

## Template Alignment: 2020-12-04\_G02\_ASAST15BSPTF

ctcaagaagatcctttgatcttttctacggcgcgcccagctgtctagggcggcggtttgtcctactcaggagagcgttcaccgacaaacaacagataaa

10 20 30 40 50 60 70 80 90 100

rrnB T1 terminator

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

acgaaaggcccagtcctttcgactgagcctttcggttttatttgatgccttgccgaattcggatccggagtttacggctagctcagtcctaggtactatgct

110 120 130 140 150 160 170 180 190 200

rrnB T1 terminator

Bba\_J23105

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

agctattgtgagcggataacaatttcacacatactagagaaaggagagaataactaaATGAGCAAGCTGGAGAAGTTTACAAACTGCTACTCCCTGTCTA

210

220

230

240

250

260

270

280

290

300

lac operator

stron...000)

synthetic parts

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

AGACCCTGAGGTTCAAGGCCATCCCTGTGGGCAAGACCCAGGAGAACATCGACAATAAGCGGCTGCTGGTGGAGGACGAGAAGAGAGCCGAGGATTATAA

310

320

330

340

350

360

370

380

390

400

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

GGGCGTGAAGAAGCTGCTGGATCGCTACTATCTGTCTTTTATCAACGACGTGCTGCACAGCATCAAGCTGAAGAATCTGAACAATTACATCAGCCTGTTC

410

420

430

440

450

460

470

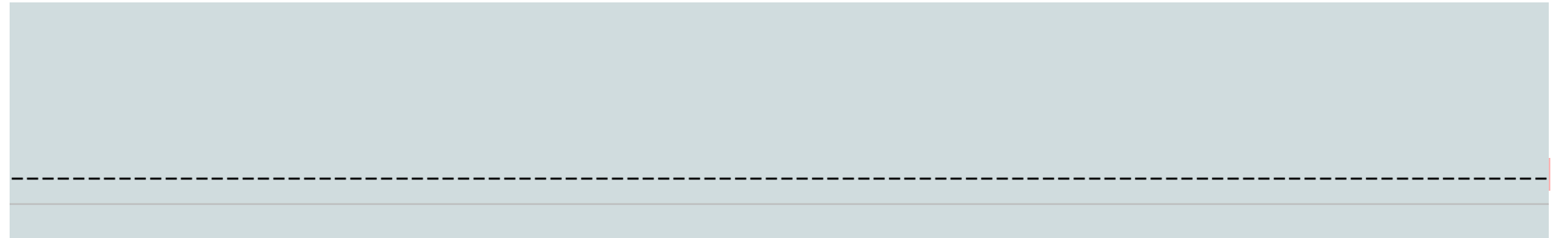
480

490

500

»» LB cpf 1 »»

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

CGGAAGAAAACCAGAACCGAGAAGGAGAATAAGGAGCTGGAGAACCTGGAGATCAATCTGCGGAAGGAGATCGCCAAGGCCTTCAAGGGCAACGAGGGCT

510

520

530

540

550

560

570

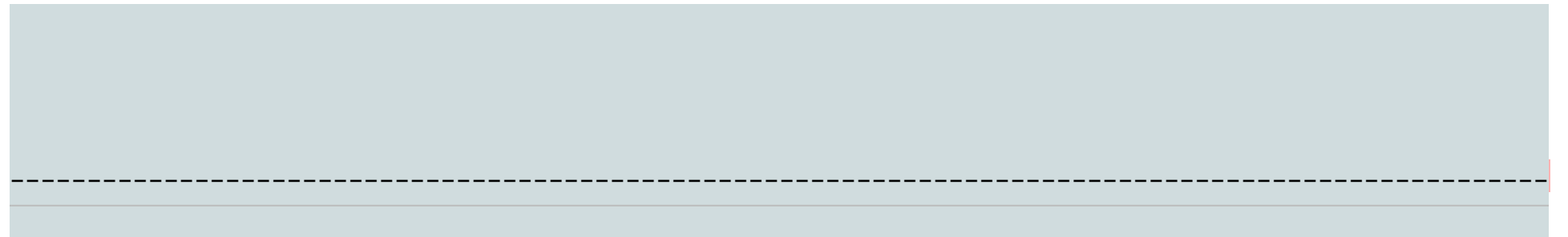
580

590

600

»» LB cpf 1 »»

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

ACAAGTCCCTGTTTAAGAAGGATATCATCGAGACAATCCTGCCAGAGTTCCTGGACGATAAGGACGAGATCGCCCTGGTGAACAGCTTCAATGGCTTTAC

610

620

630

640

650

660

670

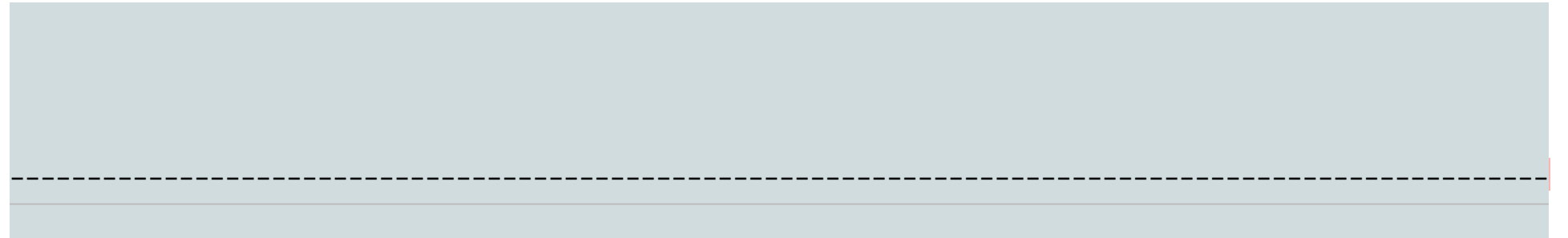
680

690

700

»» LB cpf 1 »»

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

CACAGCCTTCACCGGCTTCTTTGATAACAGAGAGAATATGTTTTCCGAGGAGGCCAAGAGCACATCCATCGCCTTCAGGTGTATCAACGAGAATCTGACC

710

720

730

740

750

760

770

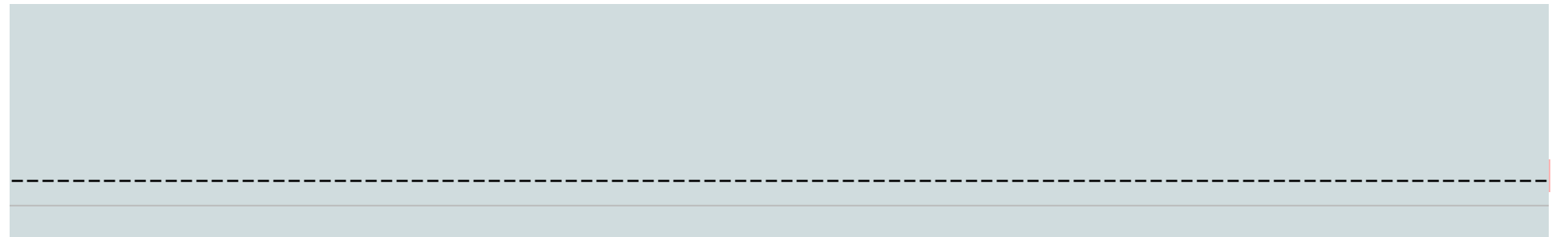
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790

800

»» LB cpf 1 »»

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

CGCTACATCTCTAATATGGACATCTTCGAGAAGGTGGACGCCATCTTTGATAAGCACGAGGTGCAGGAGATCAAGGAGAAGATCCTGAACAGCGACTATG

810

820

830

840

850

860

870

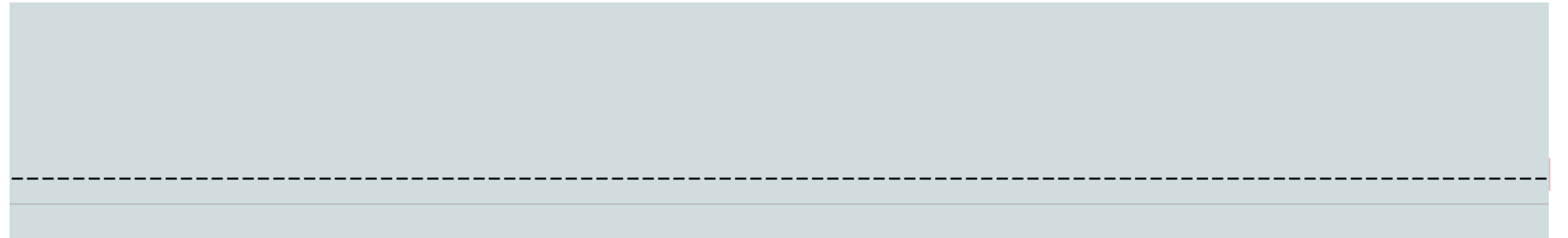
880

890

900

»» LB cpf 1 »»

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

ATGTGGAGGATTTCTTTGAGGGCGAGTTCTTTAACTTTGTGCTGACACAGGAGGGCATCGACGTGTATAACGCCATCATCGGCGGCTTCGTGACCGAGAG

910

920

930

940

950

960

970

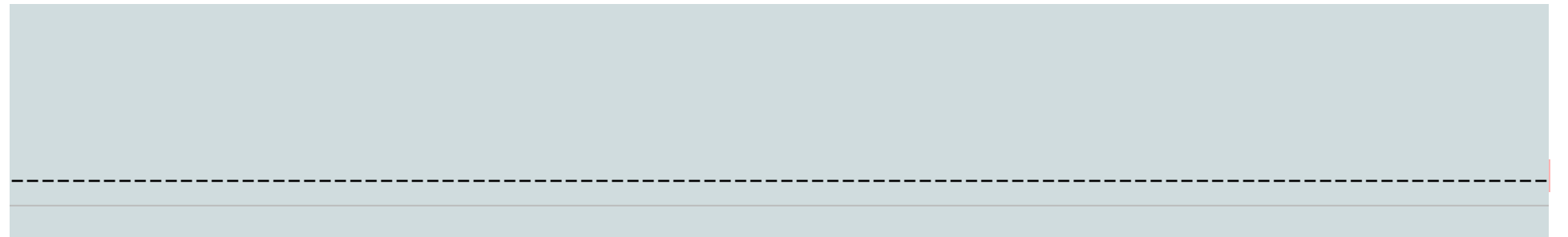
980

990

1,000

»» LB cpf 1 »»

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

CGGCGAGAAGATCAAGGGCCTGAACGAGTACATCAACCTGTATAATCAGAAAACCAAGCAGAAGCTGCCTAAGTTTAAGCCACTGTATAAGCAGGTGCTG

1,010

1,020

1,030

1,040

1,050

1,060

1,070

1,080

1,090

1,100

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

AGCGATCGGGAGTCTCTGAGCTTCTACGGCGAGGGCTATACATCCGATGAGGAGGTGCTGGAGGTGTTTAGAAACACCCTGAACAAGAACAGCGAGATCT

1,110

1,120

1,130

1,140

1,150

1,160

1,170

1,180

1,190

1,200

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

TCAGCTCCATCAAGAAGCTGGAGAAGCTGTTCAAGAATTTTGACGAGTACTCTAGCGCCGGCATCTTTGTGAAGAACGGCCCCGCCATCAGCACAATCTC

1,210

1,220

1,230

1,240

1,250

1,260

1,270

1,280

1,290

1,300

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

CAAGGATATCTTCGGCGAGTGGAACGTGATCCGGGACAAGTGGAATGCCGAGTATGACGATATCCACCTGAAGAAGAAGGCCGTGGTGACCGAGAAGTAC

1,310

1,320

1,330

1,340

1,350

1,360

1,370

1,380

1,390

1,400

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

GAGGACGATCGGAGAAAGTCCTTCAAGAAGATCGGCTCCTTTTCTCTGGAGCAGCTGCAGGAGTACGCCGACGCCGATCTGTCTGTGGTGGAGAAGCTGA

1,410

1,420

1,430

1,440

1,450

1,460

1,470

1,480

1,490

1,500

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

AGGAGATCATCATCCAGAAGGTGGATGAGATCTACAAGGTGTATGGCTCCTCTGAGAAGCTGTTCGACGCCGATTTTGTGCTGGAGAAGAGCCTGAAGAA

1,510

1,520

1,530

1,540

1,550

1,560

1,570

1,580

1,590

1,600

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF



GAACGACGCCGTGGTGGCCATCATGAAGGACCTGCTGGATTCTGTGAAGAGCTTCGAGAATTACATCAAGGCCTTCTTTGGCGAGGGCAAGGAGACAAAC

1,610

1,620

1,630

1,640

1,650

1,660

1,670

1,680

1,690

1,700

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

AGGGACGAGTCCTTCTATGGCGATTTTGTGCTGGCCTACGACATCCTGCTGAAGGTGGACCACATCTACGATGCCATCCGCAATTATGTGACCCAGAAGC

1,710

1,720

1,730

1,740

1,750

1,760

1,770

1,780

1,790

1,800

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

CCTACTCTAAGGATAAGTTCAAGCTGTATTTTCAGAACCCCTCAGTTCATGGGCGGCTGGGACAAGGATAAGGAGACAGACTATCGGGCCACCATCCTGAG

1,810

1,820

1,830

1,840

1,850

1,860

1,870

1,880

1,890

1,900

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

ATACGGCTCCAAGTACTATCTGGCCATCATGGATAAGAAGTACGCCAAGTGCCTGCAGAAGATCGACAAGGACGATGTGAACGGCAATTACGAGAAGATC

1,910

1,920

1,930

1,940

1,950

1,960

1,970

1,980

1,990

2,000

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

AACTATAAGCTGCTGCCCCGGCCCTAATAAGATGCTGCCAAAGGTGTTCTTTTCTAAGAAGTGGATGGCCTACTATAACCCCAGCGAGGACATCCAGAAGA

2,010 2,020 2,030 2,040 2,050 2,060 2,070 2,080 2,090 2,100

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

TCTACAAGAATGGCACATTCAAGAAGGGCGATATGTTTAACTGAATGACTGTCACAAGCTGATCGACTTCTTTAAGGATAGCATCTCCCGGTATCCAAA

2,110 2,120 2,130 2,140 2,150 2,160 2,170 2,180 2,190 2,200

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

GTGGTCCAATGCCTACGATTTCAACTTTTCTGAGACAGAGAAGTATAAGGACATCGCCGGCTTTTACAGAGAGGTGGAGGAGCAGGGCTATAAGGTGAGC

2,210

2,220

2,230

2,240

2,250

2,260

2,270

2,280

2,290

2,300

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

TTCGAGTCTGCCAGCAAGAAGGAGGTGGATAAGCTGGTGGAGGAGGGCAAGCTGTATATGTTCCAGATCTATAACAAGGACTTTTCCGATAAGTCTCACG

2,310

2,320

2,330

2,340

2,350

2,360

2,370

2,380

2,390

2,400

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

GCACACCCAATCTGCACACCATGTACTTCAAGCTGCTGTTTGACGAGAACAATCACGGACAGATCAGGCTGAGCGGAGGAGCAGAGCTGTTTCATGAGGCG

2,410

2,420

2,430

2,440

2,450

2,460

2,470

2,480

2,490

2,500

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

CGCCTCCCTGAAGAAGGAGGAGCTGGTGGTGCACCCAGCCAACTCCCCTATCGCCAACAAGAATCCAGATAATCCCAAGAAAACCACAACCCTGTCCTAC

2,510

2,520

2,530

2,540

2,550

2,560

2,570

2,580

2,590

2,600

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

GACGTGTATAAGGATAAGAGGTTTTCTGAGGACCAGTACGAGCTGCACATCCCAATCGCCATCAATAAGTGCCCCAAGAACATCTTCAAGATCAATACAG

2,610

2,620

2,630

2,640

2,650

2,660

2,670

2,680

2,690

2,700

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

AGGTGCGCGTGCTGCTGAAGCACGACGATAACCCCTATGTGATCGGCATCGATAGGGGCGAGCGCAATCTGCTGTATATCGTGGTGGTGGACGGCAAGGG

2,710

2,720

2,730

2,740

2,750

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2,770

2,780

2,790

2,800

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

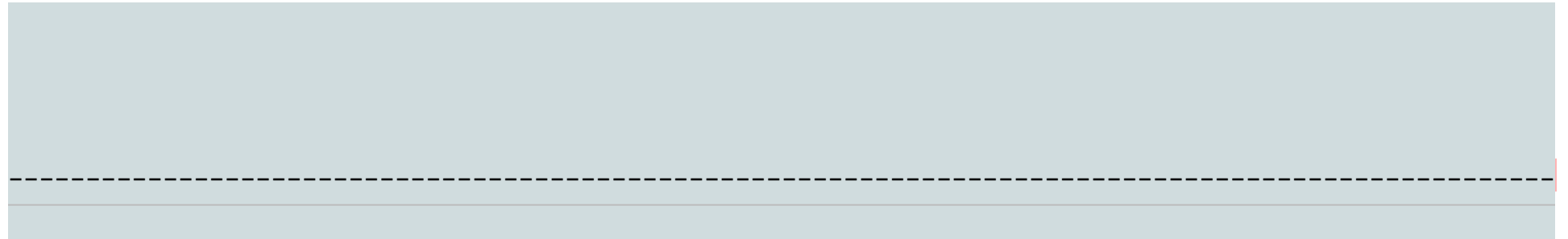
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

CAACATCGTGGAGCAGTATTCCCTGAACGAGATCATCAACAACCTTCAACGGCATCAGGATCAAGACAGATTACCACTCTCTGCTGGACAAGAAGGAGAAG

2,810 2,820 2,830 2,840 2,850 2,860 2,870 2,880 2,890 2,900

»» LB cpf 1 »»

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



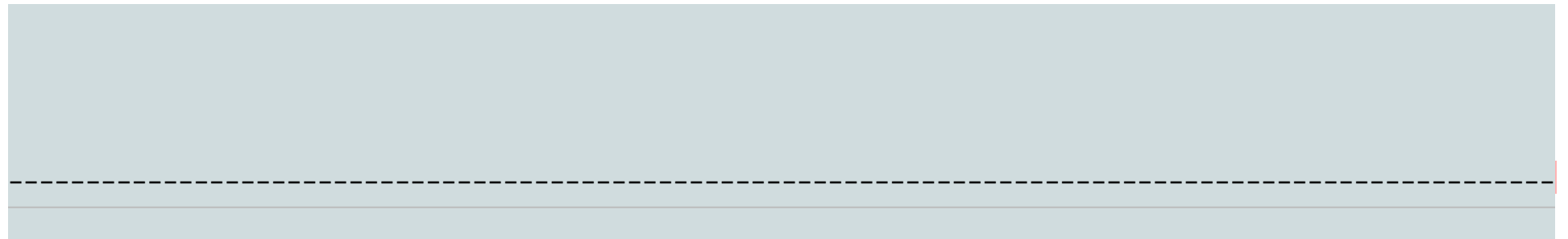
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

GAGAGGTTTCGAGGCCCGCCAGAACTGGACCTCCATCGAGAATATCAAGGAGCTGAAGGCCGGCTATATCTCTCAGGTGGTGCACAAGATCTGCGAGCTGG

2,910 2,920 2,930 2,940 2,950 2,960 2,970 2,980 2,990 3,000

»» LB cpf 1 »»

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

TGGAGAAGTACGATGCCGTGATCGCCCTGGAGGACCTGAACTCTGGCTTTAAGAATAGCCGCGTGAAGGTGGAGAAGCAGGTGTATCAGAAGTTCGAGAA

3,010

3,020

3,030

3,040

3,050

3,060

3,070

3,080

3,090

3,100

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

GATGCTGATCGATAAGCTGAACTACATGGTGGACAAGAAGTCTAATCCTTGTGCAACAGGCGGCGCCCTGAAGGGCTATCAGATCACCAATAAGTTCGAG

3,110

3,120

3,130

3,140

3,150

3,160

3,170

3,180

3,190

3,200

LB cpf 1

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

aligned sequence 2020-12-04\_G02\_ASAST15BSPTF



AGCTTTAAGTCCATGTCTACCCAGAACGGCTTCATCTTTTACATCCCTGCCTGGCTGACATCCAAGATCGATCCATCTACCGGCTTTGTGAACCTGCTGA

3,210

3,220

3,230

3,240

3,250

3,260

3,270

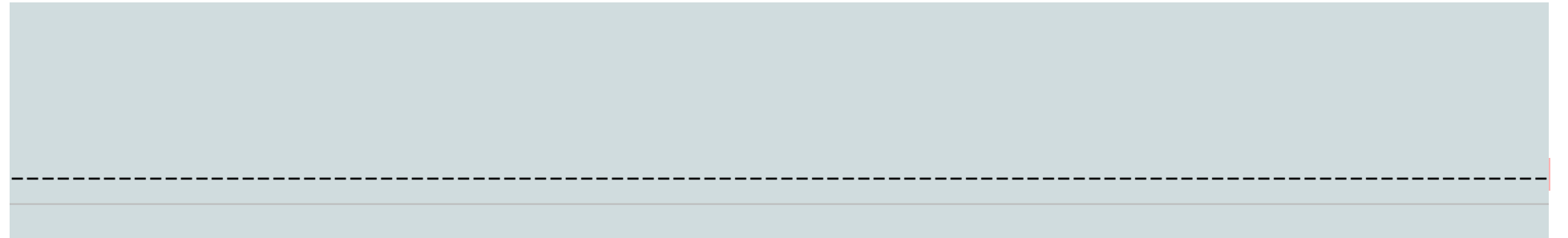
3,280

3,290

3,300

»» LB cpf 1 »»

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

AAACCAAGTATACCAGCATCGCCGATTCCAAGAAGTTCATCAGCTCCTTTGACAGGATCATGTACGTGCCCCGAGGAGGATCTGTTGAGTTTGCCTGGA

3,310

3,320

3,330

3,340

3,350

3,360

3,370

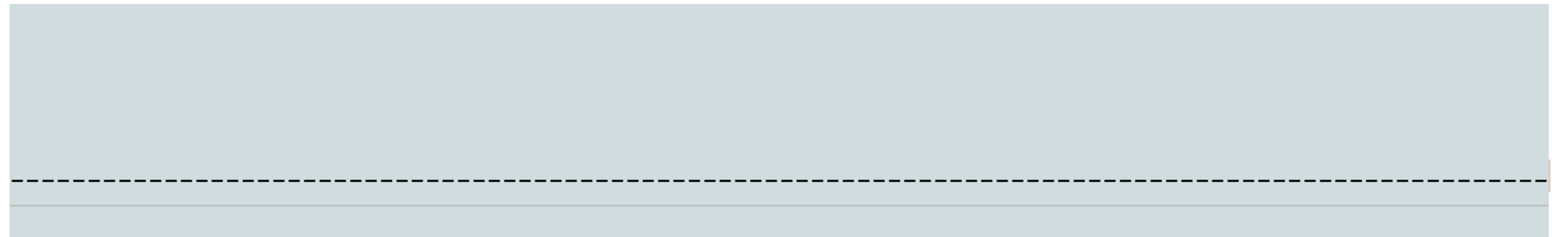
3,380

3,390

3,400

»» LB cpf 1 »»

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



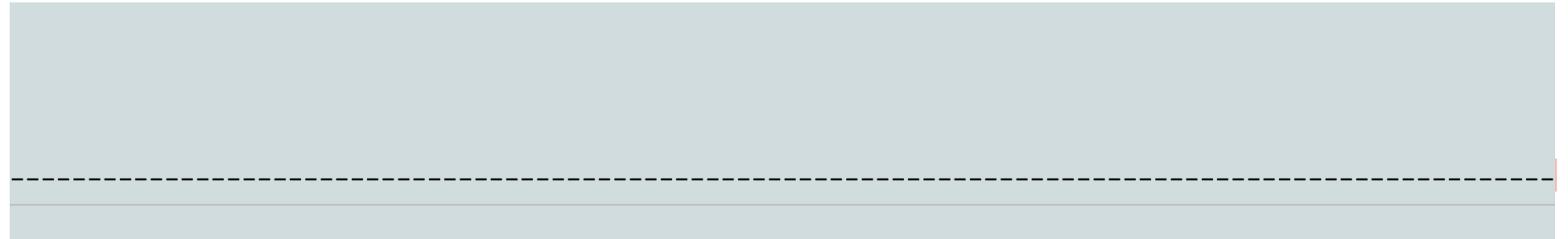
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CTATAAGAACTTCTCTCGCACAGACGCCGATTACATCAAGAAGTGAAGCTGTACTCCTACGGCAACCGGATCAGAATCTTCCGGAATCCTAAGAAGAAC

3,410 3,420 3,430 3,440 3,450 3,460 3,470 3,480 3,490 3,500

»» LB cpf 1 »»

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



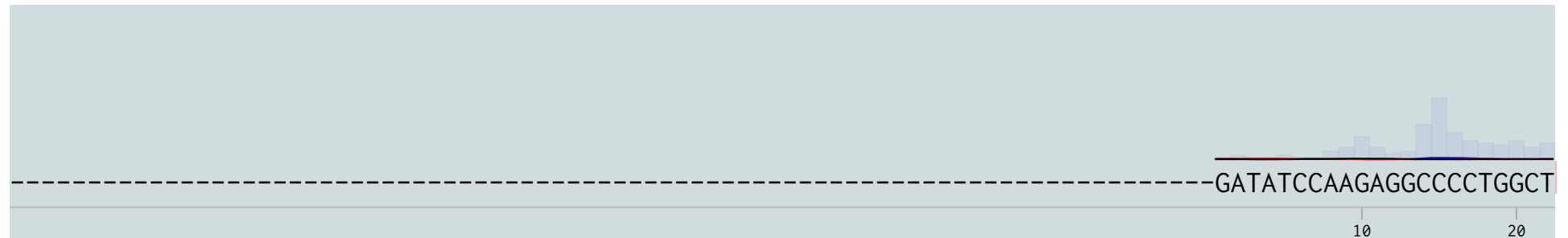
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

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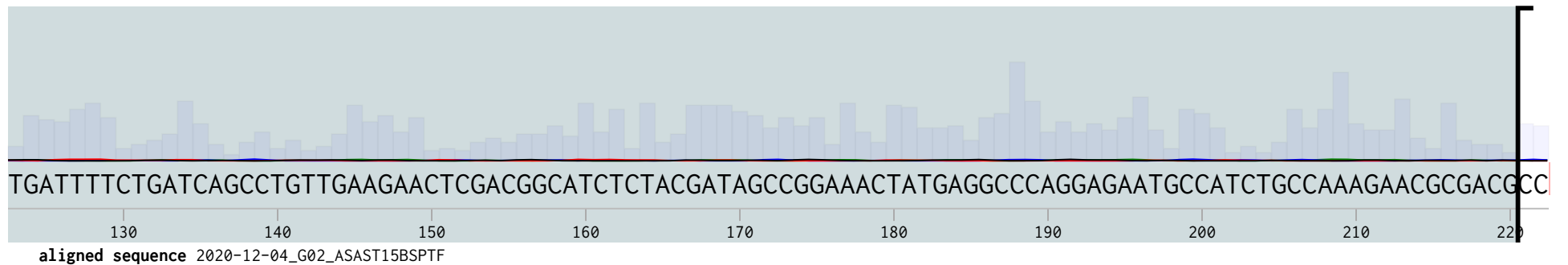
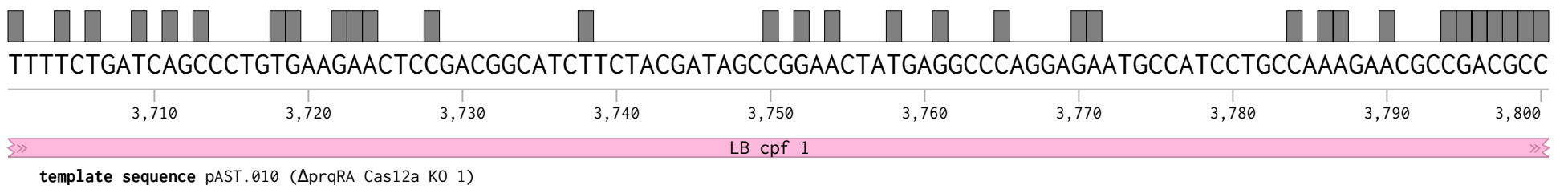
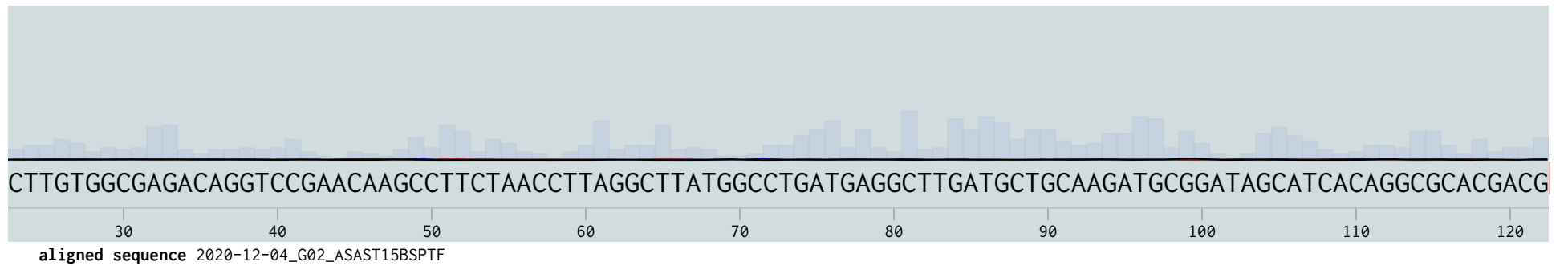
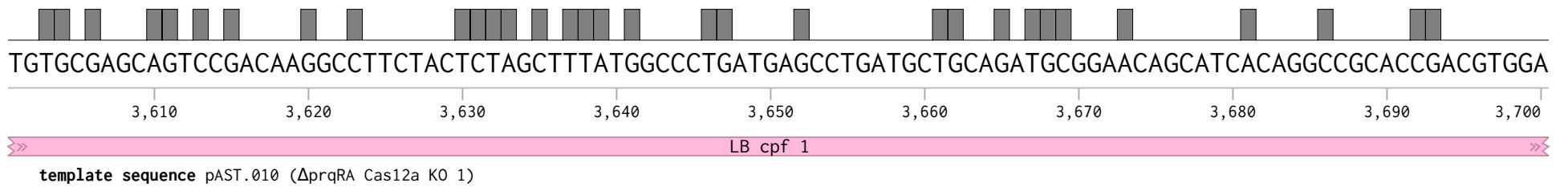
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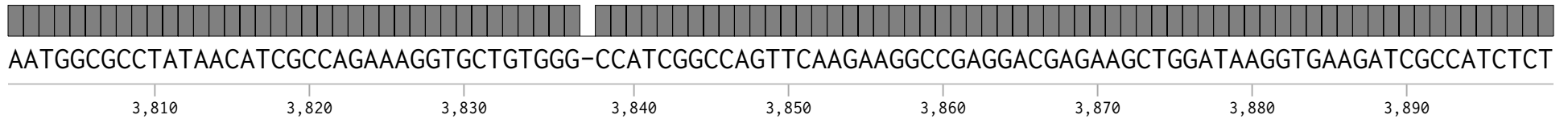
»» LB cpf 1 »»

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

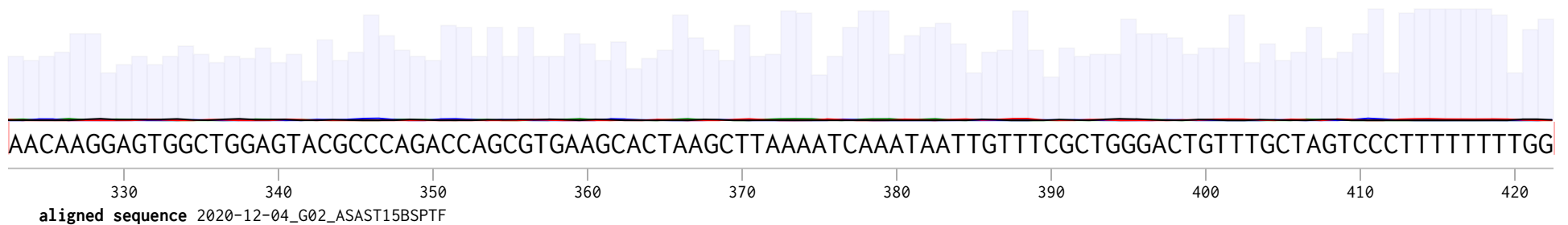
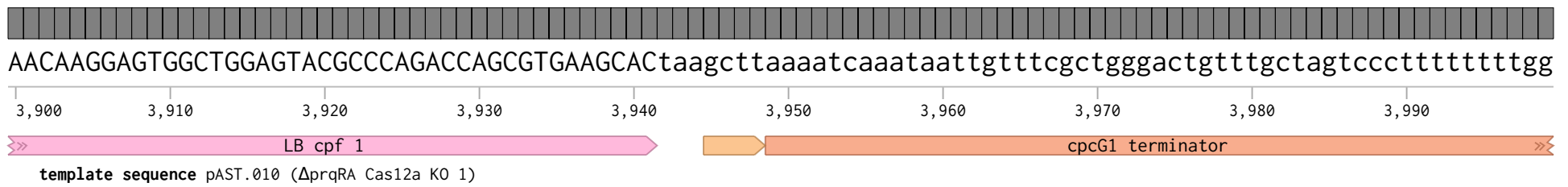
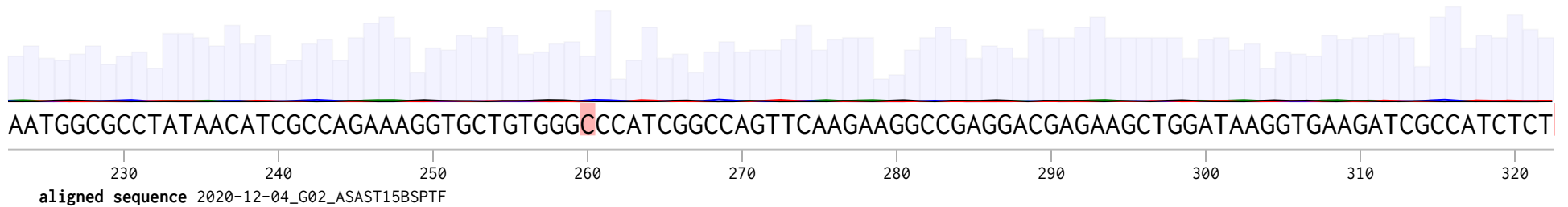


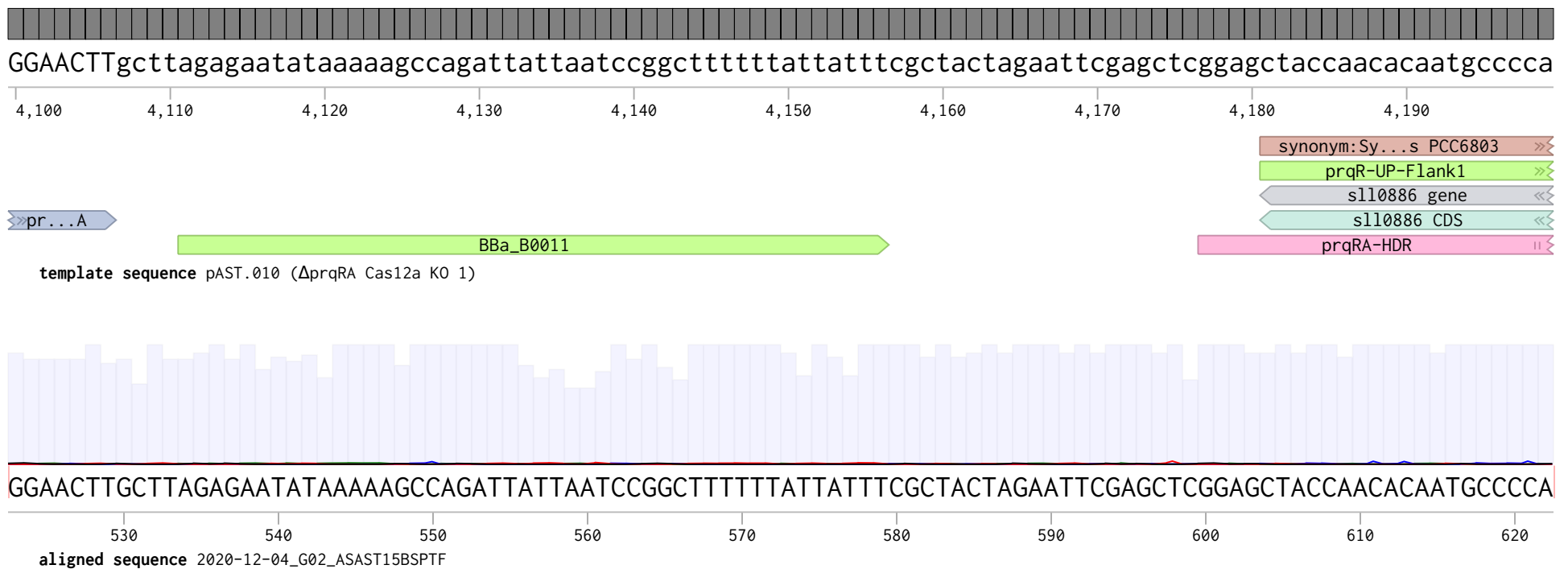
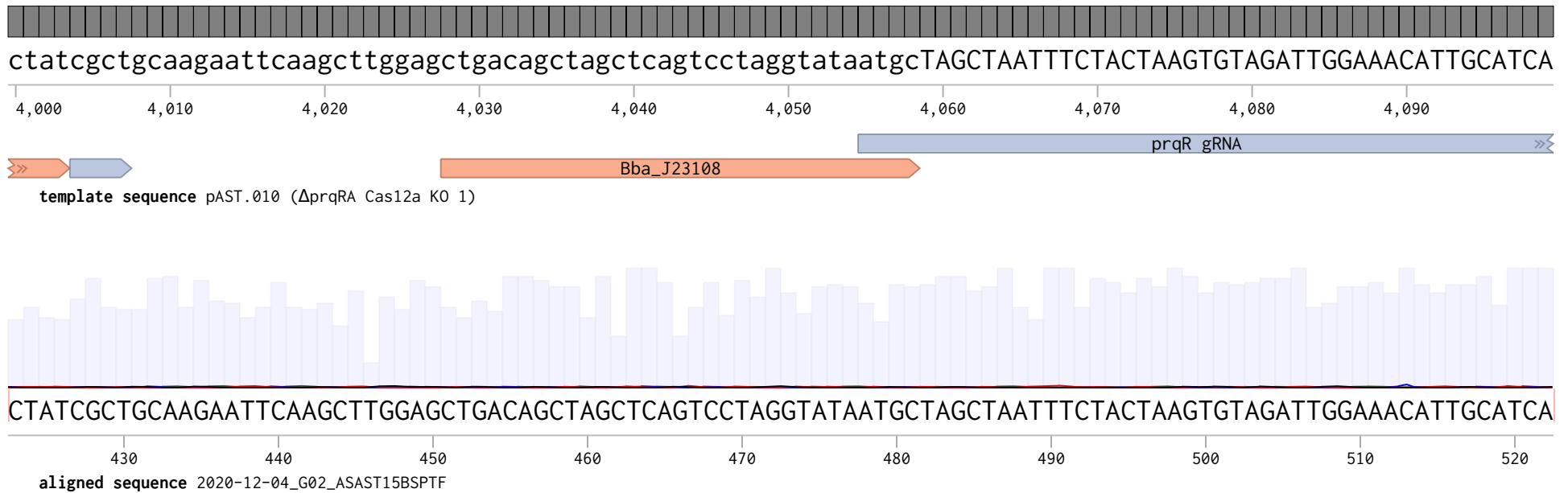
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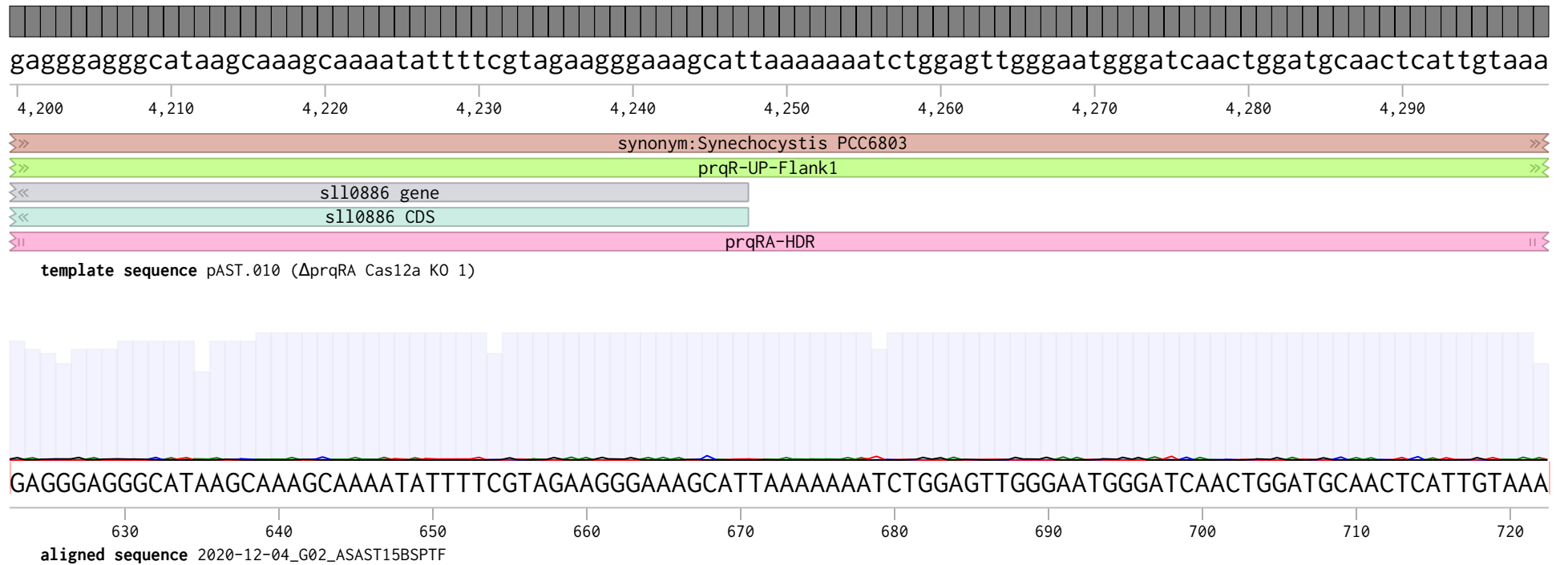


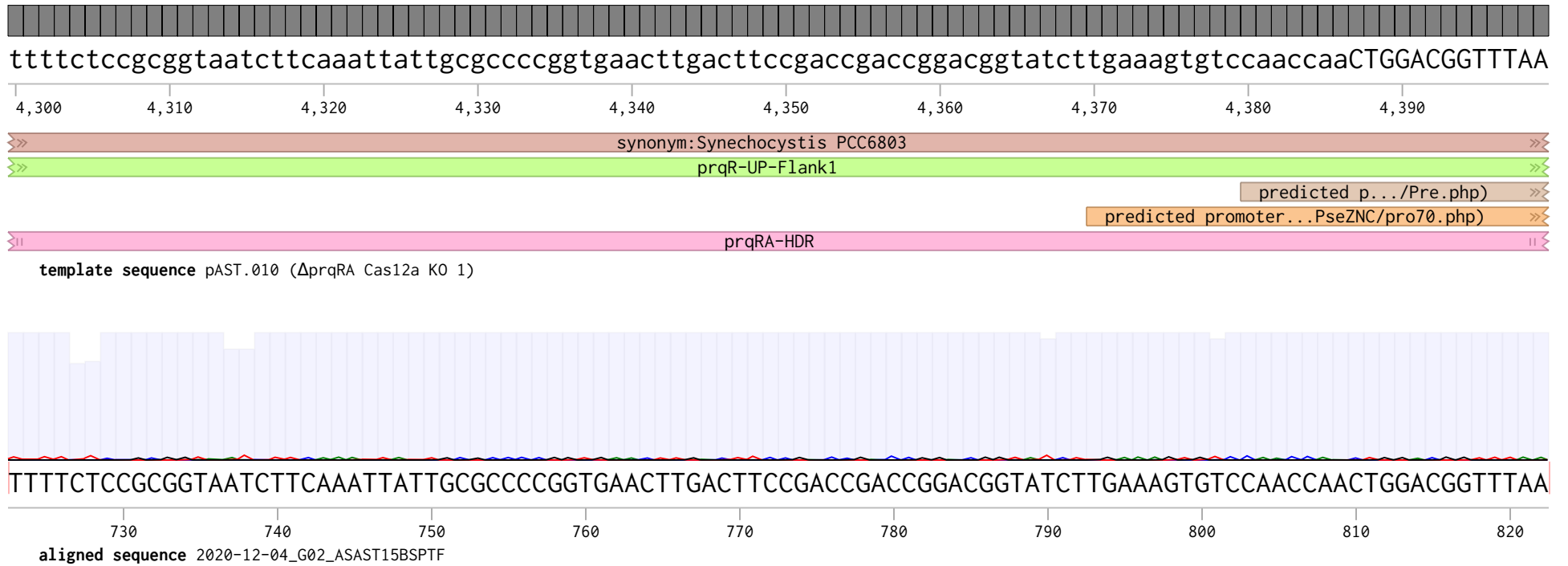


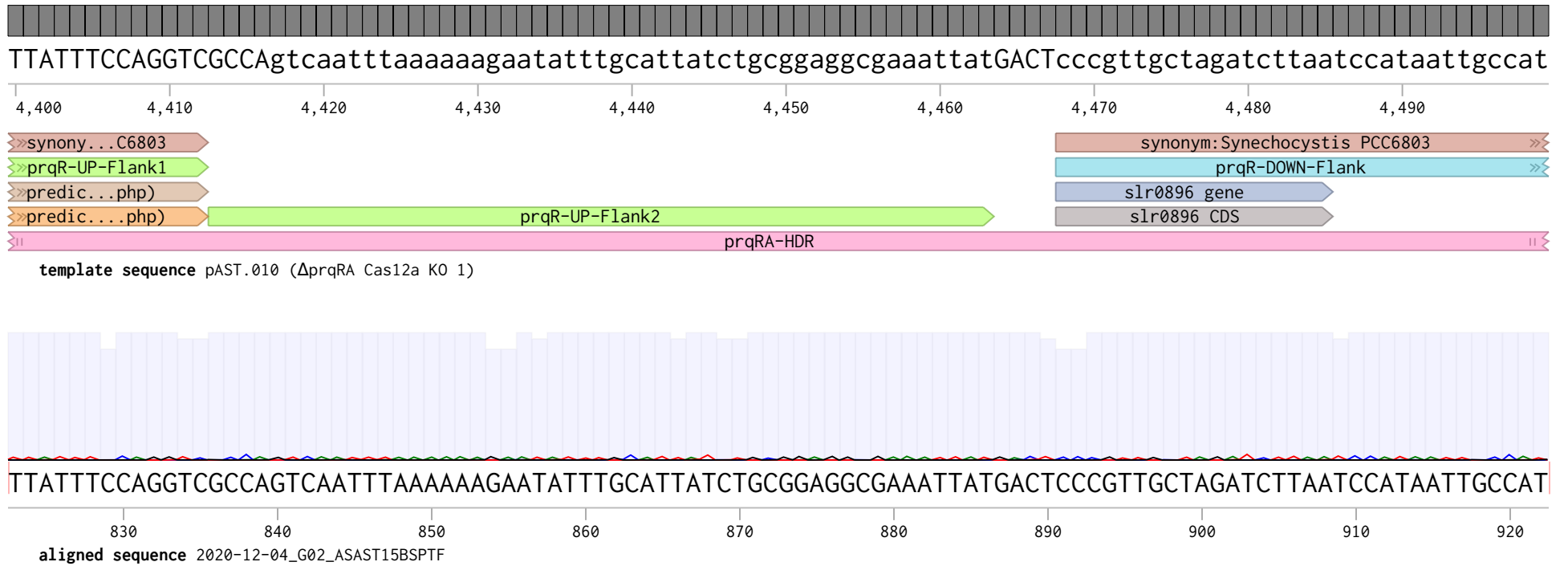
template sequence pAST.010 (ΔprqRA Cas12a KO 1)



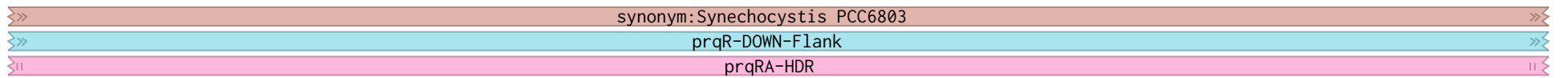
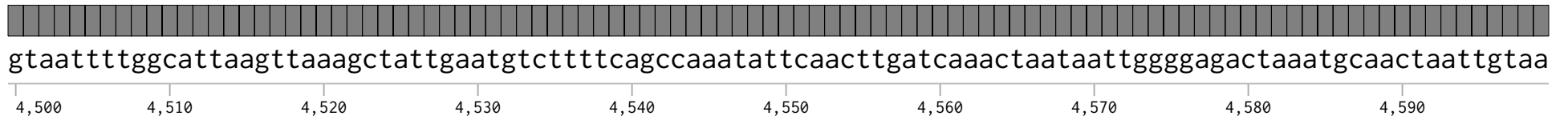




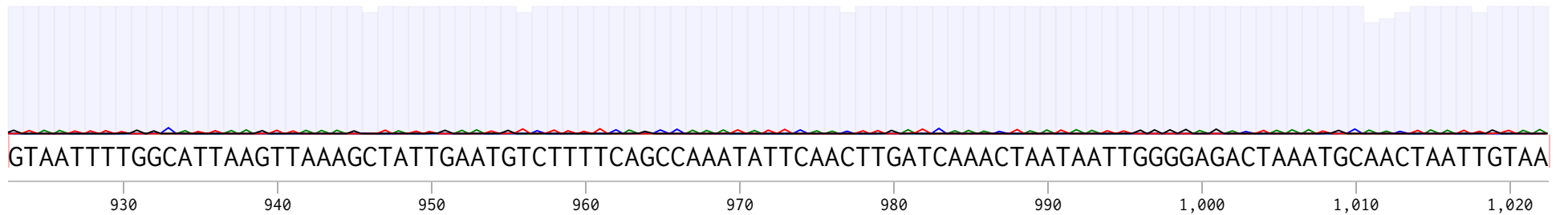




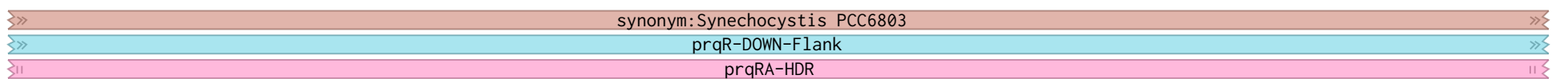
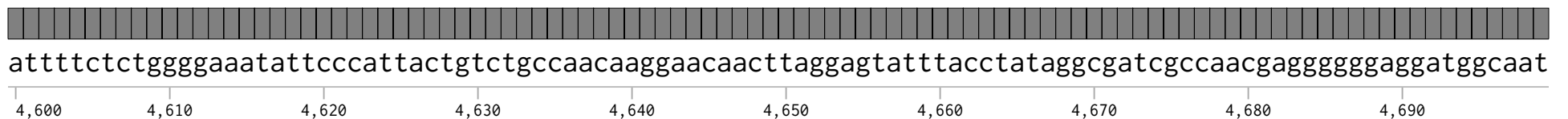




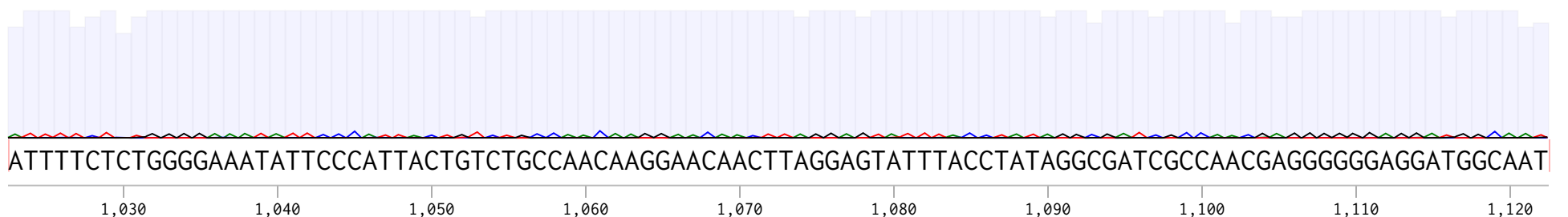
template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



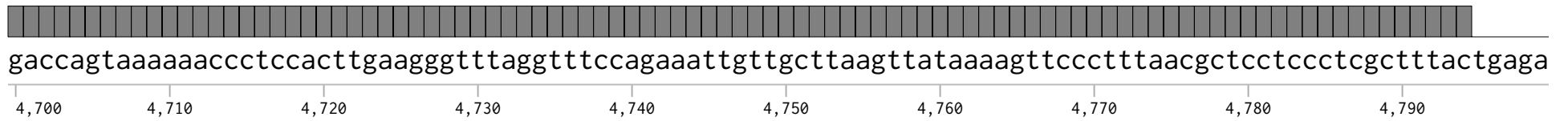
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF



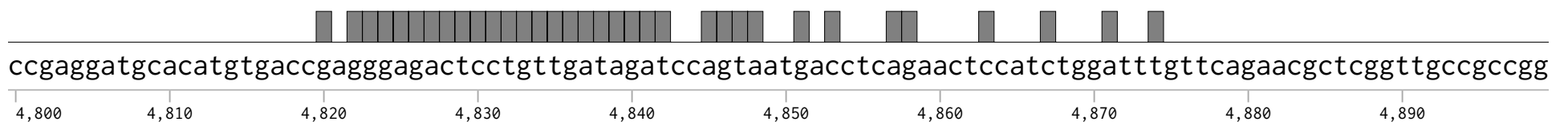
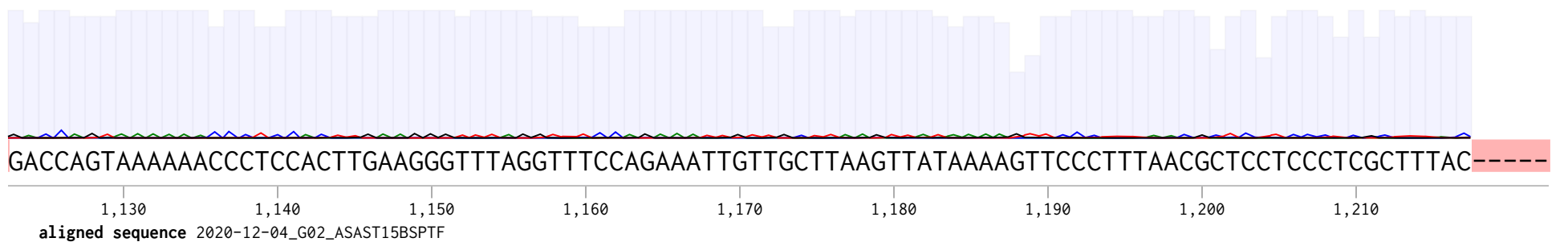
template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

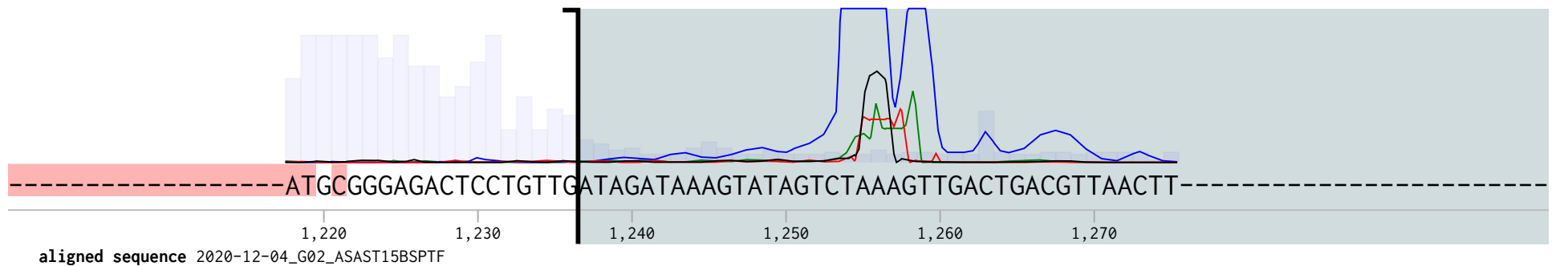


template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



lambda t0 terminator

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

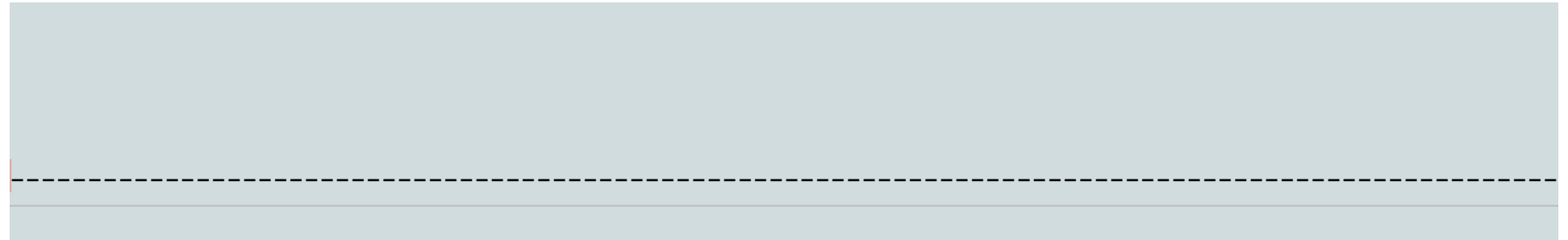


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4,900 4,910 4,920 4,930 4,940 4,950 4,960 4,970 4,980 4,990

>> lambda t0 terminator

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

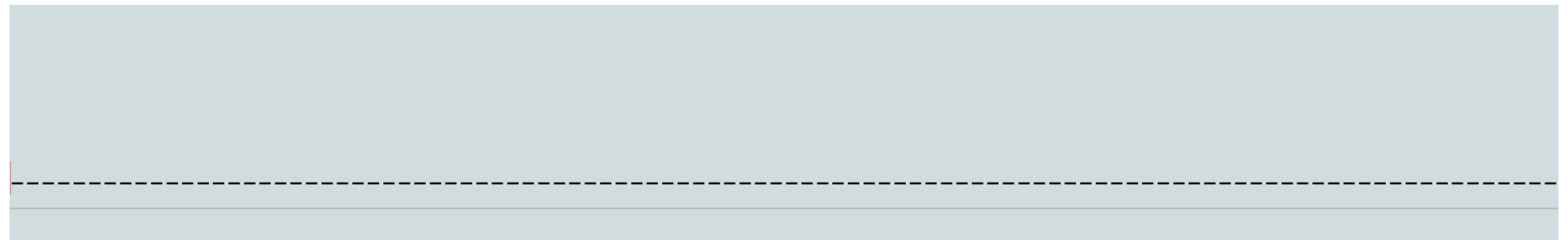


aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

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5,000 5,010 5,020 5,030 5,040 5,050 5,060 5,070 5,080 5,090

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



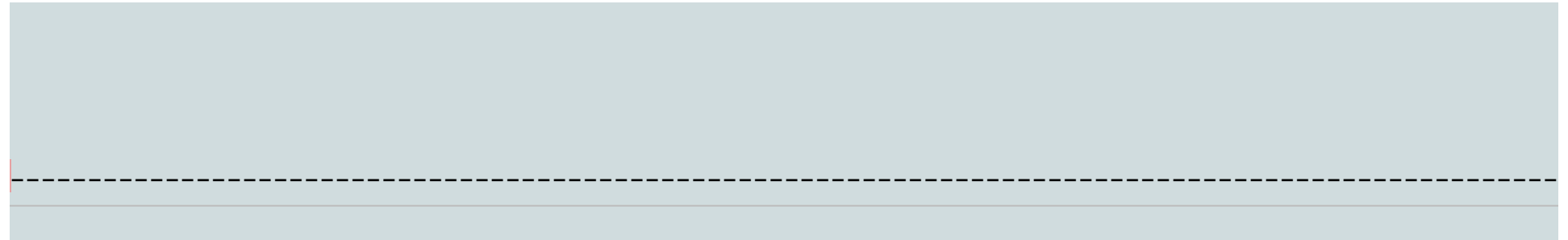
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5,100 5,110 5,120 5,130 5,140 5,150 5,160 5,170 5,180 5,190

SmR

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



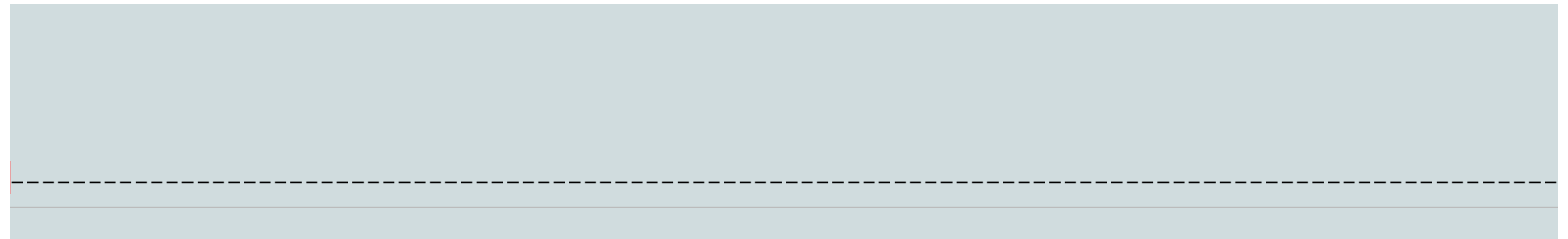
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5,200 5,210 5,220 5,230 5,240 5,250 5,260 5,270 5,280 5,290

SmR

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



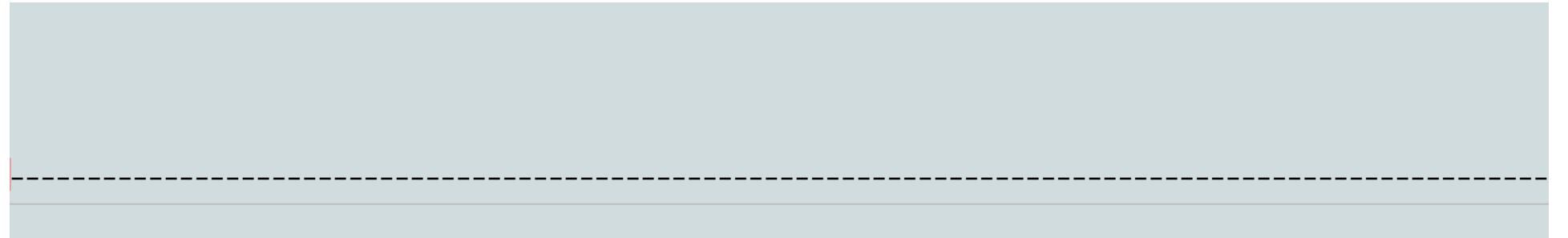
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5,300 5,310 5,320 5,330 5,340 5,350 5,360 5,370 5,380 5,390

>> SmR >>

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



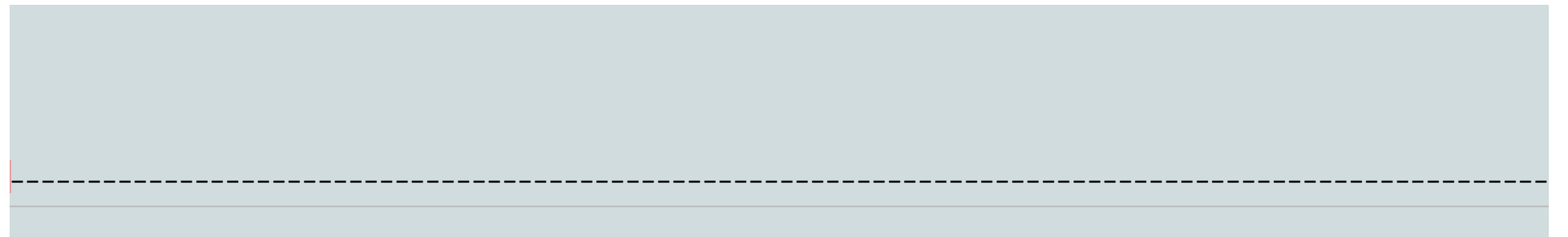
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5,400 5,410 5,420 5,430 5,440 5,450 5,460 5,470 5,480 5,490

>> SmR >>

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



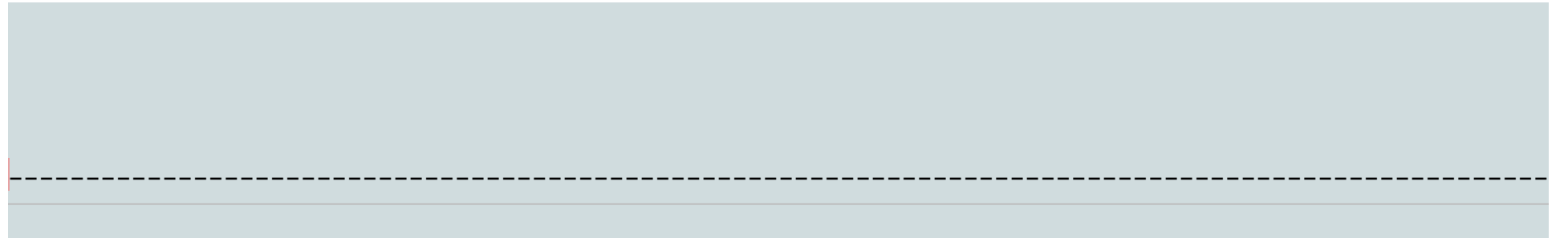
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5,500 5,510 5,520 5,530 5,540 5,550 5,560 5,570 5,580 5,590

>> SmR >>

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



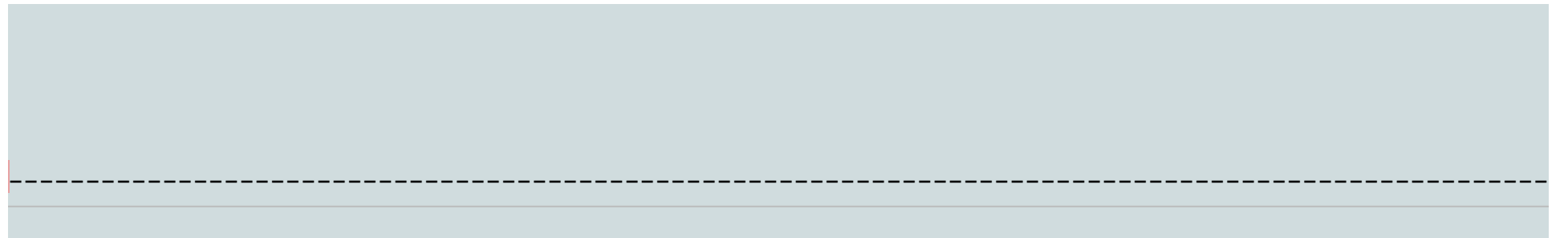
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

gcggcggaggaactctttgatccggttcctgaacaggatctatttgaggcgctaaatgaaaccttaacgctatgggaactcgccgcccgactgggctggcg

5,600 5,610 5,620 5,630 5,640 5,650 5,660 5,670 5,680 5,690

>> SmR >>

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



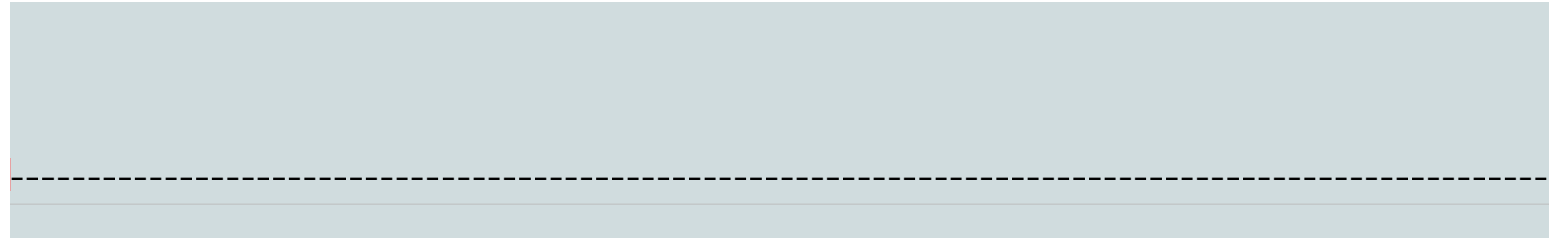
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5,700 5,710 5,720 5,730 5,740 5,750 5,760 5,770 5,780 5,790

>> SmR >>

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

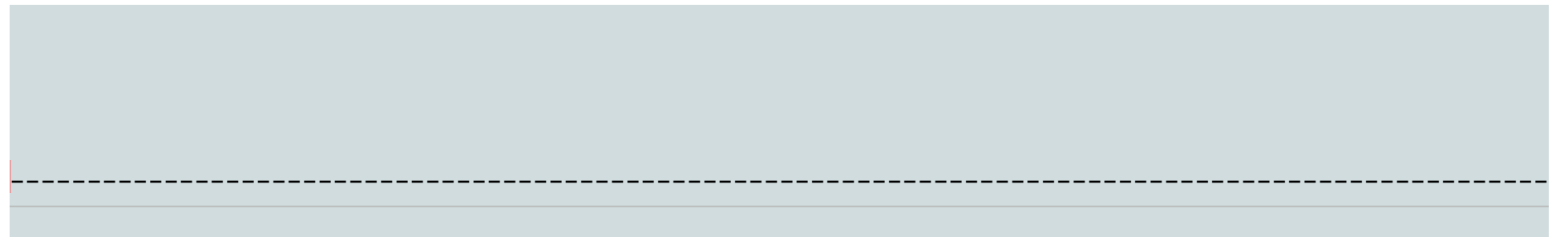


cctgccggcccagtatcagcccgctcatacttgaagctagacaggcttatcttggacaagaagaagatcgcttggcctcgcgcgagatcagttggaagaa

5,800 5,810 5,820 5,830 5,840 5,850 5,860 5,870 5,880 5,890

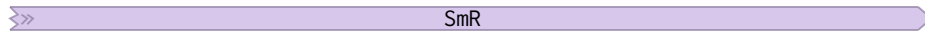
>> SmR >>

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

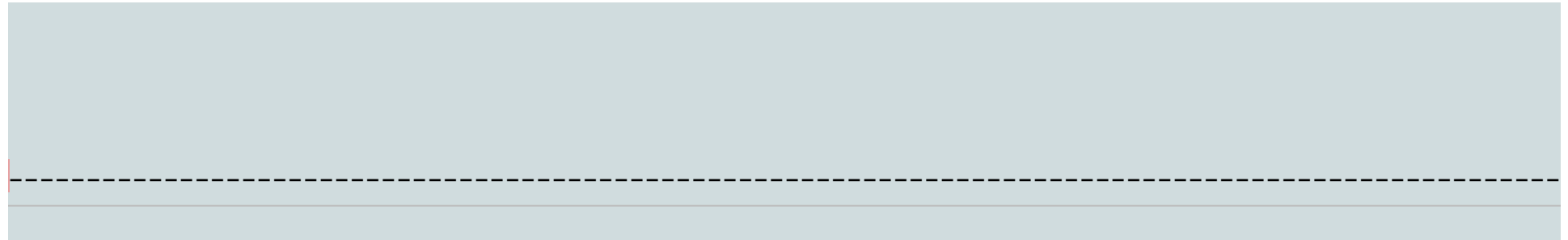


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5,900 5,910 5,920 5,930 5,940 5,950 5,960 5,970 5,980 5,990



template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



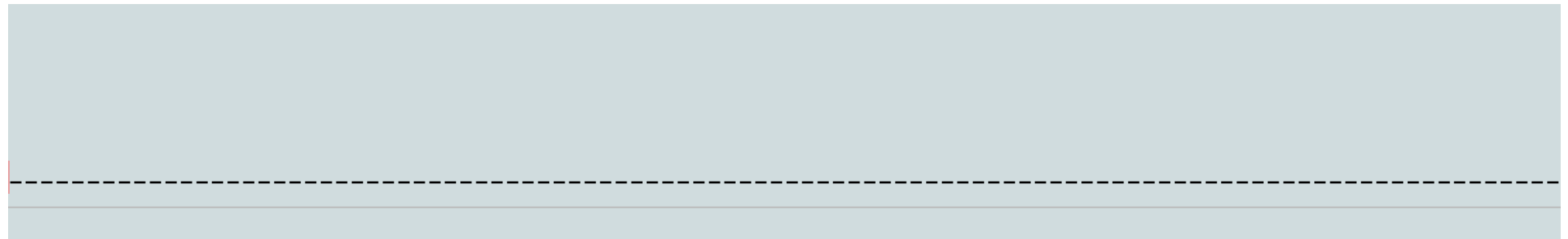
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

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6,000 6,010 6,020 6,030 6,040 6,050 6,060 6,070 6,080 6,090

in...r >>

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

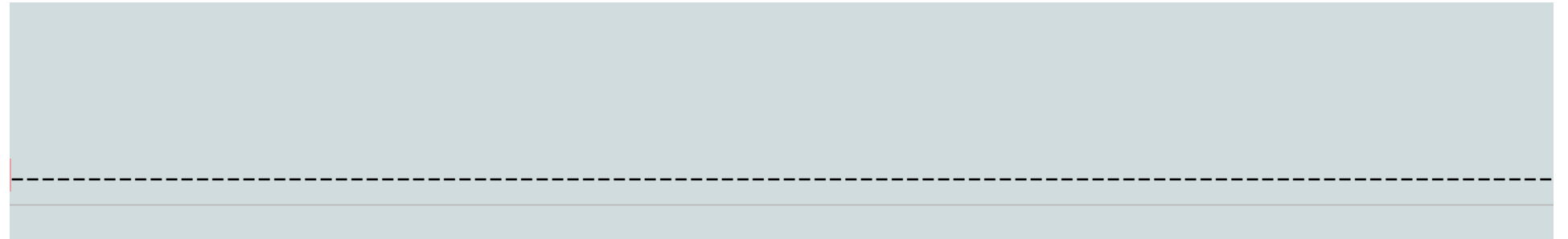


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6,100 6,110 6,120 6,130 6,140 6,150 6,160 6,170 6,180 6,190

incP origin of transfer

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



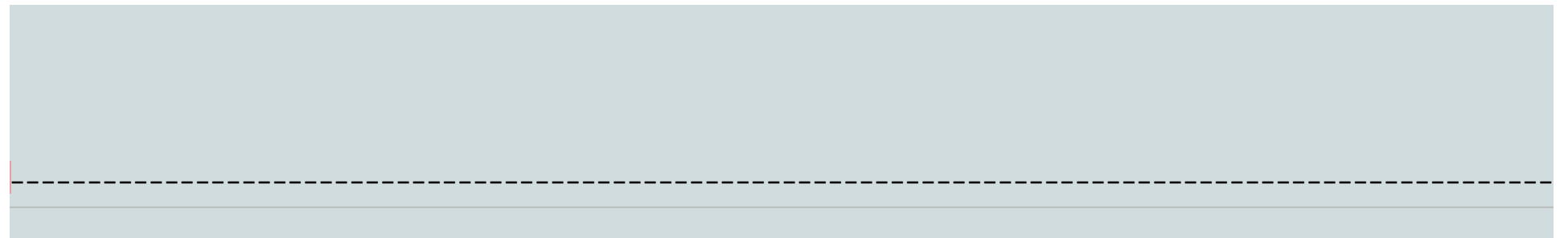
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

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6,200 6,210 6,220 6,230 6,240 6,250 6,260 6,270 6,280 6,290

trfA

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



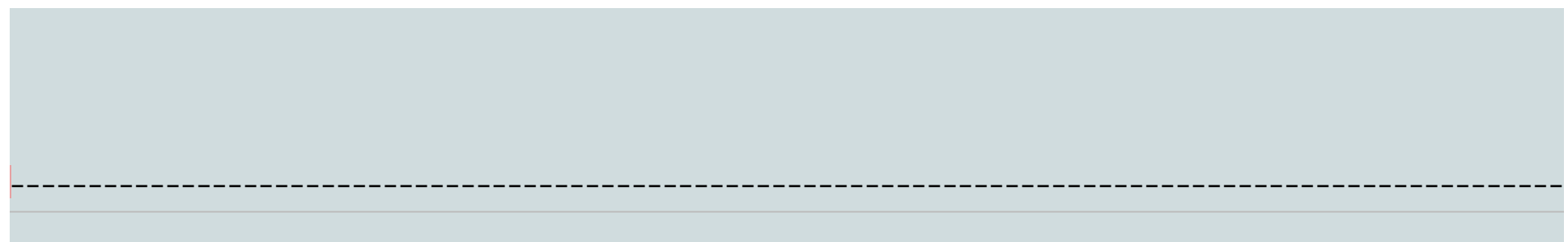
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6,300 6,310 6,320 6,330 6,340 6,350 6,360 6,370 6,380 6,390

trfA

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



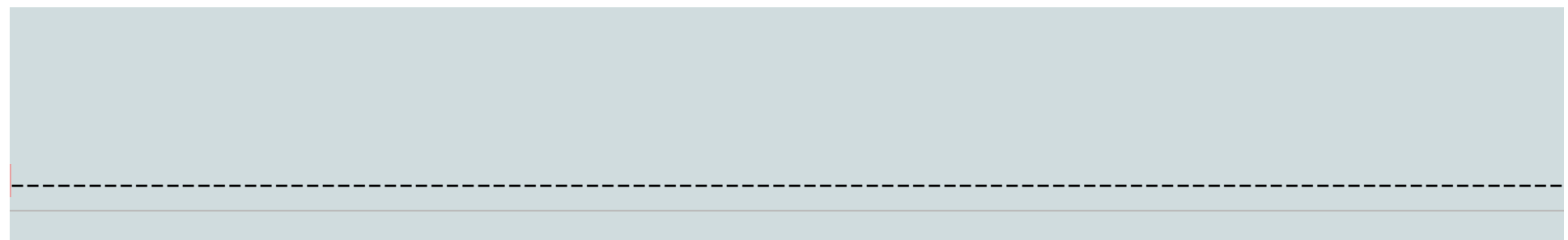
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

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6,400 6,410 6,420 6,430 6,440 6,450 6,460 6,470 6,480 6,490

trfA

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



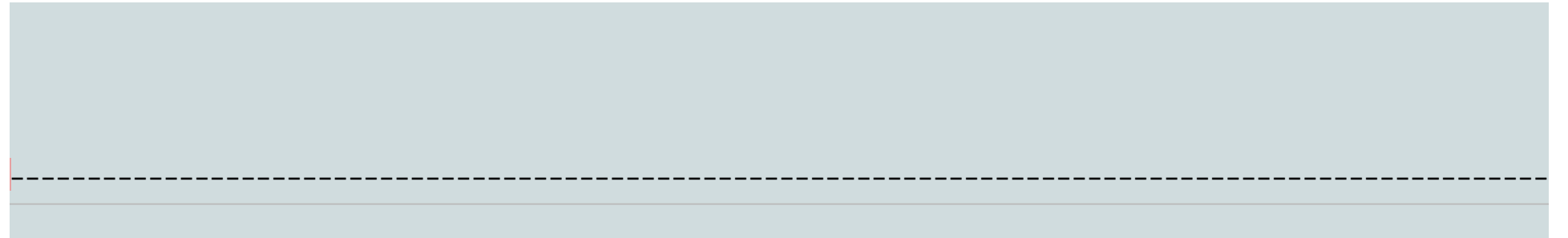
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

aatttcgtgtagtggtcgccagcaaacagcacgacgatttcctcgtcgatcaggacctggcaacgggacgttttcttgccacggtccaggacgcggaagc

6,500 6,510 6,520 6,530 6,540 6,550 6,560 6,570 6,580 6,590

trfA

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



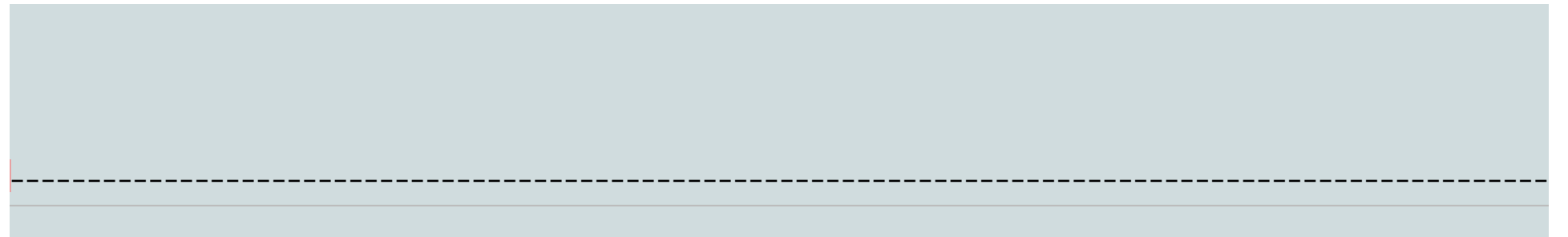
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

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6,600 6,610 6,620 6,630 6,640 6,650 6,660 6,670 6,680 6,690

trfA

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



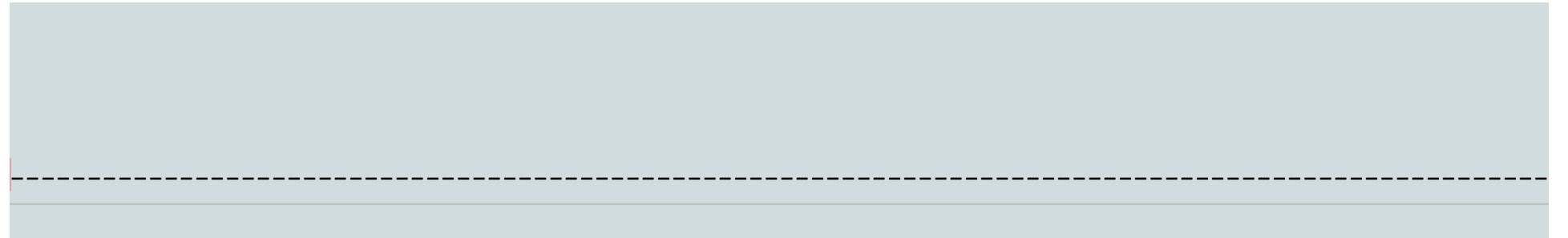
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

ataccggccattgatcgaccagcccaggctcctggcaaagctcgtagaacgtgaaggatcggtcgccgataggggtgcgcttcgcgtactccaacacc

6,700 6,710 6,720 6,730 6,740 6,750 6,760 6,770 6,780 6,790

trfA

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



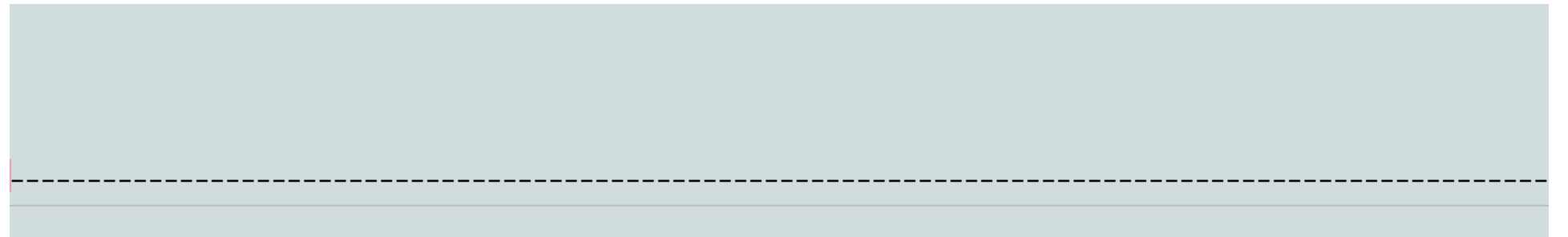
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tgctgccacaccagttcgatcatcgctggcccgagctcgacgccggtgtaggtgatcttcacgtccttggtgacgtggaaaatgaccttgttttgcagcg

6,800 6,810 6,820 6,830 6,840 6,850 6,860 6,870 6,880 6,890

trfA

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



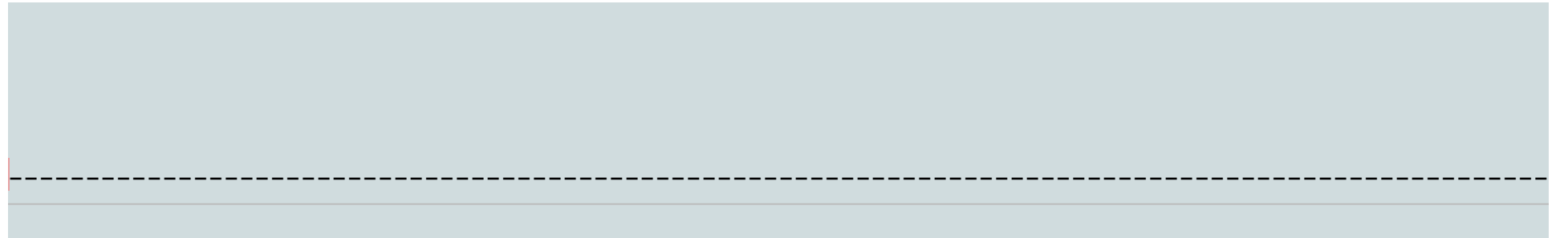
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

cctcgcgcgggattttcttggtgcgcgtggtgaacagggcagagcgggccgtgtcgtttggcatcgctcgcatcggtgtccggccacggcgcaatatcgaa

6,900 6,910 6,920 6,930 6,940 6,950 6,960 6,970 6,980 6,990

trfA

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



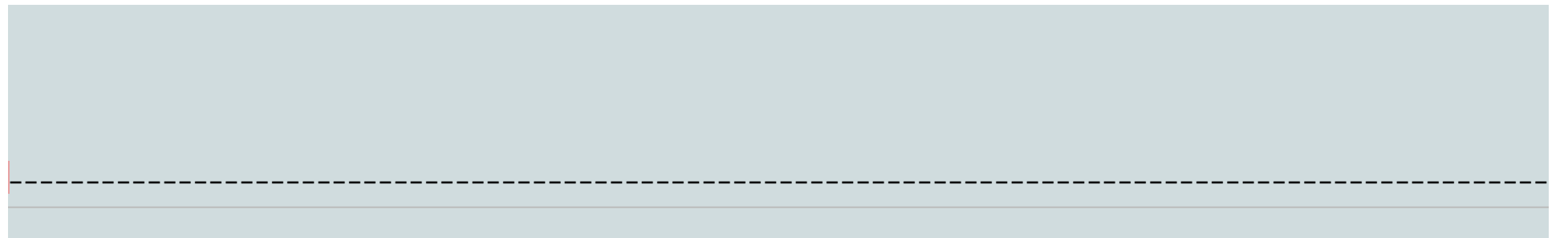
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

caaggaaagctgcatttccttgatctgctgcttcgtgtgtttcagcaacgcggcctgcttggcttcgctgacctgttttgccaggtcctcgccggcggtt

7,000 7,010 7,020 7,030 7,040 7,050 7,060 7,070 7,080 7,090

trfA

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



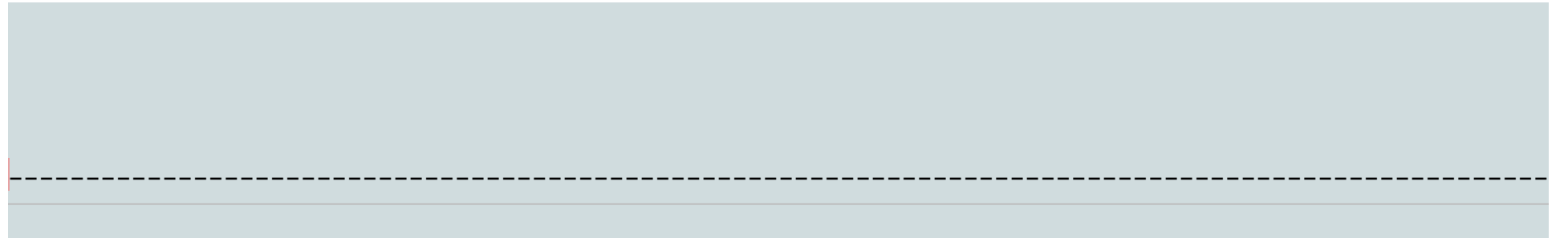
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tttcgcttcttggtcgtcatagttcctcgcggtgctgatggatcgacttcgccaacctgccgcctcctgttcgagacgacgcgaacgctccacggcgg

7,100 7,110 7,120 7,130 7,140 7,150 7,160 7,170 7,180 7,190

<< trfA >>

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



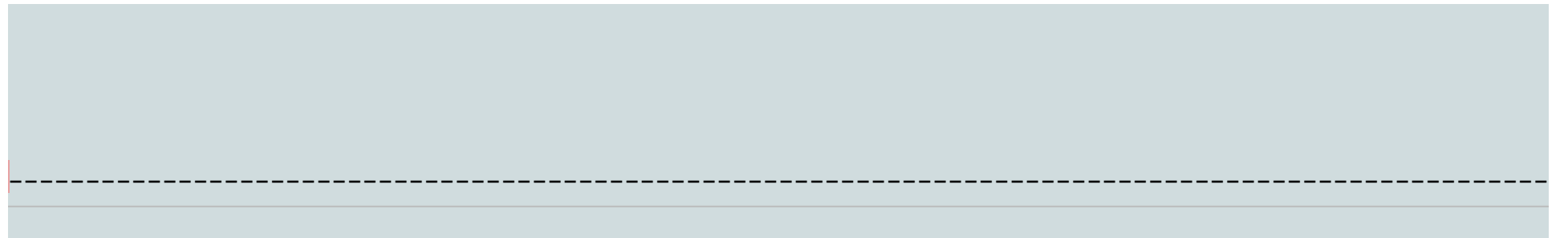
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

ccgatggcgcgggcagggcagggggagccagttgcacgctgtcgcgctcgatcttggccgtagcttgctggactatcgagccgacggactggaagggttc

7,200 7,210 7,220 7,230 7,240 7,250 7,260 7,270 7,280 7,290

<< trfA >>

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



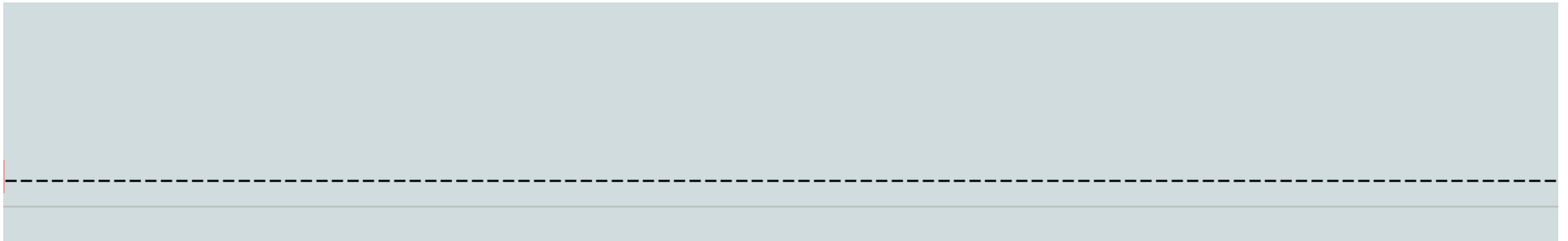
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gcggggcgacgcatgacggtgcggttcgatgggttcggcatcctcggcggaaccccgcgatcagttcttgccgtgatgccttccggtcaaac

7,300 7,310 7,320 7,330 7,340 7,350 7,360 7,370 7,380 7,390

trfA

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



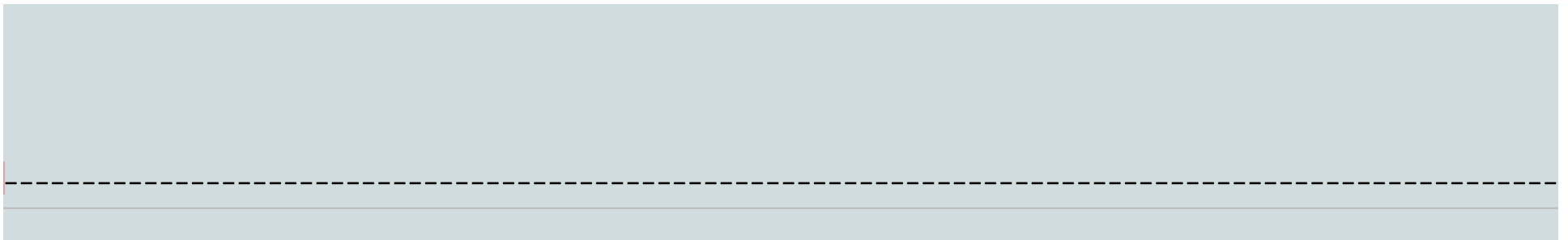
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7,400 7,410 7,420 7,430 7,440 7,450 7,460 7,470 7,480 7,490

trfA

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

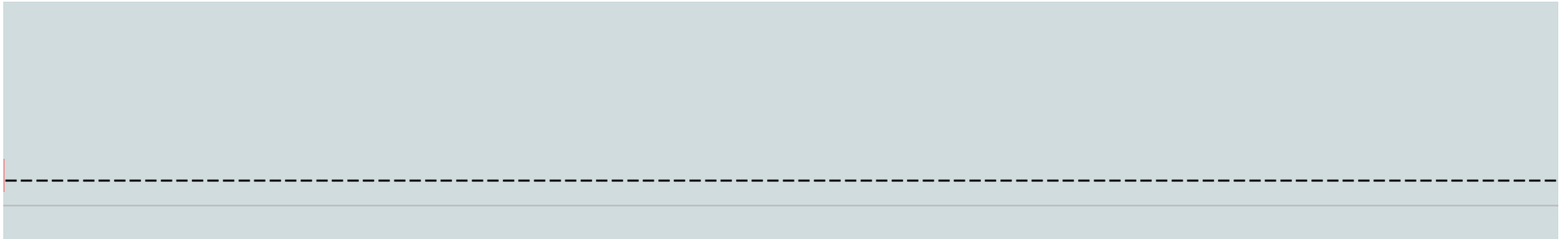


aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

gaaagccatccagtttactttgcagggttcccaaccttaccagagggcgccccagctggcaattccggttcgcttgctgtccataaaaccgcccagtct

7,500 7,510 7,520 7,530 7,540 7,550 7,560 7,570 7,580 7,590

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

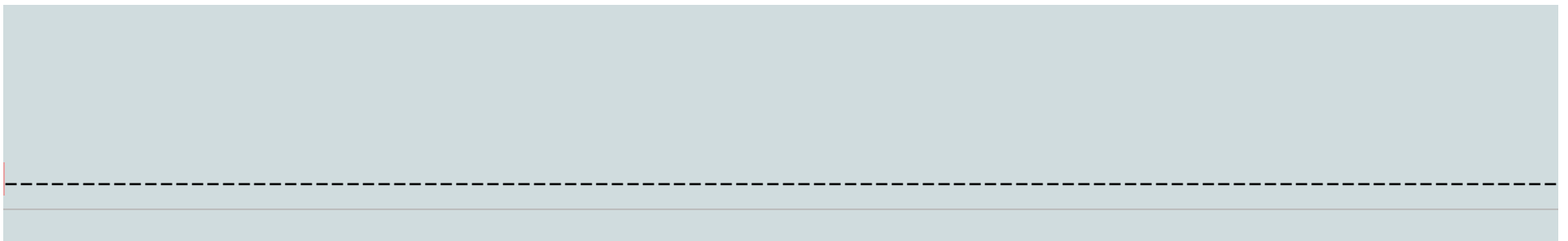


aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

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7,600 7,610 7,620 7,630 7,640 7,650 7,660 7,670 7,680 7,690

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



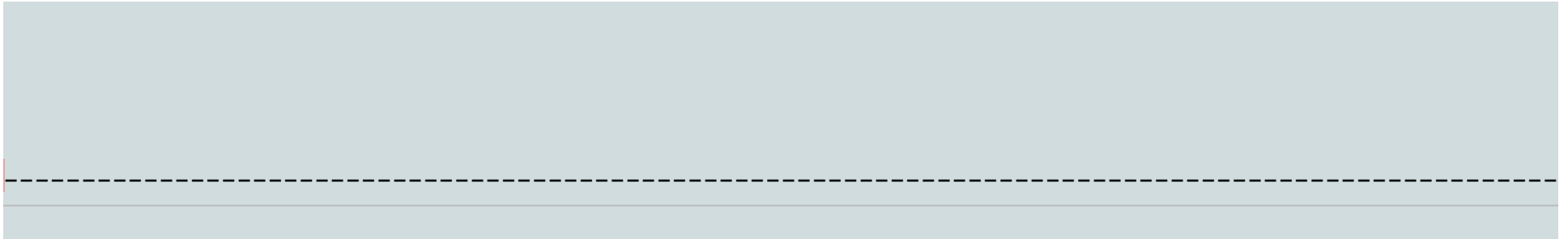
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF



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7,700 7,710 7,720 7,730 7,740 7,750 7,760 7,770 7,780 7,790

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



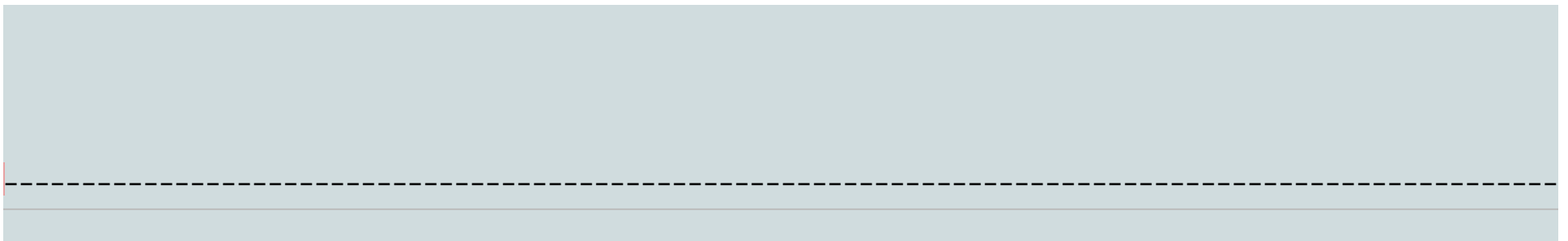
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

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7,800 7,810 7,820 7,830 7,840 7,850 7,860 7,870 7,880 7,890

oriV

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



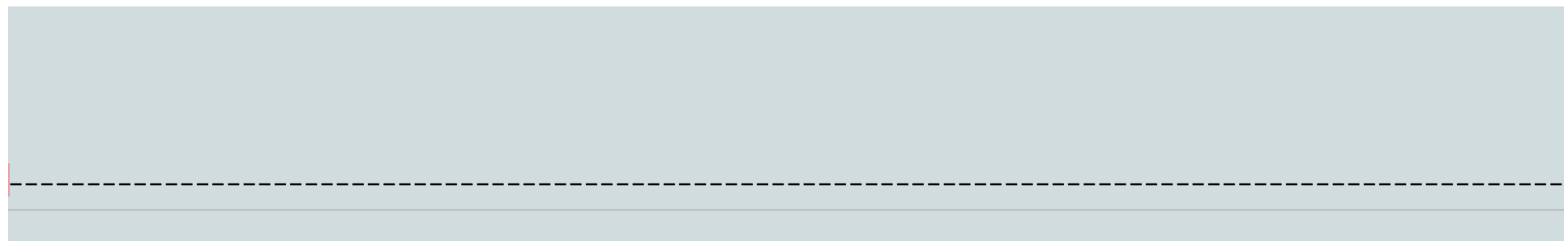
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7,900 7,910 7,920 7,930 7,940 7,950 7,960 7,970 7,980 7,990

>> oriV >>

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



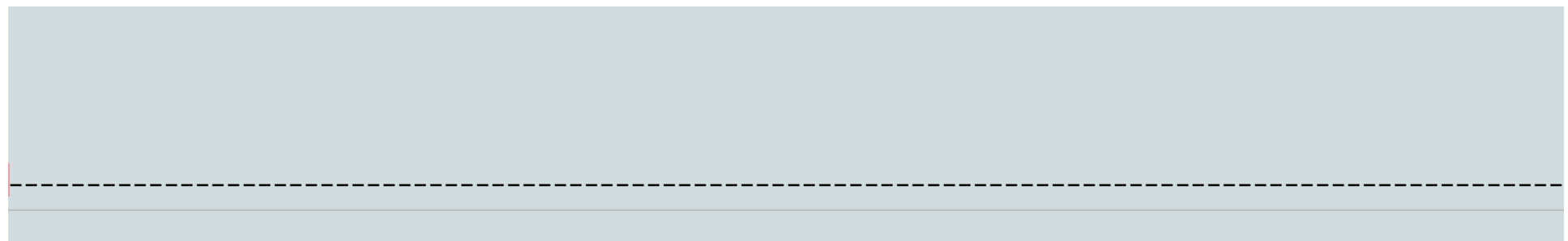
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8,000 8,010 8,020 8,030 8,040 8,050 8,060 8,070 8,080 8,090

>> oriV >>

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



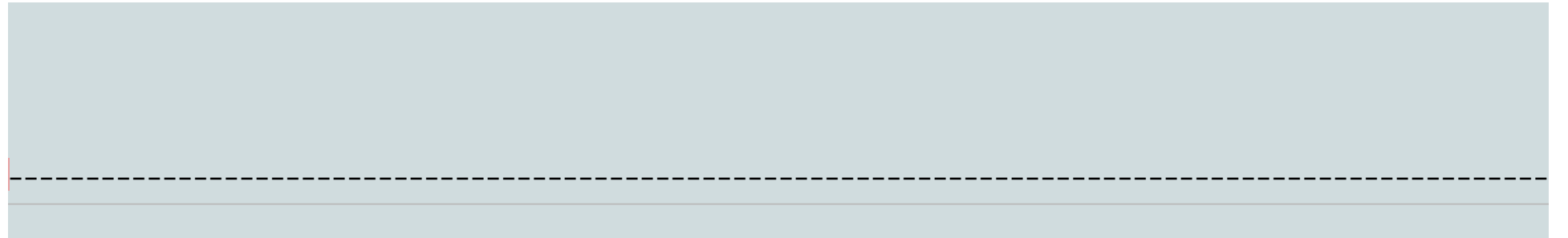
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8,100 8,110 8,120 8,130 8,140 8,150 8,160 8,170 8,180 8,190

>> oriV >>

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



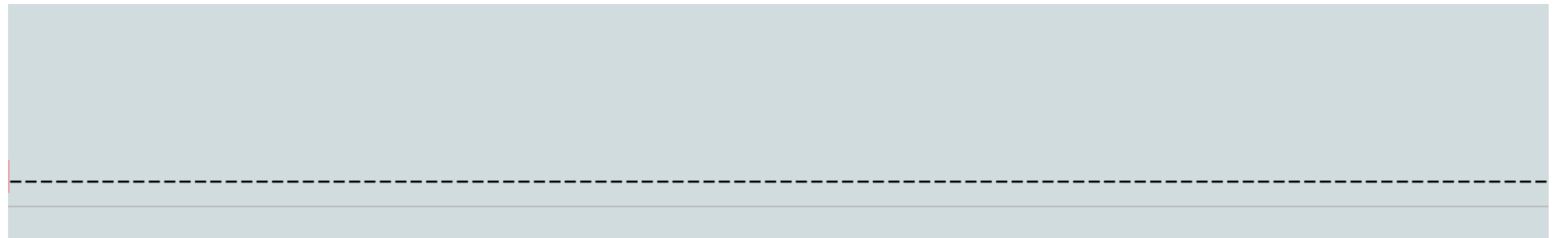
aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

cgccggccgaaatcgagcctgcccctcatctgtcaacgccgcgccgggtgagtcggcccctcaagtgtcaacgtccgcccctcatctgtcagtgagggcc

8,200 8,210 8,220 8,230 8,240 8,250 8,260 8,270 8,280 8,290

>> oriV >>

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



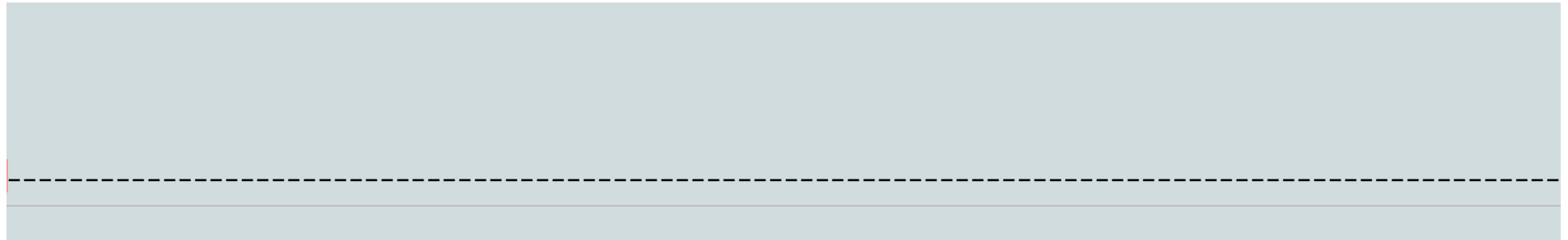
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aagttttccgcgaggtatccacaacgccggcgccctacatggctctgctgtagtgagtggggttgcgctccggcagcggtcctgatccccgcagaaaaa

8,300 8,310 8,320 8,330 8,340 8,350 8,360 8,370 8,380 8,390

>> oriV

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)

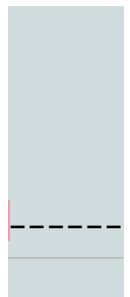


aligned sequence 2020-12-04\_G02\_ASAST15BSPTF

aaggat

8,400 405

template sequence pAST.010 ( $\Delta$ prqRA Cas12a KO 1)



aligned sequence 2020-12-04\_G02\_ASAST15BSPTF