models or experimental values from a single panning experiment. Finally, we demonstrate that our model retrieves diverse compounds during virtual screening.

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