task_04

February 19, 2018

1 Introduction

For this Task we will imagine that we are working in a lab exploring the mechanics of Cas9 binding and cleavage. We will use data from genome-wide screens to develop a novel prediction method. We will then compare this method to the previously published methods.

```
In [1]: import sys
        sys.path.append('../')
        import crisprtree
        from crisprtree import utils
        from crisprtree import estimators
        from crisprtree import annotators
        from crisprtree import preprocessing
```

```
In [2]: from Bio import SeqIO
        import gzip
        import pandas as pd
        import numpy as np
        import matplotlib.pyplot as plt
        import seaborn as sbn
        import glob

        sbn.set(style = 'white', font_scale = 1.5)
        %matplotlib inline
```

2 Load data

For this example we will use results from a set of GUIDE-Seq experiments by Tsai et al. This methodology allows for an unbaised querying of the in vivo cleavage rates across the entire genome. The relavant files, directly produced by the `guideseq` package, are included in the `data/GUIDESeq` folder.

```
In [3]: files = sorted(glob.glob('data/GUIDESeq/*/*.tsv'))

        data = pd.concat([pd.read_csv(f, sep='\t').reset_index() for f in files],
                         axis=0, ignore_index=True)
        data.columns = list(data.columns[1:]) + ['_']
        data[['Filename', 'BED Name', 'Off-Target Sequence', 'Mismatches', 'bi.sum.mi', 'Target
```

Out[3]:

	Filename	BED Name	Off-Target Sequence	Mismatches	bi.sum.mi	Target Sequence
0	EMX1	chr1_23720618_87	AAGTCCGAGGAGAGGAAGAAAGG	3.0	87	GAGTCCGAGCAGAAGAAGAANGG
9	EMX1	chr10_58848728_2	GAGCACGAGCAAGAGAAGAAGGG	4.0	2	GAGTCCGAGCAGAAGAAGAANGG
21	EMX1	chr13_27769657_8	GAGTAGGAGCAGGAGAAGAAGGA	4.0	8	GAGTCCGAGCAGAAGAAGAANGG
22	EMX1	chr15_44109763_1412	GAGTCTAAGCAGAAGAAGAAGAG	3.0	1412	GAGTCCGAGCAGAAGAAGAANGG
25	EMX1	chr15_100292479_7	AAGTCCCGGCAGAGGAAGAAGGG	4.0	7	GAGTCCGAGCAGAAGAAGAANGG

In [4]:
```
# we only care about hits
hit_data = data.dropna(subset = ['Off-Target Sequence']).copy()
# remove ones that are the wrong size
hit_data = hit_data.loc[hit_data['Off-Target Sequence'].map(len) == 23, :].copy()

# Transform bi.sum.mi into a probability to normalize out the total number of reads per
hit_data['NormSum'] = hit_data.groupby('Cells')['bi.sum.mi'].transform(lambda x: x/np.ma

# Edit Target Sequence to remove NGG
hit_data['gRNA'] = hit_data['Target Sequence'].map(lambda x: x[:-3])

print('%i observations' % len(hit_data.index))

hit_data[['BED Name', 'Off-Target Sequence', 'Mismatches', 'bi.sum.mi', 'NormSum', 'gRNA
```

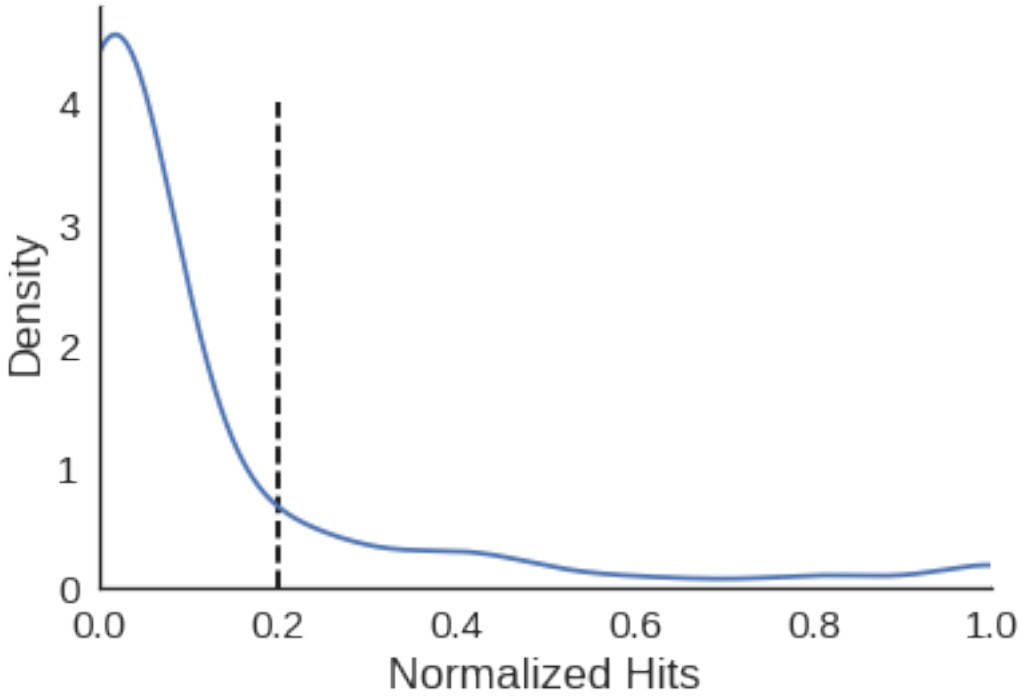
```
451 observations
```

Out[4]:

	BED Name	Off-Target Sequence	Mismatches	bi.sum.mi	NormSum	gRNA
0	chr1_23720618_87	AAGTCCGAGGAGAGGAAGAAAGG	3.0	87	0.018786	GAGTCCGAGCAGAAGAAGAA
9	chr10_58848728_2	GAGCACGAGCAAGAGAAGAAGGG	4.0	2	0.000432	GAGTCCGAGCAGAAGAAGAA
21	chr13_27769657_8	GAGTAGGAGCAGGAGAAGAAGGA	4.0	8	0.001727	GAGTCCGAGCAGAAGAAGAA
22	chr15_44109763_1412	GAGTCTAAGCAGAAGAAGAAGAG	3.0	1412	0.304902	GAGTCCGAGCAGAAGAAGAA
25	chr15_100292479_7	AAGTCCCGGCAGAGGAAGAAGGG	4.0	7	0.001512	GAGTCCGAGCAGAAGAAGAA

For this example we want to train a binary classifier (no cleavage vs cleavage) we need to converty the Normalized Sum into a binary response. For simplicity we will use a basic cutoff.

```
In [5]: fig, ax = plt.subplots(1,1)

        hit_data['NormSum'].plot(kind='kde', ax=ax)
        ax.set_xlabel('Normalized Hits')

        cutoff = 0.2
        ax.vlines(cutoff, 0, 4, linestyle = '--')

        ax.set_xlim(0, 1)
        ax.set_ylim(0, None)

        sbn.despine(ax=ax)
```



A cutoff of `0.2` will be used to distinguish between cleavage and no cleavage.

3 Training a new model

The `preprocessing.MatchingTransformer` converts gRNA-Target pairs into a binary vector that can be used as the input of and `sklearn` module.

```
In [6]: from sklearn.ensemble import GradientBoostingClassifier
        from sklearn.model_selection import cross_validate, StratifiedKFold

        # Convert gRNA-Target pairs into a binary "matching" vector
        X = preprocessing.MatchingTransformer().transform(hit_data[['gRNA', 'Off-Target Sequence
```

```
y = (hit_data['NormSum'] >= cutoff).values

# Evaluate the module using 3-fold cross-validation
grad_res = cross_validate(GradientBoostingClassifier(), X, y,
                          scoring = ['accuracy', 'precision', 'recall'],
                          cv = StratifiedKFold(random_state=0),
                          return_train_score=True)
grad_res = pd.DataFrame(grad_res)
```

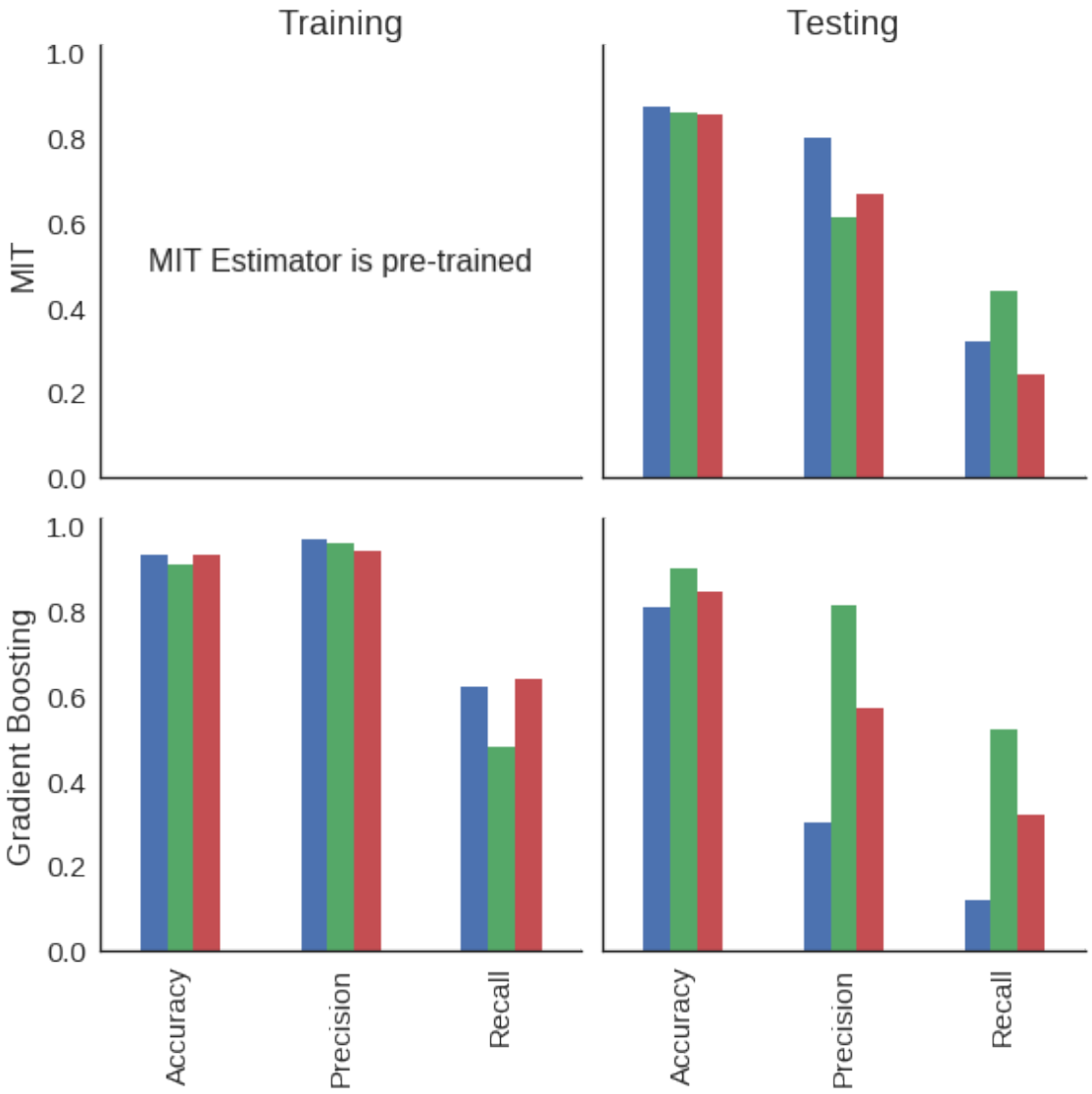
4 Evaluating built-in models

Since all `estimators` are subclasses of `sklearn` objects, they can be treated in the same way.

In [7]:
```
# Evaluate MITEstimator using 3-fold cross-validation
mit_res = cross_validate(estimators.MITEstimator(), X, y,
                         scoring = ['accuracy', 'precision', 'recall'],
                         cv = StratifiedKFold(random_state=0),
                         return_train_score=True)
mit_res = pd.DataFrame(mit_res)
```

In [8]:
```
fig, axs = plt.subplots(2, 2, figsize = (8, 8),
                        sharey=True, sharex=True)

order = ['accuracy', 'precision', 'recall']
train_cols = ['train_' + col for col in order]
test_cols = ['test_' + col for col in order]

for row, (name, df) in enumerate([('MIT', mit_res),
                                  ('Gradient Boosting', grad_res)]):

    train_ax, test_ax = axs[row, :]

    if row != 0:
        df[train_cols].T.plot(kind='bar', ax=train_ax, legend=False)
    else:
        train_ax.annotate('MIT Estimator is pre-trained',
                          xy = (0.5, 0.5), va = 'center', ha='center',
                          xycoords = 'axes fraction', fontsize = 16)
    df[test_cols].T.plot(kind='bar', ax=test_ax, legend=False)

    if train_ax.is_first_row():
        train_ax.set_title('Training')
        test_ax.set_title('Testing')
    else:
        train_ax.set_xticklabels(['Accuracy', 'Precision', 'Recall'])
        test_ax.set_xticklabels(['Accuracy', 'Precision', 'Recall'])

    train_ax.set_ylabel(name)
```

```
    sbn.despine(ax=train_ax)
    sbn.despine(ax=test_ax)

fig.tight_layout()
```



A Gradient Boosting model (using default parameters) has comparable accuracy and precision but lower recall when compared with the original MIT method.

5 Training multiple models

Using the same strategy, it is trivial to train other classifiers using the same data.

```
In [9]: from sklearn.neural_network import MLPClassifier
        from sklearn.neighbors import KNeighborsClassifier
```

```
        # Train different multiple different models
        models = [('GradientBoost', GradientBoostingClassifier()),
                  ('Neighbors', KNeighborsClassifier(n_neighbors=10)),
                  ('NeuralNetwork', MLPClassifier(hidden_layer_sizes=(100, 10),
                                                  random_state = 50,
                                                  learning_rate = 'adaptive')),
                 ]

        for name, model in models:
            classif = model.fit(X, y)
            hit_data[name] = classif.predict_proba(X)[:, 1]
```

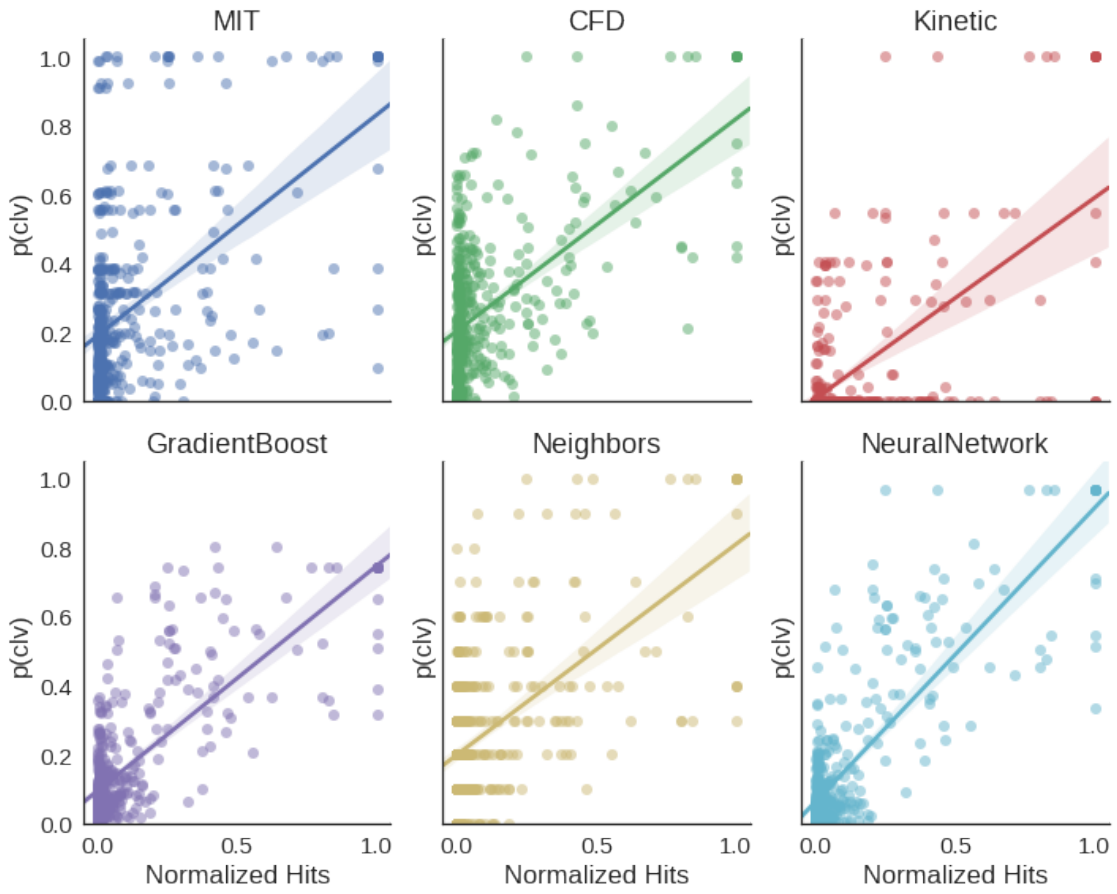
In [10]:
```
        # Evaluate the built-in models on the same data.
        ests = [('MIT', estimators.MITEstimator.build_pipeline()),
                ('CFD', estimators.CFDEstimator.build_pipeline()),
                ('Kinetic', estimators.KineticEstimator.build_pipeline())]

        for name, est in ests:
            hit_data[name] = est.predict_proba(hit_data[['gRNA', 'Off-Target Sequence']].values

        hit_data[['BED Name', 'Mismatches', 'NormSum', 'MIT', 'CFD', 'Kinetic', 'GradientBoost'
```

Out[10]:

	BED Name	Mismatches	NormSum	MIT	CFD	Kinetic	GradientBoost	Neighbors
0	chr1_23720618_87	3.0	0.018786	0.137229	0.163333	0.000151	0.104572	0.1
9	chr10_58848728_2	4.0	0.000432	0.190404	0.225064	0.000036	0.083170	0.2
21	chr13_27769657_8	4.0	0.001727	0.000000	0.012940	0.000000	0.051218	0.3
22	chr15_44109763_1412	3.0	0.304902	0.000000	0.240741	0.000000	0.734942	0.4
25	chr15_100292479_7	4.0	0.001512	0.101767	0.211750	0.000217	0.176817	0.4

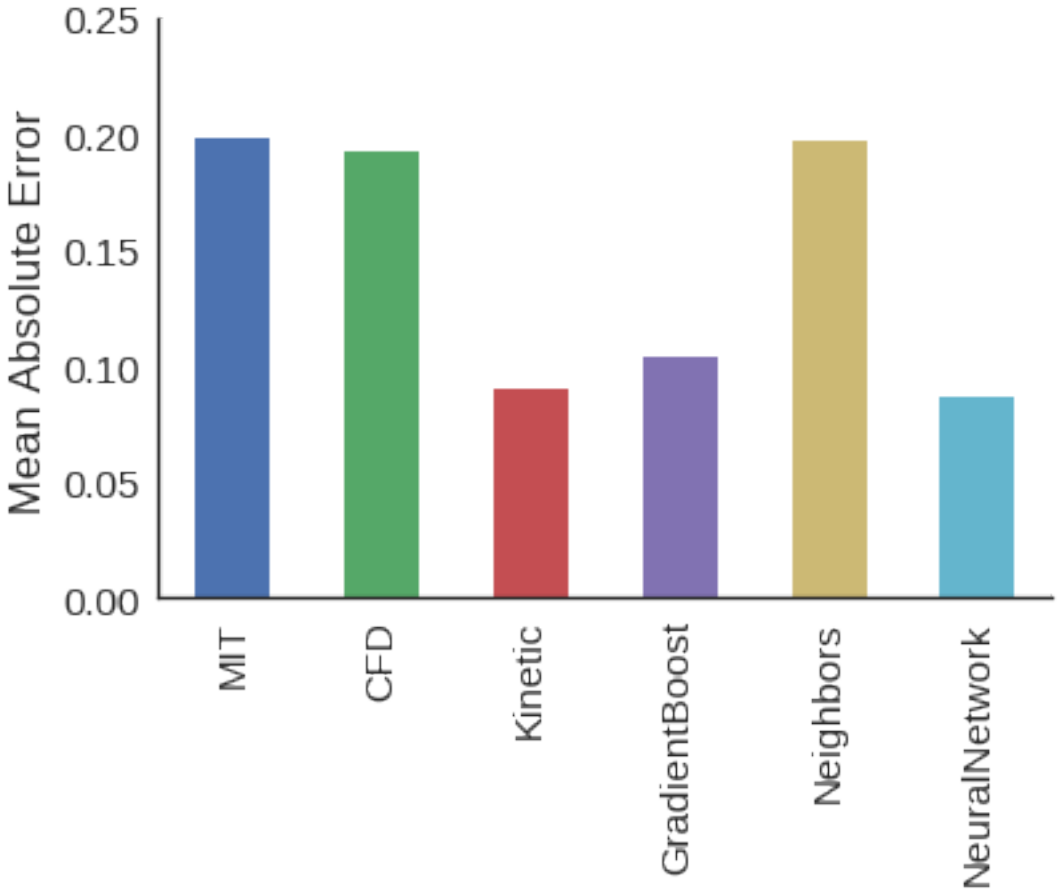
In [11]:
```
        from sklearn.metrics import mean_absolute_error

        fig, axs = plt.subplots(2, 3, figsize=(10, 8), sharex=True, sharey=True)

        cols = ['MIT',
                'CFD',
                'Kinetic',
                'GradientBoost',
                'Neighbors',
                'NeuralNetwork']
```

```
mabs = {}

for ax, col in zip(axs.flatten(), cols):

    sbn.regplot(data = hit_data,
                x = 'NormSum', y = col,
                ax = ax,
                scatter_kws = {'alpha': 0.5})
    error = mean_absolute_error(hit_data['NormSum'], hit_data[col])
    mabs[col] = error
    ax.set_ylim(0, 1.05)
    sbn.despine(ax=ax)
    if col != 'Mismatches':
        ax.set_title(col)
        ax.set_ylabel('p(clv)')

    if not ax.is_last_row():
        ax.set_xlabel('')
    else:
        ax.set_xlabel('Normalized Hits')

fig.tight_layout()

error = pd.Series(mabs)
```



From this data we can see that, qualitatively, the newly trained methods perform as well as previously published methods.

```
In [12]: fig, ax = plt.subplots(1,1)
         order = ['MIT', 'CFD', 'Kinetic', 'GradientBoost', 'Neighbors', 'NeuralNetwork']
         error[order].plot(kind='bar', ax=ax)
         ax.set_ylabel('Mean Absolute Error')
         ax.set_ylim(0, 0.25)
         sbn.despine(ax=ax)
```



Gradient Boosting and Neural Network models can "outperform" the previously published when measuring the `mean absolute error` between prediction and observation. This implies that there may be an advantage to using more advanced learning techniques.

However, keep in mind: 1. We're training on only 451 observations for 21 features. There may be some degree of over-fitting. 2. These models were trained on all 451 observations. There is no held-out dataset for this analysis. 3. We have only used data from one experiment. Training a rubust model requires multiple data sources.

In [ ]: