task_01

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1 Introduction

In this Task we will imagine that we are in a lab studying the newly sequenced Thermofilum sp. NZ13-TE1 strain. We are tasked with creating a set of gRNAs which will target an inserted plasmid (Addgene Plasmid 54622) expressing an enhanced GFP (EGFP). We will find a set of protospacers that are specific to the EGFP coding region and excluding those with a potental off-target effect within the Thermofilum genome.

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

```
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

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

        with open('data/addgene-plasmid-54622-sequence-158642.gbk') as handle:
            plasmid = list(SeqIO.parse(handle, 'genbank'))[0]
```

```
        for rec in plasmid.features:
            if rec.qualifiers.get('product', [''])[0].startswith('enhanced GFP'):
                print('Found')
                egfp_feature = rec
                break

        egfp_record = egfp_feature.extract(plasmid)
```

```
Found
```

3 Extract possible targets

Using the EGFP sequence the `crisprtree` can extract all potential protospacers by finding all sequences upstream of a PAM sequence on each strand.

```
In [4]: possible_targets = utils.extract_possible_targets(egfp_record, pams=('NGG'))
        print('%i possible targets' % len(possible_targets))
```

```
1400 possible targets
```

4 Exclude Off-targets

In order to avoid effecting the bacterium with the gRNA it is important to check for potential off-target effects in the Thermofilum genome. The `CFDEstimator` can be used to assess this likelihood taking into account the position and nucleotide specific penalties for mismatches.

```
In [5]: #Build the predictor
        estimator = estimators.CFDEstimator.build_pipeline()
```

`cas-offinder` is used to rapidly find all positions in the Thermofilum genome that are <=5 mismatches for each of the potential gRNAs.

```
In [6]: possible_binding = utils.cas_offinder(possible_targets, 5, seqs = [genome])

        # Score each hit across the genome
        possible_binding['Score'] = estimator.predict_proba(possible_binding.values)
```

Higher numbers imply a larger likelihood of binding. Since we are looking for gRNAs with no off-target effects we can summarize a gRNA by its worst-case off-target hit.

```
In [7]: results = possible_binding.groupby('gRNA')['Score'].agg('max')
        results.sort_values(inplace=True)
        results.head()
```

```
Out[7]: gRNA
        GTGATCGCGCTTCTCGTTGG    0.0
        GAGCTGCACGCTGCCGTCCT    0.0
        GCCGAGGTGAAGTTCGAGGG    0.0
        TTCATCTGCACCACCGGCAA    0.0
        TCCTTGAAGAAGATGGTGCG    0.0
        Name: Score, dtype: float64
```

Here are 5 protospacers that are identical to the EGFP plasmid gene but have no predicted effect across the Thermofilum genome.

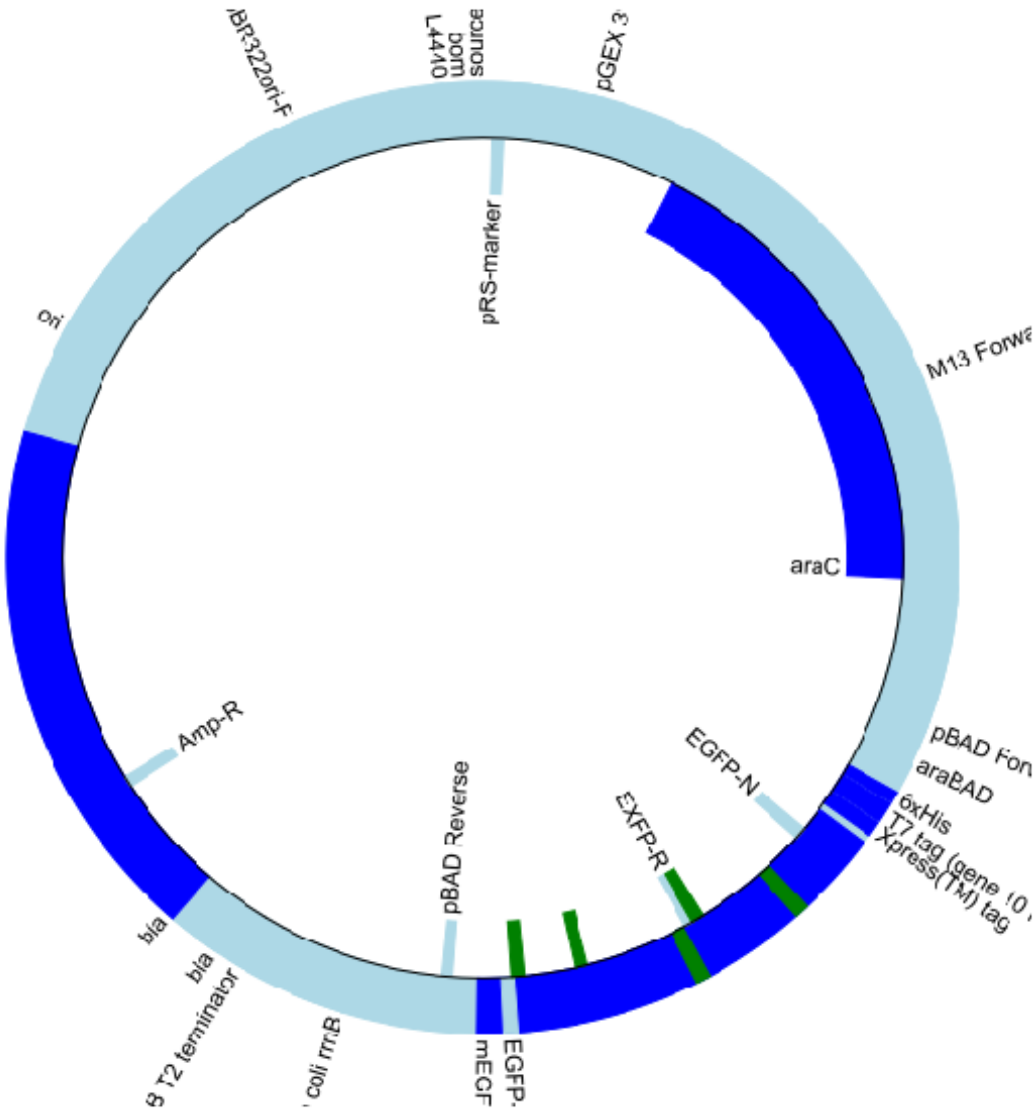
5 Visualization

The `crisprtree` module is fully back-compatable with the BioPython module. As such, building visualizations becomes trivial.

```
In [8]: from Bio.Seq import reverse_complement
        # Label the top-5 best protospacers on the plasmid backbone.

        for key, val in results.head().to_dict().items():
            annotators.annotate_grna_binding(key, plasmid,
                                             None,
                                             exhaustive = True,
                                             extra_qualifiers = {'Off Target Score': val})
```

```
In [9]: from reportlab.lib import colors
        from reportlab.lib.units import cm
        from Bio.Graphics import GenomeDiagram
        from Bio import SeqIO

        gd_diagram = GenomeDiagram.Diagram("AddGene Plasmid")
        gd_track_for_features = gd_diagram.new_track(1, name="Annotated Features")
        gd_feature_set = gd_track_for_features.new_set()

        for feature in plasmid.features:
            if feature.type == 'CDS':
                # Label genes in Blue
                color = colors.blue
            elif 'gRNA' in feature.qualifiers:
                # Label gRNAs in Green
                color = colors.green
            else:
                # Everything else in Light Blue
                color = colors.lightblue
            gd_feature_set.add_feature(feature, color=color,
                                       label=True, label_size=12)
```

```
gd_diagram.draw(format="circular",  pagesize=(20*cm,20*cm),
                fragments=4, start=0, end=len(plasmid), circle_core=0.7)

gd_diagram.write("plasmid_circular.png", "png")

fig, ax = plt.subplots(1,1, figsize= (10, 10))

img = plt.imread("plasmid_circular.png")
ax.imshow(img)
ax.set_xticks([])
ax.set_yticks([])
sbn.despine(ax=ax, left=True, bottom=True)
```



In [ ]: