

7-29-21-pcr-rxns

August 1, 2021

1 PCR reaction protocols for pFC8, pFC9 and pFC8tac

These reactions amplify regions of pFC8, 9, and 8tac (previously labeled 53 tac) for sanger sequencing.

```
[102]: import pandas as pd
from pydna.amplify import pcr
from pydna.dseqrecord import Dseqrecord
from pydna.readers import read
from pydna.tm import program # pcr program for tac pol
from pydna.primer import Primer
import sys

sys.path.append('experiments/VR-inserts/notebooks')
```

Read primers.

```
[9]: primers = pd.read_csv('data_files/Ethan_Oligos-dsDNA_oligos.tsv', sep='\t').
    ↳ set_index('Name', drop=False)
primers
```

```
[9]:
```

	Name \
Name	
pFC8tac_tac_promoter_Primer_1	pFC8tac_tac_promoter_Primer_1
pFC8tac_tac_promoter_Primer_2	pFC8tac_tac_promoter_Primer_2
pFC9_t7_primer_1	pFC9_t7_primer_1
pFC9_t7_primer_2	pFC9_t7_primer_2
pFC8_t7_primer_2	pFC8_t7_primer_2
Variable_region_insert_primer_1	Variable_region_insert_primer_1
Variable_region_insert_primer_2	Variable_region_insert_primer_2

	Sequence	Date created \
Name		
pFC8tac_tac_promoter_Primer_1	gcagaacggcacaacagc	7/29/21
pFC8tac_tac_promoter_Primer_2	gtattaccgcctttgagtgagctg	7/29/21
pFC9_t7_primer_1	cccgccgcgcttaatg	7/29/21
pFC9_t7_primer_2	gccccaatgcgagcgg	7/29/21
pFC8_t7_primer_2	gaaggggcagtagcacagtcc	7/29/21

Variable_region_insert_primer_1	tacgactcactatagggcgaattgg	7/29/21
Variable_region_insert_primer_2	cctcctcgctcggtcac	7/29/21

Name	Date ordered	Order details	Purification \
pFC8tac_tac_promoter_Primer_1	7/29/21	146853411	STD
pFC8tac_tac_promoter_Primer_2	7/29/21	146853411	STD
pFC9_t7_primer_1	7/29/21	146853411	STD
pFC9_t7_primer_2	7/29/21	146853411	STD
pFC8_t7_primer_2	7/29/21	146853411	STD
Variable_region_insert_primer_1	7/29/21	146853411	STD
Variable_region_insert_primer_2	7/29/21	146853411	STD

Name	Scale \
pFC8tac_tac_promoter_Primer_1	25nm
pFC8tac_tac_promoter_Primer_2	25nm
pFC9_t7_primer_1	25nm
pFC9_t7_primer_2	25nm
pFC8_t7_primer_2	25nm
Variable_region_insert_primer_1	25nm
Variable_region_insert_primer_2	25nm

Name	Notes \
pFC8tac_tac_promoter_Primer_1	Amplifies tac promoter of pFC
plasmids	
pFC8tac_tac_promoter_Primer_2	Amplifies tac promoter of pFC
plasmids	
pFC9_t7_primer_1	Amplifies t7 promoter of pFC
plasmids	
pFC9_t7_primer_2	Amplifies t7 promoter of pFC
plasmids	
pFC8_t7_primer_2	Amplifies t7 promoter of pFC8 when used with
p...	
Variable_region_insert_primer_1	Amplifies any variable region insert
fragment	
Variable_region_insert_primer_2	Amplifies any variable region insert
fragment	

Name	Lab notebook	Storage location	Arrival Date \
pFC8tac_tac_promoter_Primer_1	7-29-21.md	NaN	NaN
pFC8tac_tac_promoter_Primer_2	7-29-21.md	NaN	NaN
pFC9_t7_primer_1	7-29-21.md	NaN	NaN
pFC9_t7_primer_2	7-29-21.md	NaN	NaN
pFC8_t7_primer_2	7-29-21.md	NaN	NaN

Variable_region_insert_primer_1	7-29-21.md	NaN	NaN
Variable_region_insert_primer_2	7-29-21.md	NaN	NaN

	Empty
Name	
pFC8tac_tac_promoter_Primer_1	False
pFC8tac_tac_promoter_Primer_2	False
pFC9_t7_primer_1	False
pFC9_t7_primer_2	False
pFC8_t7_primer_2	False
Variable_region_insert_primer_1	False
Variable_region_insert_primer_2	False

Read template DNA for reactions.

```
[103]: pFC8 = 'data_files/resources/files/genbank/pFC8.gb'
pFC9 = 'data_files/resources/files/genbank/pFC9.gb'
pFC8tac = 'data_files/resources/files/genbank/pFC8tacT1T2.gb'

template_paths = (pFC8, pFC9, pFC8tac)
templates = [Dseqrecord(read(tp)) for tp in template_paths]
```

Setup helper PCR functions.

```
[116]: def pcr_program(primers, template):
        product = pcr(primers, template)
        return program(product)

def get_primer_seq(name, primer_table):
    return Primer(primer_table.loc[name].values[1])
```

1.1 pFC8

```
[111]: pFC8 = templates[0]
pfc8_primers = get_primer_seq('pFC9_t7_primer_1', primers),
↳ get_primer_seq('pFC8_t7_primer_2', primers)
```

```
[117]: pcr_program(pfc8_primers, pFC8)
```

```
[117]: |95°C|95°C          |          |tmf:66.0
|____|____          |          |72°C|72°C|tmr:69.5
|5min|30s \ 61.3°C ____|____|45s/kb
|      | \_____/ 0:30|5min|GC 55%
|      | 30s          |          |319bp
```

1.2 pFC9

```
[123]: pFC9 = templates[1]
pfc9_primers = get_primer_seq('pFC9_t7_primer_1', primers),
↳get_primer_seq('pFC9_t7_primer_2', primers)
pcr_program(pfc9_primers, pFC9)
```

```
[123]: |95°C|95°C          |      |tmf:66.0
|____|_____          72°C|72°C|tmr:69.1
|5min|30s \ 61.3°C _____|____|45s/kb
|      |      \_____/ 0:30|5min|GC 56%
|      |      30s          |      |300bp
```

1.3 pFC8tac

```
[124]: pFC9 = templates[2]
pfc8tac_primers = get_primer_seq('pFC8tac_tac_promoter_Primer_1', primers),
↳get_primer_seq('pFC8tac_tac_promoter_Primer_2', primers)
pcr_program(pfc8tac_primers, pFC8)
```

```
[124]: |95°C|95°C          |      |tmf:64.1
|____|_____          72°C|72°C|tmr:66.4
|5min|30s \ 60.7°C _____|____|45s/kb
|      |      \_____/ 0:30|5min|GC 55%
|      |      30s          |      |301bp
```