

Supplementary Information

Supplementary Table 1 – oligonucleotides (attached excel file)

Supplementary Table 2 – sgRNA targets (attached excel file)

Supplementary Table 3 – PAM MiSeq data (attached excel file)

Supplementary Sequences - plasmids used in this study

Supplementary Software - for analyzing PAM depletion MiSeq data

Supplementary Sequences - plasmids used in this study

Name	Addgene ID	Description
BPK764	65767	T7-humanSpCas9-NLS-3xFLAG-T7-Bsalcassette-Sp-sgRNA
MSP712	65768	T7-humanSpdCas9(D10A/H840A)-NLS-3xFLAG-T7-Bsalcassette-Sp-sgRNA
MSP1673	65769	T7-humanSt1Cas9-NLS-T7-BspMlcassette-St1-sgRNA
BPK2101	65770	T7-humanSaCas9-NLS-3xFLAG-T7-Bsalcassette-Sa-sgRNA
p11-lacY-wtx1 ¹⁵	-----	BAD-ccDB-Amp ^R -AraC-lacY(A177C)
JDS246	43861	CMV-T7-humanSpCas9-NLS-3xFLAG
MSP469	65771	CMV-T7-humanSpCas9(D1135V/R1335Q/T1337R)-NLS-3xFLAG (VQR variant)
MSP680	65772	CMV-T7-humanSpCas9(D1135E/R1335Q/T1337R)-NLS-3xFLAG (EQR variant)
MSP1101	65773	CMV-T7-humanSpCas9(D1135V/G1218R/R1335E/T1337R)-NLS-3xFLAG (VRER variant)
MSP977	65774	CMV-T7-humanSpCas9(D1135E)-NLS-3xFLAG
MSP1594	65775	CAG-humanSt1Cas9-NLS
BPK2139	65776	CAG-humanSaCas9-NLS-3xFLAG
BPK1520	65777	U6-BsmBlcassette-Sp-sgRNA
BPK2301	65778	U6-BsmBlcassette-St1-sgRNA
VVT1	65779	U6-BsmBlcassette-Sa-sgRNA

BPK764: T7-humanSpCas9-NLS-3xFLAG-T7-Bsalcassette-Sp-sgRNA

T7 promoters colored in **green**, human codon optimized *S. pyogenes* Cas9 colored in **blue**, NLS **underlined**, 3xFLAG tag in **bold**, Bsal sites **underlined**, sgRNA colored in **purple**, T7 terminator colored in **red**

TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTGTAGAAATAATTTTGAACCTTTAATAAGGAGATATACCATGGATAAAAAAGTATTCT
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GACAAGAAACATGAACGGCACCCCATCTTTGAAACATAGTAGATGAGGTGGCATATCATGAAAGTACCCAACGATTATCACCTCAGAAAAAGCTAGTTG
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CGGAGGGTTTTCAAAGGAATCGATTCTTCCAAAAAGGAATAGTGATAAGCTCATCGCTCGTAAAAAGGACTGGGACCCGAAAAAGTACGGTGGCTTCGATAG
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AATAACCTAGCATAAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTTG

MSP712: T7-humanSpCas9(D10A/H840A)-NLS-3xFLAG-T7-Bsalcassette-Sp-sgRNA

T7 promoters colored in **green**, human codon optimized *S. pyogenes* Cas9 colored in **blue**, modified codons in **pink**, NLS underlined, 3xFLAG tag in **bold**, BsaI sites underlined, sgRNA colored in **purple**, T7 terminator colored in **red**

TAATACGACTCACTATAGGGGAATTTGTAGCGGATAACAATTCCTGTAGAAATAATTTGTTAACTTTAATAAGGAGATATACCATGGATAAAAAGTATTCT
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AGCGCCCGCTCTCTAAATCCCGACGGCTAGAAAACCTGATCGCACAATTACCCGGAGAGAAGAAAAATGGGTTGTTCCGTAACCTTATAGCGCTCTCACTA
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ACACCTAGTGGTCTCGCTTTAGAGCTAGAAATAGCAAGTTAAATAAGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGCCTGCGTGAAGC
AATAACCTAGCATAAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTTG

MSP1673: T7-humanSt1Cas9-NLS-T7-BspMlcassette-St1-sgRNA

T7 promoters colored in **green**, human codon optimized *S. thermophilus*1 Cas9 colored in **blue**, NLS underlined, BspMI sites underlined, sgRNA colored in **purple**, T7 terminator colored in **red**

TAATACGACTCACTATAGGGGAATTTGTAGCGGATAACAATTCCTGTAGAAATAATTTGTTAACTTTAATAAGGAGATATACCATGGGACGCGACCTGGT
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CAGGCTGAGAACAACTGTTGCGGCCACCAACCGGAGGCTGCGCGCTTCTGCGCGGAGAAAGCACCGGCGGCTGCGGCTGAACCGCTGTTCCGAG
GAGAGCGGCTGATCAGCGACTTACCAAGATCAGCATCAACCTGAACCCCTACGCTGCGCGTGAAGGGCCTGACCGACAGCTGACCAAGCAGGAGC
TGTTTCATCGCCCTGAAGAATGTTGAAGCACCAGCGGCATCAGCTACCTGGACGACGCCAGCGACGCGCAACAGCAGCGTGGGCGACTACGCCAGAT
CGTGAAGGAGAACAGCAGCTGAGAGCAAGACCCCGGCCAGATCCAGCTGAGAGCGCTACCAGACCTACGGCCAGCTGCGCGGCGACTTACCGCT

JDS246: CMV-T7-humanSpCas9-NLS-3xFLAG

Human codon optimized *S. pyogenes* Cas9 colored in **blue**, NLS underlined, 3xFLAG tag in **bold**

ATGGATAAAAAGTATTCTATTGGTTTATAGACATCGGCACATAATTCGGTTGGATGGGCTGTCATAACCGATGAATACAAAGTACCTTCAAAGAAATTTAAGGTGTT
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MSP469: CMV-T7-humanSpCas9(D1135V/R1335Q/T1337R)-NLS-3xFLAG (VQR variant)

Human codon optimized *S. pyogenes* Cas9 colored in **blue**, modified codons in **red**, NLS underlined, 3xFLAG tag in **bold**

ATGGATAAAAAGTATTCTATTGGTTTATAGACATCGGCACATAATTCGGTTGGATGGGCTGTCATAACCGATGAATACAAAGTACCTTCAAAGAAATTTAAGGTGTT
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CATCAAAATCCACTTAGGCGAATTCGATGCTATACCTAGAAAGCAGGAGGATTTATTCGTTCTCCTCAAAAGACAATCGTGAAAGATTTGAGAAAATCCTAACCTT
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TACGTGAGCAGCGGAAAAATATTATCCATTGTGTTACTCTTACCAACCTCGGCGCTCCAGCGGCATTCAAGTATTTTGACACAACGATAGATCGCAAA**CAGTA**
CAGATCTACCAAGGAGGTGCTAGACGCGACACTGATTACCAATCCATCACGGGATTATATGAACTCGGATAGATTTGTACAGCTTTGGGGGTGACGGATC
CCCCAAGAAGAAGAGGAAAGTCTCGAGCG**ACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGTGA**

MSP680: CMV-T7-humanSpCas9(D1135E/R1335Q/T1337R)-NLS-3xFLAG (EQR variant)
Human codon optimized *S. pyogenes* Cas9 colored in **blue**, modified codons in **red**, NLS underlined, 3xFLAG tag in **bold**

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AC**AGAT**CTACCAAGGAGGTGATGAGCGGACACTGATTACCAATCCATCAGGGGATTATGAACTCGGATAGTTTGTACACGATTTGGGGGTGACGGAT
CCCCAAGAAGAAGAGGAAAGTCTCGAGCG**ACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGTGA**

MSP1101: CMV-T7-humanSpCas9(D1135V/G1218R/R1335E/T1337R)-NLS-3xFLAG (VRER variant)
Human codon optimized *S. pyogenes* Cas9 colored in **blue**, modified codons in **red**, NLS underlined, 3xFLAG tag in **bold**

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MSP977: CMV-T7-humanSpCas9(D1135E)-NLS-3xFLAG

(D1135E variant)

Human codon optimized *S. pyogenes* Cas9 colored in **blue**, modified codon in **red**, NLS underlined, 3xFLAG tag in **bold**

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MSP1594: CAG-humanSt1Cas9-NLS

Human codon optimized *S. thermophilus*1 Cas9 colored in **blue**, NLS underlined

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CTGCGAGGACACTTCGCGCCCCACAAGATCGACACCAAGGTGAGCGTGGTGCAGCGGCCAGTTTACCAGCCAGCTGCGCCGCCACTGGGGCATCGAGAAG
CAGCGCAAGCTGACGACGACGCGCCGACGAGCCTGATCATTGCGGCTTCAGCCAGCTGGAACCTGTGGAGAAGCAAGCCCTGAGCAACAAGAGTATCT
CAGCGAGGACAGCTGCTGGACATCGAGACCGCGGAGCTGATCAGCGACGACGAGTACAAGGAGAGCGTGTTCAGGCCCTTACCAGCACTTCGTGGAC
ACCCTGAAGAGCAAGGAGTTCGAGGACAGCATCCTGTTGAGCTACCAGGTGGACAGCAAGTTCAACCGCAAGATCAGCGACGCCACCATCTACGCCACCCG
CCAGGCCAAGCTGAGGACGACGCGCCGACGAGACCTACGCTGGGCAAGTCAAGGACATCTACCCAGGACGCGCTACGACGCGCTTTCATGAAGAT
TACAAGAAGGACAAGAGCAAGTTCCTGATGTACCGCCACGACCCCGAGACCTTCGAGAAGGTGATCGAGCCATCCTGGAGAATACCCCAACAAGCAGAT
CAACGAGAAGGCAAGGAGGTGCCCTGCAACCCCTTCCTGAAGTACAAGGAGGAGCAGCGCTACATCCGAAGTACAGCAAGAAGGGCAACGCGCCCGAG
ATCAAGAGCCTGAAGTACTAGCAGCAAGCTGGGCAACCATCTGACATCACCCCAAGGACGACCAACAAGGTGGTGGTGCAGACGCTGAGCCCTTCAAGGAGT
GGCGCGCGCAGCTGTACTTCAACAAGACCAACCGGCAAGTACGAGATCCTGGGCGTGAAGTACGCCGATCTGCAGTTTGAGAAAGGCACAGGCACCTACAA
GATCAGCCAGGAGAAGTACAACGACATCAAGAAGAAGGAGGCGTGGACGCGACGAGTTCAGTTTCAACCTGTACAAGAACGACCTTCTGCTGGTGA
AGGAGACGCTGAGGACGACGACGCTGTTCCGCTTCCTGAGCGCAGCTGCCCCAGGACGACGAGTTCAGGAGTGAAGGACGCTGAGGAGTGAAGGAGTGA
GTTGAGGGCGCGAGGCCCTGATCAAGGTGCTGGGCAACGTGGCCAACAGCGGCCAGTGAAGAAGGCCCTGGGCAAGAGCAACATCAGCATCTACAA
GGTGCAGCACCGAGCTGCTGGGCAACCAGCAGCATCATCAAGAACGAGGCGGACAGGCCAAGTTGGACTTCAGCAGGGCTGACCCCAAGAAGAAGAGGAAG
GTGTGA

BPK2139: CAG-humanSaCas9-NLS-3xFLAG

Human codon optimized *S. aureus* Cas9 colored in **blue**, NLS underlined, 3xFLAG tag in **bold**

ATGGGGAAACGGAACATCATCCTGGGGCTTGACATTGGGATAACCAGCGTTGGCTACGGAATTATTGATTATGAGACACGCGATGTGATTGACGCCGGGGT
TAGGCTGTTCAAAGAGGCCACCGTTGAAAACAACGAGGGAAGCAGGAGTAAGCGCGGAGCAAGAAGACTCAAGCGCAGCAGCGAGACGATTCAGAGG
GTGAAAAAGCTGCTCTTCGATTACAATCTCTGACCGATCATATGAGCTGAGCGGAATCAACCCCTACGAGGCGCGAGTGAAGAGGCTTTCCAGAGAGT
GTCCGAAGAGGAGTTCTCCGCGCGCTTGTGCACTGGCCAAACGGAAGGGGGTTCACAATGTAACGCAAGTGAAGGAGGACACGGGCAATGAACCTAGT
ACGAAAGAACAGATCAGTAGGAACCTAAGGCTCTCGAAGAGAATACGTGCTGAGTTGACGCTTGAGAGCTGAAAAAAGACGGCGAAGTACGCGGATC
TATTAATAGTTTCAAGACTTCAGATTACGTAAAGGAAGCCAGCAGCTCCTGAAAGTACAGAAAGCGTACCATCAGCTCGATCAGAGCTTCATCGATACCTAC
ATAGATTTGCTGGAGACACGAGGACATACTACGAGGGCCAGGGGAAGGATCTCCTTTTGGGTGGAAGGACATCAAGGAATGGTACGAGATGCTTATGGG
ACATTGTACATATTTCCGAGGAGCTCAGGAGCGTCAAGTACGCTACAATGCCACCTGTACAATGCCCTCAATGACCTCAATAACCTCGTGATTACCAG
GGACGAGAAGCAGGAAGCTGGAGTACTGAAAAGTTCCAGATTACGAGTACGAGTGAAGTGTGTTTGAAGCAGAAGAAGGCCGACACTTAAAGCAAGTTGCAAGGAAAT
CCTCGTGAATGAGGAAGATATCAAGGATACAGAGTGACAAGTACAGGCAAGCCCGAGTTCAACAATCTGAAGGTGTACCAGATATTAAGGACATAACCCG
ACGAAAGGAGATAATCGAAAACGCTGAGCTCCTCGATCAGATCGCAAAAATTTTACCATCTACCAGTCTAGTGAGGACATTCAGGAGGAACCTGACTAATCTG
AACAGTGAGCTCACCCAAAGAGGAAATGAGCAGATTTCAAACCTGAAAGCTACACCGGAGCGACAATCTGAGCCTCAAGCAATCAACCTCATTTCTGGAT
GAACCTTTGGCACACAAATGACAACCAAAATGCCATATTAACCCGCTGAAACTGGTGCCAAAAAAGTGGATCTGTACAGCAAAAGGAAATCCCTACAACCT
TGGTTGACGATTTTATTCTGTCCCGCTTGTCAAGCGGAGCTTCATCCAGTCAATCAAGGTGATCAATGCCATCATTAAAAAATACGGATTGCCAAACGATATA
ATTACGAGCTTTGACGAGGAAAGTGGAAATTTAAGAAGGAACGAAACAAAGGATACAAGCACCATGCTGAGGATGCTTTGATCATCGTCAACGCGGACTT
TATCTTTAAGGAATGAAAAAGCTGGATAAGGCAAGAAAGTGATGAAAAACCAGATGTTGAGGAGAAGCAGGCAGAGTCAATGCTGAGATCGAGACAG
AGCAGGAATACAAGGAAATTTTATCACCCCTCATCAGATTAACACATAAAGGACTTCAAAGACTATAAATCTCTCATAGGGTGGACAAAAAACCCAAATCGC
GAGCTCATTATGACACCCTGTACTCAACACGGAAGGATGATAAAGGTAAATACCTTGATTGTGAATAATCTTAATGGATTGTATGACAAGATAACGACAAGCT
CAAGAAGCTGATCAACAAGTCTCCAGAGAAGCTCCTTATGTATCACACGACCCACAGACTTATCAGAAATTTGAACTGATCATGGAGCAATACGGGGATGA
GAAGAACCCTCTACAAATATTATGAGGAAACAGGTAATTACCTGACCAAGTACTCCAAGAAGGATAACGGACCAAGTATCAAAAAGATAAAGTACTATGGC
AACAACTTAATGCGCATTTGGACATAACTGACGATTACCCCAATTCGAAAAAGGTTGTGAAGCTCTCCCTGAAGCCTTATAGATTGACGTGTACCTGG
ATAATGGGGTTTATAAATTCGTACCGTGAAAAATCTGGACGTGATCAAAAAGGAGAATATTATGAAGTAAACTCAAAGTGCTATGAGGAGGCGAAGAAGCT
GAAGAAGATCTCCAATCAGGCCGAGTTCATCGCTTCTCTATAATAACGATCTCATCAAGTCAATGGAGAGCTTTATCGCGTCATTGGTGTGAACAATGAC
TTGCTGAACAGGATCGAAGTCAATATGATAGACATTACCTACCGGGAGTATCTCGAAACATGAATGATAAACCGGCCCTCGCATCATCAAGCAATCGCAT
CTAAACTCAGTCAATAAAAAAGTACTCTACCGATATCTGGGAATCTCTATGAAGTGAAGTCAAAGAAGCACCACAAATCATTAAAAAAGGTGGATCCCG
CAAGAAGAAGAGGAAAGTCTCGAGCG**GACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGTAA**

BPK1520: U6-BsmBcassette-Sp-sgRNA

U6 promoter in **green**, BsmBI sites underlined, *S. pyogenes* sgRNA colored in **purple**, U6 terminator underlined
TGTACAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGTTCGGGCAAGGAGGGCCATTTCCCATGATTCTTCATATTTG
CATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAACACCAAGGATATTAGTACAAAATACGTGACGTAGAAAAGTAATAATTTCTTGGG
TAGTTTGCAAGTTTAAATATGTTTAAATGGACTATCATGCTTACCGTAACTTGAAGTATTTTCGATTCTTGGCTTTATATCTTGTGGAAGGACGCA
ACACCGGAGACGATTAATCGGCTCC**GTTTTAGAGGTAGAATAGCAAGTTAAATAGGGCTAGTCCGTTACAACTTGAAAAAGTGGCACCGGAGCGGT**
TTTTT

BPK2301: U6-BsmBIcassette-St1-sgRNA

U6 promoter in **green**, BsmBI sites underlined, *S. thermophilus*1 sgRNA colored in **purple**, U6 terminator underlined

TGTACAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTG
CATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG
TAGTTTGCAGTTTTAAATATGTTTTAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTGATTTCTTGGCTTTATATATCTTGTGGAAGGACGAA
ACACCCGAGACGATTAATGCGTCTCGGTTTTGTACTCTCAAGATTTAGTAACTGTACAACGAACTTACACAGTTACTTAAATCTTGCAAGCTACAAAGA
TAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGGTGTTTTT

VVT1: U6-BsmBIcassette-Sa-sgRNA

U6 promoter in **green**, BsmBI sites underlined, *S. aureus* sgRNA colored in **purple**, U6 terminator underlined

TGTACAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTG
CATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGG
TAGTTTGCAGTTTTAAATATGTTTTAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTGATTTCTTGGCTTTATATATCTTGTGGAAGGACGAA
ACACCCGAGACGATTAATGCGTCTCGGTTTTAGTACTCTGTAATTTAGGTATGAGGTAGACGAAAATTGTACTTATACCTAAAATTACAGAATCTACTAAACA
AGGC AAAATGCCGTGTTTATCTCGTCAACTTGTTGGCGAGATTTTTTT

Supplementary Software - for analyzing PAM depletion MiSeq data

Run in the command prompt (in the directory containing the file) using the command "python PAM_depletion.py"

```
-----
import numpy as np
import pandas as pd
import glob
import fnmatch
import os
from collections import Counter
from Bio.Seq import Seq
from Bio import SeqIO
import itertools
import re
from pandas import ExcelWriter
import Tkinter, tkFileDialog

__author__ = "Ved V. Topkar"
__version__ = "1.0"

"""
IUPAC_notation_regex describes a mapping between certain base characters and the relevant regex string
(Useful for parsing out ambiguous base strings)
"""
IUPAC_notation_regex = {
    'N': '[ATCG]',
    'Y': '[CT]',
    'R': '[AG]',
    'W': '[AT]',
    'S': '[CG]',
    'A': 'A',
    'T': 'T',
    'C': 'C',
    'G': 'G'
}

def ambiguous_PAMs(length):
    """
    Given an inputted length, return a list of strings describing all possible PAM sequences
    NOTE: Returned strings include ambiguous base characters
    """
    permutations = itertools.product(['N', 'A', 'T', 'C', 'G'], repeat=length)
    PAMs = []

    for item in permutations:
        PAMs.append(''.join(item))

    return PAMs

def unambiguous_PAMs(length):
    permutations = itertools.product(['A', 'T', 'C', 'G'], repeat=length)
    PAMs = []

    for item in permutations:
        PAMs.append(''.join(item))

    return PAMs

def regex_from_seq(seq):
    """
    Given a sequence with ambiguous base characters, returns a regex that matches for
    the explicit (unambiguous) base characters
    """
    regex = ''

    for c in seq:
        regex += IUPAC_notation_regex[c]

    return regex

def regex_match_count(regex, list_of_counts):
    """
    Given a list of strings and a regex, return the number of strings in the list that the regex matches.
    """
```

```

"""
c = 0

for item in list_of_counts:
    if re.search(regex, item):
        c += 1

return c

def tabulate_substring_frequencies(pams, indices):
    """
    Given a list of raw pams and substring indices, tabulates the frequency of tabulate_substring_frequencies
    RETURNS a Pandas Series
    """
    base_PAMs = unambiguous_PAMs(indices[1] - indices[0])
    tmp_PAMs = Counter([pam[indices[0]:indices[1]] for pam in pams])

    c = Counter()
    for base_PAM in base_PAMs:
        c[base_PAM] = tmp_PAMs[base_PAM]

    PAMs = pd.Series(c)
    PAMs.sort(ascending=False)
    excel_PAMs = pd.DataFrame()
    excel_PAMs['PAM'] = PAMs.index
    excel_PAMs['Count'] = PAMs.values
    excel_PAMs['Frequency'] = PAMs.values.astype(float)/sum(PAMs.values)

    return excel_PAMs

def generate_raw_PAM_counts(filepaths, targetsites, PAM_length):
    """
    Here, we get all of our relevant PAM sequences from the inputted files
    by searching for the targetsites and looking at the flanking region
    """

    reverse_target_sequences = {targetsite: str(Seq(targetsites[targetsite]).reverse_complement()) for targetsite in targetsites}
    all_pams = {targetsite: [] for targetsite in targetsites}

    # Iterate through each file and collect the PAMs of each sequence
    # Checks both forward and reverse reads
    for filepath in filepaths:
        print 'Scanning file: ' + os.path.basename(filepath)
        pams = []
        records = SeqIO.parse(filepath, filepath.split('.')[1])
        for record in records:
            seq = str(record.seq)
            for targetsite in targetsites:
                target_seq = targetsites[targetsite]
                target = seq.find(targetsites[targetsite])
                if target > -1:
                    index = target + len(target_seq)
                    all_pams[targetsite].append(seq[index:index + PAM_length])
            else:
                target = seq.find(reverse_target_sequences[targetsite])
                if target > -1:
                    index = target
                    all_pams[targetsite].append(str(Seq(seq[index - PAM_length:index]).reverse_complement()))

    return all_pams

def analyze_PAM_depletion_data(filepaths, targetsites, PAM_length=3):
    """
    Given a directory that contains a given file extension and a target sequence, do the entire PAM depletion analysis
    """

    # Make sure that dirnames and target sequences are inputted
    if filepaths is None:
        raise Exception('Please specify a directory name')

    if targetsites is None:
        raise Exception('Please specify a target sequence')

    if PAM_length is None or PAM_length < 3:
        raise Exception('Please enter a valid PAM length')

    all_pams = generate_raw_PAM_counts(filepaths, targetsites, PAM_length)

```

```

letters = ['A', 'T', 'C', 'G']

all_counters = {targetsite: Counter(all_pams[targetsite]) for targetsite in targetsites}

for targetsite in targetsites:
    pams = all_pams[targetsite]

    base_counters = [Counter() for x in range(PAM_length)]

    for pam in pams:
        for i, c in enumerate(pam):
            base_counters[i][c] += 1

    raw_PAM_counts = pd.Series(all_counters[targetsite])
    raw_PAM_counts.sort(ascending=False)
    raw_counts_df = pd.DataFrame()
    raw_counts_df['PAM'] = raw_PAM_counts.index
    raw_counts_df['Count'] = raw_PAM_counts.values

    single_base_counts = pd.DataFrame(base_counters)
    single_base_frequencies = single_base_counts.divide(single_base_counts.sum(axis=1).ix[0])

    # Prepare substring counts and frequencies
    writer = ExcelWriter('out/' + os.path.basename(filepath).split('.')[0] + '_' + targetsite + '.xlsx')
    single_base_counts.to_excel(writer, 'Single Base Counts')
    single_base_frequencies.to_excel(writer, 'Single Base Frequencies')
    raw_counts_df.to_excel(writer, 'Raw PAM Counts')

    # Designate which windows should be analyzed and name them
    settings = {
        'XXXNNN': [0,3],
        'NXXXNN': [1,4],
        'NNXXXN': [2,5],
        'NNNXXX': [3,6],
        'XXXXNN': [0,4],
        'NXXXXN': [1,5],
        'NNXXXX': [2,6],
        'XXNNNN': [0,2],
        'NXXNNN': [1,3],
        'NNXXNN': [2,4],
        'NNNXXN': [3,5],
        'NNNNXX': [4,6],
        'XXXXXX': [0,5],
        'NXXXXX': [1,6],
        'XXXXXX': [0,6],
    }

    for item in settings:
        df = tabulate_substring_frequencies(pams, settings[item])
        df.to_excel(writer, item)

    writer.save()

    print 'Saved excel output for ' + targetsite

if __name__ == "__main__":

    # Display the filepicker, accepting only FASTQ files
    root = Tkinter.Tk()
    root.withdraw()
    file_paths = tkFileDialog.askopenfilenames(parent=root, title='Choose FASTQ files', filetypes=[("FastQ files", "*.fastq")])

    # Describe the targetsite(s) to search for
    targetsites = {'EGFP site 1': 'GTCGCCCTCGAACTTCACCT'}

    # Run the analysis on the inputted filepaths and targetsite for a given variable nucleotide region length
    analyze_PAM_depletion_data(file_paths, targetsites, PAM_length=6)

```
