task_02

February 19, 2018

1 Introduction

In this Task we will continue to imagine that we are in a lab studying the newly sequenced Thermofilum sp. NZ13-TE1 strain. After the success of the EGFP knockdown experiment we are tasked with creating a protospacer library containing 5 protospacers targeting each gene with minimal off-target effects.

```
In [13]: import sys
         sys.path.append('../')
         import crisprtree
         from crisprtree import utils
         from crisprtree import estimators
         from crisprtree import annotators
```

```
In [22]: from Bio import SeqIO
         from Bio.Seq import reverse_complement
         import gzip
         import pandas as pd
         import numpy as np
         import matplotlib.pyplot as plt
         import seaborn as sbn
         from scipy.stats import hmean

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

2 Load sequences

Standard Biopython tools can be used to load the Thermofilum sp. NZ13-TE1 genome and the AddGene plasmid.

```
In [15]: with open('data/GCA_002855745.1_ASM285574v1_genomic.gbff') as handle:
             genome = list(SeqIO.parse(handle, 'genbank'))[0]
```

3 Algorithm

The `crisprtree` library allows us to use a simple algorithm.

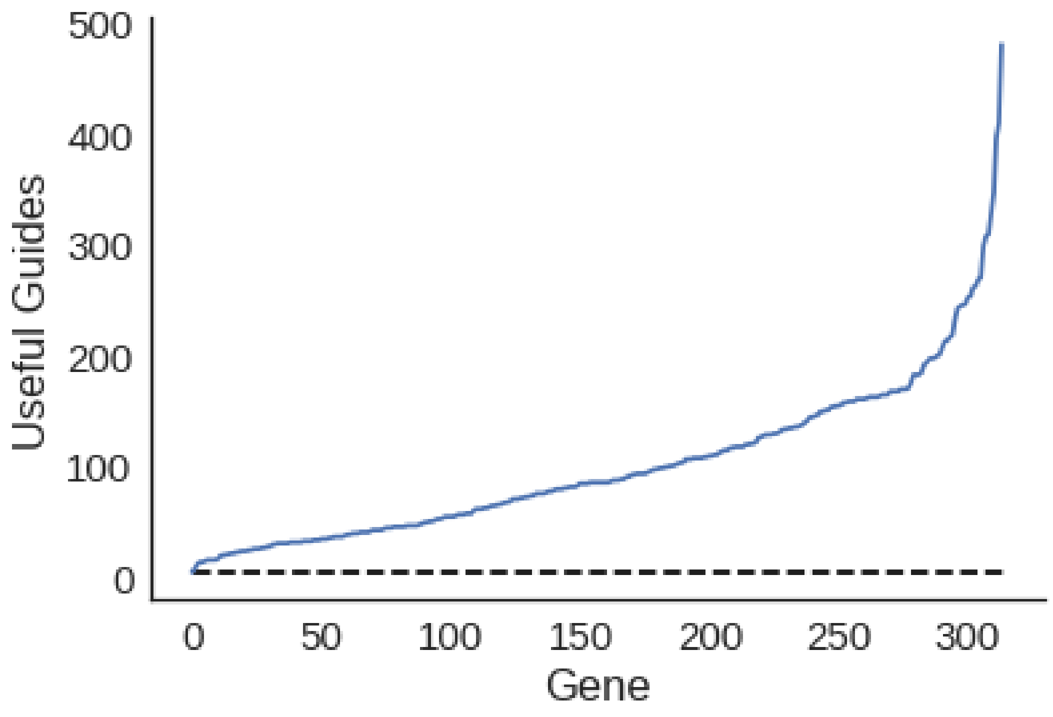
for each gene in Thermofilum: 1. Extract possible targets 2. Scan the genome (minus the target gene) for off-target hits 3. Exclude high-risk protospacers 4. Save 5 that pass the filter

```
In [31]: estimator = estimators.CFDEstimator.build_pipeline()

         library_grnas = []

         gene_info = []

         for feat in genome.features:
             if feat.type == 'CDS':
                 # Only target genes

                 # Get info about this gene
                 product = feat.qualifiers['product'][0]
                 tag = feat.qualifiers['locus_tag'][0]
                 gene_record = feat.extract(genome)

                 # Get protospacers
                 possible_targets = utils.extract_possible_targets(gene_record)

                 # Score off-target hits
                 genome_minus_gene = genome[:feat.location.start] + genome[feat.location.end:]
                 possible_binding = utils.cas_offinder(possible_targets, 5, seqs = [genome_minus
                 possible_binding['Score'] = estimator.predict_proba(possible_binding.values)

                 # Aggregate and sort off-target scan
                 offtarget_scores = possible_binding.groupby('gRNA')['Score'].agg('max')
                 offtarget_scores.sort_values(inplace=True)


                 # Save information
                 gene_info.append({'Product': product,
                                   'Tag': tag,
                                   'UsefulGuides': (offtarget_scores<=0.25).sum()})


                 for protospacer, off_score in offtarget_scores.head().to_dict().items():
                     location = genome.seq.find(protospacer)
                     strand = '+'
                     if location == -1:
                         location = genome.seq.find(reverse_complement(protospacer))
                         strand = '-'

                     library_grnas.append({'Product': product,
                                           'Tag': tag,
                                           'Protospacer': protospacer,
                                           'Location': location,
                                                   'Strand': strand,
                                                   'Off Target Score': off_score})

        gene_df = pd.DataFrame(gene_info)

        library_df = pd.DataFrame(library_grnas)
```

4 Sucess?

Our goal was to create 5 protospacers for each gene. Were we able to?

```
In [35]: useful_guides = gene_df['UsefulGuides'].sort_values()
         fig, ax = plt.subplots(1,1)
         ax.plot(useful_guides.values)
         ax.set_ylabel('Useful Guides')
         ax.set_xlabel('Gene')
         ax.hlines(5, 0, len(useful_guides), linestyle = '--')
         sbn.despine(ax=ax)
```



```
In [34]: gene_df.query('UsefulGuides < 5')

Out[34]:                   Product          Tag  UsefulGuides
         150  hypothetical protein  B7L53_00760             4
```

Only 1 hypothetical protein had fewer than 5 useful targets.

```
In [ ]:
```