A deep learning framework for high-throughput mechanism-driven phenotype compound screening and its application to COVID-19 drug repurposing

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ABSTRACT

This supplementary document includes supplementary notes, algorithms, figures, and tables that support the manuscript "A deep learning framework for high-throughput mechanism-driven phenotype compound screening".

Supplementary Notes

Data processing

L1000 data processing for *de novo* chemical and imputation settings In this section, we present the method of constructing training, development, and testing sets from Bayesian-based peak deconvolution L1000 dataset which has been shown to generate more robust z-score profiles from L1000 assay data, and therefore, gives better representation for perturbagens. To achieve that, they propose a peak deconvolution algorithm based on Bayes' theorem that gives unbiased likelihood estimations for peak locations and they characterize the peaks with probability-based z-scores.

Bayesian-based peak deconvolution L1000 dataset consists of three levels of the data (corresponding to level 2, 4, and 5 of the original L1000 dataset): level 2 data presents the marginal distributions of peak locations computed from Bayesian-based method, level 4 data presents the z-score of each gene for each bio-replicates by comparing the probability distribution for each gene with its background distribution, and level 5 data combines z-score profiles from bio-replicates into one signature by weighted average. In our study, we conduct experiments on level 5 data and use level 4 data for filtering out unreliable experiments in level 5 data. In particular, average Pearson correlation (APC) score for each experiment in level 5 data is calculated from Pearson correlation scores among its bio-replicates' gene expression profiles in level 4 data and experiments that have APC scores less than a threshold are considered as unreliable experiments and filtered out. In our setting, this threshold is set at 0.7. The APC score density function and cumulative distribution of L1000 data are shown in Supplementary Figure 1. Level 4 data is also used in our data augmentation method described in the following sections. From this filtered dataset, we select the gene signatures measured after 24h of the 7 most popular cell lines (MCF7, A375, HT29, PC3, HA1E, YAPC, HELA) and 6 most popular dose sizes ($0.04\,\mu m$, $0.12\,\mu m$, $0.37\,\mu m$, $1.11\,\mu m$, $3.33\,\mu m$, $10.0\,\mu m$) to create the dataset used in our experiments. We split this dataset into training, development, and testing sets w.r.t. chemical by the ratio $0.6 : 0.2 : 0.2$ respectively. This training set is called high-quality training set. We also create the training set without removing unreliable experiments and call it as original training set. The statistics of these training, development, and testing sets are shown in Supplementary Table 2.

Besides constructing training, development, and testing sets for *de novo* chemical setting, we also generate these set for traditional imputation setting from high-quality dataset. In particular, new training, development, and testing sets are constructed from high-quality dataset with the same ratio as *de novo* chemical setting, but at this time, we split this dataset by experiment instead of chemical. Therefore, chemicals in the testing set can appear in the training set. The statistics of the training, development, and testing sets for traditional imputation setting are shown in Supplementary Table 3

Data processing for ATC code and drug-target prediction We extract gene expression profiles of chemicals that do not have reliable experiments in L1000 dataset (i.e. 1258 chemicals) and call them a original feature set. We only extract gene expression profiles induced by chemicals with dose size of 10 um because gene expression profiles induced by the larger amount of chemicals can differentiate chemicals better than them induced by the smaller amount of chemicals. Note that, each chemical compound is experimented on 7 cell lines so it is represented by 7 gene expression profiles. Similarly, we use DeepCE trained on the high-quality training set to generate gene expression profiles for these chemicals and call them a predicted feature set. Next, we extract the ATC codes and drug-targets of these chemicals from Drugbank database to create labels for these chemicals. We also filter out labels that have low frequencies in the dataset (i.e. $< 3\%$ of the number of chemicals) to avoid unreliable evaluation. After that, 10 ATC codes ((i.e. *N, C, A, J, S, L, D, R, G, M*) and 4 drug-targets (HRH1, DRD2, HTR2A, ADRA1A) are selected to construct 14 binary classification datasets. The details of these datasets are shown in Supplementary Table 7.

Feature engineering

Our models predict gene expression values based on tuples of chemical and biological objects including chemical compounds, cell lines, dose sizes, and L1000 genes. In the following paragraphs, we present the way to transform these chemical and biological objects into numerical representations that can be put into our models.

Chemical fingerprints The canonical SMILES strings which are the raw representation of chemicals are transformed to the chemical fingerprints using the open-source cheminformatics software RCDK[1] (i.e. *get.fingerprint()* function). chemical fingerprints are binary (bit) vectors that represent the presence or absence of particular substructures in chemicals. In our settings, we experiment with PubChem and circular (ECFP6) fingerprints which have lengths (i.e. number of substructures) of 881 and 1024 respectively. We also use neural fingerprints which are continuous vectors to represent chemicals. The neural fingerprints are described in detail later.

Drug-target features Besides using molecule structure information, bioactivity information of chemicals (i.e. drug-target interaction knowledge) can be used to represent chemicals. In particular, vector representations for chemicals are calculated from drug-target interaction and human protein-protein interaction networks extracted from Drugbank and STRING databases respectively. To increase the quality of generated vectors, all edges (interactions) in the human protein-protein interaction network have their combined scores of less than 700 are removed. If chemical compound interacts with L1000 genes (i.e. L1000 genes are 1-hop neighborhoods of chemical compound in the interaction network), values at indexes corresponding to these genes are set at 1.0. If L1000 genes interact with targets of chemicals (i.e. L1000 genes are 2-hop neighborhoods of chemical compound in interaction network), their values are set at 0.1. Similarly, the values of L1000 genes which are 3-hop neighborhoods of chemical compound are set at 0.01. The values of remaining L1000 genes are set at 0. The generated vectors are normalized to have Euclidean norm to be 1. The length of these vectors is 978 which is equal to the number of L1000 genes. Besides our proposed drug-target interaction-based representation, we also experiment with another chemical representation using drug-target interaction information named latent target interaction profile (LTIP) which has been shown to be an effective representation for chemical compound in many bioactivity prediction tasks, especially for the setting that the biological information is important for prediction[2]. In particular, LTIP maps chemicals into low dimensional continuous vectors which are embeddings of nodes in the drug-target interaction network (i.e. bipartite graph where drugs and targets are nodes, and their interactions are edges).

Gene features Vector representations for L1000 genes are generated from the human protein-protein interaction network extracted from STRING database using node2vec method[3]. The length of these vectors is set at 128.

Cell line and chemical dose size features We represent cell lines and chemical dose sizes using one-hot encoding. The lengths of these vectors are 7 and 6 corresponding to the numbers of cell lines and chemical dose sizes used in our experiments respectively.

Metrics

Root mean squared error Root mean squared error (RMSE) captures the differences between the ground-truth and predicted z-scores for all L1000 genes. This score is defined as the square root of the mean square error and is computed as follows:

$$RMSE = \sqrt{\frac{1}{NM}\sum_{i=1}^{N}\sum_{j=1}^{M}(z_{i,j} - y_{i,j})^2}$$

where N and M are the number of gene expression profiles in a batch and number of L1000 genes respectively. $z_{i,j}$ and $y_{i,j}$ are ground-truth and predicted gene expression values of j^{th} gene in i^{th} gene expression profile. The lower value of RMSE shows the better performance.

Gene set enrichment analysis While Pearson correlation and RMSE captures the variations among all L1000 genes, gene set enrichment analysis (GSEA)[4,5] only focuses on the most significant up and down regulated genes. In particular, GSEA, which is often used in the biological science literature, is calculated based on the enrichment score that represents the amount to which the genes in the set are over-represented at either the top (up-regulated) or bottom (down-regulated) of the ranked gene list. The higher GSEA score shows the better performance.

Precision@k Similar to GSEA, Precision@k (P@k), which is commonly used information retrieval field, only focuses on top-k significant p and down regulated genes. In particular, P@k is a fraction of intersection of top-k up or down-regulated genes between predicted and ground-truth genes to the ground-truth genes. By definition, there are 2 kinds of P@k scores that measure the most significant positive and negative genes. For example, positive P@100 measures the overlap between top 100 positive genes (i.e. genes that have their z-scores in top 100 positive z-scores) in ground-truth and predicted gene expressions and is computed as follows:

$$Positive\ \ P@100 = \frac{|A_{ground-truth}^{100-positive} \cap |A_{predicted}^{100-positive}|}{||A_{predicted}^{100-positive}|}$$

where $A_{ground-truth}^{100-positive}$ and $A_{predicted}^{100-positive}$ are the sets of top 100 positive genes in ground-truth and predicted gene expression respectively. Similarly, negative P@100 is computed as follows:

$$Negative\ \ P@100 = \frac{|A_{ground-truth}^{100-negative} \cap |A_{predicted}^{100-negative}|}{||A_{predicted}^{100-negative}|}$$

where $A_{ground-truth}^{100-negative}$ and $A_{predicted}^{100-negative}$ are the sets of top 100 negative genes in ground-truth and predicted gene expression respectively.

Implementation details

All neural network-based models including DeepCE, vanilla neural network, and linear models are implemented with Pytorch[6]. The training process lasts at most 100 epochs for all training sets and all models. We use Adam optimizer[7] with the learning rate of 0.0005 and the batch size is set at 16. The development set is used for tuning hyperparameters and stopping the training process. kNN model is implemented by Python 3 while we use authors' implementation for TT-WOPT. Binary classification models used in downstream task evaluation including logistic regression, support vector machine and kNN are implemented by scikit-learn package[8]. All experiments are conducted on Intel Xeon E5-2680 v4 processor with 128 GB of RAM and Tesla P100 with 16 GB of VRAM and are repeated three times with different random seeds.

Supplementary Tables

Dataset	Description	Usage
LINCS L1000	This dataset includes differential gene expression signature induced by chemical compound. Each example contains gene expression signature and its experiment setting (i.e. chemical compound, cell line, and dosage)	This dataset is used to train and evaluate DeepCE and other models for predicting differential gene expression signature from experiment setting
STRING	The protein-protein interaction network	This dataset is used to compute vector representation for L1000 genes by network embedding method (i.e. node2vec) and to compute drug-target features for chemical compounds
DrugBank	The online database contains information of drugs and their targets	We extract drugs and their properties from this dataset to create datasets for downstream tasks including ATC code and drug-target predictions. We also generate gene expression profiles for all drugs in DrugBank for virtual screening for finding COVID-19 treatment
COVID-19 Patient Gene Expression (NGDC, NCBI)	The transcriptomics data of COVID-19 patients and healthy control samples	We compute differential gene expression profiles for COVID-19 patients from both patient and healthy samples in this dataset. This profile is then used to compared with chemical-induced gene expression profiles for finding COVID-19 treatment

Supplementary Table 1. The summary of datasets used in our study.

	#chemicals	Cell lines (#gene expression profile)							Chemical doses (#gene expression profile)					
		A375	HA1E	HELA	HT29	MCF7	PC3	YAPC	0.04 um	0.12 um	0.37 um	1.11 um	3.33 um	10.00 um
Train (Original)	1553	9225	9233	8443	9243	9240	9247	8440	10587	10504	10526	10512	10502	10440
Train (High-quality)	284	397	296	209	287	250	279	177	136	175	227	322	402	633
Train (Augmented)	626	707	953	399	552	519	514	321	327	414	497	639	817	1271
Dev	92	120	81	50	89	77	69	58	40	57	72	93	89	193
Test	92	121	69	39	82	61	72	52	33	48	57	83	91	184

Supplementary Table 2. Statistics of (original/high-quality/augments) training, development, and testing sets generated from Bayesian-based peak deconvolution L1000 dataset for *de novo* chemical setting.

	#chemicals	Cell lines (#gene expression profile)							Chemical doses (#gene expression profile)					
		A375	HA1E	HELA	HT29	MCF7	PC3	YAPC	0.04 um	0.12 um	0.37 um	1.11 um	3.33 um	10.00 um
Train	394	396	258	177	288	229	251	162	133	169	235	292	332	600
Dev	268	132	89	54	90	76	82	64	44	51	61	99	129	203
Test	257	110	99	67	80	83	87	61	32	60	60	107	121	207

Supplementary Table 3. Statistics of training, development, and testing sets generated from Bayesian-based peak deconvolution L1000 dataset for traditional imputation setting.

	Hyperparameter	Value
Training	Batch size	16
	Learning rate	0.0002
	Optimizer	Adam
	Maximum number of epochs	100
	Loss function	MSE
	Initializer	Xavier Uniform
Feature mapping	Atom feature size	62
	Bond feature size	6
	Convolution size	16
	Number of convolutional layers	2
	Neural fingerprint size	128
	Chemical dose feature size	6
	Cell line feature size	7
	L1000 gene feature size	128
	Chemical dose hidden size	4
	Cell line hidden size	4
	L1000 gene hidden size	128
Interaction component	Number of attention heads	4
	Number of attention layers	2
	Attention hidden size	512
	Batch normalization	yes
	Dropout	0.1
Prediction component	Number of feed-forward layers	2
	Feed-forward layer sizes	128, 978
	Activation function	ReLU

Supplementary Table 4. Hyperparameter used in DeepCE model.

Models	PubChem	ECFP	Drug-target	LTIP	Random
Vanilla neural network	0.4086 ± 0.0306	0.4138 ± 0.0227	0.4275 ± 0.0280	0.4311 ± 0.0321	0.3187 ± 0.0245
kNN	0.3988 ± 0.0177	0.4009 ± 0.0187	0.3975 ± 0.0188	0.3896 ± 0.0140	-
Linear regression	0.1757 ± 0.0099	0.1758 ± 0.0100	0.1758 ± 0.0100	0.1759 ± 0.1101	-
Lasso	0.1755 ± 0.0100	0.1756 ± 0.0101	0.1756 ± 0.0100	0.1757 ± 0.0101	-
Ridge regression	0.1757 ± 0.0099	0.1758 ± 0.0100	0.1758 ± 0.0100	0.1759 ± 0.0101	-

(a)

Models	Performances
TT-WOPT	0.0075 ± 0.0106
DeepCE w/o interaction component	0.4424 ± 0.0213
DeepCE w/o chemical substructure-gene attention	0.4601 ± 0.0345
DeepCE w/o gene-gene attention	0.4617 ± 0.0259
DeepCE	0.4869 ± 0.0130

(b)

Supplementary Table 5. Performances (Pearson correlation) under 5-fold cross-validation setting of DeepCE and other baseline models. **(a)** shows performances of vanilla neural network, kNN, and linear models with different chemical features. **(b)** shows performances of TT-WOPT, and DeepCE and its simpler versions.

Setting	Performance
DeepCE	0.4907
DeepCE w/o cell line feature	0.3723
DeepCE w/o dose size feature	0.4453
DeepCE w/o cell line and dose size features	0.3567

Supplementary Table 6. Performances (Pearson correlation) on testing set of DeepCE when removing cell line and dose size information.

ATC code	Frequency	ATC code	Frequency
N	143	L	57
C	124	D	54
A	84	R	53
J	80	G	48
S	62	M	43

(a)

Drug-target	Frequency
HRH1	47
DRD2	45
HTR2A	42
ADRA1A	40

(b)

Supplementary Table 7. Data statistics for downstream task evaluation. **(a)** shows frequencies of ATC codes across all low-quality drugs. **(b)** shows frequencies of drug-targets across all low-quality drugs.

	Original feature	Predicted feature
A375	0.5712	0.6292
HA1E	0.5441	0.6173
HELA	0.5527	0.6262
HT29	0.5522	0.6182
MCF7	0.5518	0.6203
PC3	0.5586	0.6206
YAPC	0.5805	0.6191

(a)

	Original feature	Predicted feature
N	0.5534	0.6511
C	0.5260	0.6077
A	0.5047	0.5312
J	0.5199	0.7116
S	0.5678	0.6385
L	0.5153	0.5219
D	0.5923	0.5806
R	0.4969	0.5955
G	0.5436	0.5698
M	0.5436	0.5890

(b)

	Original feature	Predicted feature
HRH1	0.6015	0.7746
DRD2	0.6219	0.5792
HTR2A	0.6538	0.7115
ADRA1A	0.5813	0.6399

(c)

	Original feature	Predicted feature
LR	0.5958	0.6809
kNN	0.5471	0.6110
SVM	0.5850	0.6655
DT	0.5070	0.5288

(d)

Supplementary Table 8. AUC of experiments with original and predicted gene expression profiles. **(a)** shows results per cell-specific profile, across experiments for different classification tasks and models. **(b)** shows results per ATC code, across experiments for different cell-specific profiles and models. **(c)** shows results per drug-target, across experiments for different cell-specific profiles and models. **(d)** shows results per model, across experiments for different cell-specific profiles and classification tasks.

Supplementary Algorithms

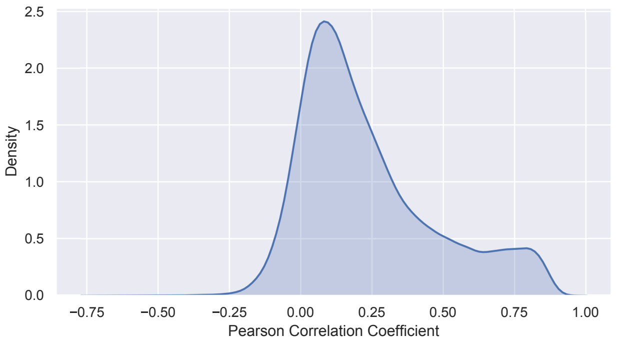
Algorithm 1: Pseudo-code of GCN

```
**Input:** Chemical graph = $(V,E)$, radius $R$, hidden weights $(H_1^1,...,H_R^5),(U_1,...,U_l),(W_1,...,W_l)$
**Output:** $v_R^{(1)},...,v_R^{(|V|)}$
for $l$ = 1 to $R$ do
    for $i$ = 1 to —V— do
        $V_{neighbor}, E_{neighbor} \leftarrow Neighbors(\mathbf{v}^{(i)})$;
        $\mathbf{v}_l^{(i)} \leftarrow \sum_{\mathbf{v}^{(j)} \in V_{neighbor}} \mathbf{v}_{l-1}^{(j)}$;
        $\mathbf{e}_l^{(i)} \leftarrow \sum_{\mathbf{e}^{(j)} \in E_{neighbor}} \mathbf{e}_0^{(j)}$;
        $\mathbf{v}_l^{(i)} \leftarrow concat(\mathbf{v}_l^{(i)}, \mathbf{e}_l^{(i)})$;
        $\mathbf{v}_l^{(i)} \leftarrow ReLU(\mathbf{v}_{l-1}^{(i)}\mathbf{U}_l + (\mathbf{v}_l^{(i)}\mathbf{W}_l)$;
        $\mathbf{v}_l^{(i)} \leftarrow softmax(\mathbf{v}_l^{(i)}\mathbf{H}_l^{|V_{neighbor}|})$
    end
end
```

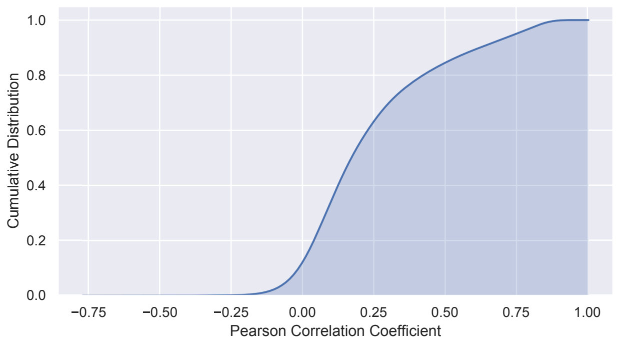
Algorithm 2: Pseudo-code of data augmentation method

```
**Input:** High-quality training set $D_{high}^{LV5}$, level 4 low-quality training set $D_{low}^{LV4}$, model $F(\Theta)$, threshold $t$
**Output:** Augmented training set $D_{augment}$
$D_{augment} \leftarrow D_{high}^{LV5}$;
$F(\Theta_{train}) \leftarrow$ train $F(\Theta)$ on $D_{high}^{LV5}$;
$D_{predict} \leftarrow$ predicted gene expression profiles for unreliable experiments by $F(\Theta_{train})$;
for each profile $d$ in $D_{predict}$ do
    $D_{bio} \leftarrow$ set of bio-replicate profiles corresponding to $d$ in $D_{low}^{LV4}$;
    $S \leftarrow$ set of similarity scores of $D_{bio}$ and $d$;
    $d_{max} \leftarrow argmax_{d \in D_{bio}} s_d \in S$;
    if $d_{max} \geq t$ then
        $D_{augment} \leftarrow D_{augment} \cup d_{max}$
    end
end
```

Supplementary Figures



(a)



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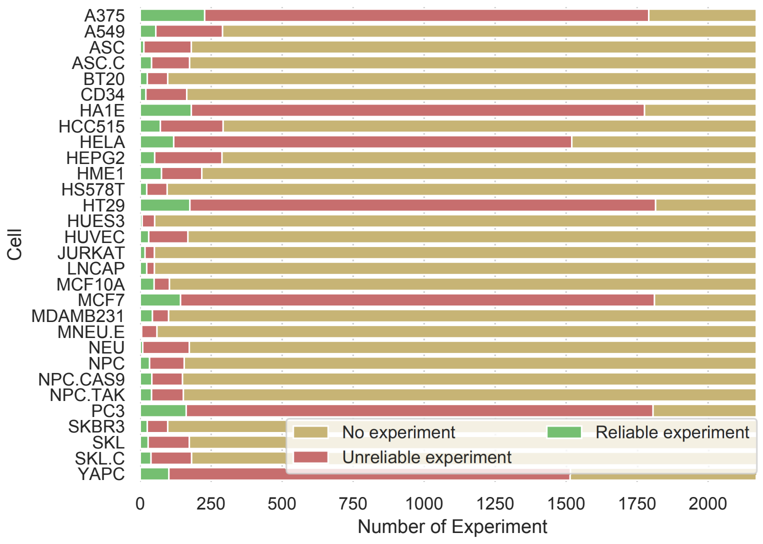
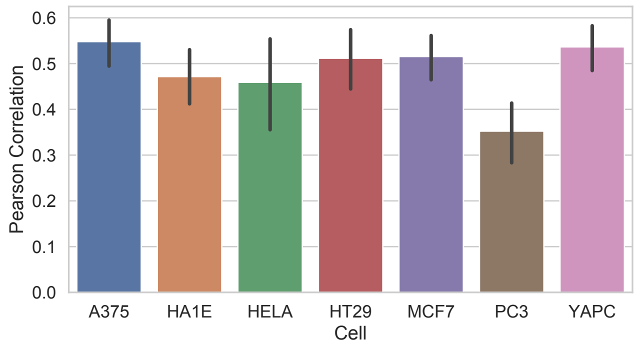


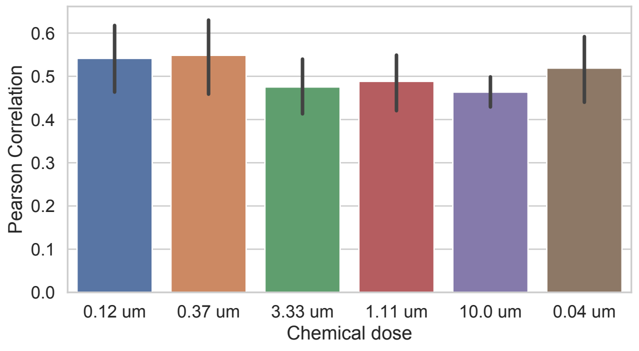
(c)

Supplementary Figure 1. Statistics of Bayesian-based peak deconvolution L1000 dataset (Experiments that have APC score < 0.7 are considered as unreliable experiments). **(a)** shows the density function of APC score for level 5 data. **(b)** shows the cumulative distribution of APC score for level 5 data. **(c)** shows the experiment statistics per cell line (time = 24h and dose size = 10 um).

Results of DeepCE on the testing test per cell line, dose size, and L1000 gene

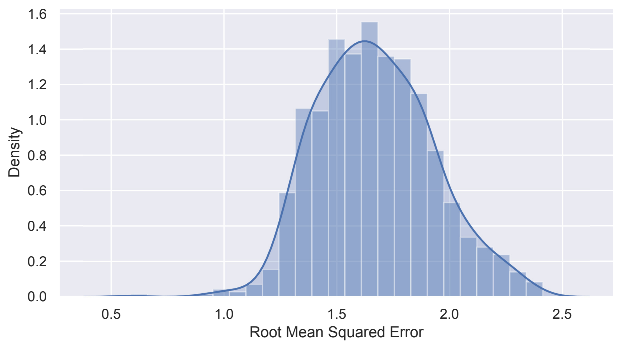


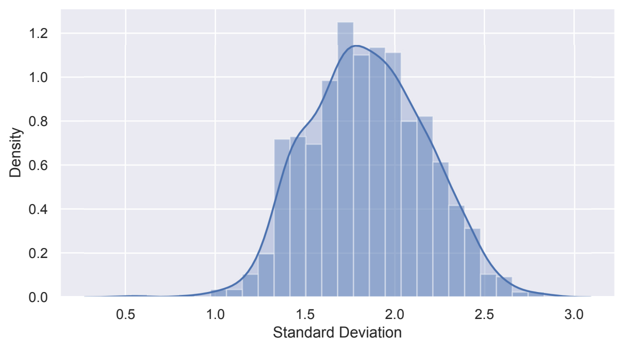
(a)



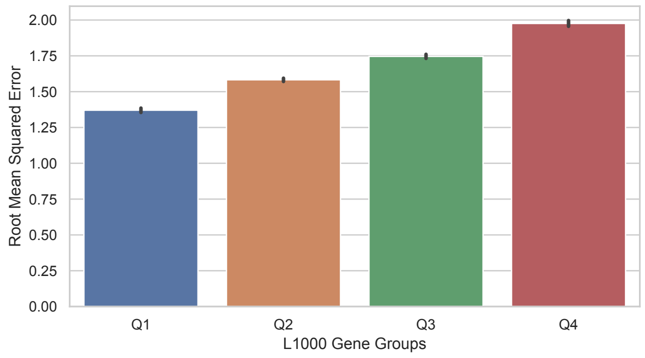
(b)

Supplementary Figure 2. Performance (Pearson correlation) of DeepCE model on the testing set per cell line and chemical dose size. **(a)** shows performance of DeepCE model per cell line. **(b)** shows performance of DeepCE model per chemical dose size





(a) **(b)**



(c)

Supplementary Figure 3. Performance (RMSE) of DeepCE over L1000 genes on the testing set. Note: We sort L1000 genes by their standard deviation on the testing set and divide them into 4 groups: Q1 (genes that have their standard deviation scores $<$ the first quartile in the sorted list), Q2 (genes that have their standard deviation scores from the first quartile to the second quartile in the sorted list), Q3 (genes that have their standard deviation scores from the second quartile to the third quartile in the sorted list), and Q4 (genes that have their standard deviation scores $>$ the third quartile in the sorted list). **(a)** shows the density function of prediction performance of DeepCE (RMSE) over L1000 genes on the testing set. **(b)** shows the density function of standard deviation of z-scores over L1000 genes on the testing set. **(c)** shows the average performance (RMSE) of DeepCE for each L1000 gene group. DeepCE achieves better performances on groups of genes that have lower standard deviation scores.

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