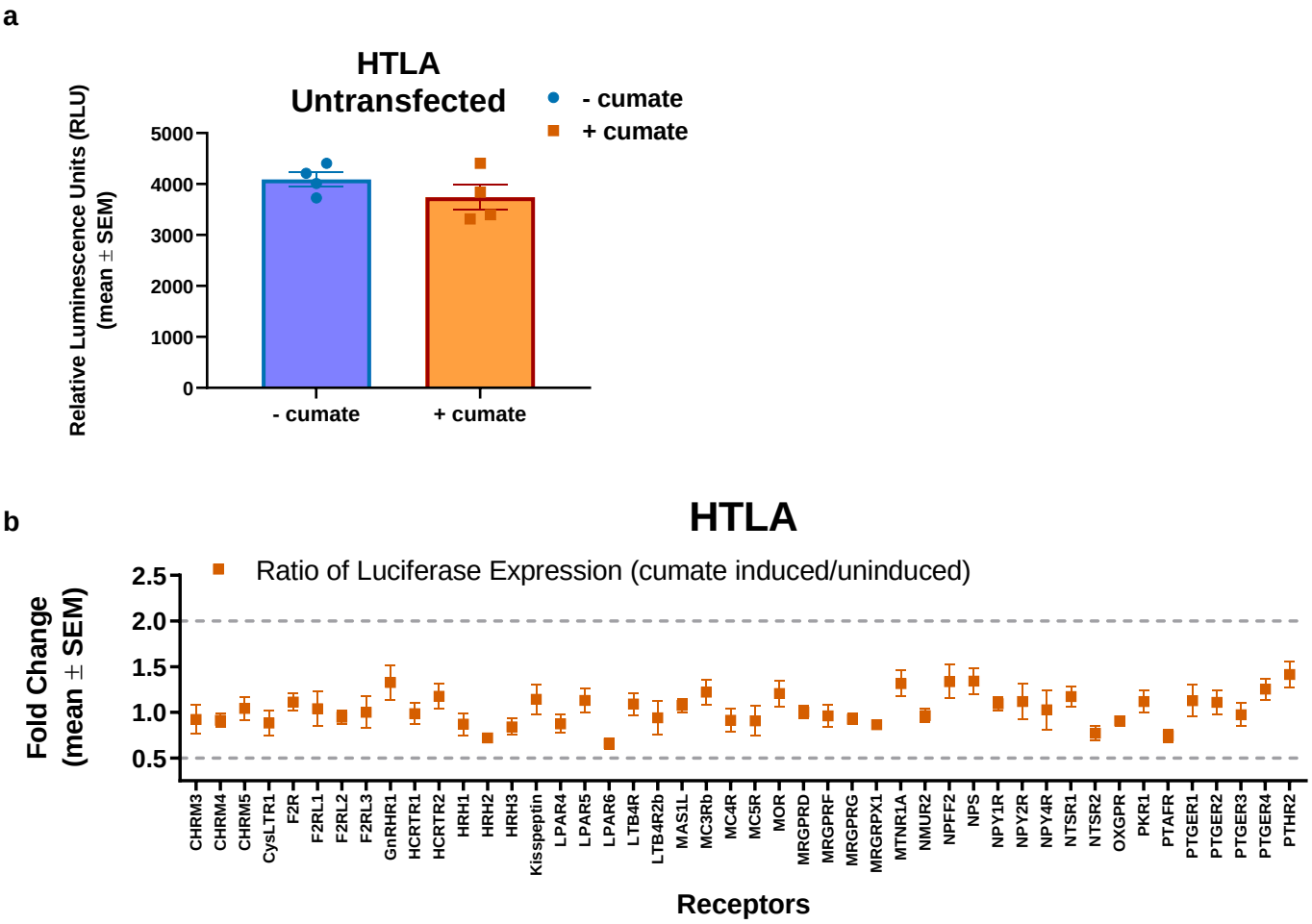
Supplementary Information for

Profiling of basal and ligand-dependent GPCR activities by means of a polyvalent cell-based high-throughput platform

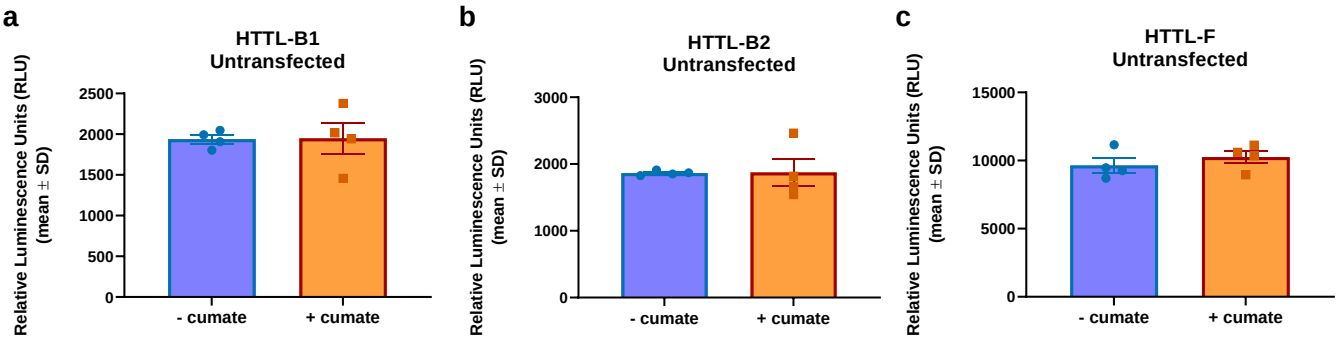
Manel Zeghal[1], Geneviève Laroche[1], Julia Douglas Freitas[1], Rebecca Wang[1], Patrick M. Giguère[1,2*]

[1] Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, ON, K1H8M5, Canada
[2] Brain and Mind Research Institute, University of Ottawa, Ottawa, ON, K1H8M5, Canada
*Correspondence to Patrick M. Giguère. Email: patrick.giguere@uottawa.ca

Supplemental Figures

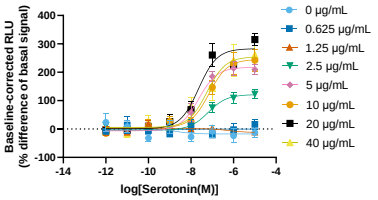


Supplementary Figure 1. Assessment of the effect of cumate in the PRESTO-Tango (cumate-independent system). To confirm that cumate itself does not possess any agonistic or antagonistic properties in a cumate-independent system, HTLA cells from the PRESTO-Tango platform were plated in the presence and absence of cumate (30 µg/mL) (a) and were transfected with a panel of diverse GPCR Tango constructs (b). 96 hours following its initial addition, fold changes in basal arrestin recruitment were calculated between the wells in the absence or presence of cumate. Data are presented as mean values, with error bars representing SEM (Supplementary Fig 1A: n = 12, with 3 technical replicates from 4 biological samples; Supplementary Fig 1B: n = 4, with 4 technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.



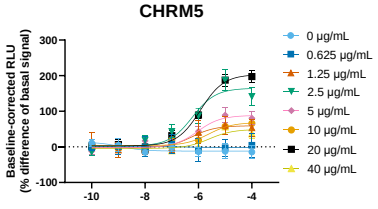
Supplementary Figure 2. Baseline signal of Tango-Trio cell lines. (A-C) HTTL-B1, HTTL-B2, and HTTL-F cells were plated in the presence or absence of cumate (30 µg/mL), which was maintained throughout (tot alling approximately 72 hours). As per standard protocol, cells were serum starved for the last 24 hours of the experiment, and luminescence was subsequently read to compare the differences in baseline sig nal of untransfected cells. Data are presented as mean values, with error bars representing SD (n = 12, with 3 te chnical replicates from 4 biological samples).

a



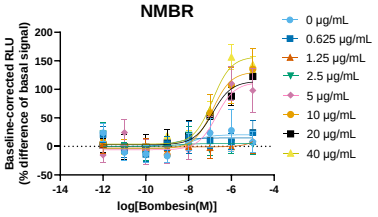
	EC50	Fold
0 µg/mL	1.522e-009	0.7986
0.625 µg/mL	6.476e-009	1.032
1.25 µg/mL	5.666e-007	0.8598
2.5 µg/mL	6.493e-008	2.302
5 µg/mL	2.277e-008	3.396
10 µg/mL	7.106e-008	3.305
20 µg/mL	2.281e-008	3.797
40 µg/mL	5.685e-008	3.347

b



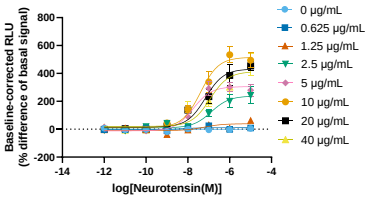
	EC50	Fold
0 µg/mL	1.261e-009	0.7768
0.625 µg/mL	2.616e-007	0.9735
1.25 µg/mL	5.848e-007	1.630
2.5 µg/mL	5.948e-007	2.585
5 µg/mL	9.187e-007	1.923
10 µg/mL	2.424e-006	1.626
20 µg/mL	1.187e-006	2.952
40 µg/mL	2.552e-006	1.560

c



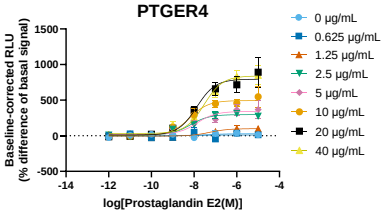
	EC50	Fold
0 µg/mL	1.333e-008	1.255
0.625 µg/mL	2.705e-009	1.146
1.25 µg/mL	0.1617	Unstable
2.5 µg/mL	2.063e-009	1.053
5 µg/mL	2.005e-007	2.256
10 µg/mL	1.295e-007	2.403
20 µg/mL	1.348e-007	2.077
40 µg/mL	1.316e-007	2.475

d



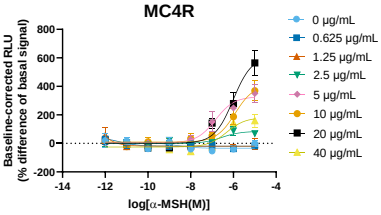
	EC50	Fold
0 µg/mL	0.03127	Unstable
0.625 µg/mL	5.844e-009	1.140
1.25 µg/mL	6.036e-008	1.545
2.5 µg/mL	1.300e-007	3.099
5 µg/mL	1.779e-008	4.610
10 µg/mL	4.025e-008	5.504
20 µg/mL	6.903e-008	4.617
40 µg/mL	1.153e-007	4.251

e



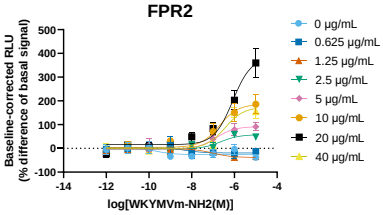
	EC50	Fold
0 µg/mL	5.024e-008	1.309
0.625 µg/mL	2.567e-009	1.143
1.25 µg/mL	5.236e-008	2.099
2.5 µg/mL	6.696e-009	3.636
5 µg/mL	1.387e-008	3.731
10 µg/mL	6.337e-009	5.511
20 µg/mL	1.327e-008	8.103
40 µg/mL	2.933e-008	7.132

f



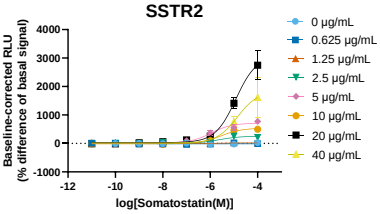
	EC50	Fold
0 µg/mL	2.220e-009	0.6302
0.625 µg/mL	4.753e-012	0.5670
1.25 µg/mL	2.018e-015	Unstable
2.5 µg/mL	2.823e-007	1.864
5 µg/mL	1.459e-007	4.093
10 µg/mL	1.204e-006	4.687
20 µg/mL	9.759e-007	7.012
40 µg/mL	4.858e-007	3.775

g



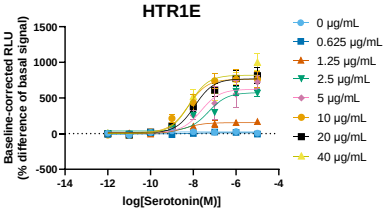
	EC50	Fold
0 µg/mL	2.372e-010	0.7269
0.625 µg/mL	5.153e-009	0.7824
1.25 µg/mL	1.013e-007	0.6304
2.5 µg/mL	2.296e-007	1.621
5 µg/mL	1.377e-007	1.970
10 µg/mL	1.981e-007	2.739
20 µg/mL	8.317e-007	4.177
40 µg/mL	3.618e-007	2.758

h



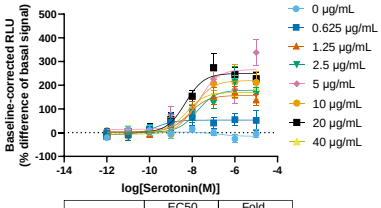
	EC50	Fold
0 µg/mL	1.306e-007	0.9568
0.625 µg/mL	1.517e-010	0.7628
1.25 µg/mL	2.058e-006	1.253
2.5 µg/mL	2.399e-006	3.481
5 µg/mL	1.086e-006	7.144
10 µg/mL	2.812e-006	7.369
20 µg/mL	1.202e-005	24.43
40 µg/mL	1.403e-005	16.52

i



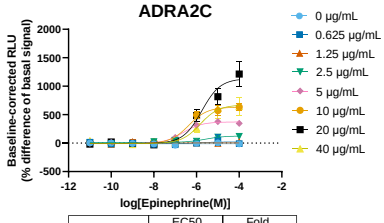
	EC50	Fold
0 µg/mL	6.643e-011	1.347
0.625 µg/mL	9.436e-009	1.154
1.25 µg/mL	4.096e-009	2.500
2.5 µg/mL	5.166e-008	4.929
5 µg/mL	2.304e-008	6.100
10 µg/mL	5.882e-009	7.516
20 µg/mL	1.179e-008	7.945
40 µg/mL	6.688e-009	8.827

j



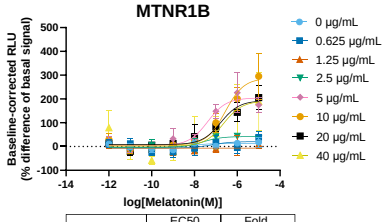
	EC50	Fold
0 µg/mL	1.711e-007	0.8261
0.625 µg/mL	1.945e-010	1.660
1.25 µg/mL	7.274e-009	2.478
2.5 µg/mL	1.703e-008	2.768
5 µg/mL	2.194e-008	3.205
10 µg/mL	1.199e-008	3.403
20 µg/mL	4.799e-009	3.491
40 µg/mL	5.383e-009	2.669

k



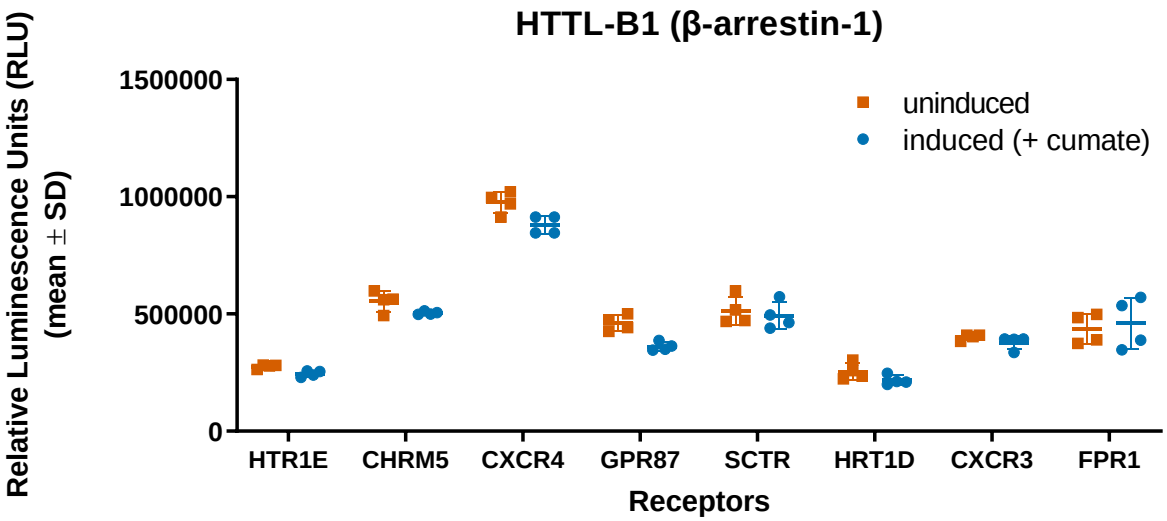
	EC50	Fold
0 µg/mL	6.870e-010	0.8535
0.625 µg/mL	1.161e-006	1.332
1.25 µg/mL	6.461e-016	Unstable
2.5 µg/mL	2.674e-006	2.013
5 µg/mL	2.320e-007	4.982
10 µg/mL	4.337e-007	7.992
20 µg/mL	1.761e-006	12.92
40 µg/mL	1.491e-006	8.250

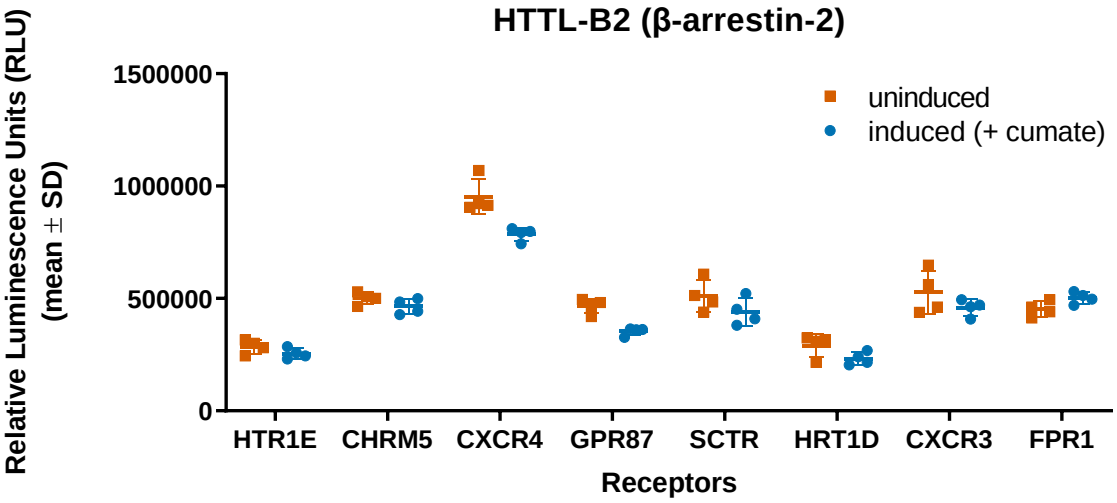
l

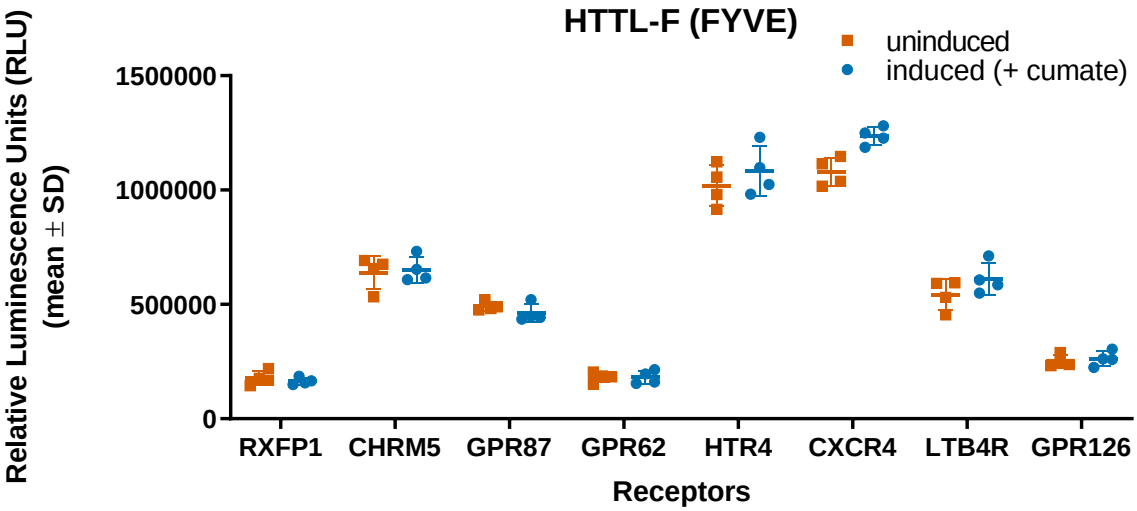


	EC50	Fold
0 µg/mL	8.045e-009	1.165
0.625 µg/mL	6.530e-008	1.287
1.25 µg/mL	90711713	Unstable
2.5 µg/mL	2.453e-008	1.478
5 µg/mL	4.129e-008	2.906
10 µg/mL	2.752e-007	3.653
20 µg/mL	1.686e-007	2.702
40 µg/mL	2.283e-007	2.870

Supplementary Figure 3. Changes in baseline signals and fold windows due to cumate induction. HTTL-B1, HTTL-B2 and HTTL-F cells were plated in increasing concentrations of cumate (0, 0.625, 1.25, 2.5, 5, 10, 20, and 40 µg/mL), which was maintained throughout the entire experiment, and were transfected with select GPCRs: HTR4 (a), CHRM5 (b), and NMBR (c) in HTTL-F; NTSR1 (d), PTGER4 (e), MC4R (f), FPR2 (g), and SSTR2 (h) in HTTL-B2; HTR1E (i), HTR1D (j), ADRA2C (k), and MTNR1B (l) in HTTL-B1. Transfected cells were stimulated with the receptor specific agonist and dose-response curves were built using XY analysis for non-linear regression curve and the 3-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with three technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.

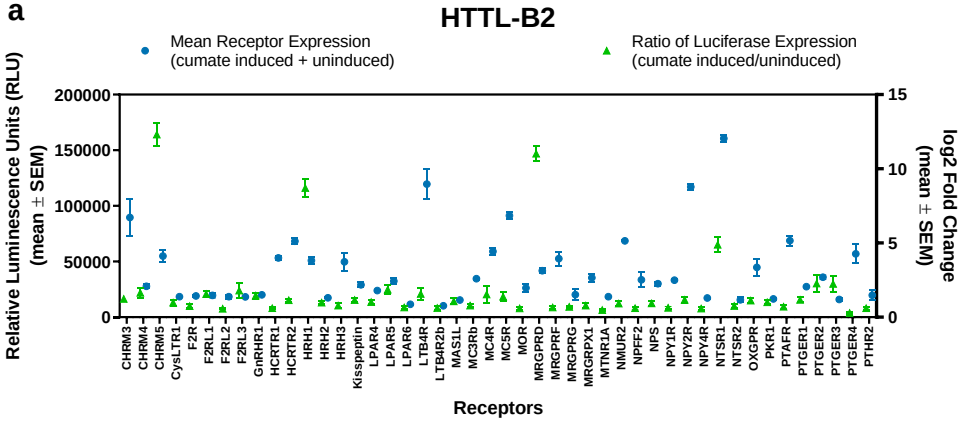

HTTL-B1 (β-arrestin-1)
Relative Luminescence Units (RLU)
(mean ± SD)
1500000
1000000
500000
0
uninduced
induced (+ cumate)
HTR1E
CHRM5
CXCR4
GPR87
SCTR
HRT1D
CXCR3
FPR1
Receptors


HTTL-B2 (β-arrestin-2)
Relative Luminescence Units (RLU)
(mean ± SD)
1500000
1000000
500000
0
uninduced
induced (+ cumate)
HTR1E
CHRM5
CXCR4
GPR87
SCTR
HRT1D
CXCR3
FPR1
Receptors


HTTL-F (FYVE)
Relative Luminescence Units (RLU)
(mean ± SD)
1500000
1000000
500000
0
uninduced
induced (+ cumate)
RXFP1
CHRM5
GPR87
GPR62
HTR4
CXCR4
LTB4R
GPR126
Receptors

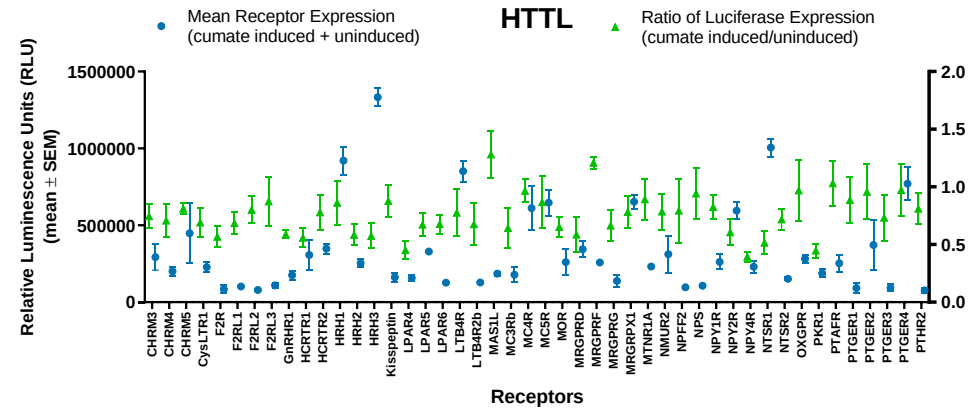
Supplementary Figure 4. Receptor surface expression following cumate induction of fusion protein expression HTTL-B1, HTTL-B2 and HTTL-F were plated in the presence or absence of cumate (30 µg/mL) and were transfected with a select number of positive GPCR hits from the constitutive HTS. ELISA experiments were subsequently carried out on transfected cells to determine receptor surface expression. Data are presented as mean values, with error bars representing SD (n = 4, with four technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.

a



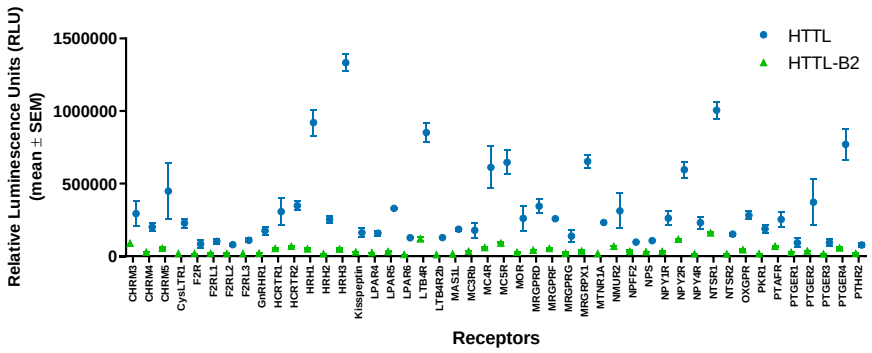
Pearson r	
r	0.2084
95% confidence interval	-0.08714 to 0.4703
R squared	0.04344
P value	
P (two-tailed)	0.1645
P value summary	ns
Significant? (alpha = 0.05)	No
Number of XY Pairs	46

b



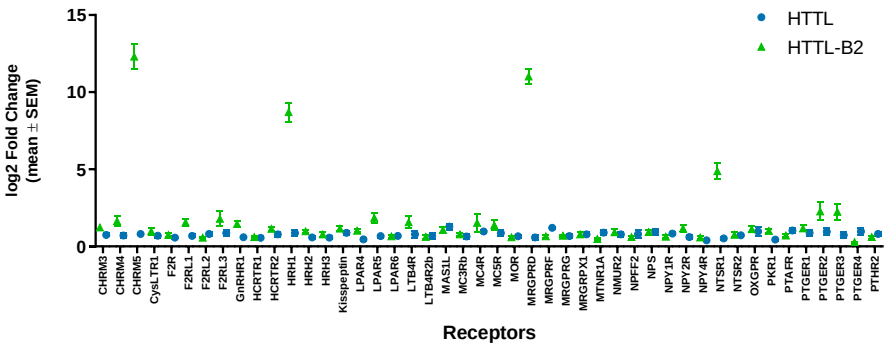
Pearson r	
r	-0.03125
95% confidence interval	-0.3187 to 0.2614
R squared	0.0009767
P value	
P (two-tailed)	0.8366
P value summary	ns
Significant? (alpha = 0.05)	No
Number of XY Pairs	46

c



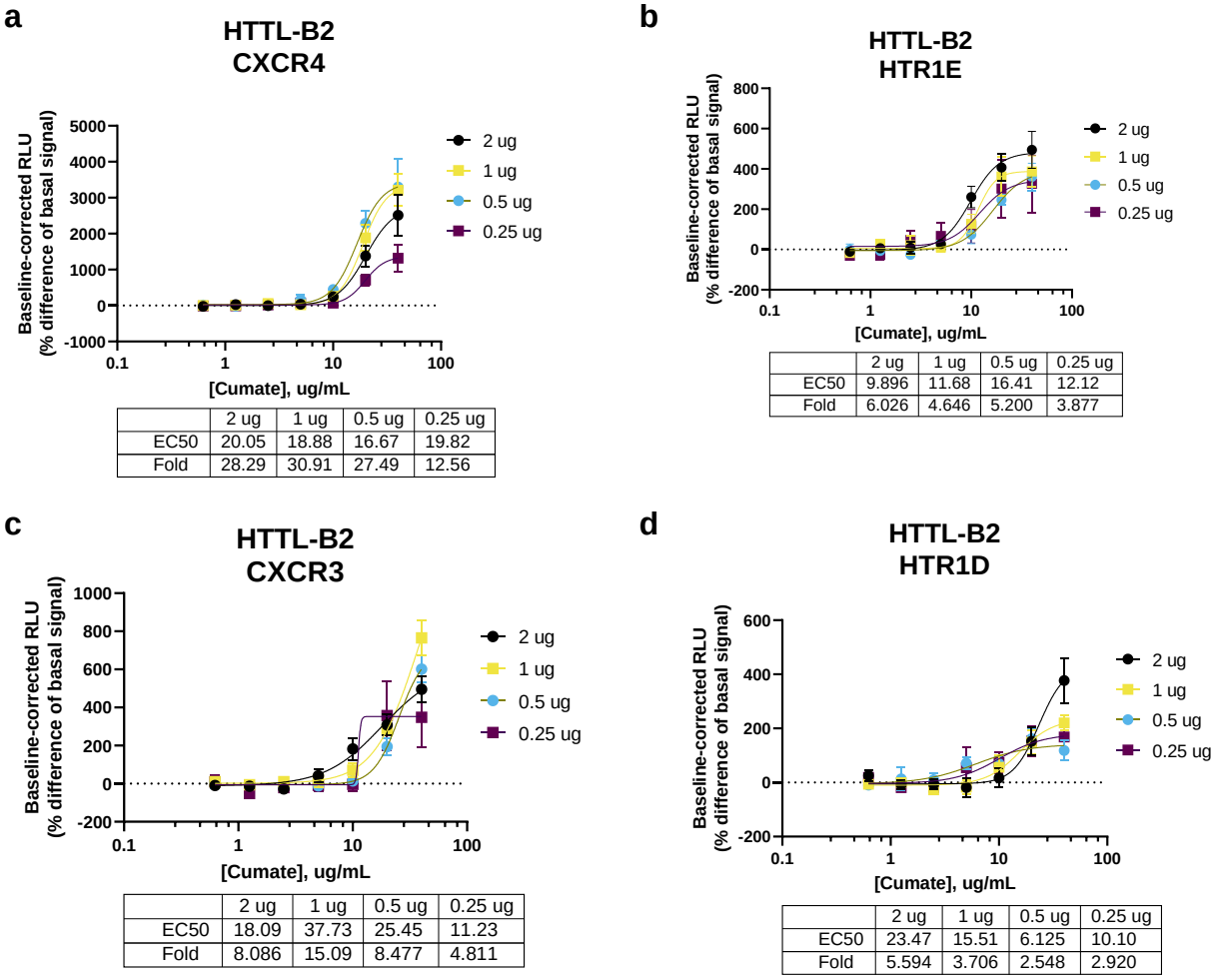
Pearson r	
r	0.6659
95% confidence interval	0.4656 to 0.8013
R squared	0.4434
P value	
P (two-tailed)	<0.0001
P value summary	****
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	46

d



Pearson r	
r	-0.04866
95% confidence interval	-0.3342 to 0.2451
R squared	0.002368
P value	
P (two-tailed)	0.7481
P value summary	ns
Significant? (alpha = 0.05)	No
Number of XY Pairs	46

Supplementary Figure 5. Comparison of receptor expression and constitutive activity across panel of GPCRs. HTTL-B2 (a) and HTTL (b) were plated in the presence or absence of cumate (30 µg/mL) and were transfected with a panel of GPCRs with a varying range of constitutive activities. ELISA experiments were subsequently carried out on transfected cells to determine receptor surface expression, and log2 fold changes in constitutive arrestin recruitment were calculated between the wells in the absence or presence of cumate. Mean receptor expression (c) and log2 fold changes in constitutive arrestin recruitment (d) were also compared between the HTTL and HTTL-B2 cell lines. Pearson correlation coefficients (r) and corresponding p values (two-tailed) were computed between data sets using GraphPad Prism. Data are presented as mean values, with error bars representing SEM (n = 4, with four technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.



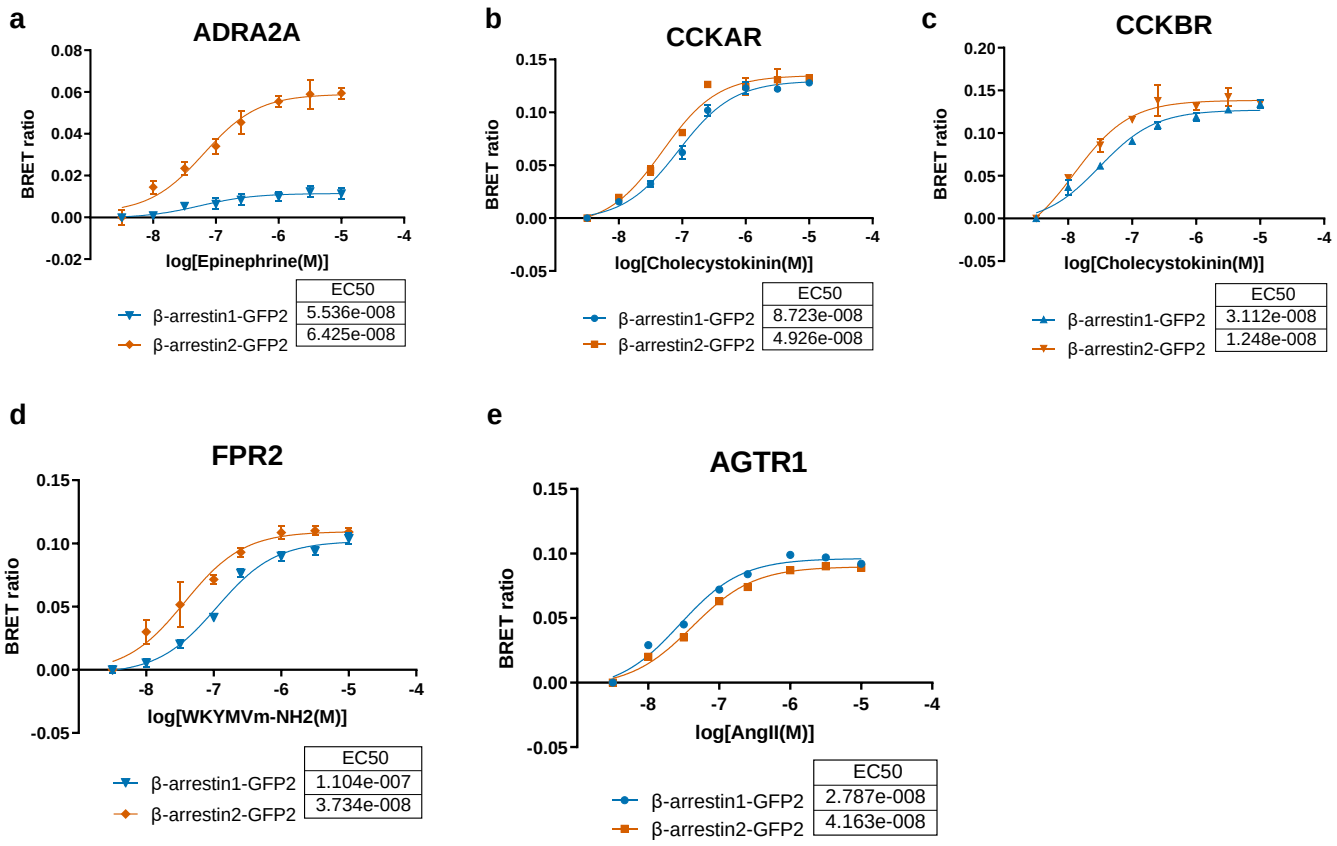
	2 ug	1 ug	0.5 ug	0.25 ug
EC50	20.05	18.88	16.67	19.82
Fold	28.29	30.91	27.49	12.56

	2 ug	1 ug	0.5 ug	0.25 ug
EC50	9.896	11.68	16.41	12.12
Fold	6.026	4.646	5.200	3.877

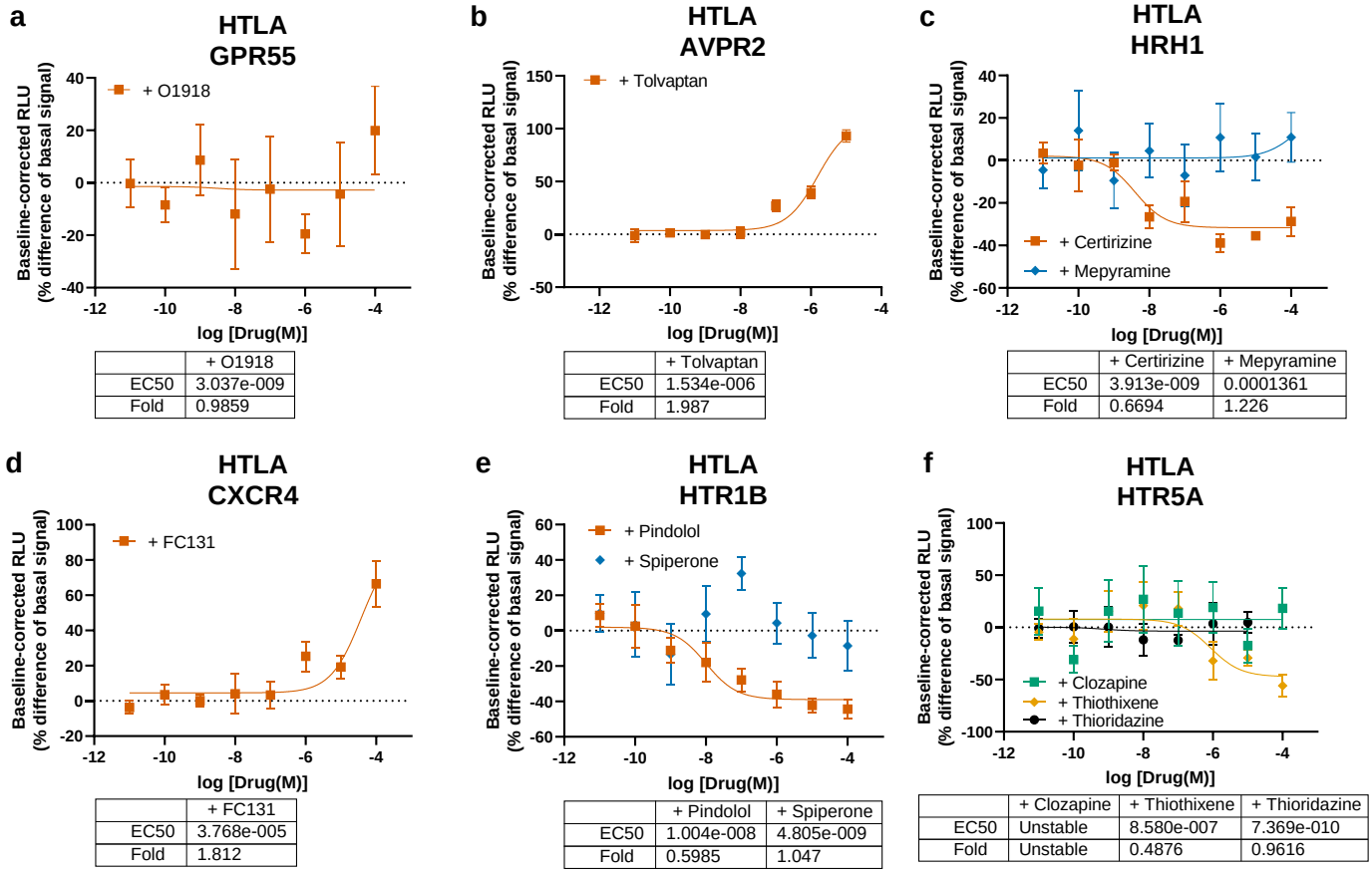
	2 ug	1 ug	0.5 ug	0.25 ug
EC50	18.09	37.73	25.45	11.23
Fold	8.086	15.09	8.477	4.811

	2 ug	1 ug	0.5 ug	0.25 ug
EC50	23.47	15.51	6.125	10.10
Fold	5.594	3.706	2.548	2.920

Supplementary Figure 6. Titration of GPCR Tango DNA and consequent effects on constitutive activity. HTTL-B2 cells were seeded in 6-well plates and transfected with various amount of CXCR4 (a), HTR1E (b), CXCR3 (c), and HTR1D (d) Tango DNA, ranging from 0.25 ug – 2 ug total per well. Transfected cells were stimulated with cumate, and dose- response curves were built using XY analysis for non-linear regression curve and the 4-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with three technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.



Supplementary Figure 7. Orthogonal determination of arrestin isoform selectivity using BRET2. HEK293T cells were transfected with β-arrestin1/2-GFP2, as well as the following GPCRs: ADRA2A (a), CCKAR (b), CCKBR (c), FPR2 (d), AGTR1 (e). Following transfection, cells were stimulated with serial dilutions of selective agonist, and read with 405 nm (RLuc8-Coelenterazine 400a) and 500 nm (GFP2) emission filters. Dose- response curves were built using XY analysis for non-linear regression curve and the 3-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 2, with two technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Rluc8 constructs.



	+ O1918
EC50	3.037e-009
Fold	0.9859

	+ Tolvaptan
EC50	1.534e-006
Fold	1.987

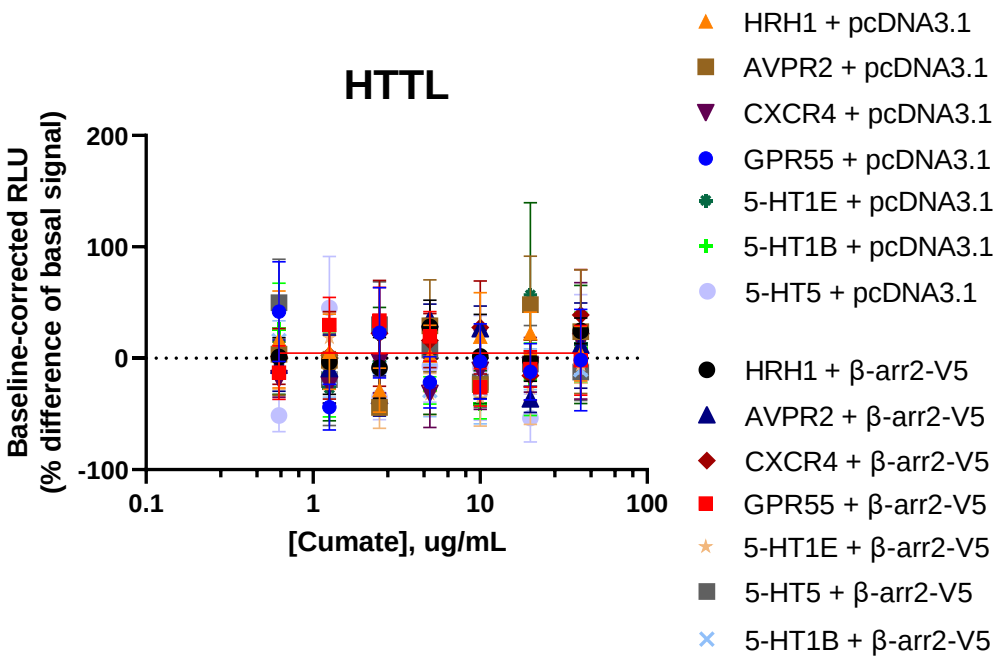
	+ Certirizine	+ Mepyramine
EC50	3.913e-009	0.0001361
Fold	0.6694	1.226

	+ FC131
EC50	3.768e-005
Fold	1.812

	+ Pindolol	+ Spiperone
EC50	1.004e-008	4.805e-009
Fold	0.5985	1.047

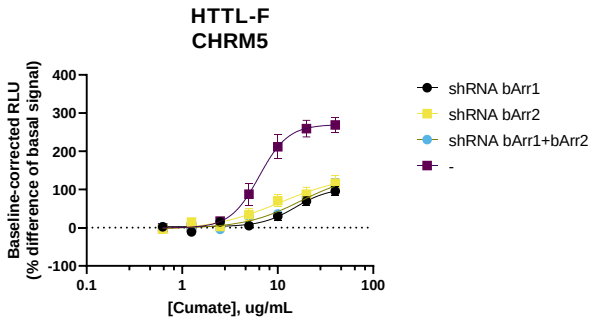
	+ Clozapine	+ Thiothixene	+ Thioridazine
EC50	Unstable	8.580e-007	7.369e-010
Fold	Unstable	0.4876	0.9616

Supplementary Figure 8. Orthogonal characterization of select inverse agonists and antagonists using PRESTO-Tango. HTLA cells were transfected with GPCRs exhibiting strong constitutive arrestin recruitment from the primary Tango-Trio screen, serving to validate the findings shown in Figure 7. Transfected cells were stimulated with a dose-response curve using the following inverse agonists/antagonists: O-1918 at GPR55 (a), Tolvaptan at AVPR2 (b), Cetirizine and Mepyramine at HRH1 (c), FC131 at CXCR4 (d), Pindolol and Spiperone at HTR1E (e), and Clozapine, Thiothexene, and Thioridazine at HTR5A (f). Stimulation curves were built using XY analysis for non-linear regression curve and the 3-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (Supplementary Figs 8a, 8c-f: n = 3, with three technical replicates from one biological sample; Supplementary Fig 8b: n = 6, with three technical replicates from two biological samples). Generic receptor codes refer to the GPCR-Tango constructs.



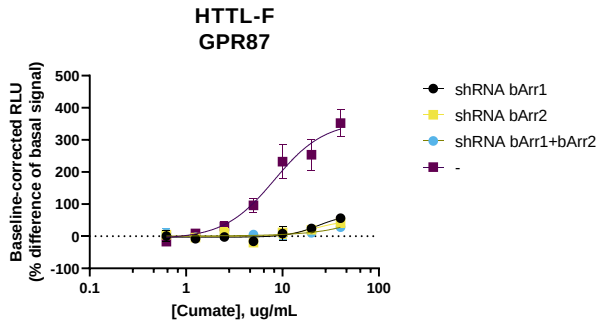
Supplementary Figure 9. Expression of GPCR-Tango constructs in the absence of TEV protease. HTTL cells were co-transfected with select GPCR constructs (tested with antagonists/inverse agonists from Fig 7), and either β-arrestin-2 (not tagged to TEV protease) or pcDNA3.1 as a control. Transfected cells were stimulated as a cumate dose-response, and stimulation curves were built using XY analysis for non-linear regression curve and the 4-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with three technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.

a



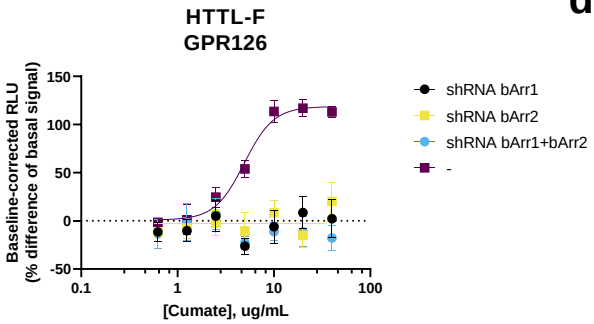
	EC50	logEC50	Span	Fold
shRNA bArr1	15.38	1.187	459303	1.993
shRNA bArr2	10.60	1.025	669083	2.622
shRNA bArr1+bArr2	17.20	1.236	564353	2.461
-	6.405	0.8065	139806	3.725

b



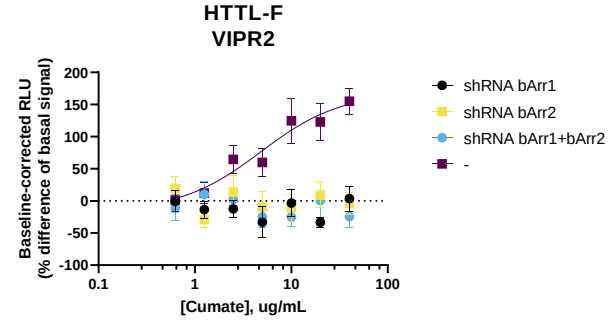
	EC50	logEC50	Span	Fold
shRNA bArr1	23.39	1.369	30849	1.744
shRNA bArr2	21.66	1.336	29759	1.513
shRNA bArr1+bArr2	557592	5.746	Unstable	Unstable
-	8.221	0.9149	48577	4.983

c



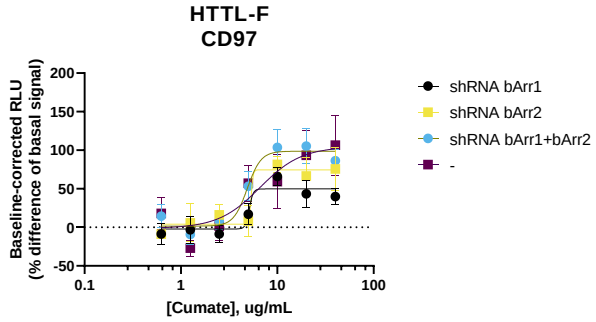
	EC50	logEC50	Span	Fold
shRNA bArr1	3.069	0.4871	30035	1.651
shRNA bArr2	42.20	1.625	Unstable	Unstable
shRNA bArr1+bArr2	3.419	0.5339	21766	1.486
-	4.921	0.6920	38098	2.169

d



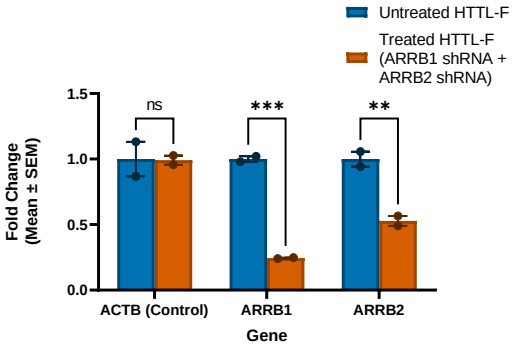
	EC50	logEC50	Span	Fold
shRNA bArr1	8.957	0.9522	63180	1.713
shRNA bArr2	4.508	0.6540	46960	1.685
shRNA bArr1+bArr2	3.386	0.5297	43671	1.472
-	4.821	0.6831	57025	3.172

e



	EC50	logEC50	Span	Fold
shRNA bArr1	5.126	0.7098	59654	1.532
shRNA bArr2	5.352	0.7285	71885	1.678
shRNA bArr1+bArr2	4.878	0.6883	94061	1.956
-	6.606	0.8200	67253	2.072

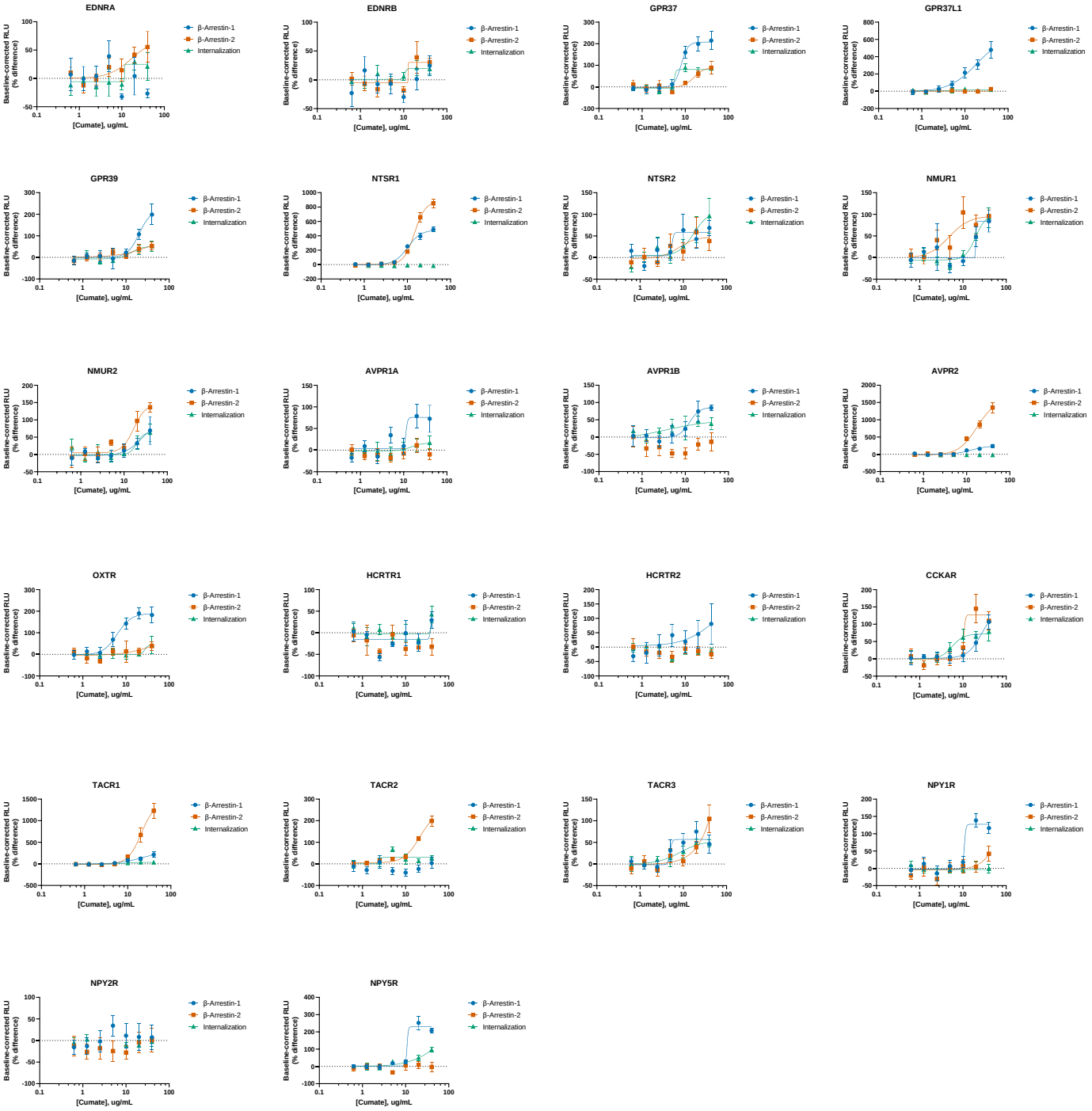
f



	ACTB (Control)	ARRB1	ARRB2
P value	0.930008	0.000147	0.001847
Mean of Untreated HTTL-F	1.000	1.000	1.000
Mean of Column B	0.9918	0.2444	0.5285
Difference	0.008163	0.7556	0.4715
SE of difference	0.08913	0.08913	0.08913
t ratio	0.09159	8.477	5.290
df	6.000	6.000	6.000
Adjusted P Value	0.930008	0.000442	0.003691

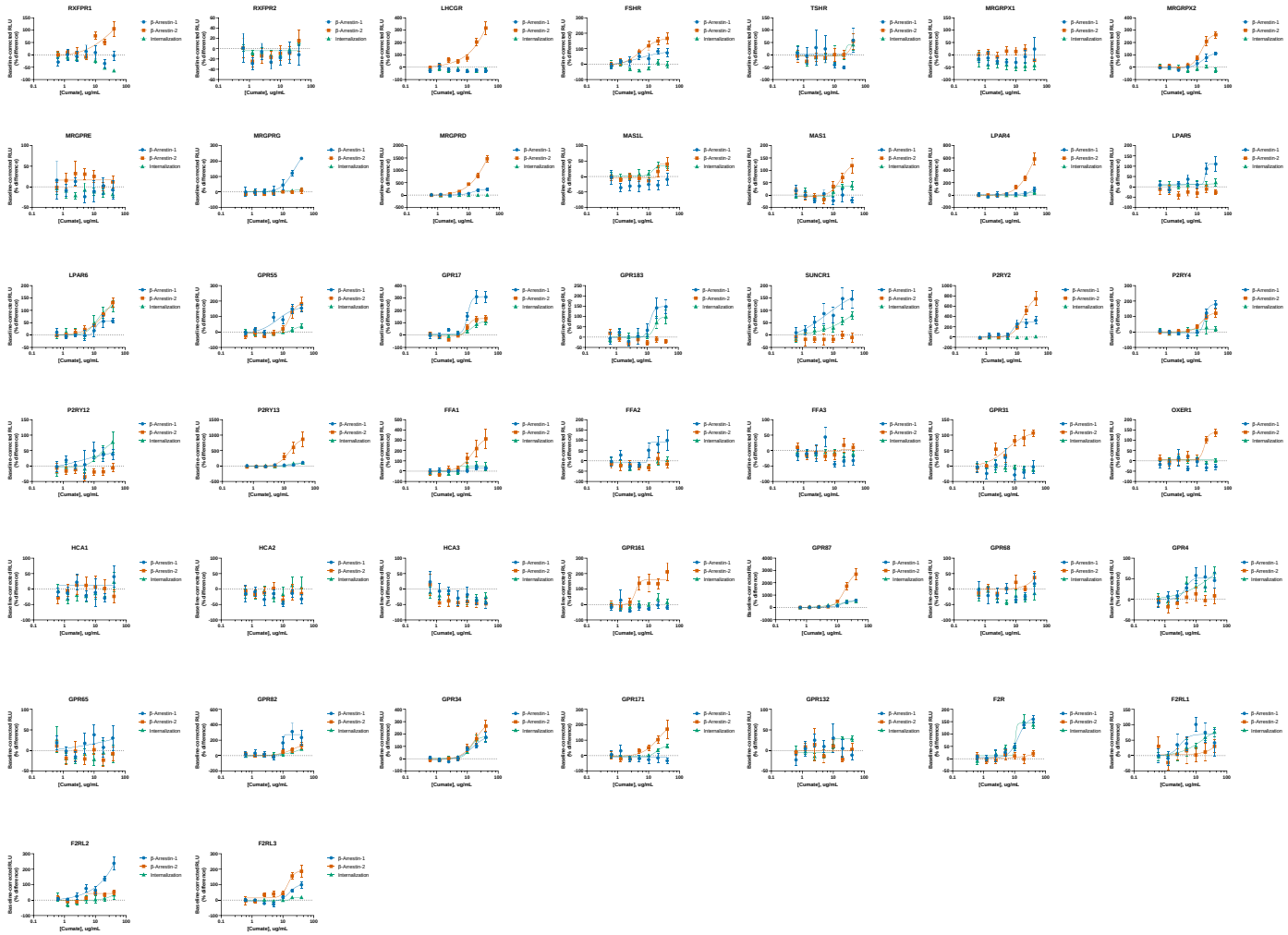
Supplementary Figure 10. Internalization of GPCRs in HTTL-F following β-arrestin-1 and β-arrestin-2 knockdown. Lentiviral β-arrestin-1 and -2 shRNA plasmids were transfected in HEK293T cells, along with psPAX2 and VSV-G vectors. The medium was replaced the following day with complete fresh medium, and lentiviral shRNA medium was collected following 48 hours transfection. For the knockdown experiment, HTTL-F cells were seeded in either complete medium or in the previously prepared lentiviral β-arrestin-1 and -2 shRNA medium (combined at a 1:1 ratio), with infection of cells facilitated with polybrene at 8 μg/mL. HTTL-F cells were transfected with GPCRs demonstrating high constitutive internalization: CHRM5 (a), GPR87 (b), GPR126 (c), VIPR2 (d), and CD97 (e). Transfected cells were stimulated as a cumate dose-response, and stimulation curves were built using XY analysis for non-linear regression curve and the 4-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with three technical replicates from one biological sample), and generic receptor codes refer to the GPCR-Tango constructs. qPCR was performed on untreated and infected HTTL-F cells to confirm sufficient knockdown of β-arrestin-1 and -2 (f). Data are presented as mean values, with error bars representing SEM. Fold change of gene expression and corresponding p values (two-tailed) were assessed with multiple unpaired t test using the FDR method of Benjamini & Yekutieli (n = 2, with two technical replicates from one biological sample).

Supplementary Figure 11. Basal profiles of β-arrestin-1 and β-arrestin-2 translocation, and receptor internalization generated using Tango-Trio (Class A, α-branch). To profile basal activities, HTTL-B1, HTTL-B2 and HTTL-F cells were transfected with GPCR Tango constructs. Transfected cells were stimulated with cumate in a dose-dependent manner. Dose-response curves were built using XY analysis for non-linear regression curve and the 4-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with 3 technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.

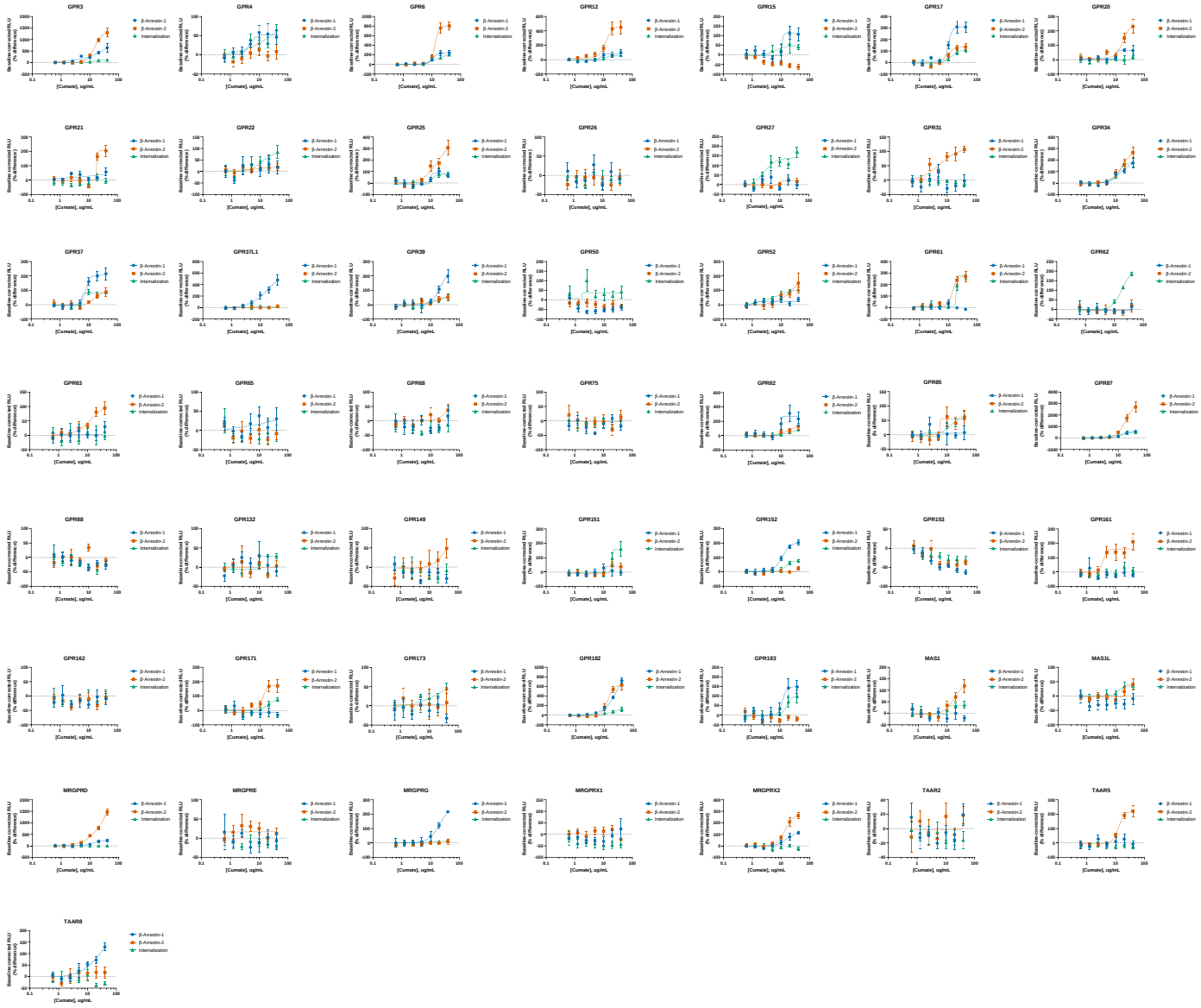


Supplementary Figure 12. Basal profiles of β-arrestin-1 and β-arrestin-2 translocation, and receptor internalization generated using Tango-Trio (Class A, β-branch). To profile basal activities, HTTL-B1, HTTL-B2 and HTTL-F cells were transfected with GPCR Tango constructs. Transfected cells were stimulated with cumate in a dose-dependent manner. Dose-response curves were built using XY analysis for non-linear regression curve and the 4-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with 3 technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.

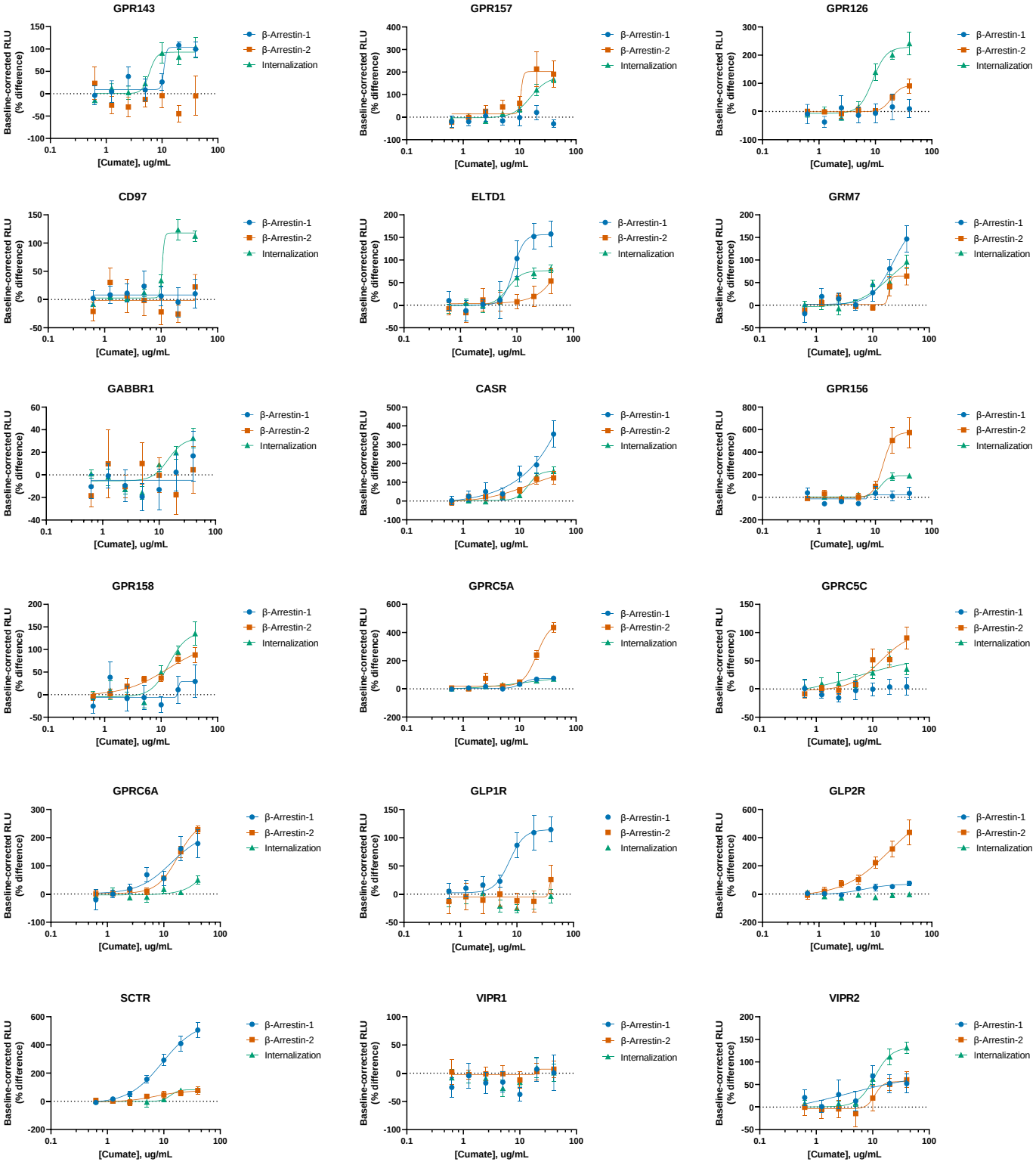
Supplementary Figure 13. Basal profiles of β-arrestin-1 and β-arrestin-2 translocation, and receptor internalization generated using Tango-Trio (Class A, γ-branch). To profile basal activities, HTTL-B1, HTTL-B2 and HTTL-F cells were transfected with GPCR Tango constructs. Transfected cells were stimulated with cumate in a dose-dependent manner. Dose-response curves were built using XY analysis for non-linear regression curve and the 4-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with 3 technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.



Supplementary Figure 14. Basal profiles of β-arrestin-1 and β-arrestin-2 translocation, and receptor internalization generated using Tango-Trio (Class A, δ-branch). To profile basal activities, HTTL-B1, HTTL-B2 and HTTL-F cells were transfected with GPCR Tango constructs. Transfected cells were stimulated with cumate in a dose-dependent manner. Dose-response curves were built using XY analysis for non-linear regression curve and the 4-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with 3 technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.



Supplementary Figure 15. Basal profiles of β-arrestin-1 and β-arrestin-2 translocation, and receptor internalization generated using Tango-Trio (Class A, orphan receptors). To profile basal activities, HTTL-B1, HTTL-B2 and HTTL-F cells were transfected with GPCR Tango constructs. Transfected cells were stimulated with cumate in a dose-dependent manner. Dose-response curves were built using XY analysis for non-linear regression curve and the 4-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with 3 technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.



Supplementary Figure 16. Basal profiles of β-arrestin-1 and β-arrestin-2 translocation, and receptor internalization generated using Tango-Trio (Class B/C). To profile basal activities, HTTL-B1, HTTL-B2 and HTTL-F cells were transfected with GPCR Tango constructs. Transfected cells were stimulated with cumate in a dose-dependent manner. Dose-response curves were built using XY analysis for non-linear regression curve and the 4-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with 3 technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.


MTNR1A
log[Melatonin(M)]
MTNR1B
log[Melatonin(M)]
GPR119
log[AS-1269574(M)]
PTGER3
log[Prostaglandin E2(M)]
PTGER1
log[Prostaglandin E2(M)]
PTGER2
log[Prostaglandin E2(M)]
PTGDR
log[Prostaglandin D2(M)]
PTGER4
log[Prostaglandin E2(M)]
ADORA3
log[NECA(M)]
ADORA2A
log[NECA(M)]
DRD1
log[Dihydrexidine HCl(M)]
DRD2
log[Quinpirole(M)]
DRD3
log[Quinpirole(M)]
DRD4
log[Quinpirole(M)]
5-HT6
log[Serotonin(M)]
HRH2
log[HTMT dimaleate(M)]
5-HT4
log[Serotonin(M)]
ADRB1
log[Isoproterenol(M)]
ADRB1
log[Isoproterenol(M)]
ADRB3
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5-HT7
log[Serotonin(M)]
5-HT5
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5-HT1D
log[Serotonin(M)]
5-HT1E
log[Serotonin(M)]
5-HT1F
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5-HT2A
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5-HT2B
log[Serotonin(M)]
5-HT2C
log[Serotonin(M)]
ADRA2A
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ADRA2B
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ADRA2C
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ADRA1A
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ADRA1B
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ADRA1D
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HRH1
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HRH3
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HRH4
log[Histamine dihydrochloride(M)]
CHRM1
log[Carbachol(M)]
CHRM2
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CHRM3
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CHRM4
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CHRM5
log[Carbachol(M)]
TAAR1
log[Tyramine(M)]
S1PR1
log[SIP(M)]
S1PR2
log[SIP(M)]
S1PR3
log[SIP(M)]
S1PR4
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S1PR5
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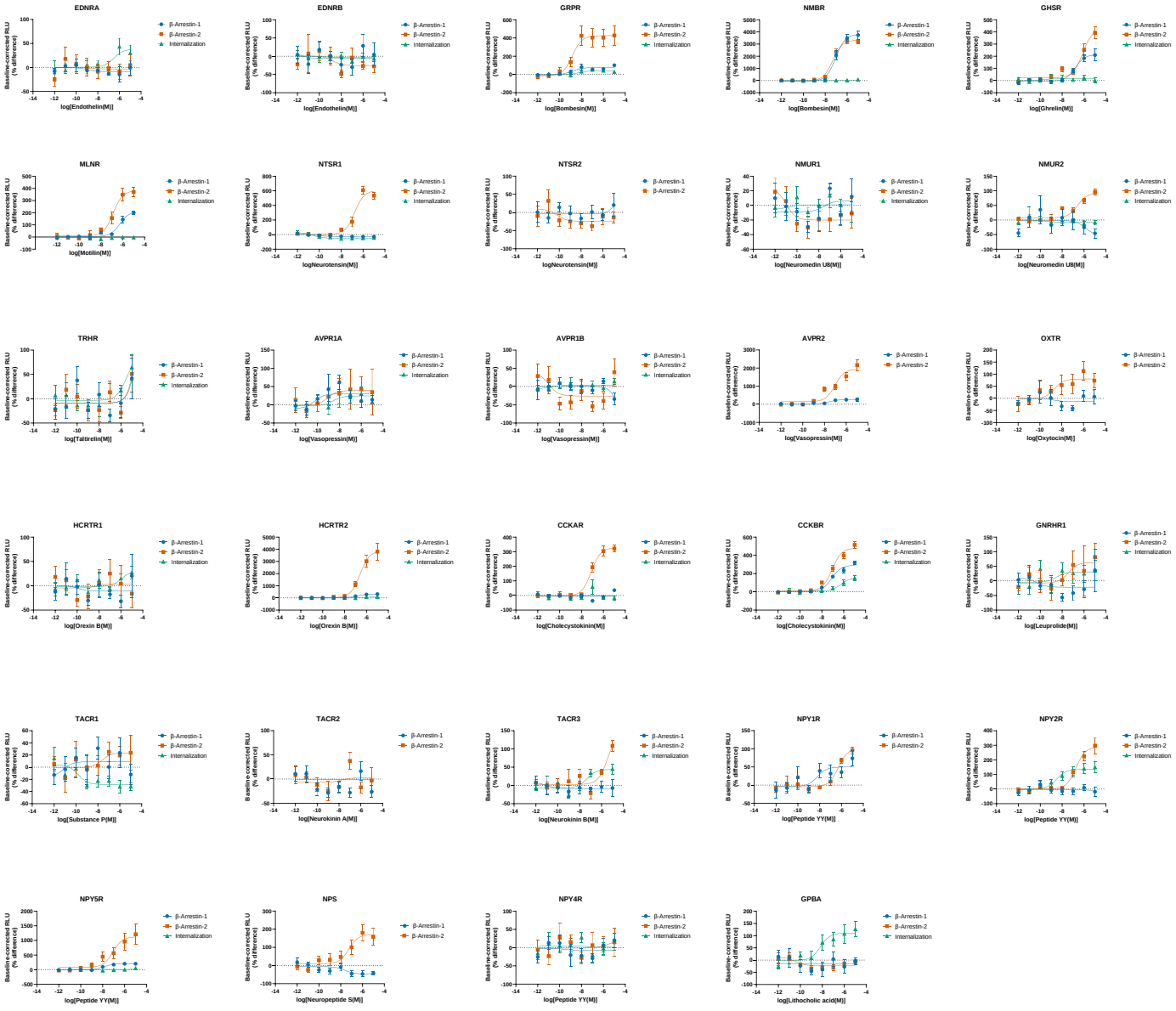
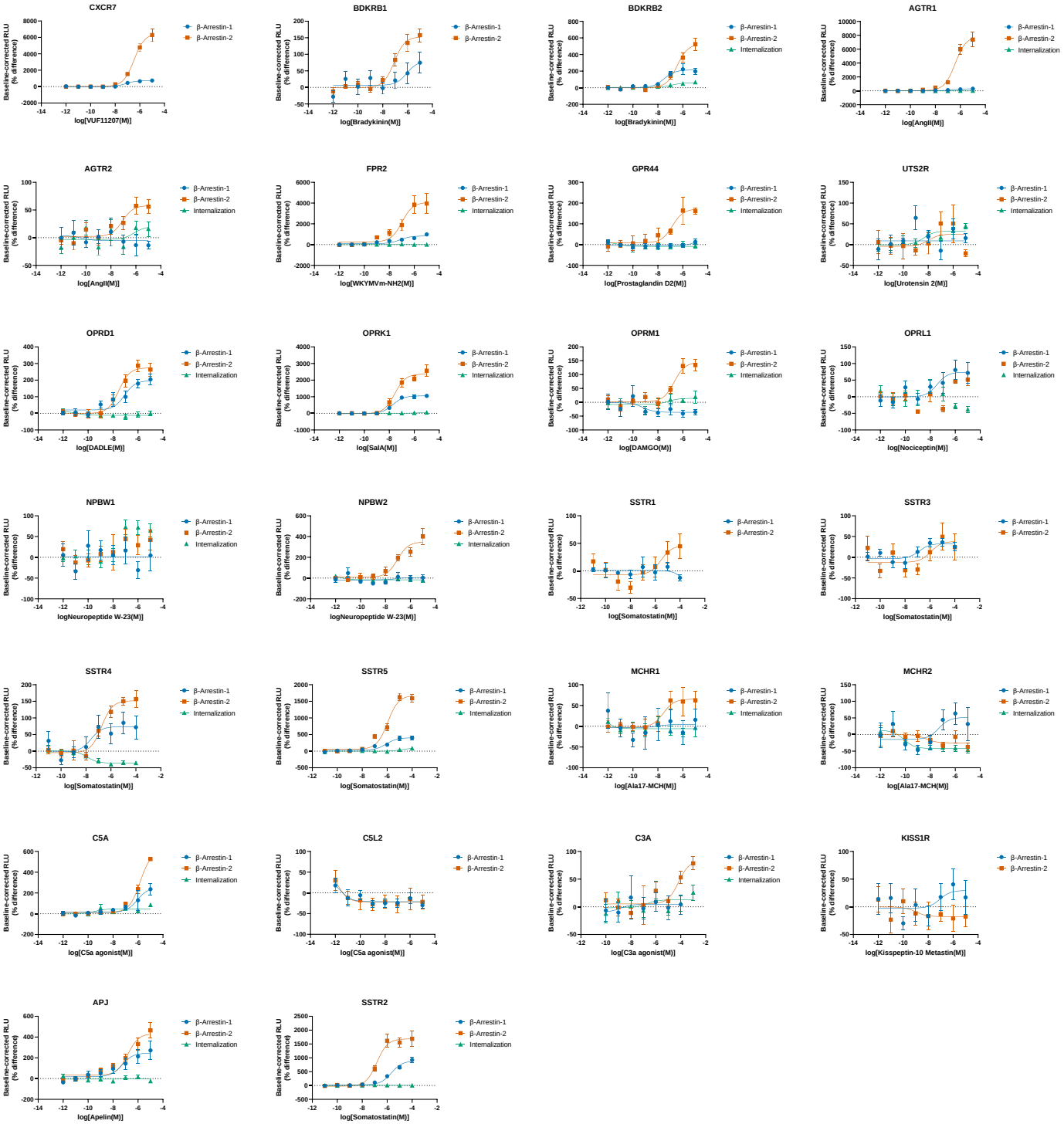
LPAR2
log[LPA(M)]
MC1R
log[Melanotan II(M)]
MC2R
log[ACTH(M)]
MC4R
log[α-MSH(M)]
MC5R
log[Melanotan II(M)]
MC3R
log[α-MSH(M)]
GPR119
log[Oleylethanolamide(M)]
ADORA1
log[NECA(M)]
ADORA2B
log[NECA(M)]
β-Arrestin-1
β-Arrestin-2
Internalization
Baseline-corrected RLU
(% difference)

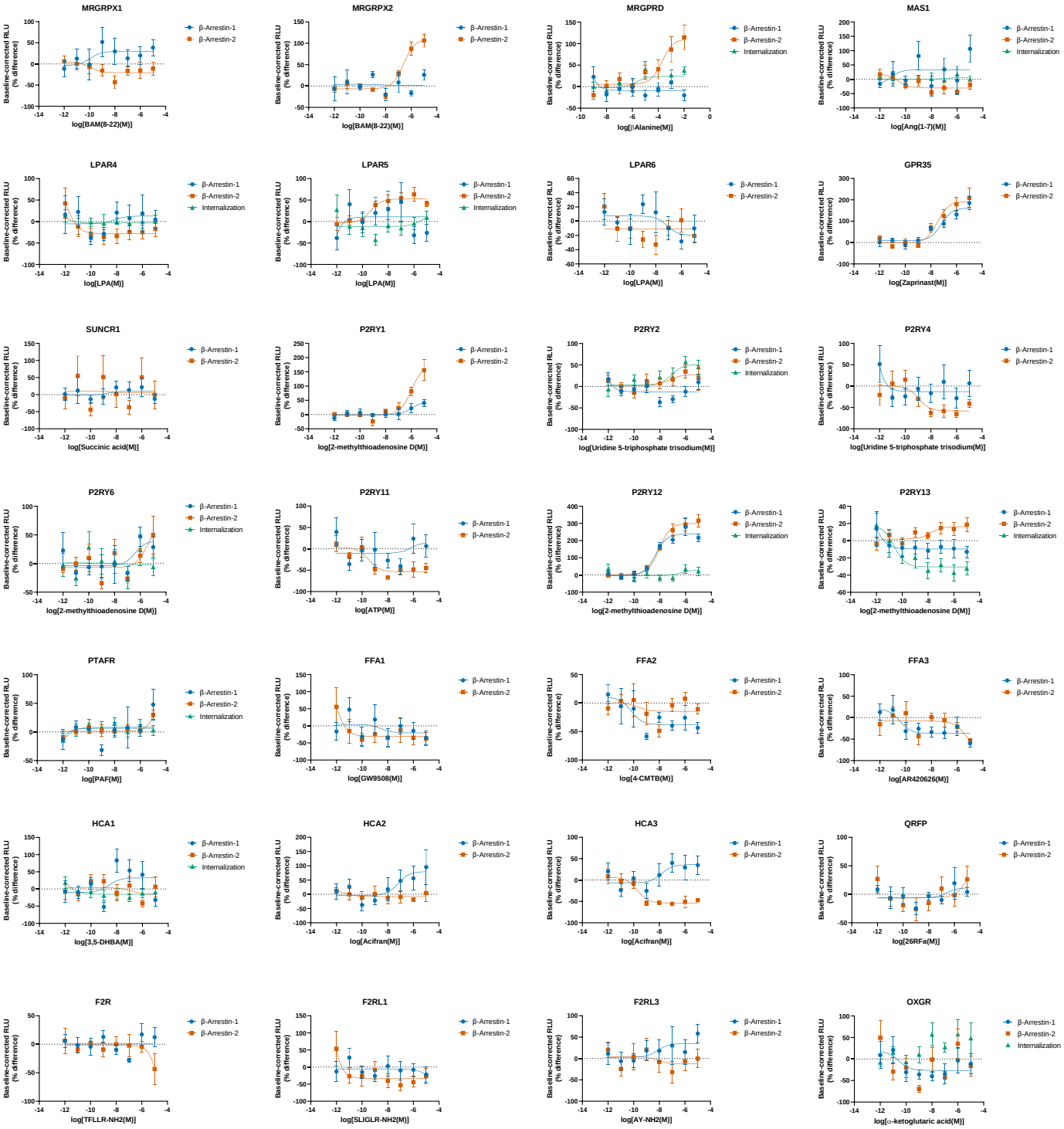
Supplementary Figure 17. Agonist-induced profiles of β-arrestin-1 and β-arrestin-2 translocation, and receptor internalization generated using Tango-Trio (Class A, α-branch). To quantify agonist-dependent activities, HTTL-B1, HTTL-B2 and HTTL-F cells were plated in cumate-containing (30 µg/mL) medium and transfected with non-orphan GPCR Tango constructs. Transfected cells were stimulated with the receptor-specific agonist, and dose-response curves were built using XY analysis for non-linear regression curve and the 3-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with 3 technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.



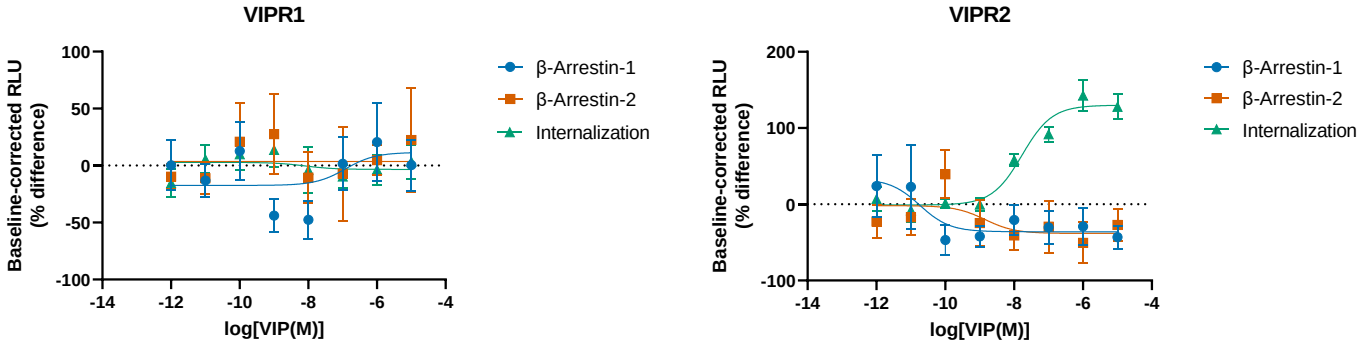
Supplementary Figure 18. Agonist-induced profiles of β-arrestin-1 and β-arrestin-2 translocation, and receptor internalization generated using Tango-Trio (Class A, β-branch). To quantify agonist-dependent activities, HTTL-B1, HTTL-B2 and HTTL-F cells were plated in cumate-containing (30 µg/mL) medium and transfected with non-orphan GPCR Tango constructs. Transfected cells were stimulated with the receptor-specific agonist, and dose-response curves were built using XY analysis for non-linear regression curve and the 3-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with 3 technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.



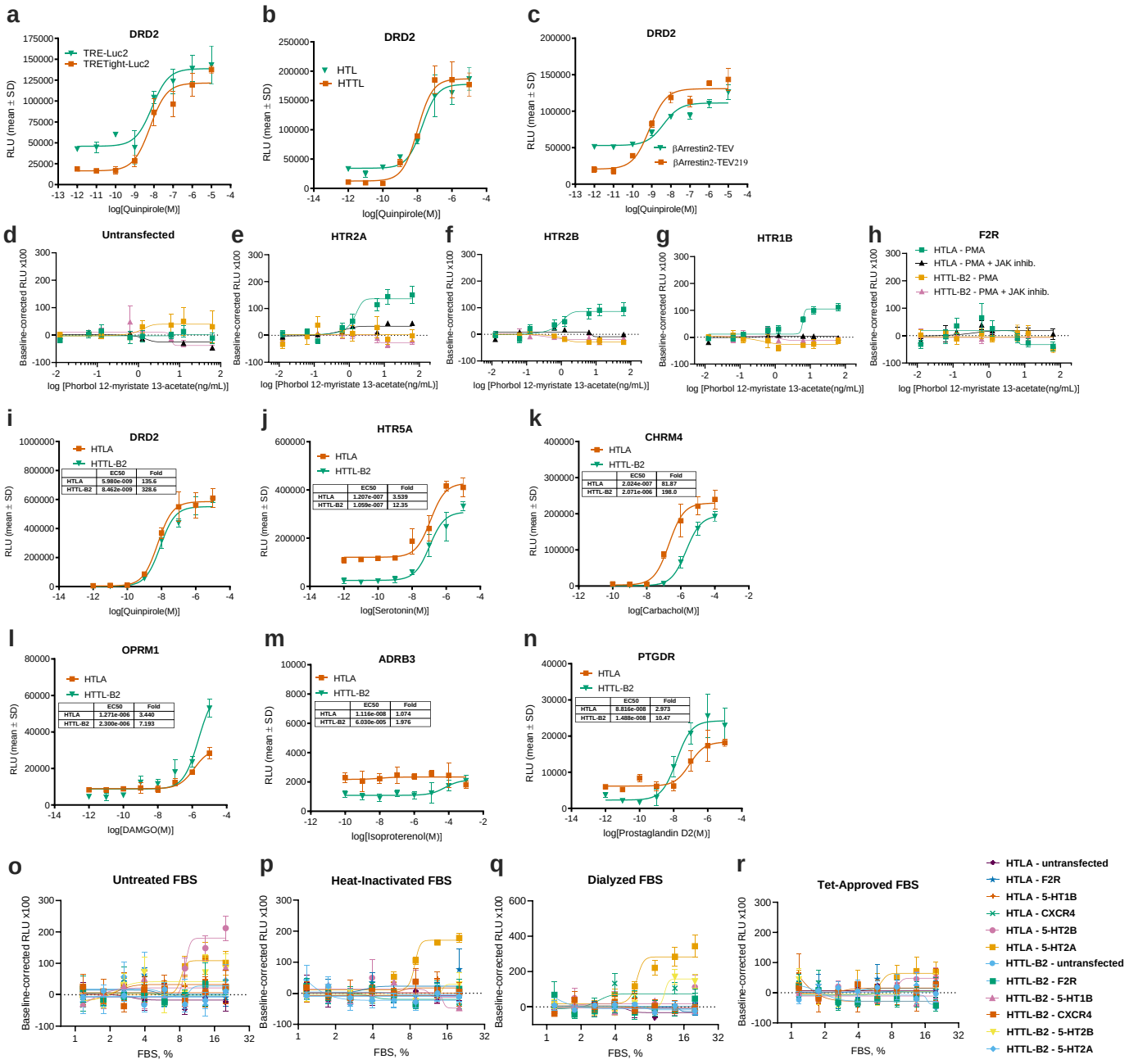
Supplementary Figure 19. Agonist-induced profiles of β-arrestin-1 and β-arrestin-2 translocation, and receptor internalization generated using Tango-Trio (Class A, γ-branch). To quantify agonist-dependent activities, HTTL-B1, HTTL-B2 and HTTL-F cells were plated in cumate-containing (30 µg/mL) medium and transfected with non-orphan GPCR Tango constructs. Transfected cells were stimulated with the receptor-specific agonist, and dose-response curves were built using XY analysis for non-linear regression curve and the 3-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with 3 technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.



Supplementary Figure 20. Agonist-induced profiles of β-arrestin-1 and β-arrestin-2 translocation, and receptor internalization generated using Tango-Trio (Class A, δ-branch). To quantify agonist-dependent activities, HTTL-B1, HTTL-B2 and HTTL-F cells were plated in cumate-containing (30 µg/mL) medium and transfected with non-orphan GPCR Tango constructs. Transfected cells were stimulated with the receptor-specific agonist, and dose-response curves were built using XY analysis for non-linear regression curve and the 3-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with 3 technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.

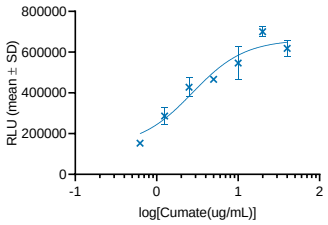


Supplementary Figure 21. Agonist-induced profiles of β-arrestin-1 and β-arrestin-2 translocation, and receptor internalization generated using Tango-Trio (Class B/C). To quantify agonist-dependent activities, HTTL-B1, HTTL-B2 and HTTL-F cells were plated in cumate-containing (30 µg/mL) medium and transfected with non-orphan GPCR Tango constructs. Transfected cells were stimulated with the receptor-specific agonist, and dose-response curves were built using XY analysis for non-linear regression curve and the 3-parameters dose-response stimulation function, followed by baseline correction. Data are presented as mean values, with error bars representing SEM (n = 3, with 3 technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.



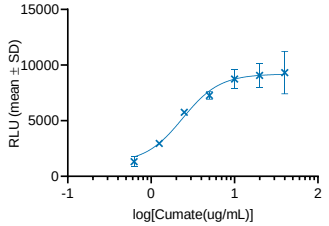
Supplementary Figure 22. Optimization of the dynamic range, sensitivity, and specificity of the Tango-Trio platform - independent biological replicate of the main manuscript Figure 1. (A-R) Data are representative of 2 biological replicates, with 3 technical replicates each.

a



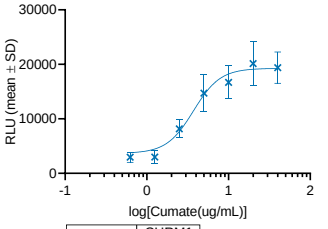
	CHRM1
EC50	2.736
Fold	4.821

b



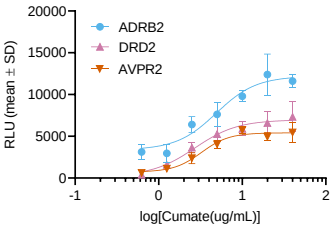
	CHRM1
EC50	2.378
Fold	7.682

c



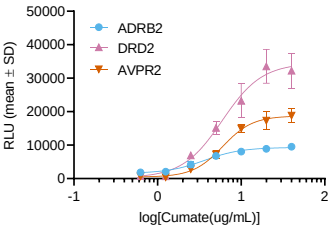
	CHRM1
EC50	3.722
Fold	5.273

d



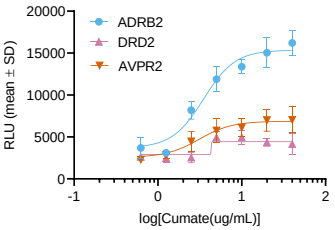
	AVPR2	ADRB2	DRD2
EC50	3.253	4.904	2.712
Fold	7.515	3.624	29.51

e



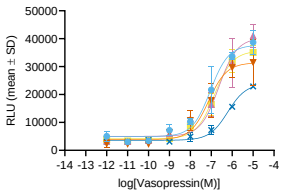
	AVPR2	ADRB2	DRD2
EC50	6.015	3.643	5.947
Fold	41.84	5.936	149.9

f



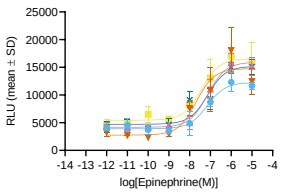
	AVPR2	ADRB2	DRD2
EC50	3.094	3.507	~ 4.328
Fold	2.693	4.171	~ 1.532

g



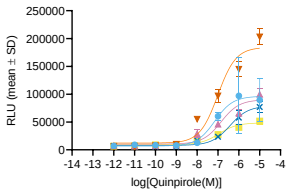
	EC50	Fold
I	9.071e-008	7.630
II	7.444e-008	8.169
III	2.299e-007	8.001
IV	6.147e-007	6.607
V	1.422e-007	8.551

h



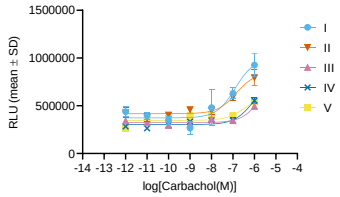
	EC50	Fold
I	7.146e-008	3.137
II	2.072e-008	5.343
III	7.349e-008	3.808
IV	7.287e-008	3.216
V	3.743e-008	3.041

i



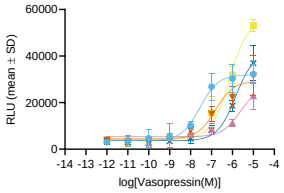
	EC50	Fold
I	7.025e-008	14.31
II	9.855e-008	15.18
III	1.410e-007	8.674
IV	3.766e-007	9.855
V	1.119e-007	5.311

j



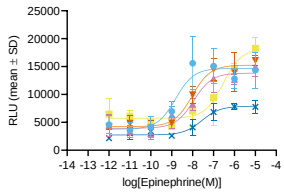
	EC50	Fold
I	1.282e-007	2.664
II	1.441e-007	2.032
III	2.072e-006	2.608
IV	8.548e-007	2.528
V	4.036e-007	1.790

k



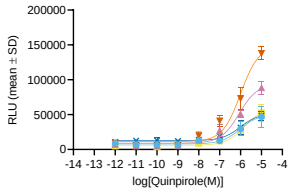
	EC50	Fold
I	2.792e-008	8.642
II	1.552e-007	6.720
III	2.365e-006	5.057
IV	1.427e-006	11.39
V	7.205e-007	12.74

l



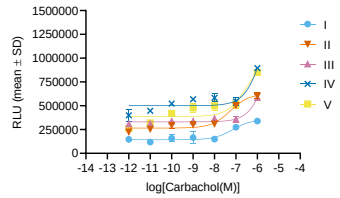
	EC50	Fold
I	1.538e-009	3.887
II	9.604e-009	3.590
III	1.129e-008	3.504
IV	2.785e-008	2.875
V	2.878e-007	3.163

m



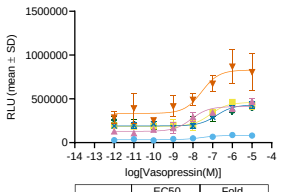
	EC50	Fold
I	9.339e-007	6.346
II	1.010e-006	13.10
III	8.610e-007	10.36
IV	9.850e-007	4.053
V	1.561e-006	9.810

n



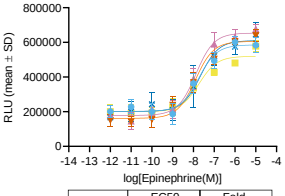
	EC50	Fold
I	7.266e-008	2.498
II	5.417e-008	2.352
III	4.336e-006	5.100
IV	2.258e-006	3.572
V	3.019e-007	2.550

o



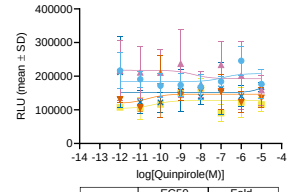
	EC50	Fold
I	2.786e-008	2.483
II	2.786e-008	2.483
III	8.515e-009	3.194
IV	1.576e-007	2.260
V	7.677e-008	2.463

p



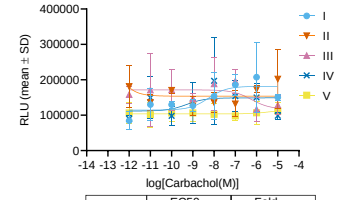
	EC50	Fold
I	1.803e-008	2.906
II	1.242e-008	3.745
III	1.305e-008	3.664
IV	2.178e-008	3.024
V	1.830e-008	2.596

q



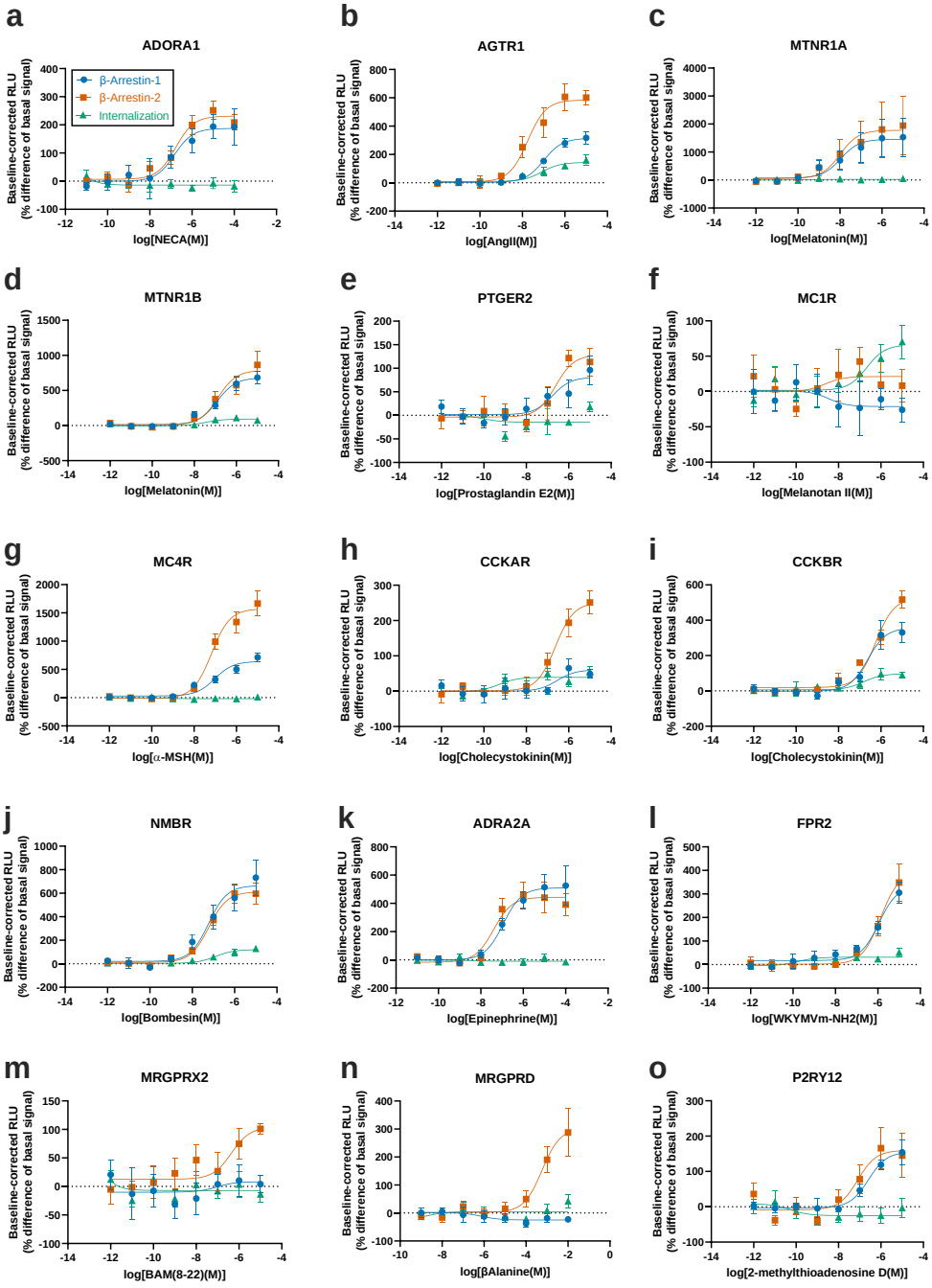
	EC50	Fold
I	1.297e-007	1.131
II	2.274e-011	1.231
III	4.310e-009	0.8847
IV	~ 0.6455	~ 6739
V	5.073e-011	1.224

r

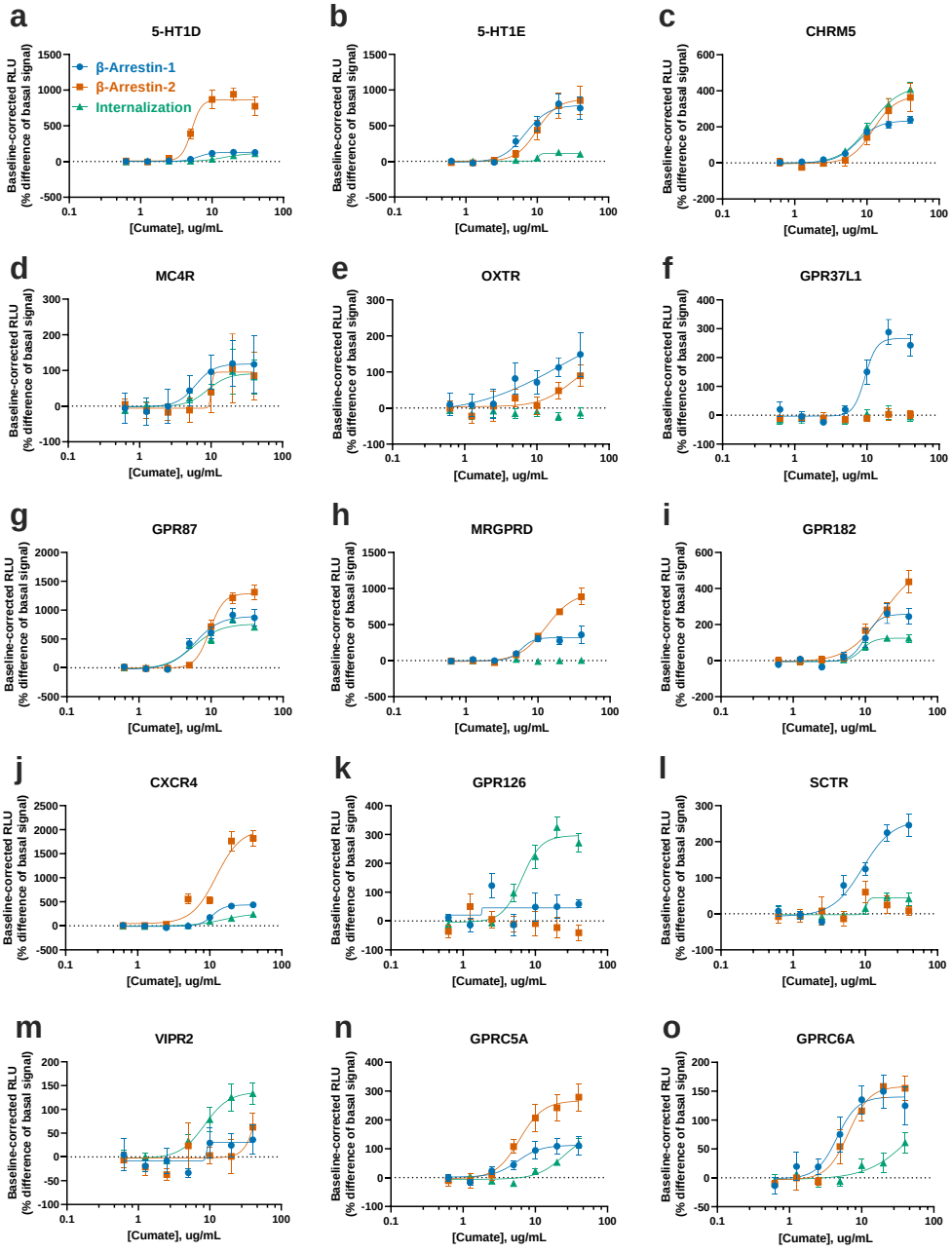


	EC50	Fold
I	5.467e-009	1.587
II	~ 6.878e-018	~ 4.130e-005
III	5.793e-007	0.6826
IV	5.126e-010	1.334
V	4.013e-006	1.112

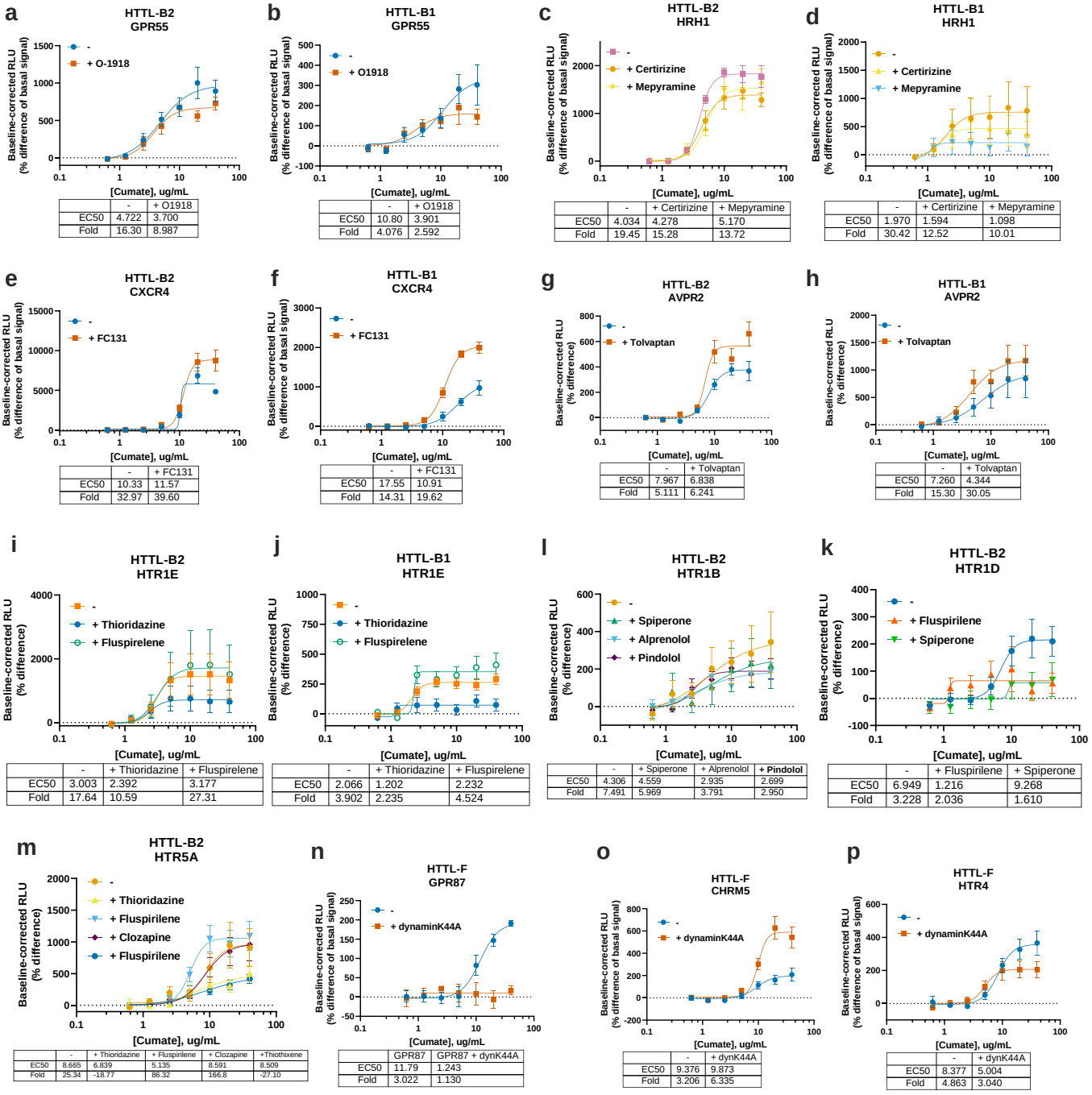
Supplementary Figure 23. Dose-response and time-course verification of cumate-induced expression - independent biological replicate of the main manuscript Figure 2. (A-R) Data are representative of 2 biologi cal replicates, with 3 technical replicates each.



Supplementary Figure 24. Validation of compiled positive hits from agonist-dependent HTS in dose response - independent biological replicate of the main manuscript Figure 5. (A-O) Data are representative of 2 biological replicates, with 3 technical replicates each.



Supplementary Figure 25. Validation of compiled positive hits from basal activity HTS in dose-response - independent biological replicate of the main manuscript Figure 6. (A-O) Data are representative of 2 biologi cal replicates, with 3 technical replicates each.



	-	+ O1918
EC50	4.722	3.700
Fold	16.30	8.987

	-	+ O1918
EC50	10.80	3.901
Fold	4.076	2.592

	-	+ Certirizine	+ Mepyramine
EC50	4.034	4.278	5.170
Fold	19.45	15.28	13.72

	-	+ Certirizine	+ Mepyramine
EC50	1.970	1.594	1.098
Fold	30.42	12.52	10.01

	-	+ FC131
EC50	10.33	11.57
Fold	32.97	39.60

	-	+ FC131
EC50	17.55	10.91
Fold	14.31	19.62

	-	+ Tolvaptan
EC50	7.967	6.838
Fold	5.111	6.241

	-	+ Tolvaptan
EC50	7.260	4.344
Fold	15.30	30.05

	-	+ Thioridazine	+ Fluspirelene
EC50	3.003	2.392	3.177
Fold	17.64	10.59	27.31

	-	+ Thioridazine	+ Fluspirelene
EC50	2.066	1.202	2.232
Fold	3.902	2.235	4.524

	-	+ Spiperone	+ Alprenolol	+ Pindolol
EC50	4.306	4.559	2.935	2.699
Fold	7.491	5.969	3.791	2.950

	-	+ Fluspirilene	+ Spiperone
EC50	6.949	1.216	9.268
Fold	3.228	2.036	1.610

	-	+ Thioridazine	+ Fluspirilene	+ Clozapine	+Thiothixene
EC50	8.665	6.839	5.135	8.591	8.509
Fold	25.34	-18.77	86.32	166.8	-27.10

	GPR87	GPR87 + dynK44A
EC50	11.79	1.243
Fold	3.022	1.130

	-	+ dynK44A
EC50	9.376	9.873
Fold	3.206	6.335

	-	+ dynK44A
EC50	8.377	5.004
Fold	4.863	3.040

Supplementary Figure 26. Applications and further investigations into basal activities revealed by Tango-Trio - independent biological replicate of the main manuscript Figure 7. (A-P) Data are representative of 2 biological replicates, with 3 technical replicates each.

Supplemental Tables

Supplementary Table 1. Comparison of the pharmacological parameters extracted from EMTA and Tango-Trio. Absolute pEC50 and Emax values of β-arrestin-1/2 activity at GPCRs stimulated with common ligands were extracted from EMTA (Avet et al. 2022) and Tango-Trio studies. Tango-Trio pEC50 data was extracted from the non-linear least-squares regression analysis using the sigmoidal dose-response function (3-parameters modeled using Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X))) and Emax values from the baseline correction as percentage difference using 100* (Value-Baseline)/Baseline), both provided in GraphPad Prism 9.5.1. (n = 3, with three technical replicates from one biological sample). Generic receptor codes refer to the GPCR-Tango constructs.

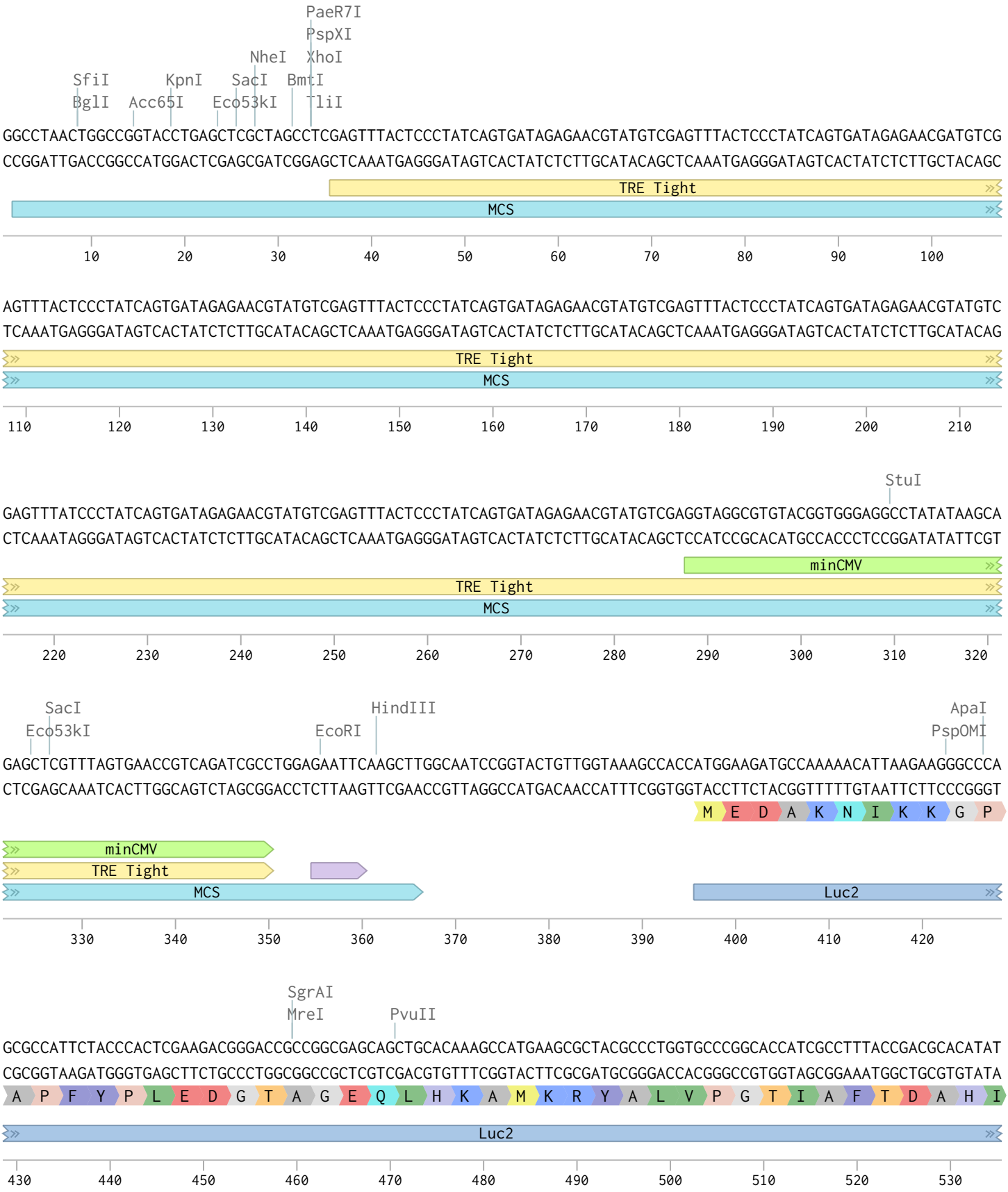
		pEC50				Emax (in % of vehicle response for EMTA & % difference from baseline for Tango-Trio)			
Receptor (Gene Name)	**Ligand**	**βArrestin1 /GRK2 EMTA**	**βArrestin1 Tango-Trio**	**βArrestin2 /GRK2 EMTA**	**β-Arrestin2 Tango-Trio**	**βArrestin1 /GRK2 EMTA**	**βArrestin1 Tango-Trio**	**βArrestin2 /GRK2 EMTA**	**β-Arrestin2 Tango-Trio**
HTR1B	Serotonin	7.6	6.96	7.685	6.49	36.35	490	57.78	393.4
HTR1D	Serotonin		7.66		6.89		-14.78		347.9
HTR2A	Serotonin	6.709	8.22	7.028	5.76	518.1	24.81	778.6	29.24
HTR2B	Serotonin	7.809	6.5	8.168	11.31	171.6	80.54	247.2	-26.67
HTR2C	Serotonin	7.995	8.76	8.456	9.33	1564	-28.38	705	14.12
AGTR1	Angiotensin II	8.64	6.62	8.84	6.41	207.8	289.2	220.6	7802
CCKAR	Cholecystokinin	8.329	unstable	8.721	7.11	1374	unstable	1520	332.9
PTGER1	PGE2		6.95		unstable		71.02		unstable
PTGER2	PGE2	6.515	6.38	6.925	5.95	107.8	400.8	100.5	363
PTGER3	PGE2		8.53		6.324		-24.13		19.44
PTGER4	PGE2	8.921	8.62	9.25	8.35	251.5	326.9	146.8	1192
EDNRA	Endothelin-1	8.232	8.9	8.381	8.7	588.1	-8.86	1581	-2.98
GHSR	Ghrelin	8.249	6.81	8.515	6.14	68.41	217.8	169.6	396.8
GNRHR	GnRH		10.32		7.43		-31.52		70.49
GPBAR1	Litocholic acid		unstable		10.41		unstable		-34.66
LPAR1	O-LPA	7.528	11.93	7.464	11.21	289.4	-90.04	232	-32.09
LPAR2	O-LPA	6.773	6.13	7.078	11.33	715.2	-41.64	938.9	-64.29
MC4R	α-MSH		7.01	7.449	7.28		346.3	76.44	1351
OPRM1	DAMGO	7.099	9.19	7.424	6.7	1008	-35.21	1198	142.9
MTNR1A	Melatonin	8.937	8.03	8.75	9.11	78.64	754.1	157.5	425.5
MTNR1B	Melatonin		7.12		8.38		290		168
OPRL1	Nociceptin	8.249	6.15	8.716	6.17	147.5	73.78	238.1	-50.64
OXTR	Oxytocin	8.091	8.78	8.6	8.67	128.3	-13.08	229.8	82.31
HCRTR2	Orexin-A	8.471	6.9	8.558	6.58	852.9	315.7	863.9	3850
P2RY2	UTP	5.235	unstable	5.36	7.29	184.7	unstable	94.68	24.6
F2R	TFLLR-NH2	5.082	unstable	5.177	-2.55	445.5	unstable	469.5	unstable
S1PR1	Sphingosine 1-phosphate	7.269	6.47	7.459	6.5	477	233.6	557.1	182.8
SSTR2	Somatostatin	8.815	5.66	8.918	6.83	2867	866	1820	1714
AVPR1A	AVP	7.743	10.25	7.824	9.08	890.1	40.7	934.7	40.67
AVP2R	AVP	7.603	7.52	7.846	7.03	353.3	258.6	334.2	1804
VIPR1	VIP	9.109	6.92	9.254	unstable	1367	28.97	1060	unstable
NPY1R	NPY		8.2	8.815	6.28		53.76	42.09	106.1
NPY5R	NPY		7.92		7.07		210.3		1046

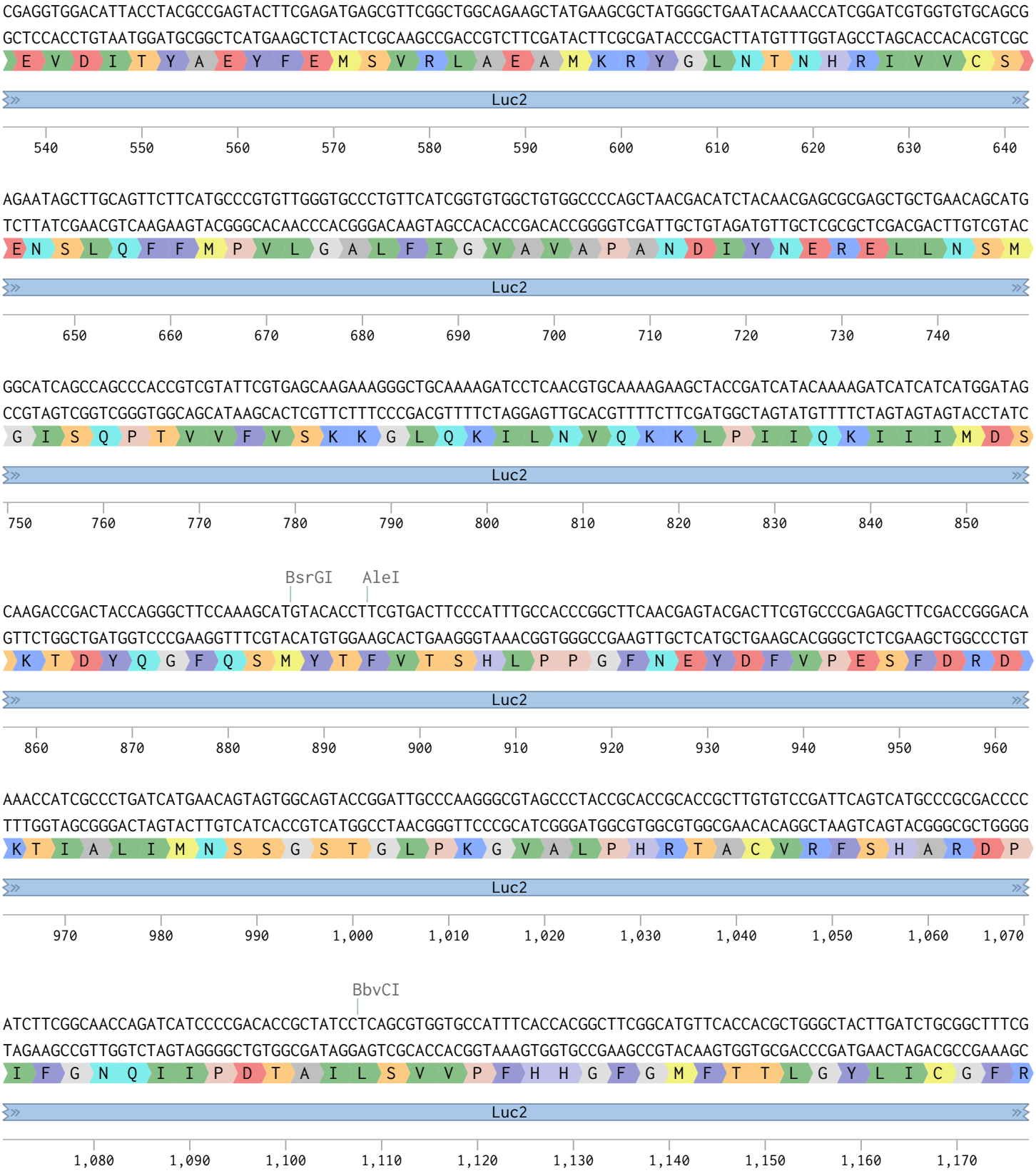
Supplemental Notes

Supplementary Note 1. Sequences of Tango-Trio constructs stably expressed in HTTL, HTTL-B1, HTTL-B2, and HTTL-F cell lines. Information regarding the cloning of the plasmids is provided in the Methods.

(from 1-535 bp)

Tight-pNLCoI1-Luc2 (6844 bp)

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E V D I T Y A E Y F E M S V R L A E A M K R Y G L N T N H R I V V C S
Luc2
540 550 560 570 580 590 600 610 620 630 640
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TCTTATCGAACGTCAAGAAGTACGGGCACAACCCACGGGACAAGTAGCCACACCGACACCGGGGTCGATTGCTGTAGATGTTGCTCGCGCTCGACGACTTGTCGTAC
E N S L Q F F M P V L G A L F I G V A V A P A N D I Y N E R E L L N S M
Luc2
650 660 670 680 690 700 710 720 730 740
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G I S Q P T V V F V S K K G L Q K I L N V Q K K L P I I Q K I I I M D S
Luc2
750 760 770 780 790 800 810 820 830 840 850
BsrGI AleI
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K T D Y Q G F Q S M Y T F V T S H L P P G F N E Y D F V P E S F D R D
Luc2
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K T I A L I M N S S G S T G L P K G V A L P H R T A C V R F S H A R D P
Luc2
970 980 990 1,000 1,010 1,020 1,030 1,040 1,050 1,060 1,070
BbvCI
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I F G N Q I I P D T A I L S V V P F H H G F G M F T T L G Y L I C G F R
Luc2
1,080 1,090 1,100 1,110 1,120 1,130 1,140 1,150 1,160 1,170


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Luc2
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PluTI
SfoI
NarI
KasI
BlpI
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Luc2
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L P G I R Q G Y G L T E T T S A I L I T P E G D D K P G A V G K V V P F
Luc2
1,400 1,410 1,420 1,430 1,440 1,450 1,460 1,470 1,480 1,490
AgeI
DraIII
HpaI
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F E A K V V D L D T G K T L G V N Q R G E L C V R G P M I M S G Y V N
Luc2
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N P E A T N A L I D K D G W L H S G D I A Y W D E D E H F F I V D R L K
Luc2
1,610 1,620 1,630 1,640 1,650 1,660 1,670 1,680 1,690 1,700 1,710
SexAI
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S L I K Y K G Y Q V A P A E L E S I L L Q H P N I F D A G V A G L P D D
Luc2
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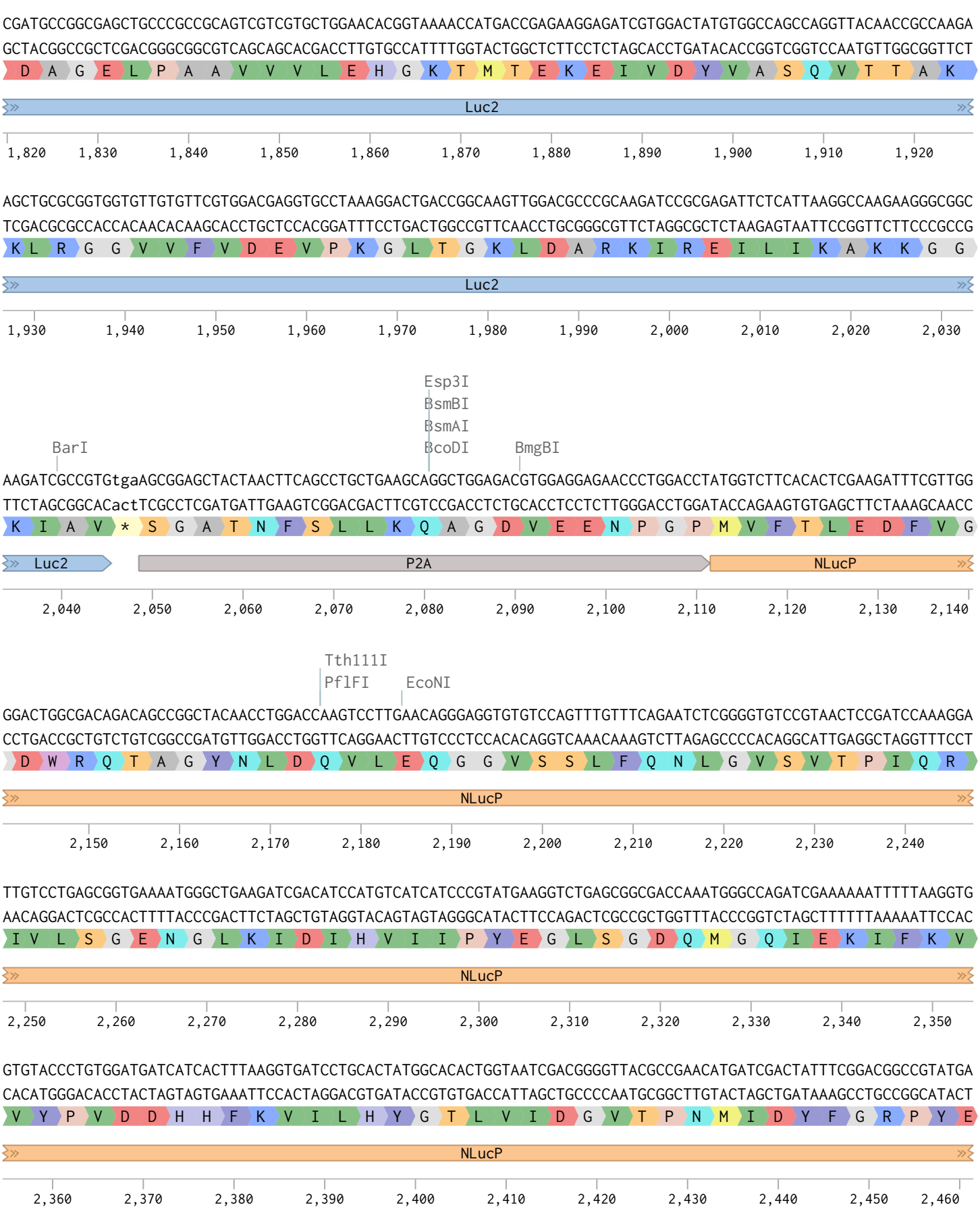
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D A G E L P A A V V V L E H G K T M T E K E I V D Y V A S Q V T T A K
Luc2
1,820 1,830 1,840 1,850 1,860 1,870 1,880 1,890 1,900 1,910 1,920
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K L R G G V V F V D E V P K G L T G K L D A R K I R E I L I K A K K G G
Luc2
1,930 1,940 1,950 1,960 1,970 1,980 1,990 2,000 2,010 2,020 2,030
Esp3I
BsmBI
BsmAI
BarI
BcoDI
BmgBI
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Luc2
P2A
NLucP
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Tth111I
PflFI
EcoNI
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D W R Q T A G Y N L D Q V L E Q G G V S S L F Q N L G V S V T P I Q R
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NLucP
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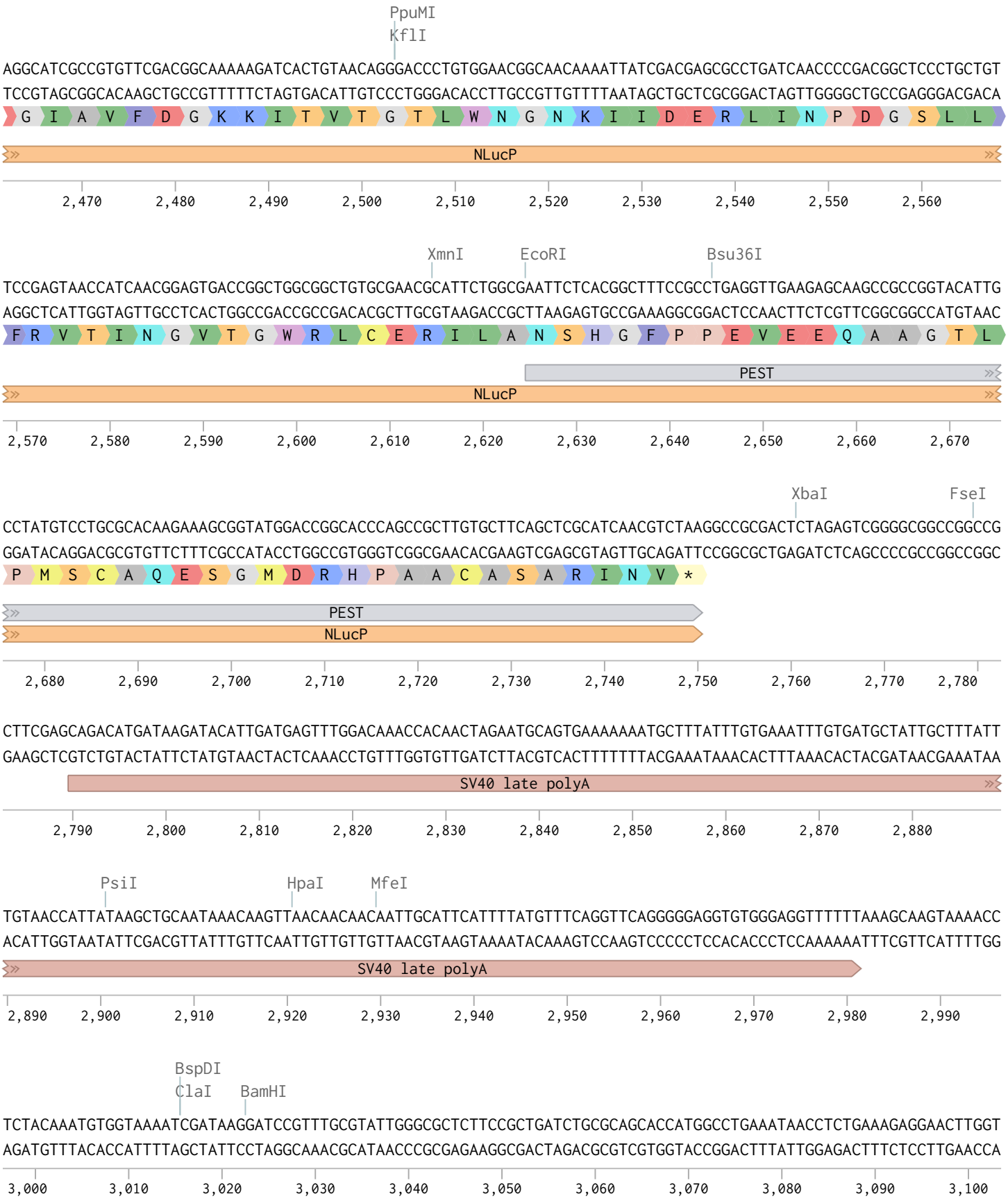
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G I A V F D G K K I T V T G T L W N G N K I I D E R L I N P D G S L L
NLucP
2,470 2,480 2,490 2,500 2,510 2,520 2,530 2,540 2,550 2,560
XmnI
EcoRI
Bsu36I
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F R V T I N G V T G W R L C E R I L A N S H G F P P E V E E Q A A G T L
PEST
NLucP
2,570 2,580 2,590 2,600 2,610 2,620 2,630 2,640 2,650 2,660 2,670
XbaI
FseI
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P M S C A Q E S G M D R H P A A C A S A R I N V *
PEST
NLucP
2,680 2,690 2,700 2,710 2,720 2,730 2,740 2,750 2,760 2,770 2,780
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SV40 late polyA
2,790 2,800 2,810 2,820 2,830 2,840 2,850 2,860 2,870 2,880
PsiI
HpaI
MfeI
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SV40 late polyA
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BspDI
ClaI
BamHI
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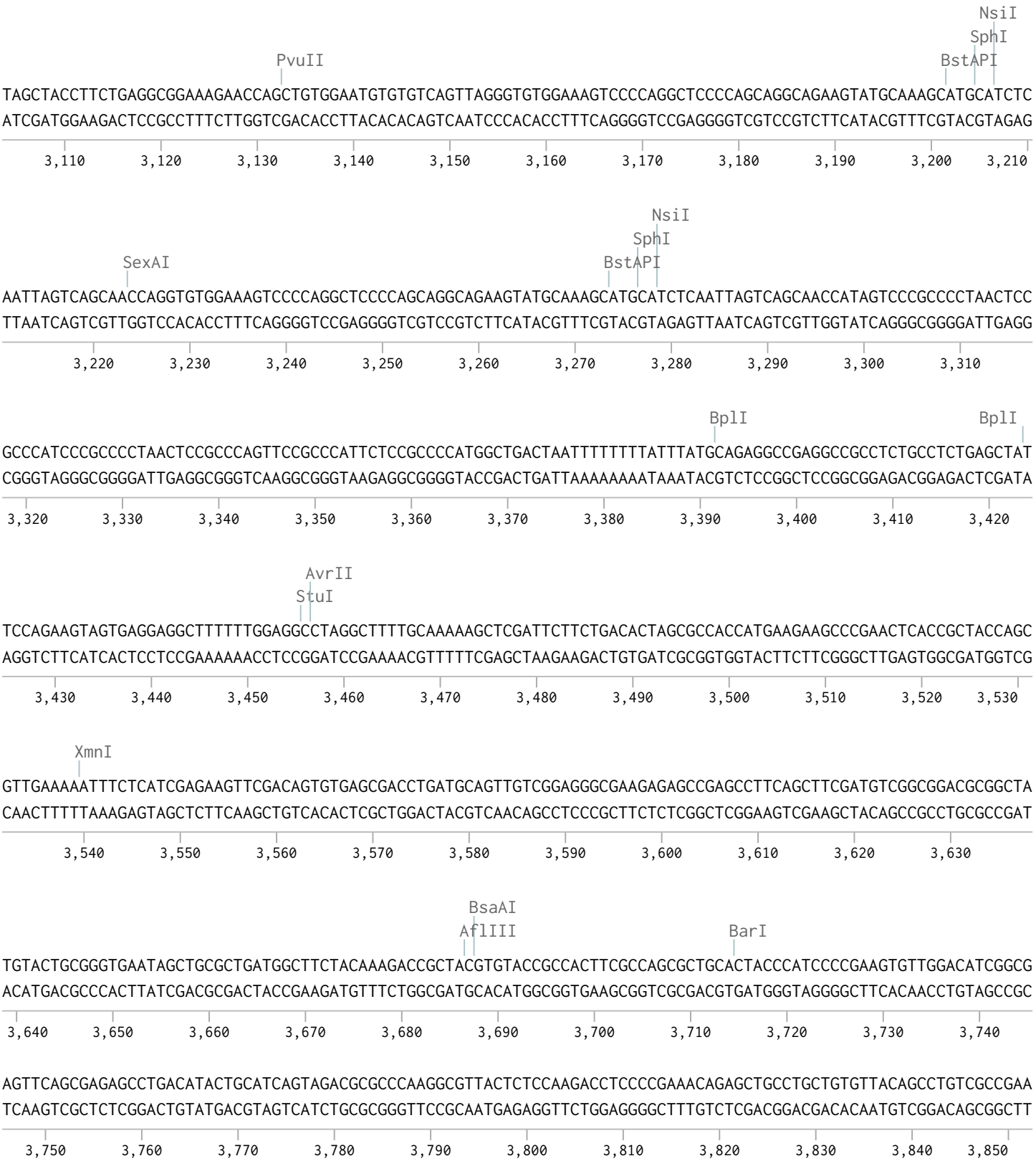
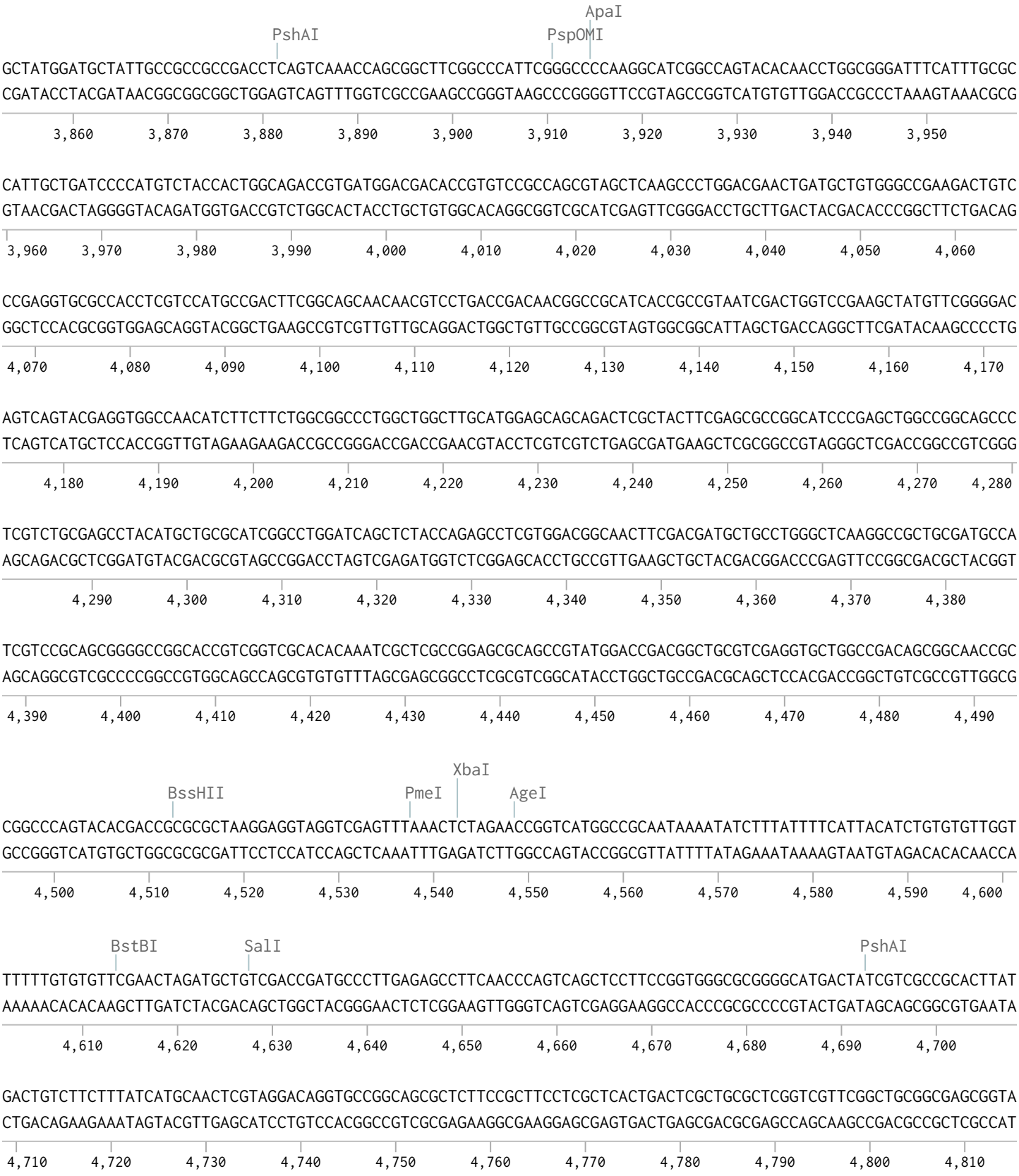
Tight-pNLCoI1-Luc2 (6844 bp) (from 3104-3852 bp)

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BssHII
PmeI
AgeI
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SalI
PshAI
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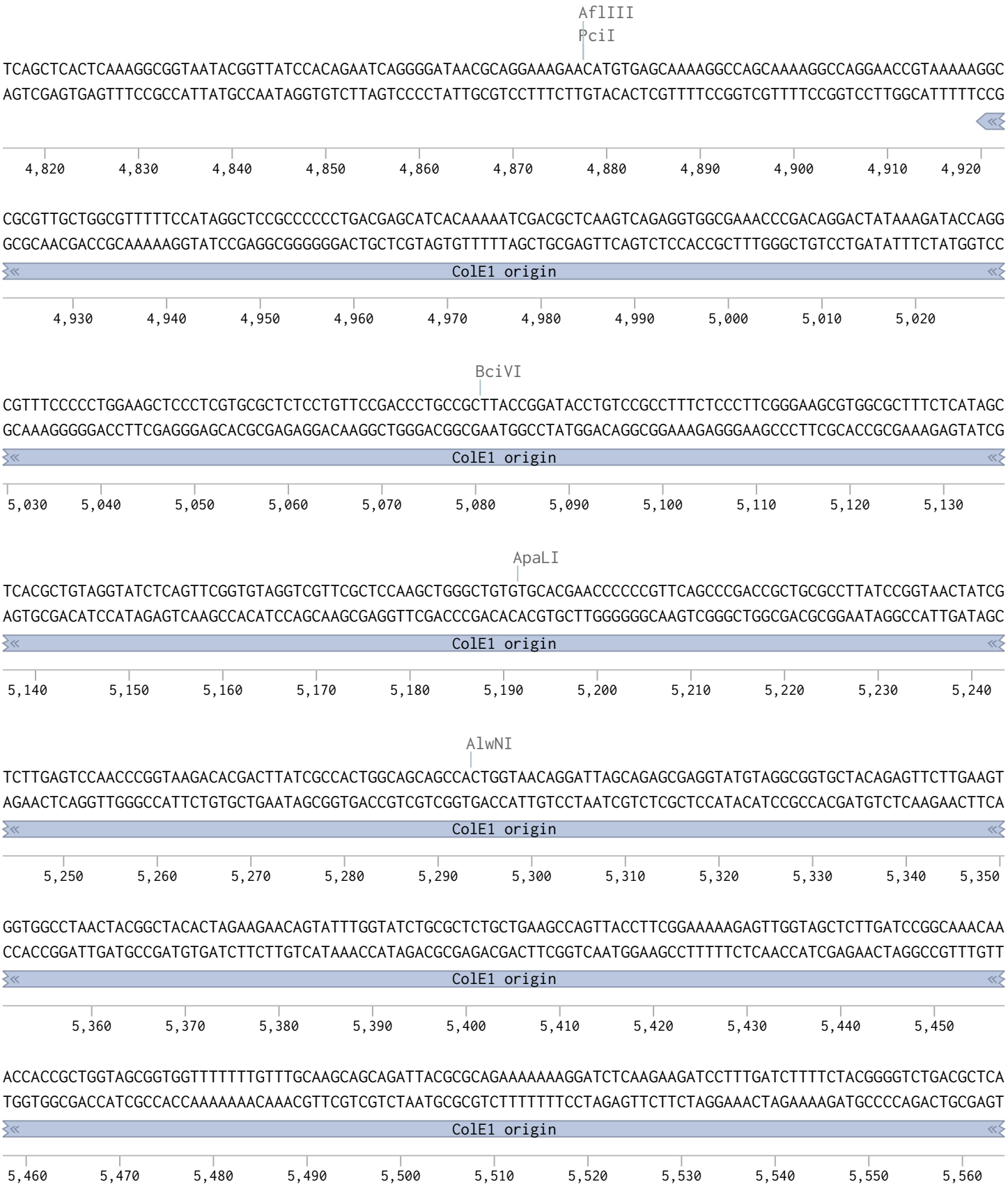
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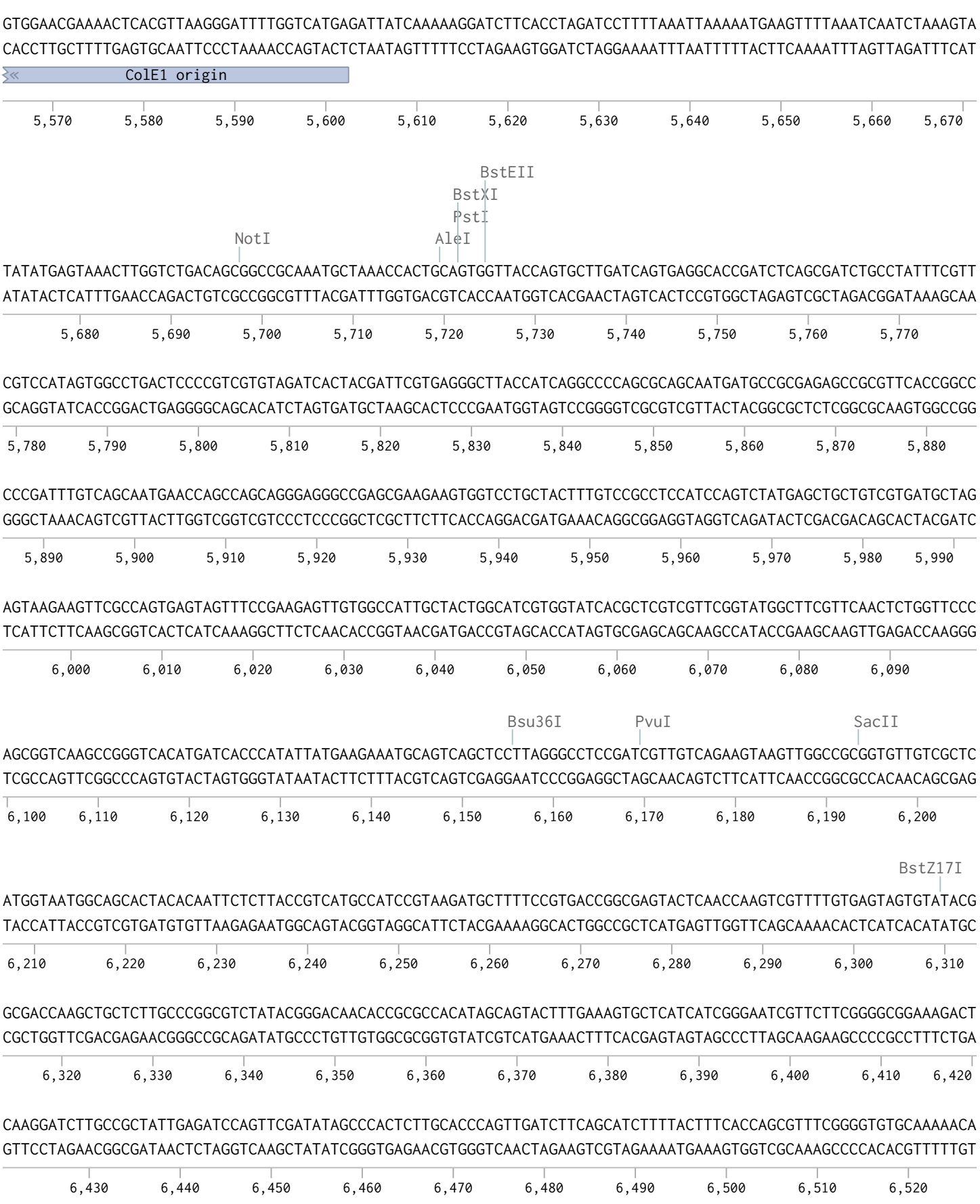
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BstEII
BstXI
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NotI
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PvuI
SacII
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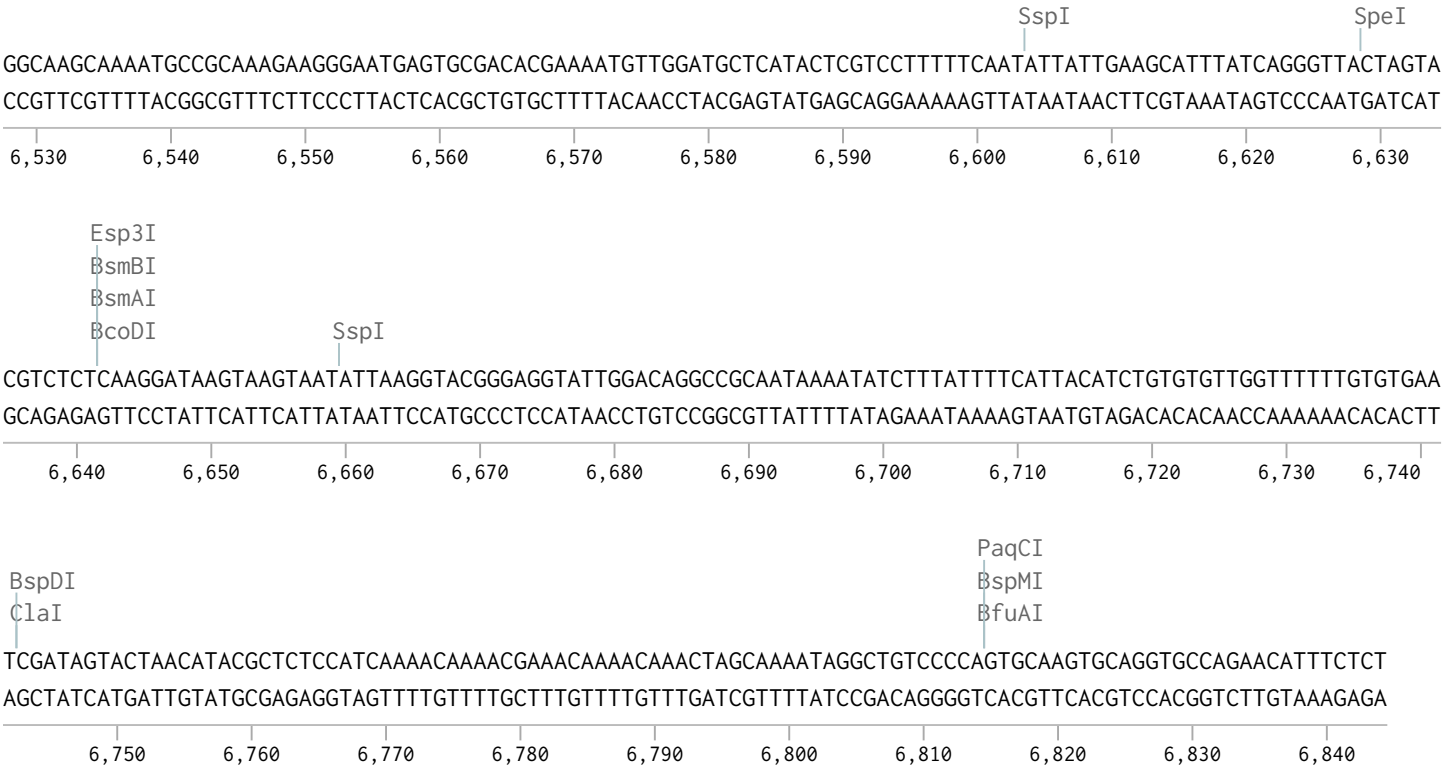
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BsmAI
BcoDI
SspI
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6,810
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(from 1-642 bp)

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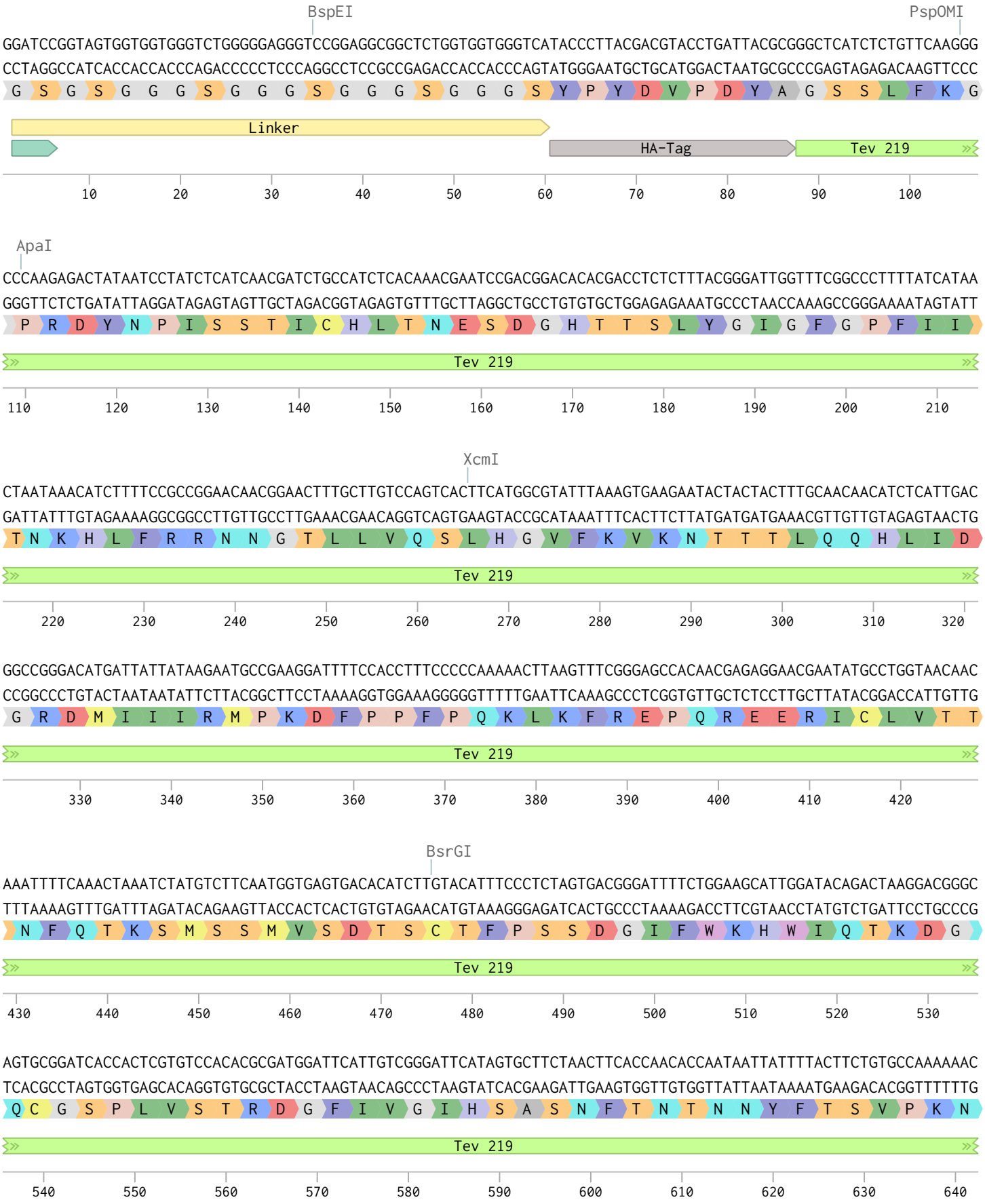


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SwaI
NotI
AsiSI
MfeI
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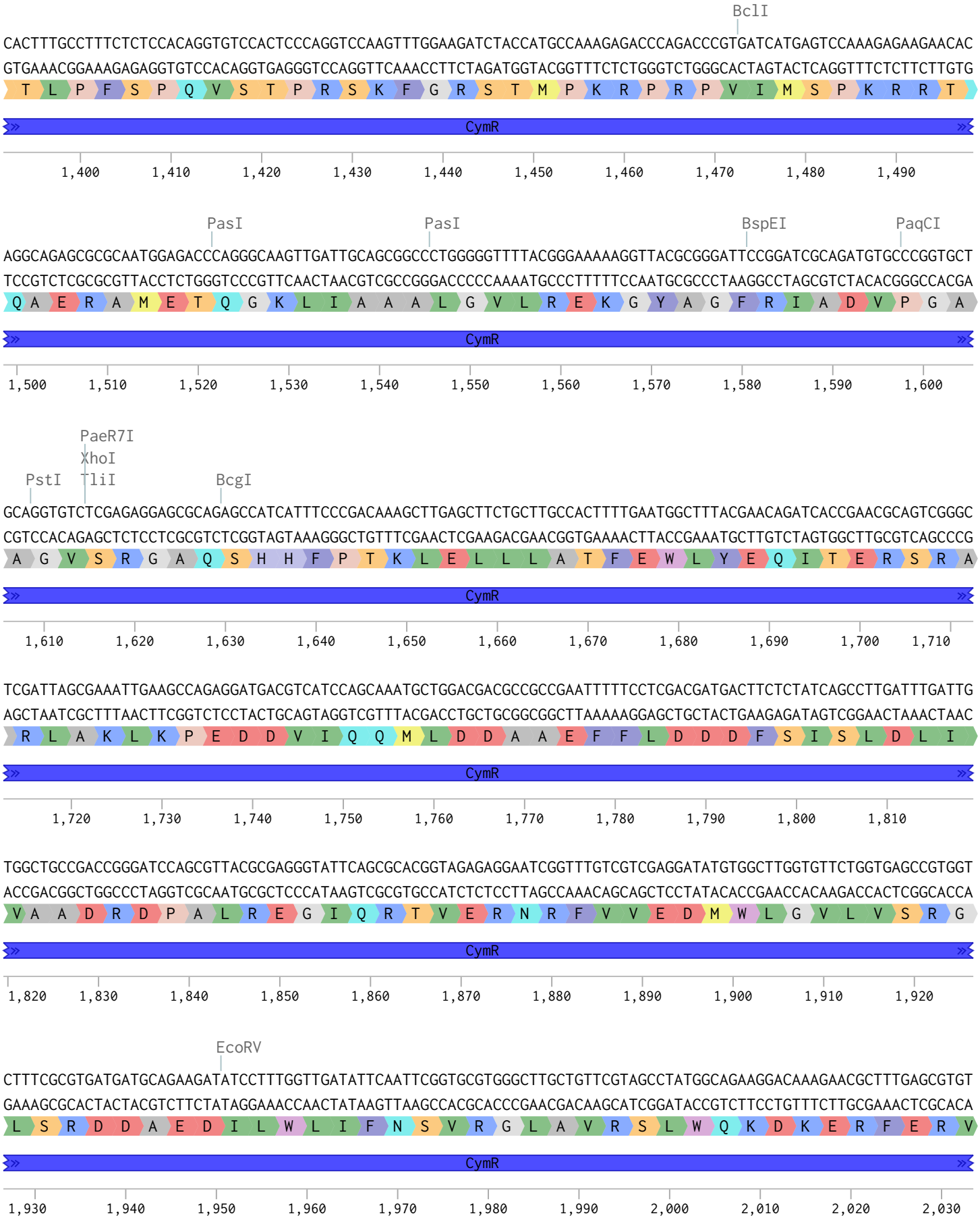
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PasI
PasI
BspEI
PaqCI
CymR
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CymR
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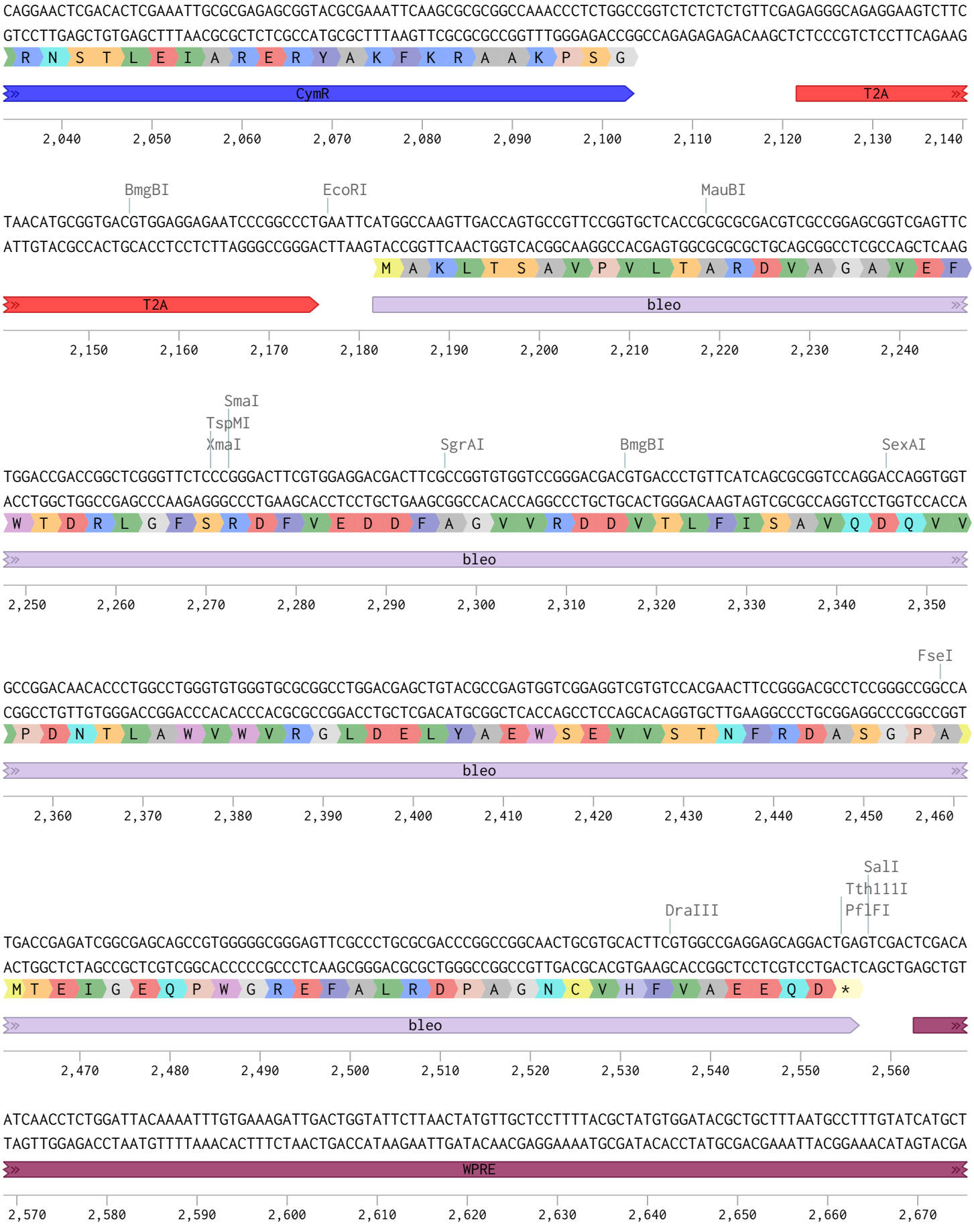
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CymR
T2A
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BmgBI
EcoRI
MauBI
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bleo
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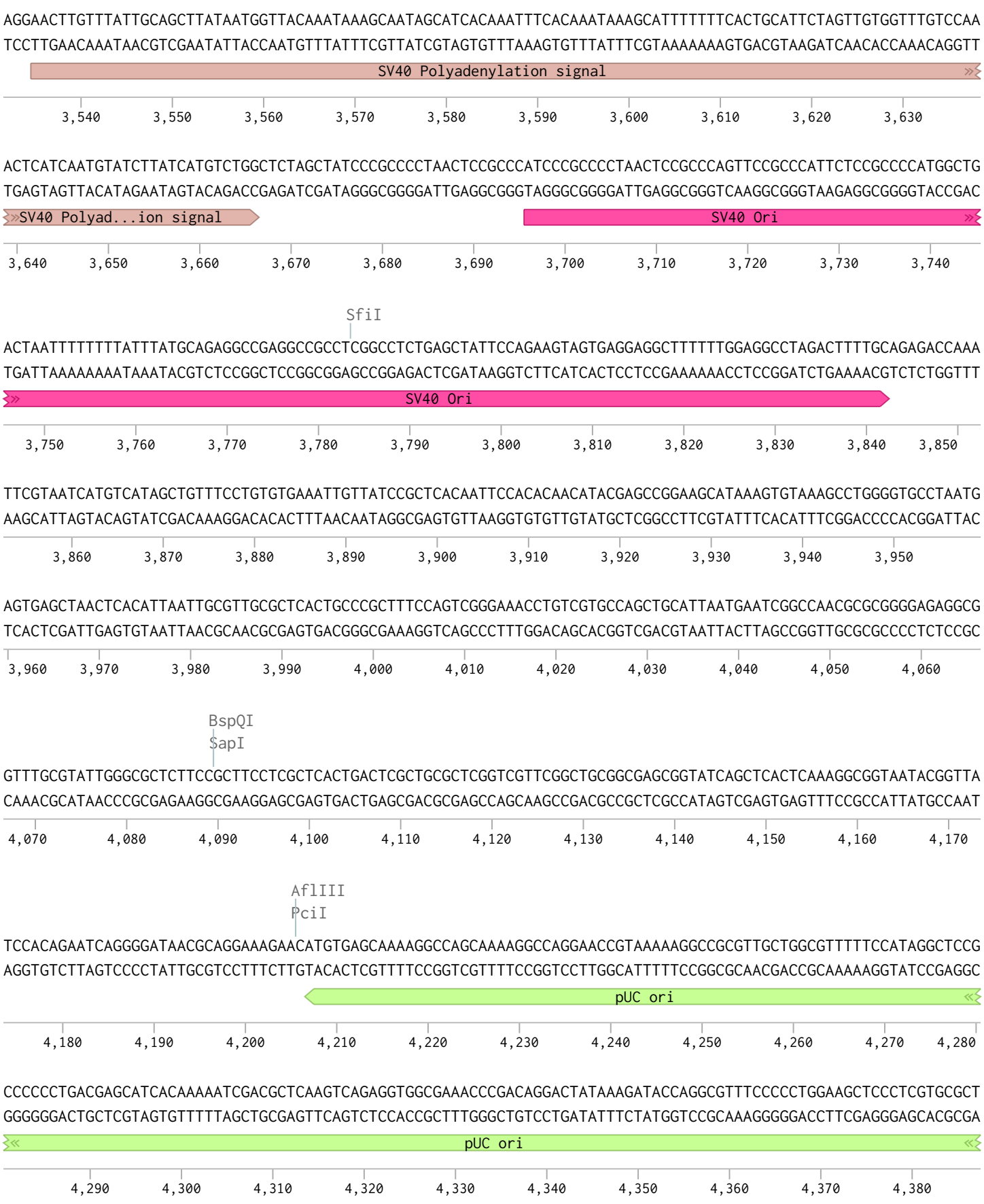
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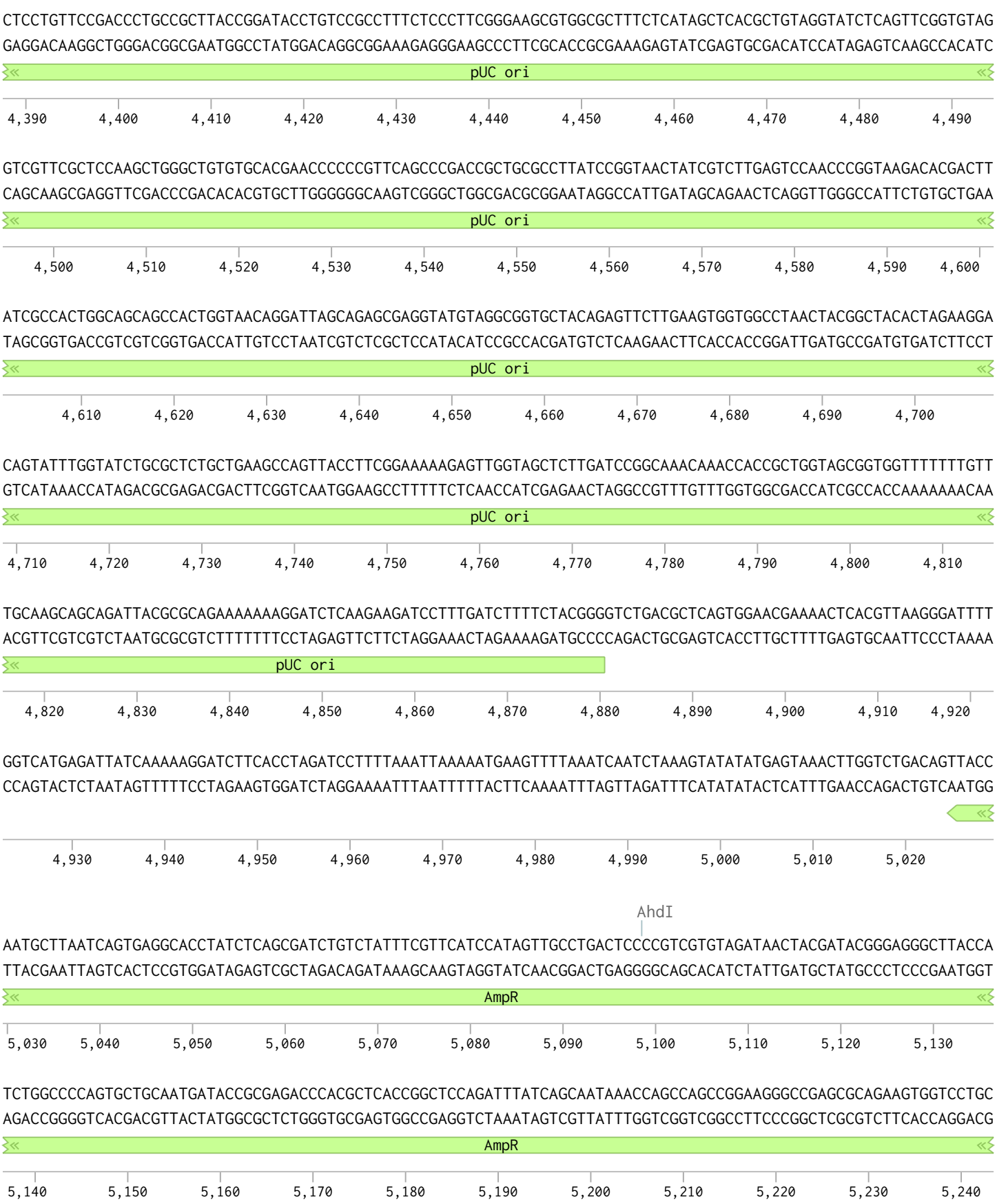
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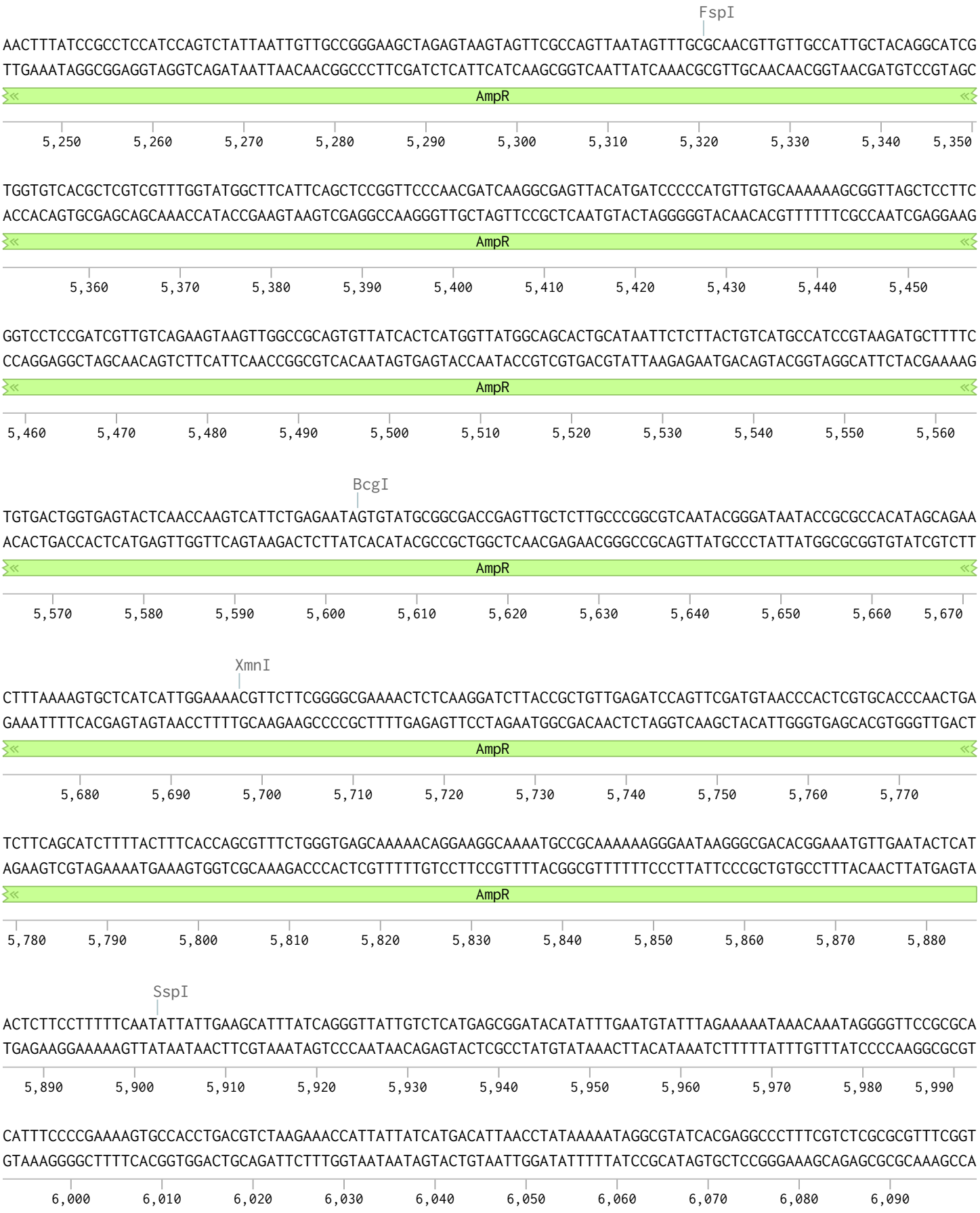
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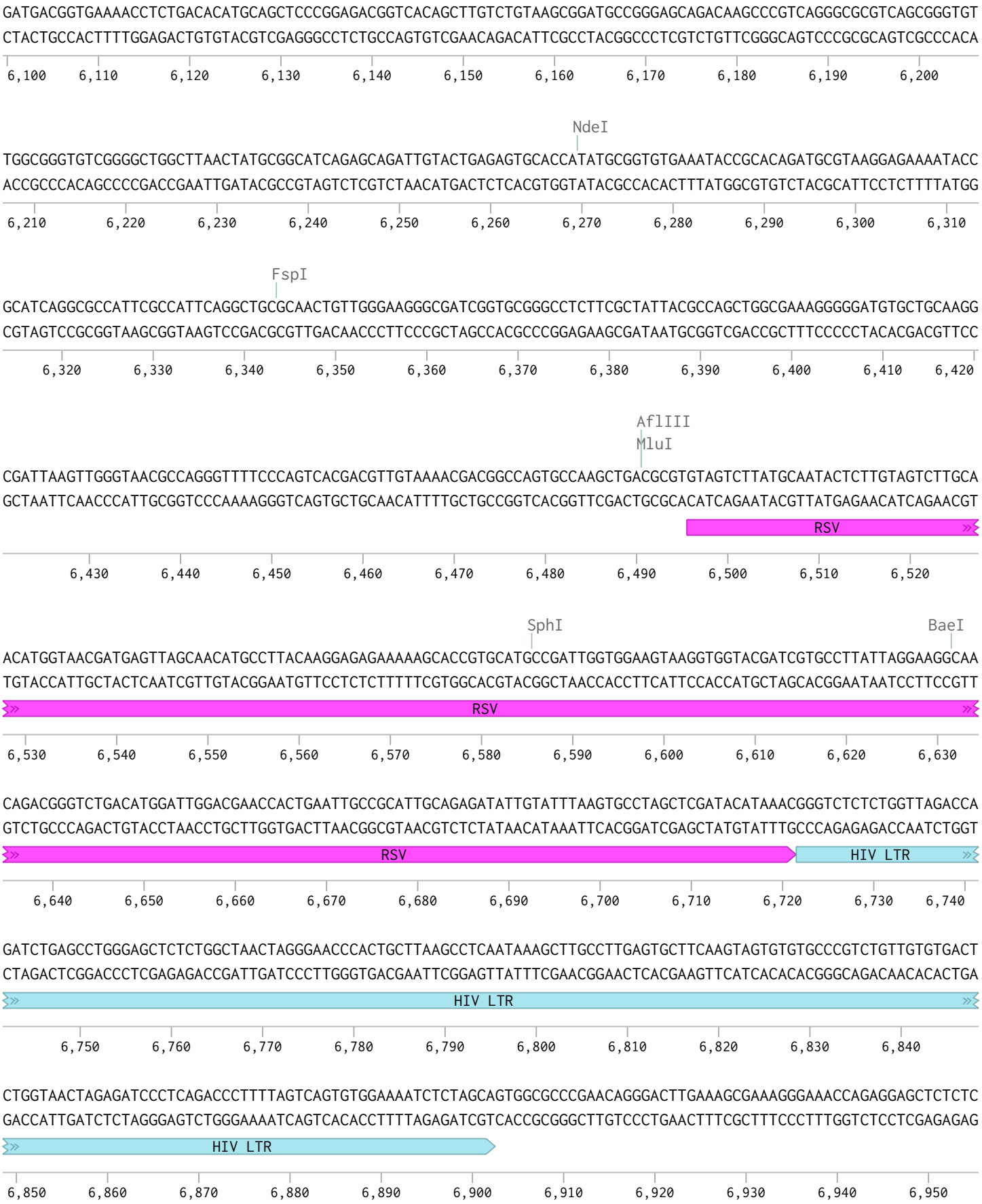
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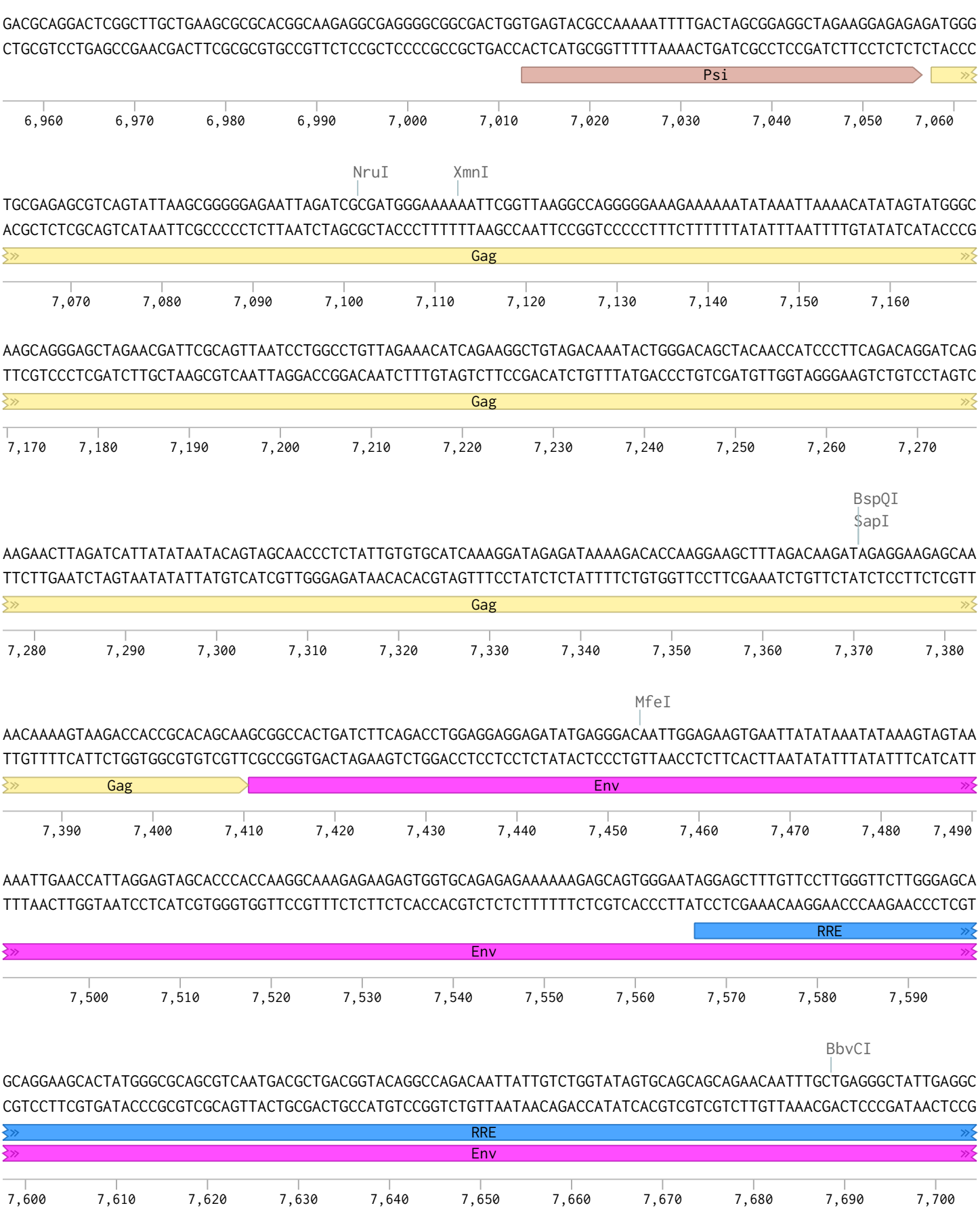
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Env
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Env
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Env
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Env
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cPPT
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ClaI
SpeI
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CMV + CUO
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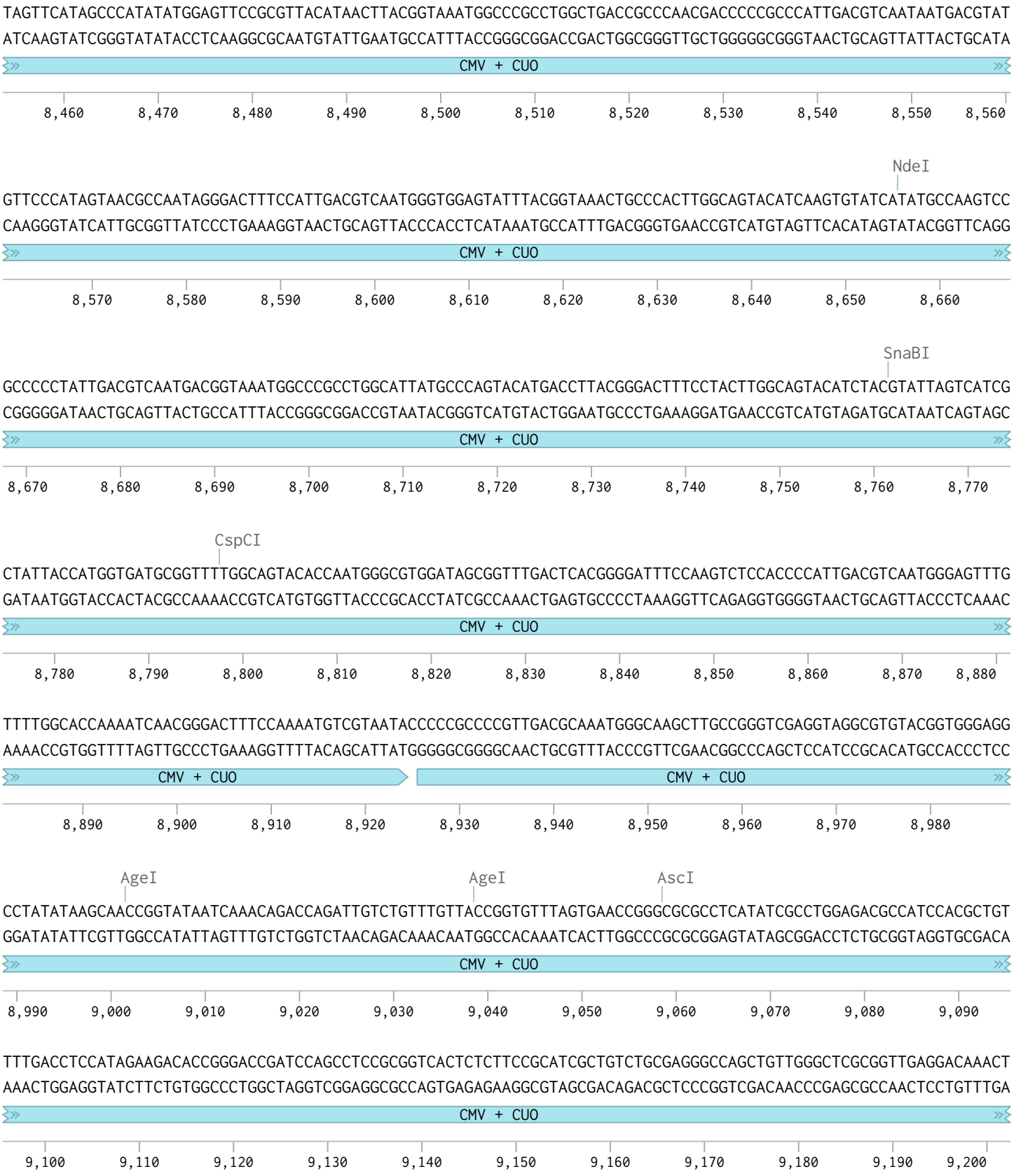
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CMV + CUO
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8,550
8,560
NdeI
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CMV + CUO
CMV + CUO
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AgeI
AgeI
AscI
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CMV + CUO
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CMV + CUO
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9,190
9,200


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CMV + CUO
CMV + CUO
9,210
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XhoI
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CMV + CUO
CMV + CUO
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CMV + CUO
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CMV + CUO
Arrb1 optimized
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BspDI
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Arrb1 optimized
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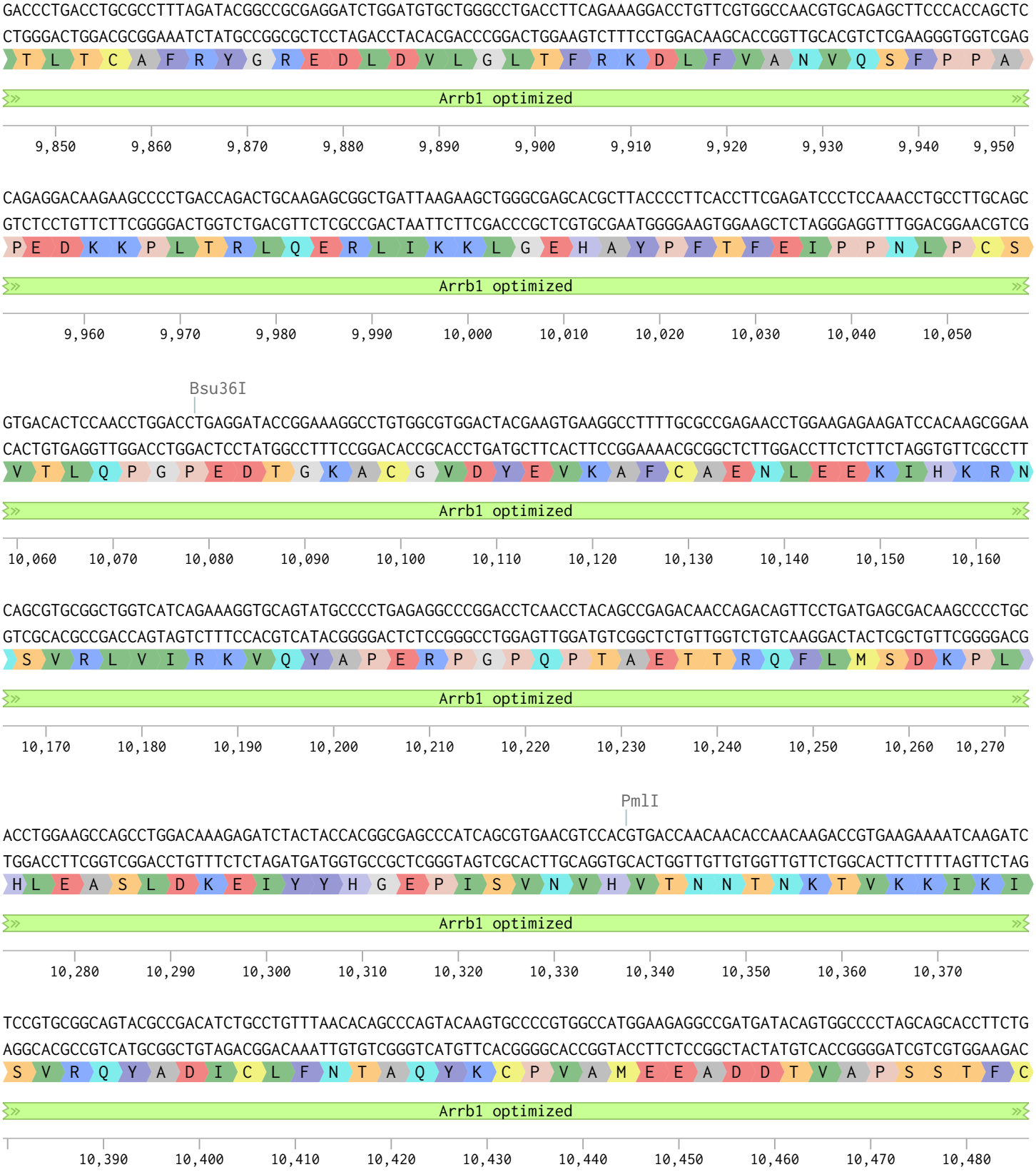
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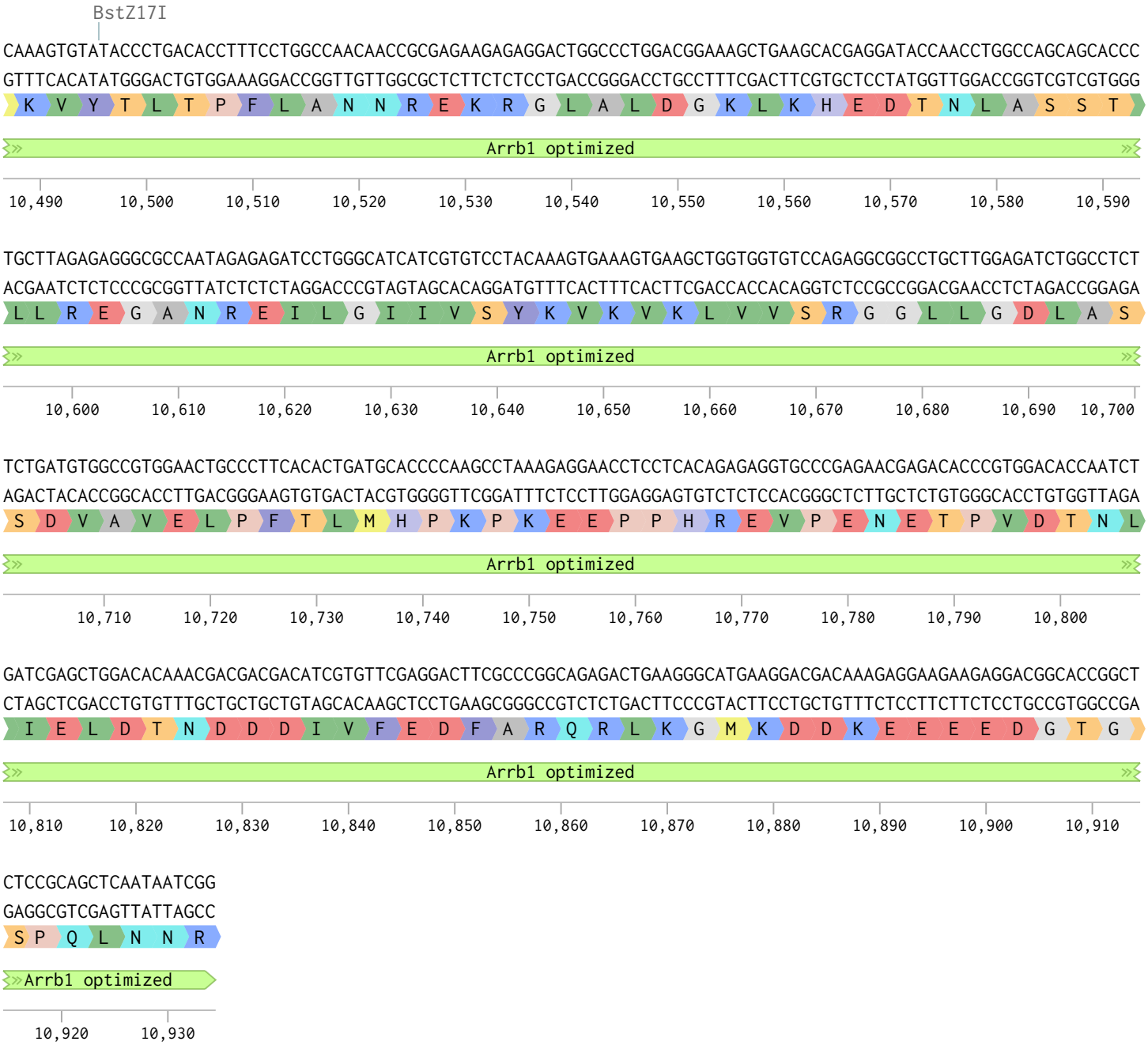
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Arrb1 optimized
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Arrb1 optimized
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Arrb1 optimized
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Arrb1 optimized
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S P Q L N N R
Arrb1 optimized
10,920 10,930

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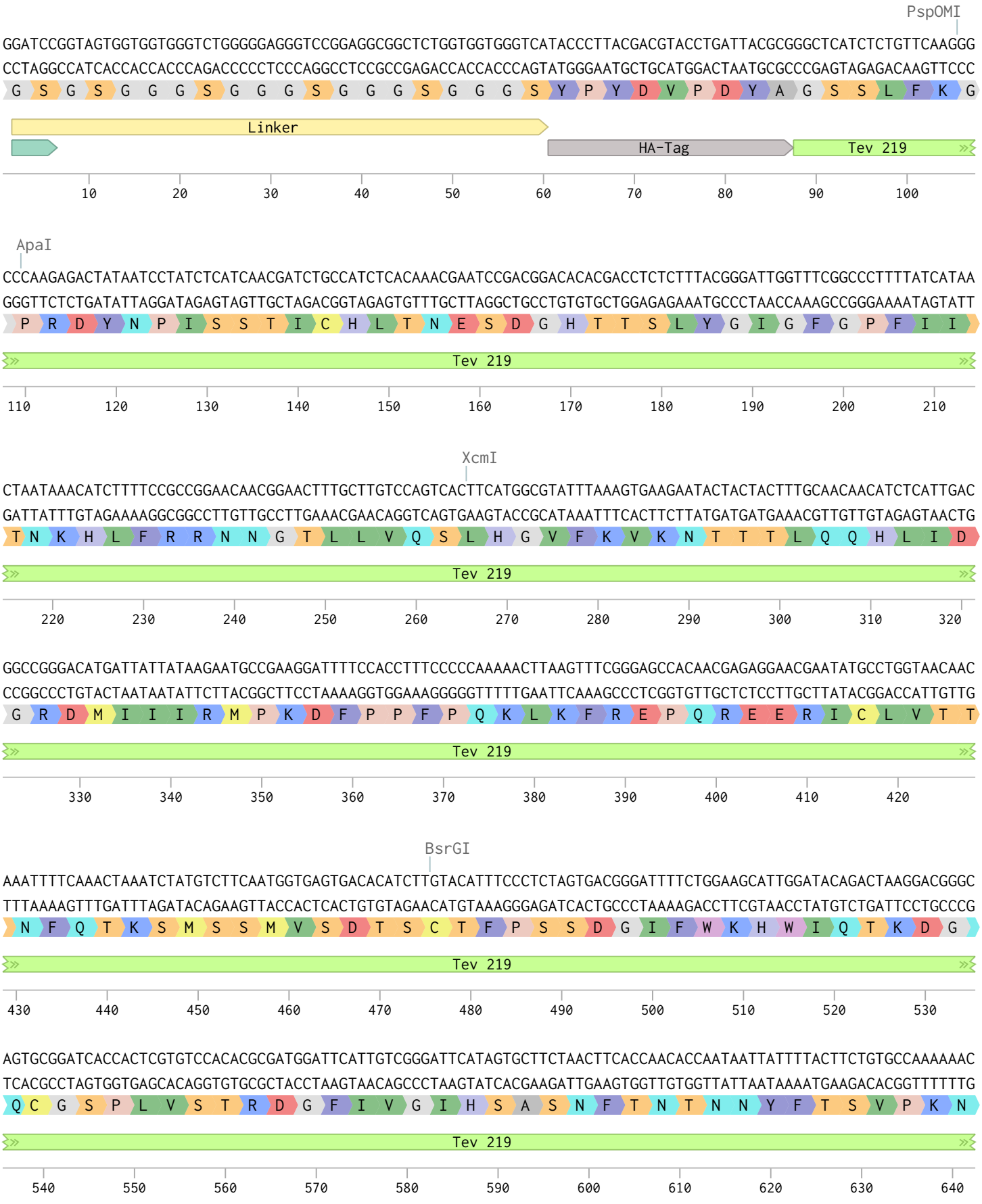


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NotI
AsiSI
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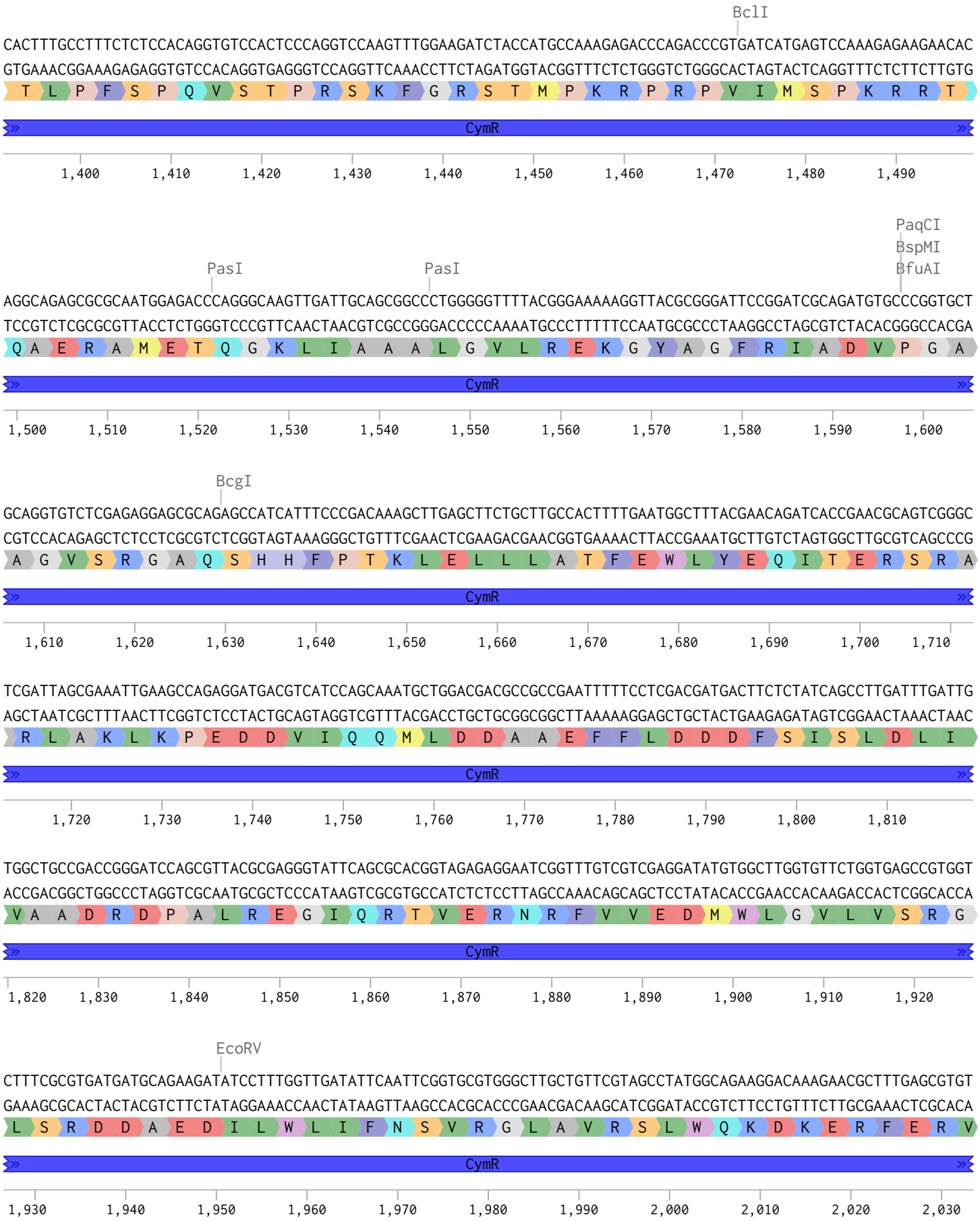
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PasI
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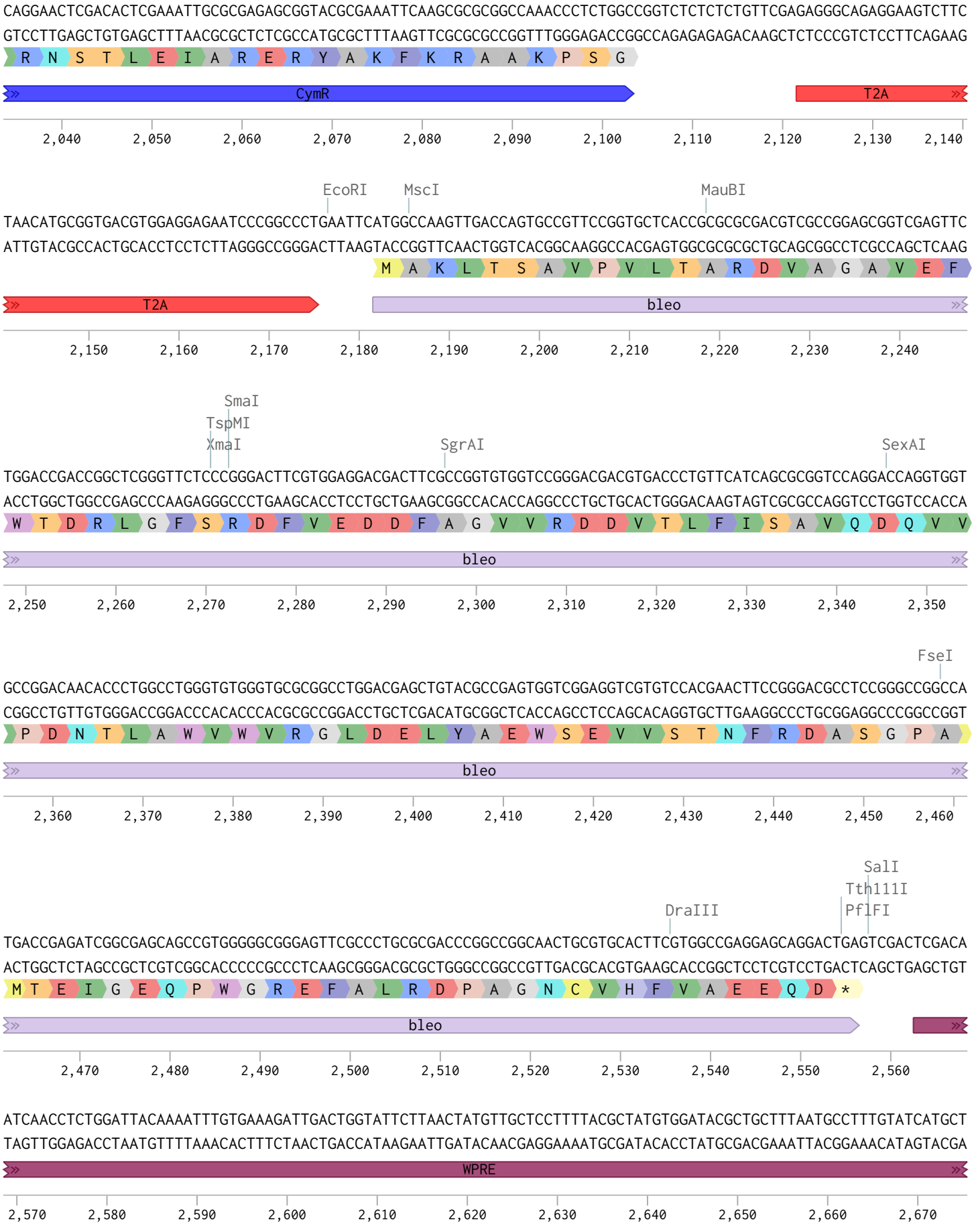
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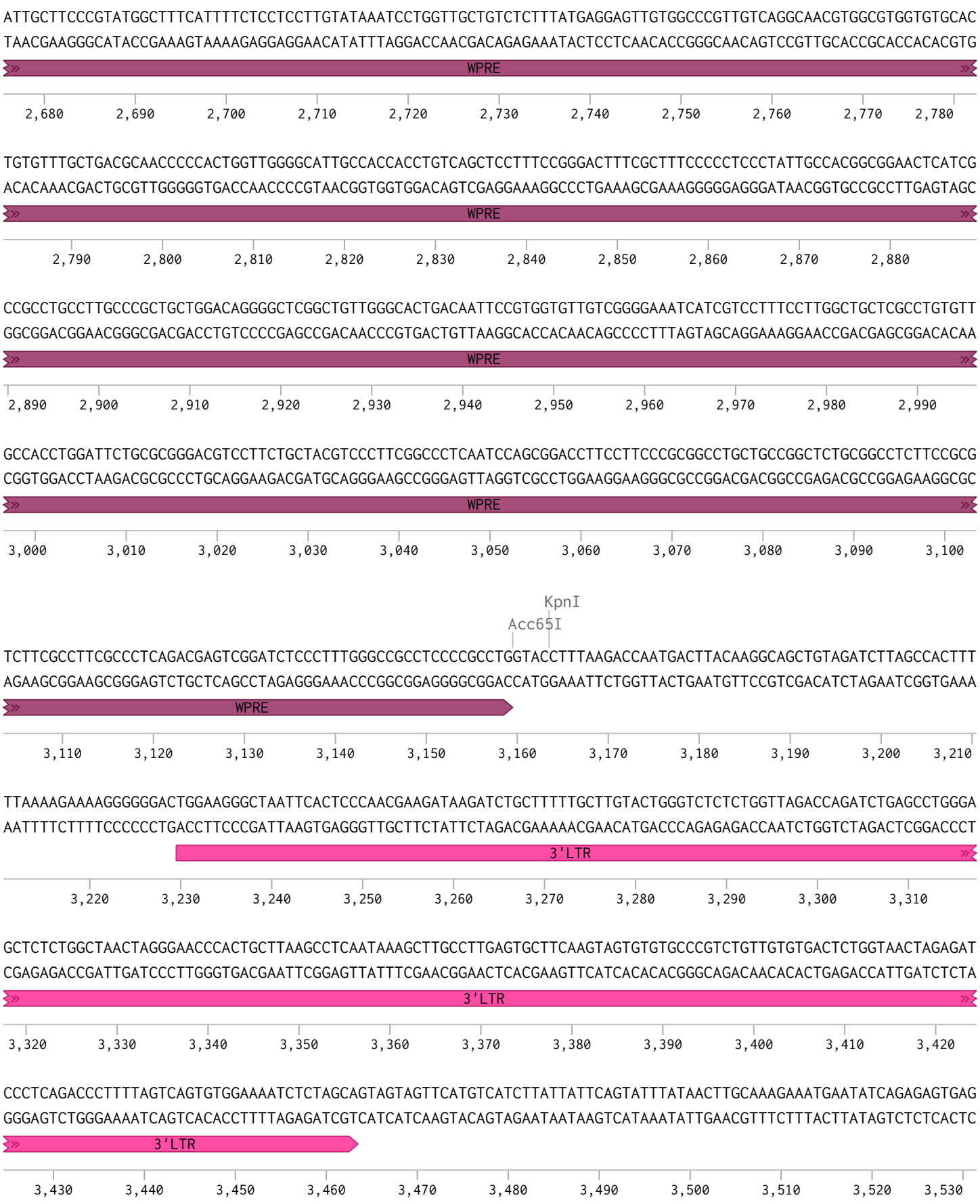
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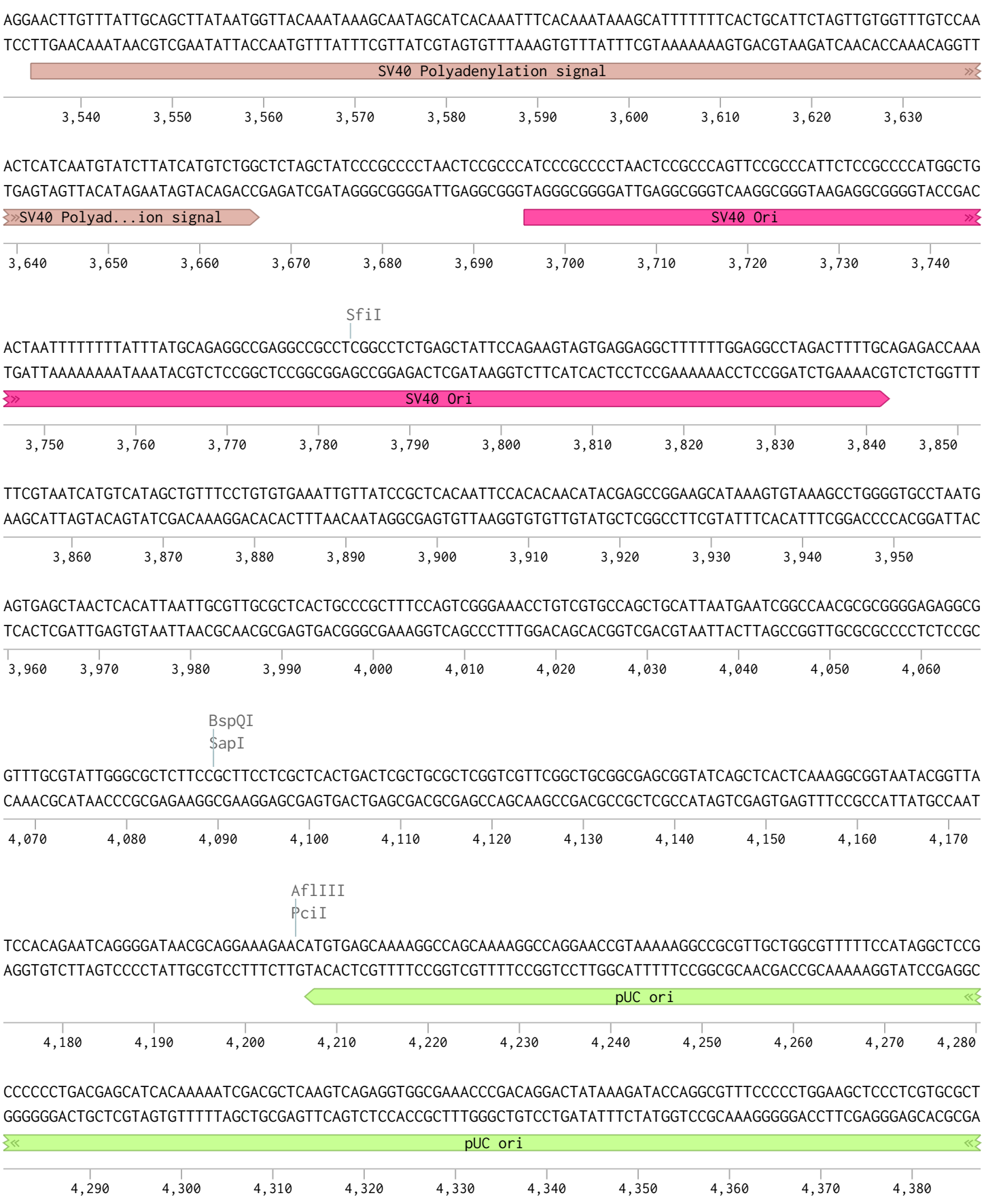
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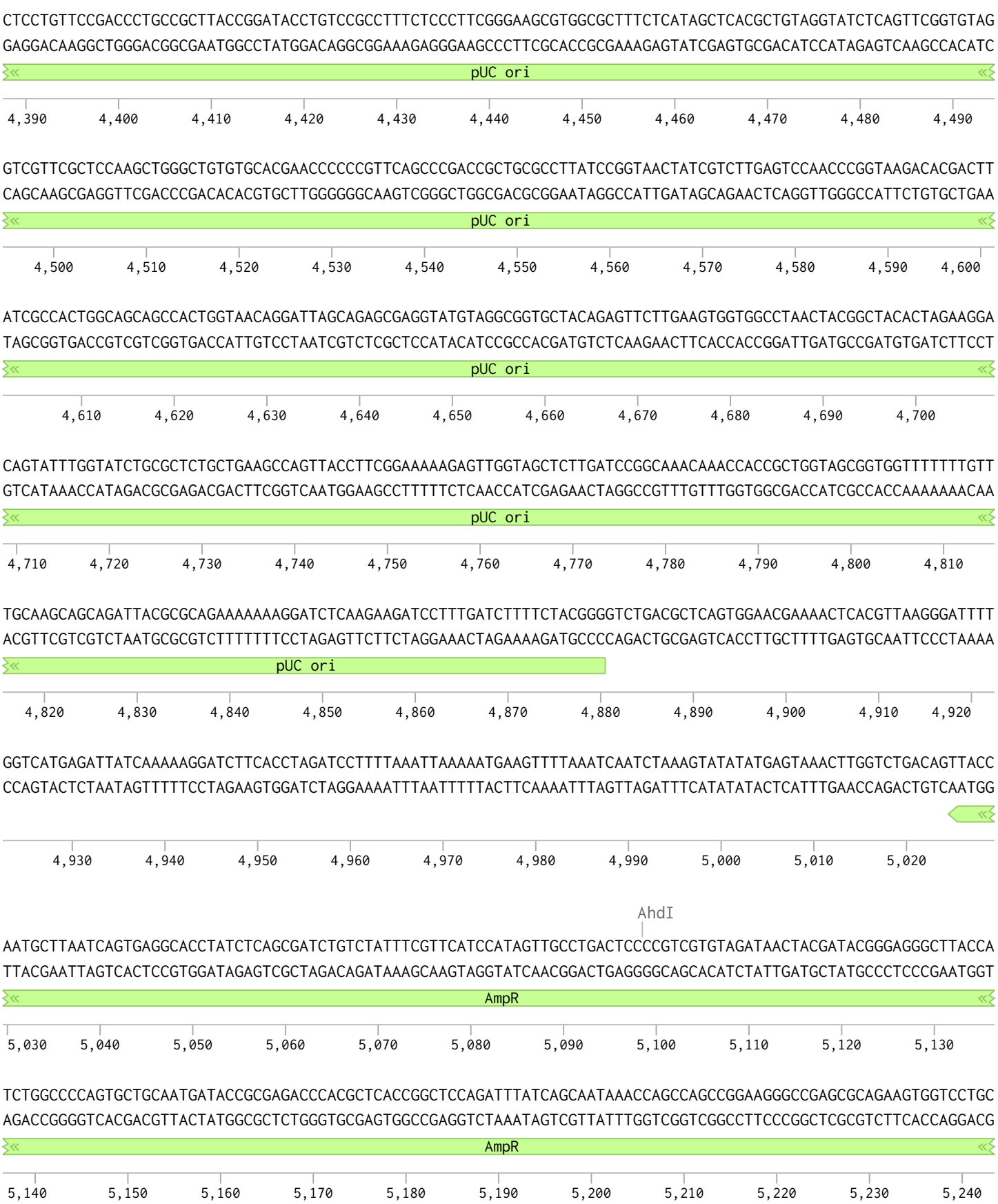
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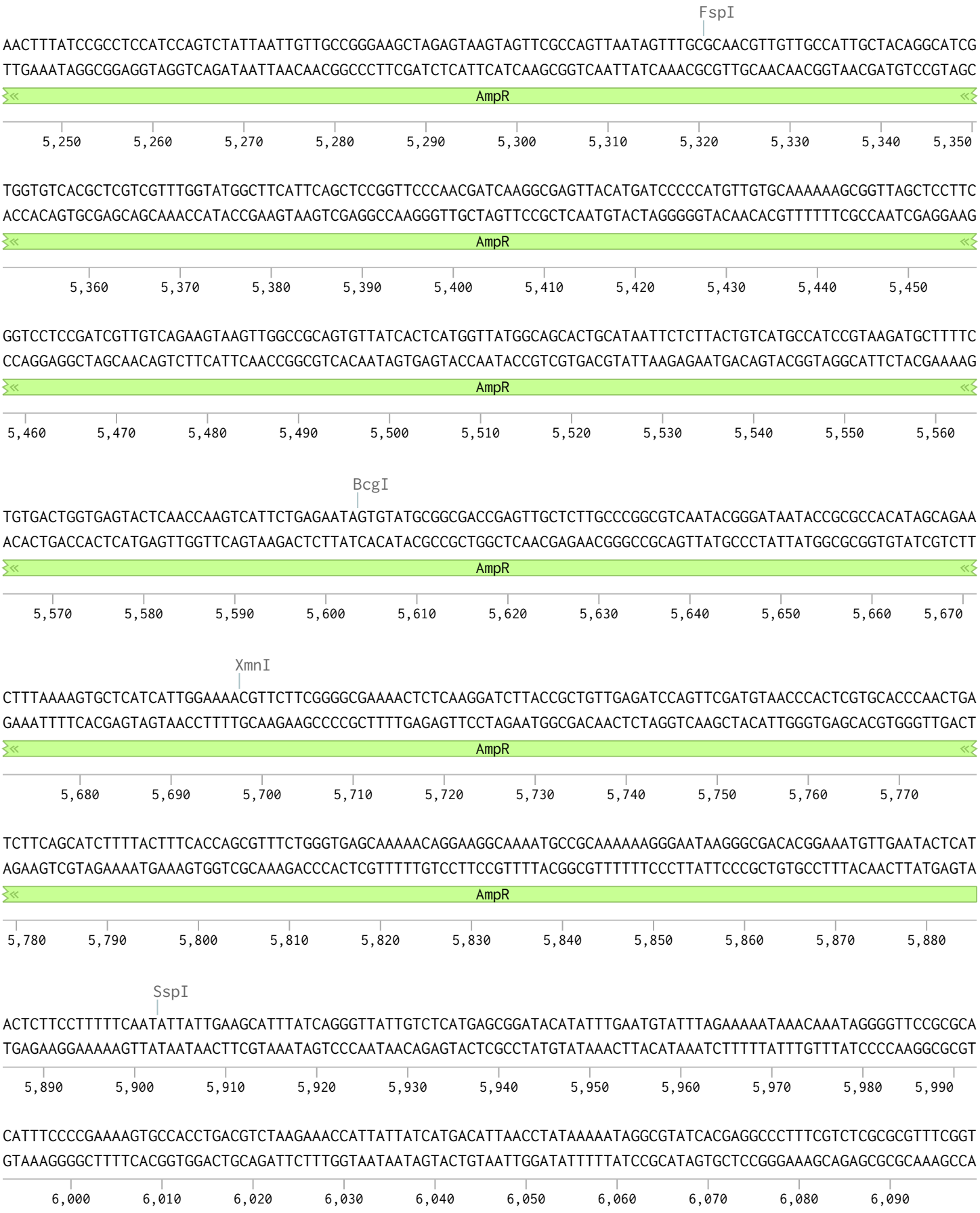
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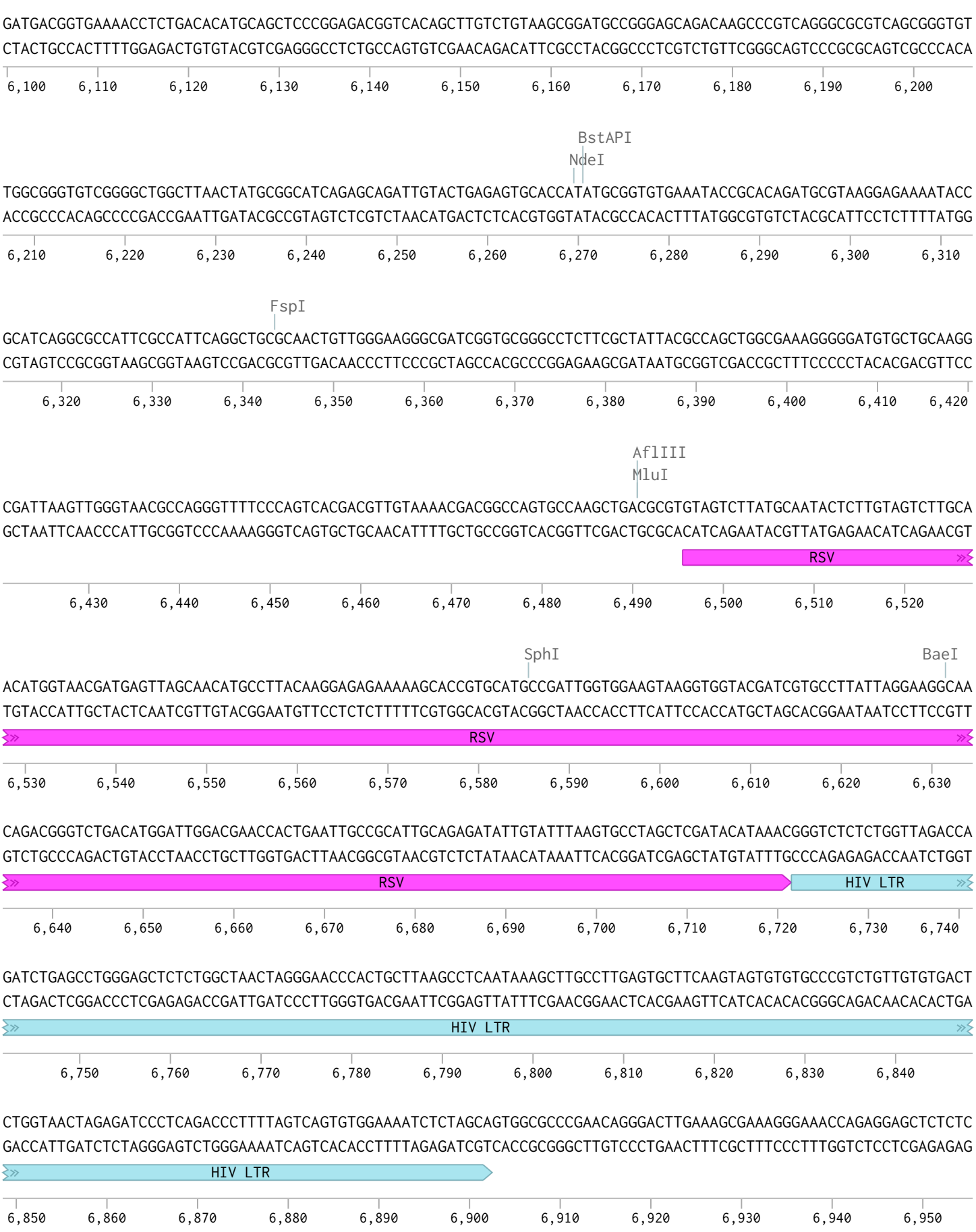
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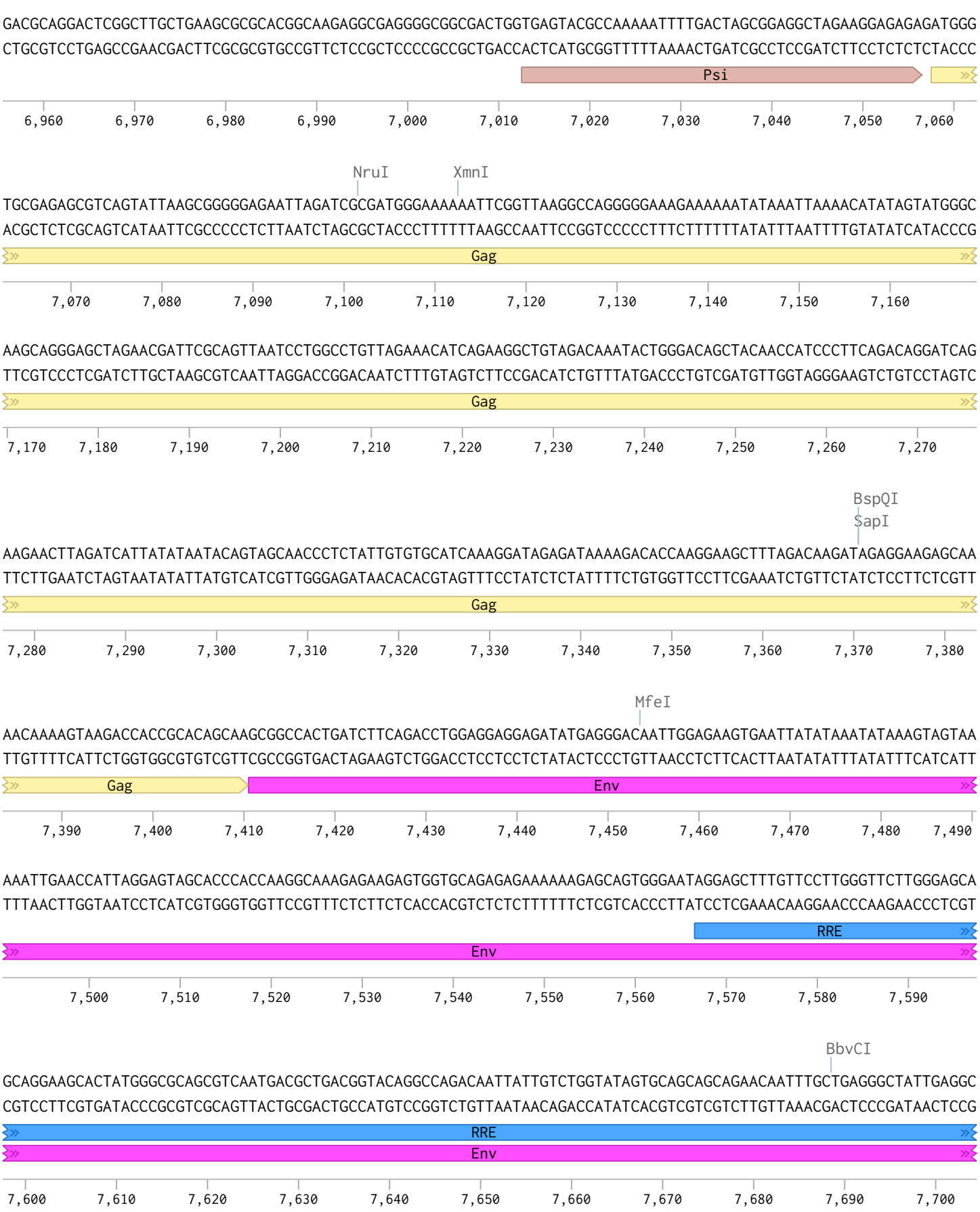
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Env
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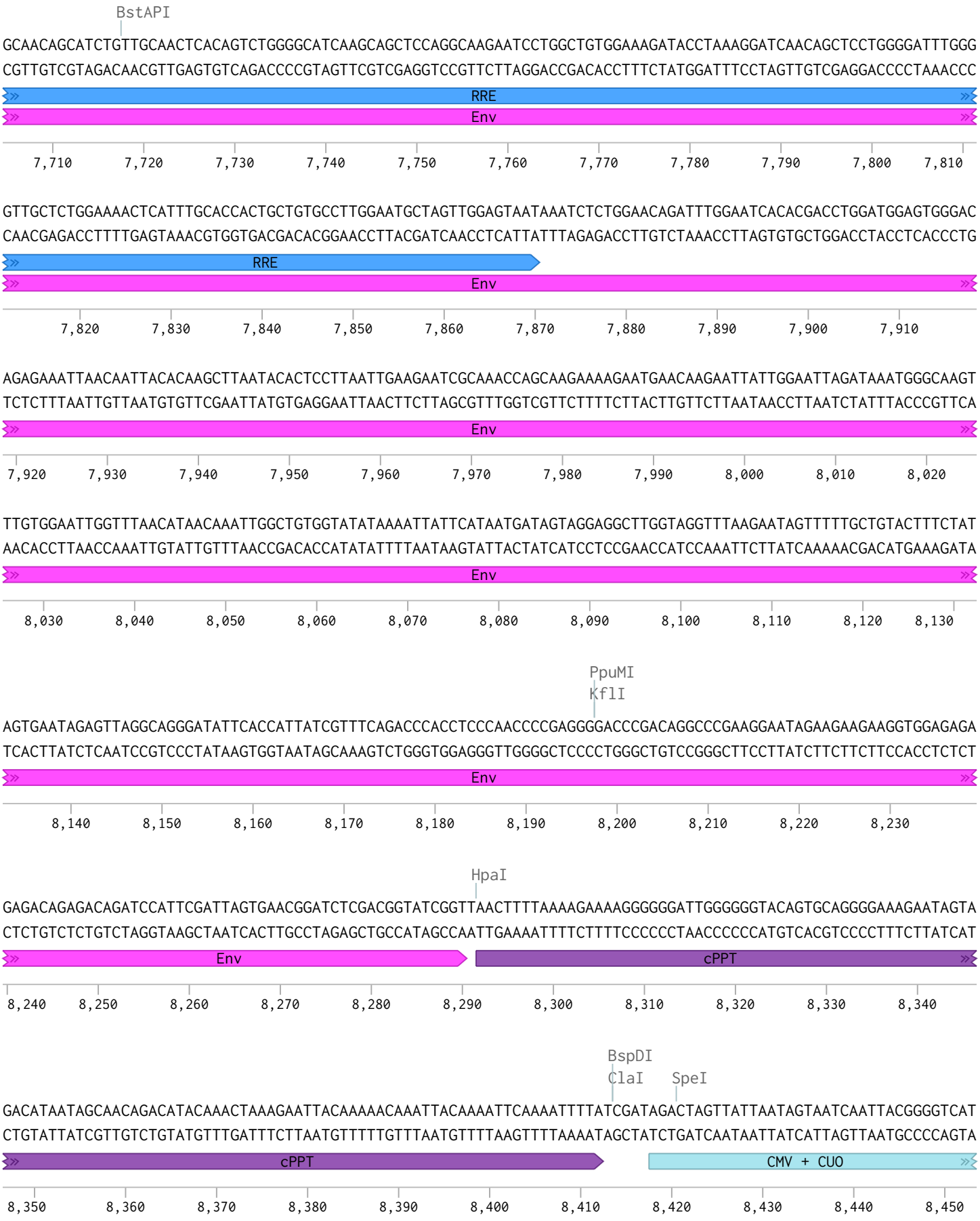
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SpeI
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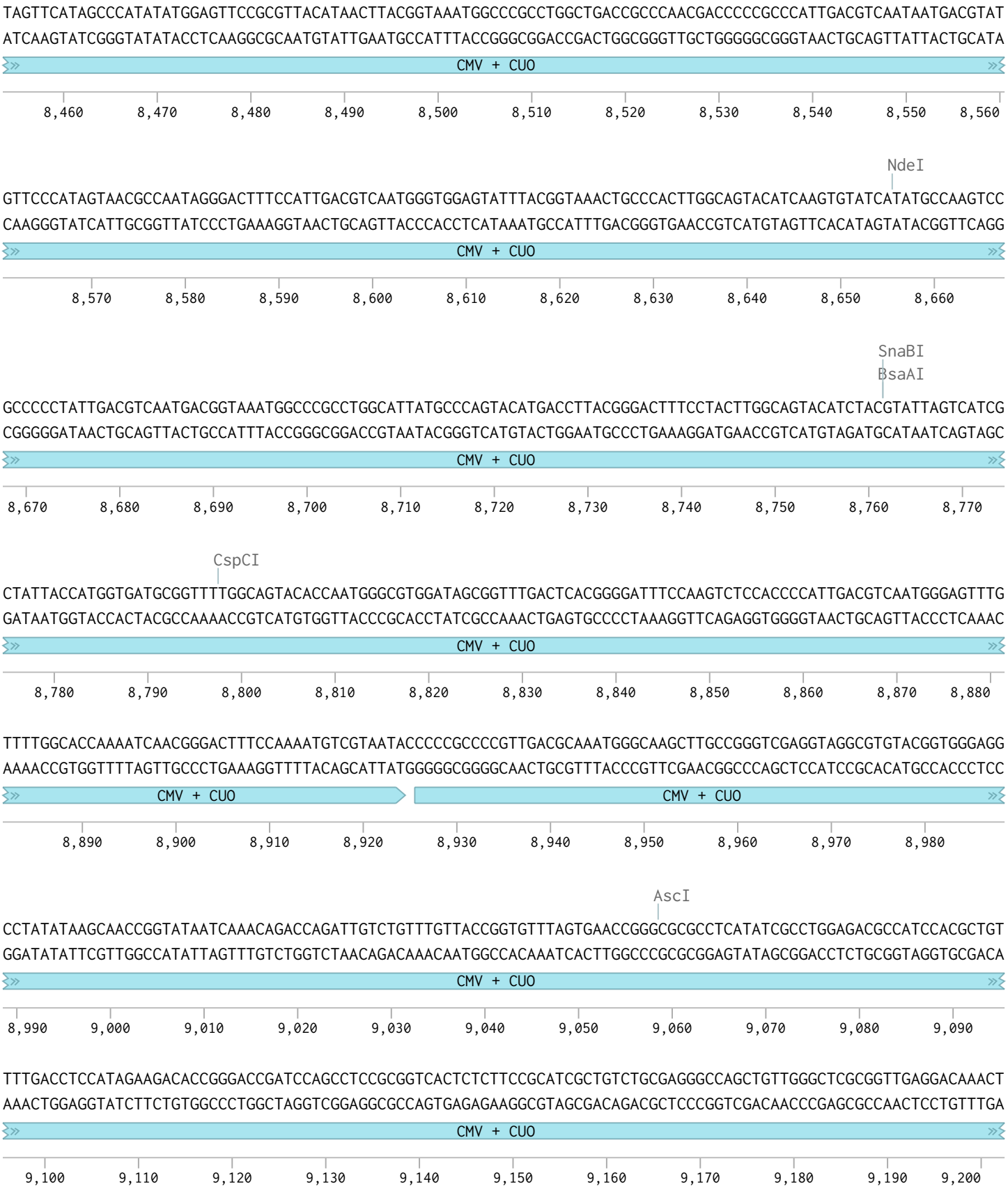
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CMV + CUO
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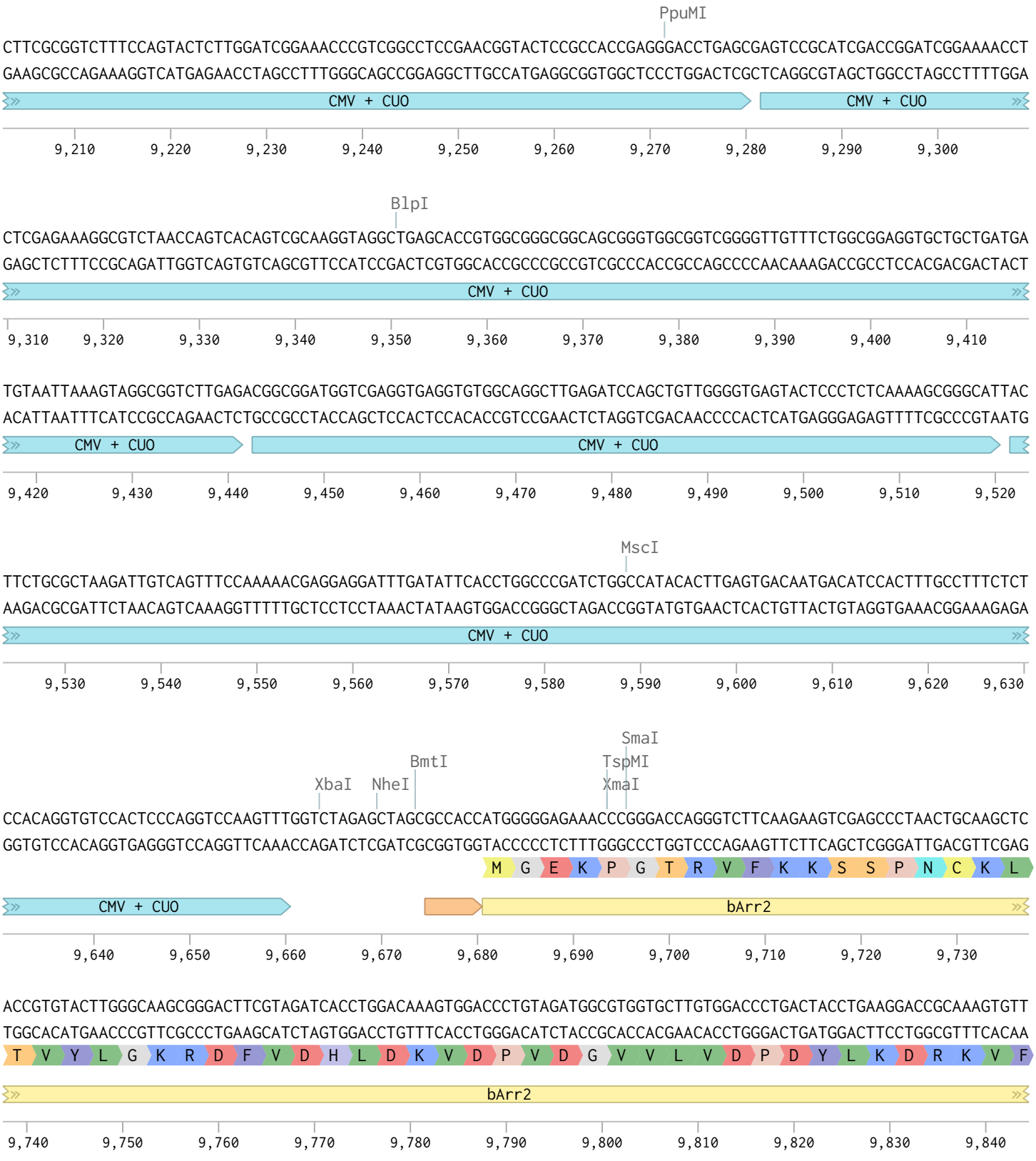
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CMV + CUO
CMV + CUO
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CMV + CUO
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BmtI
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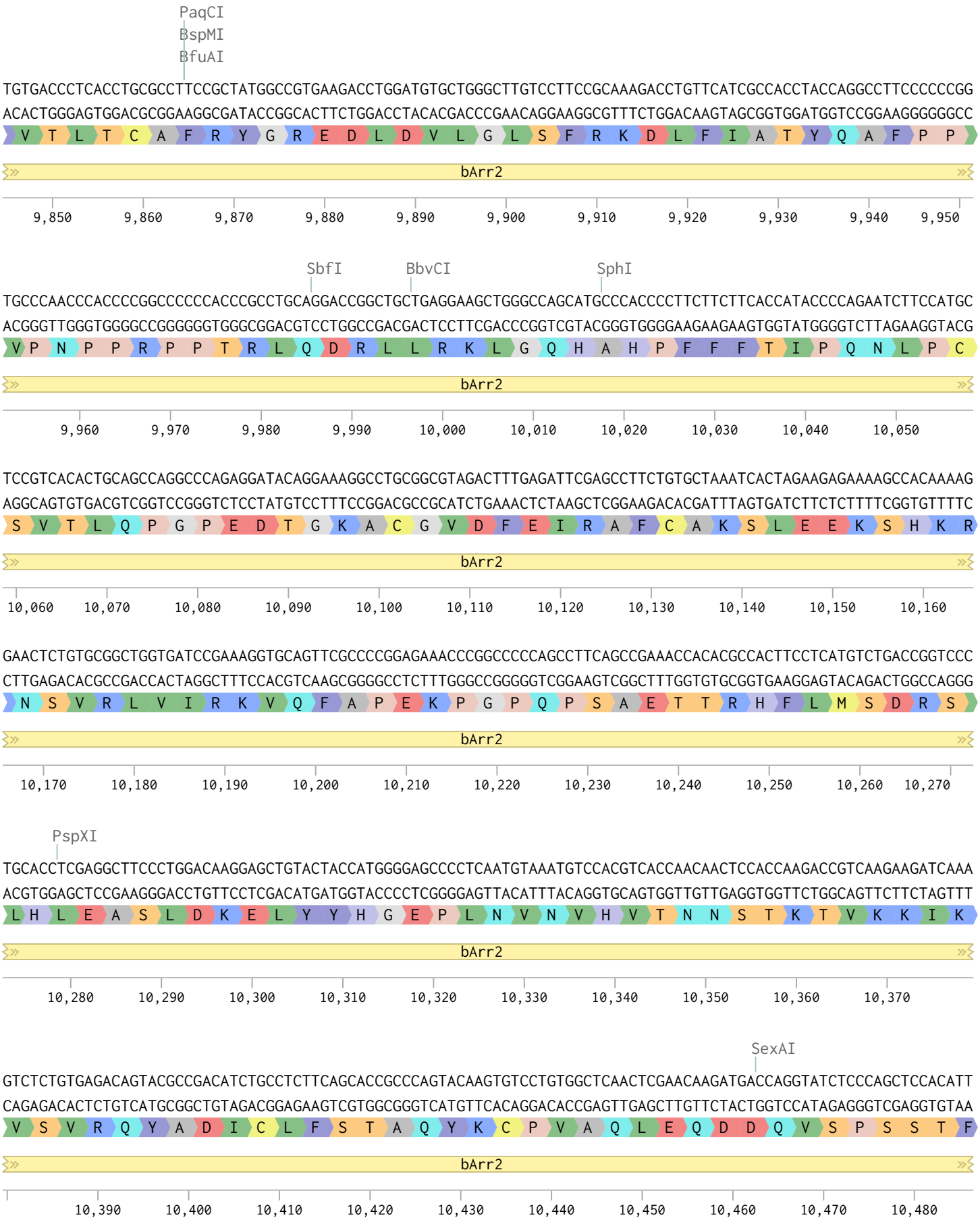
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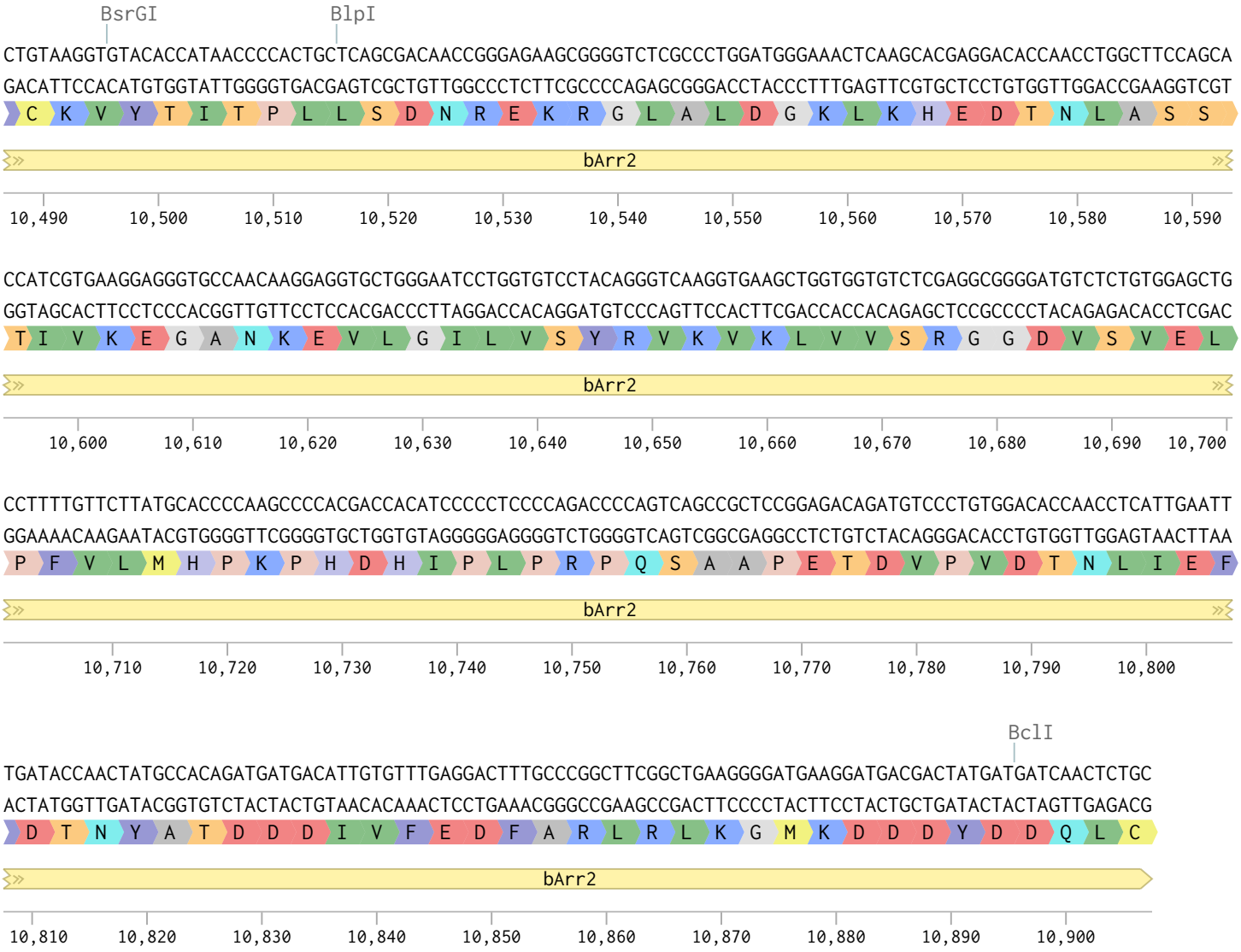
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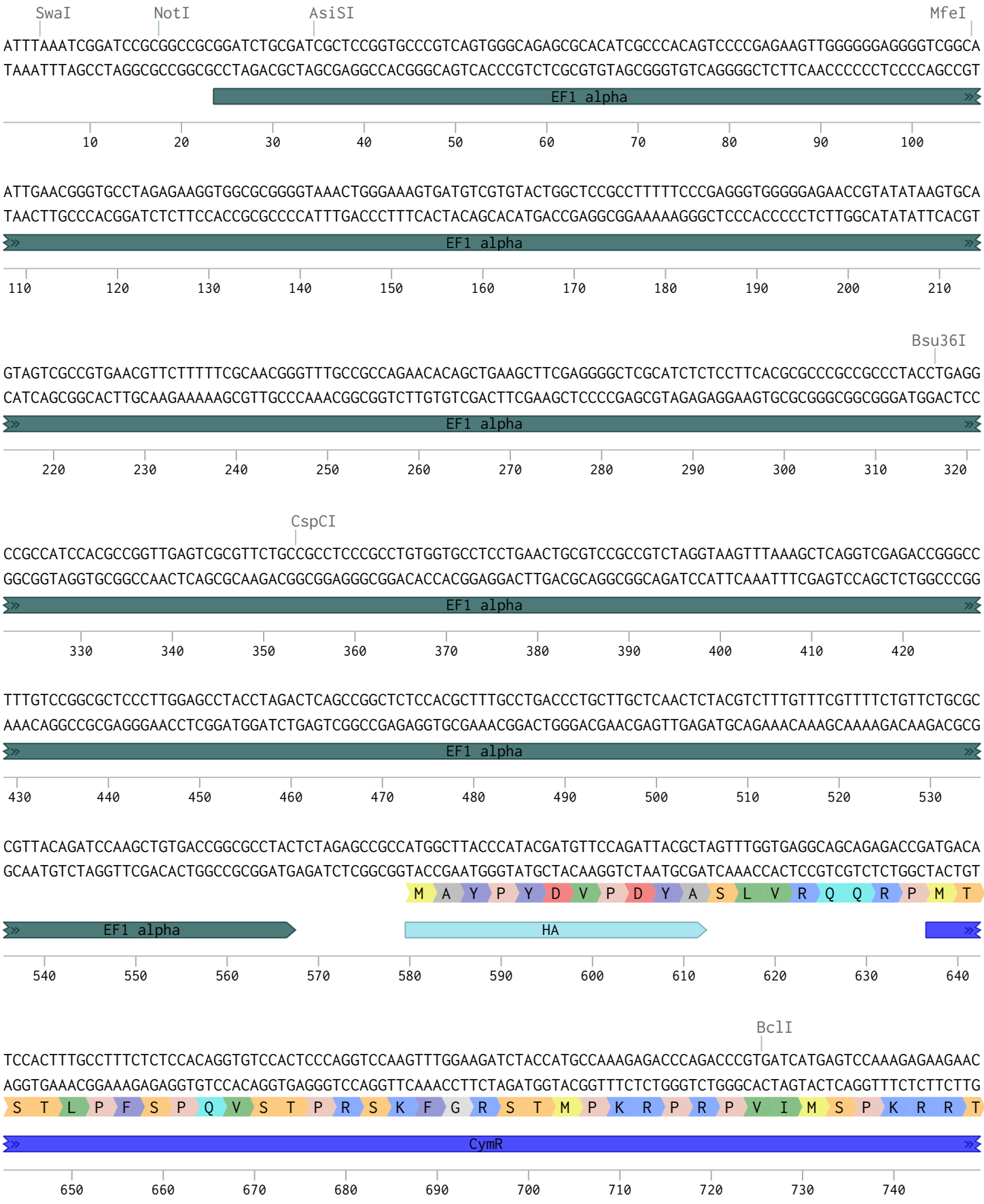

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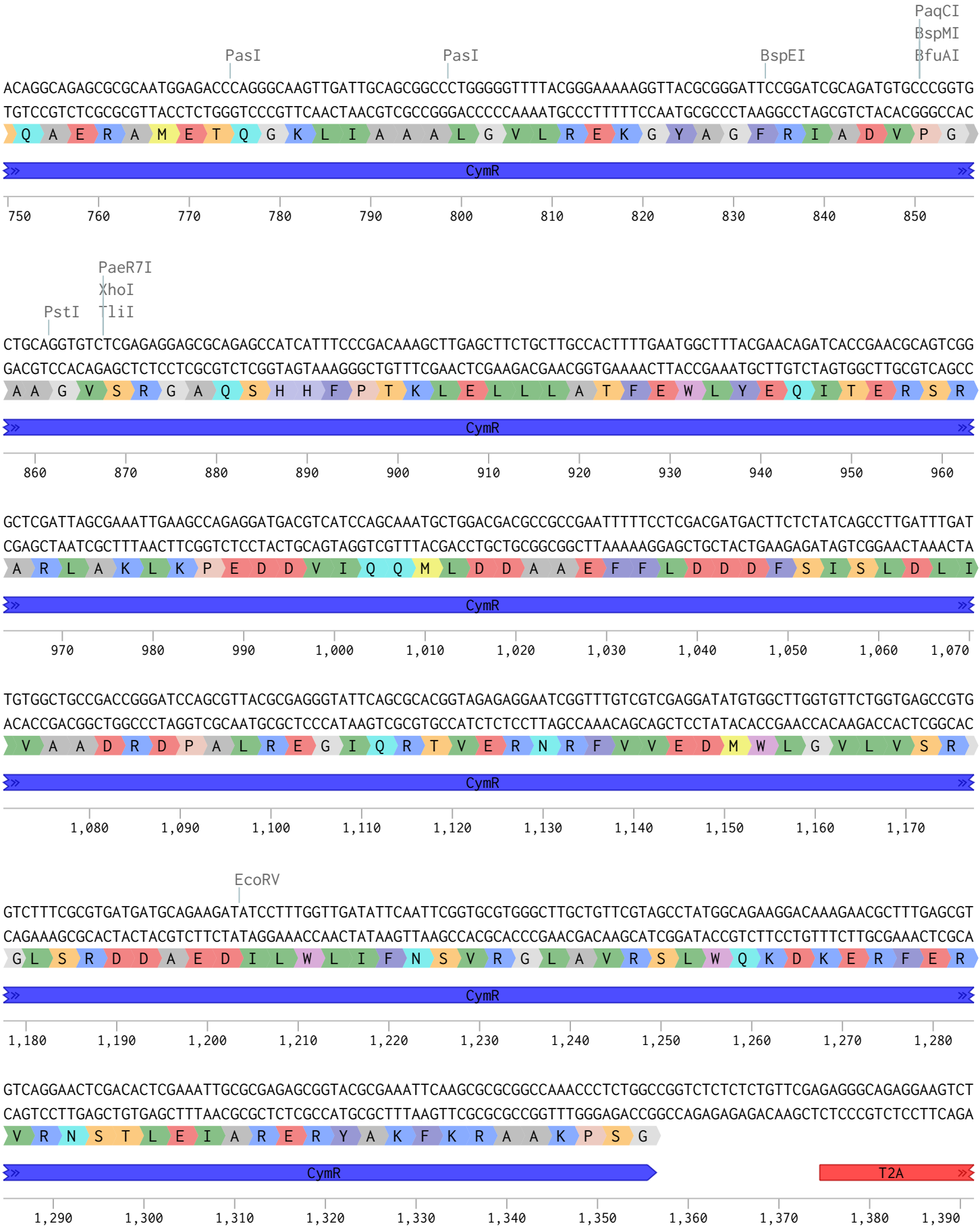

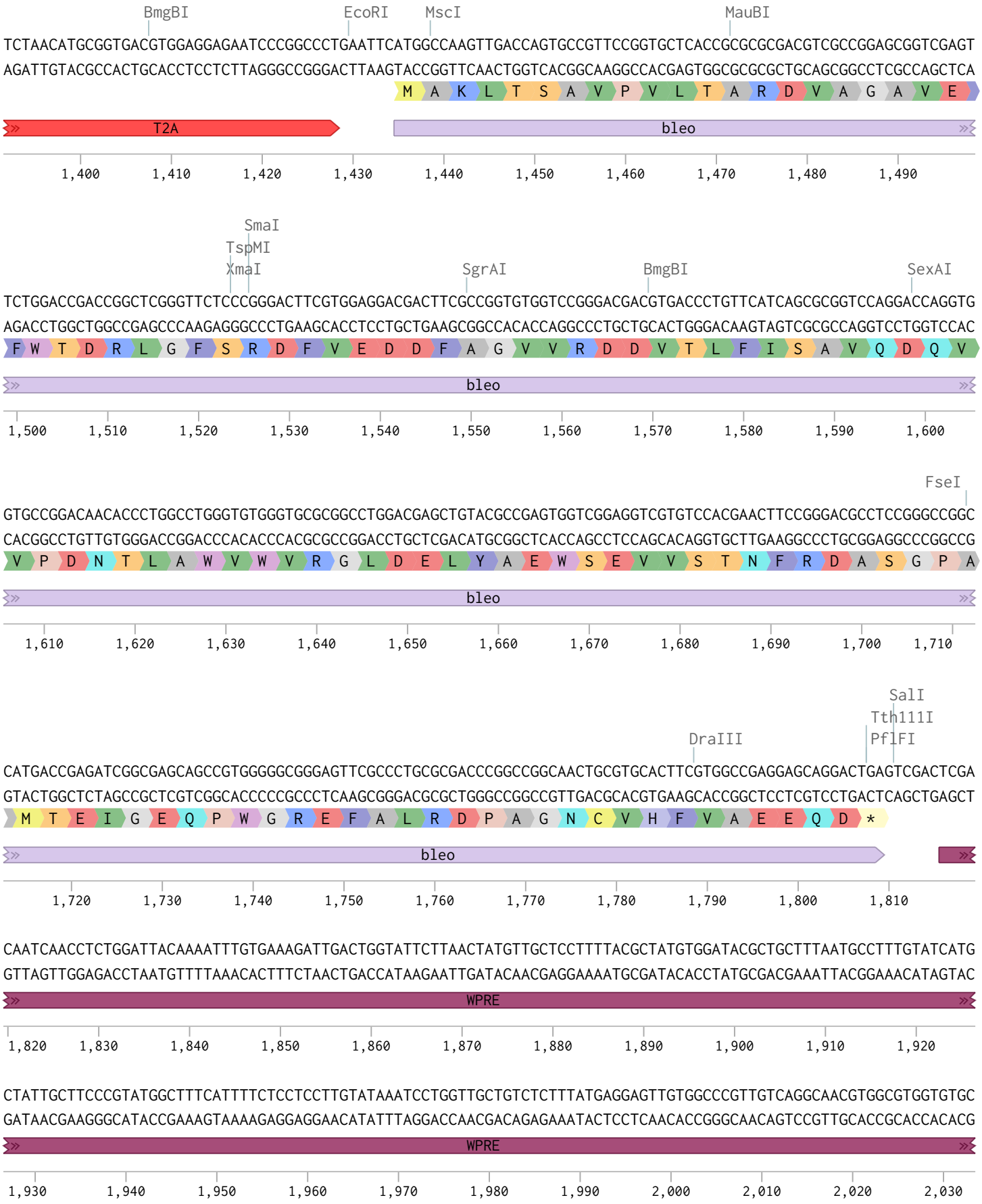
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bleo
WPRE
WPRE


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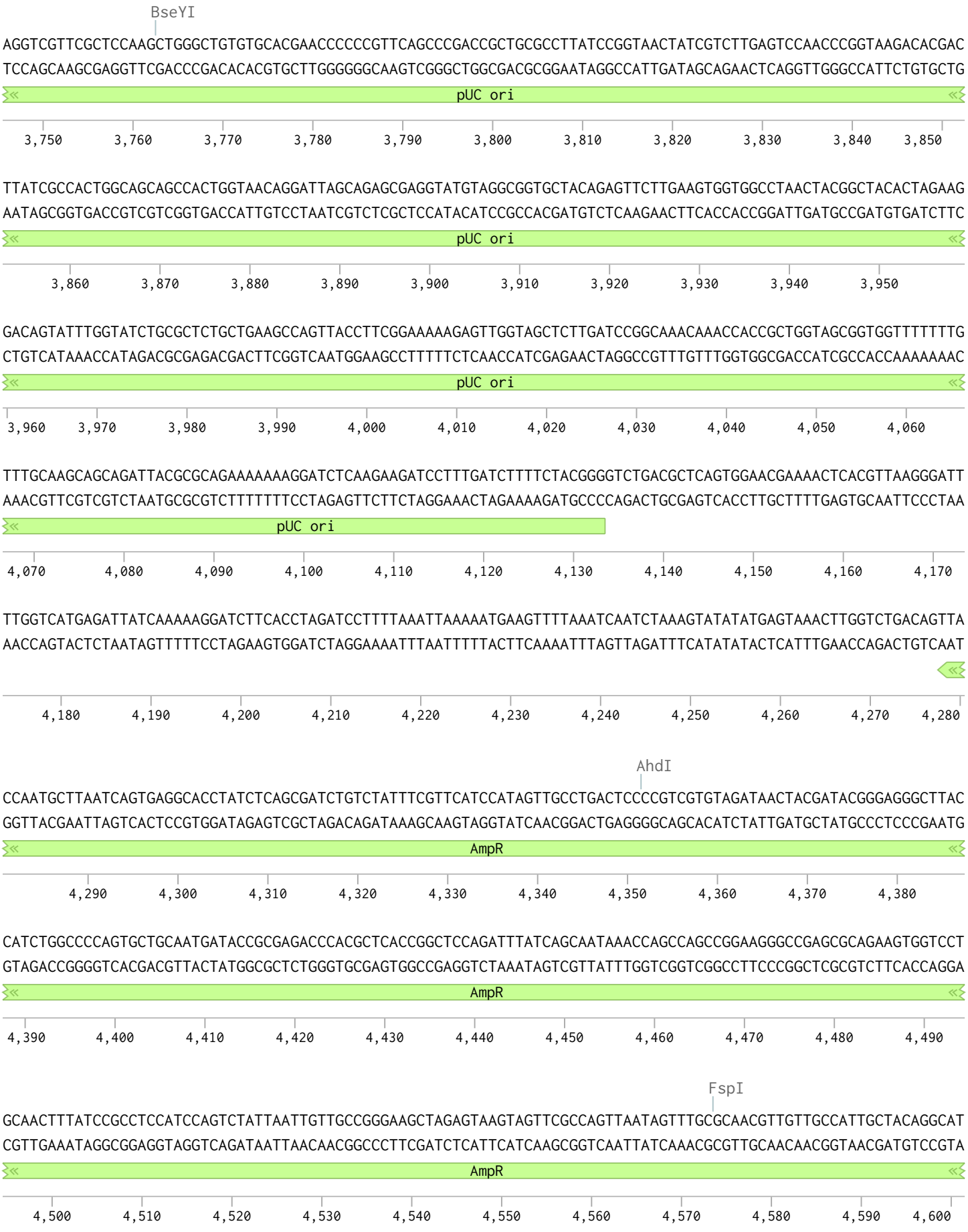
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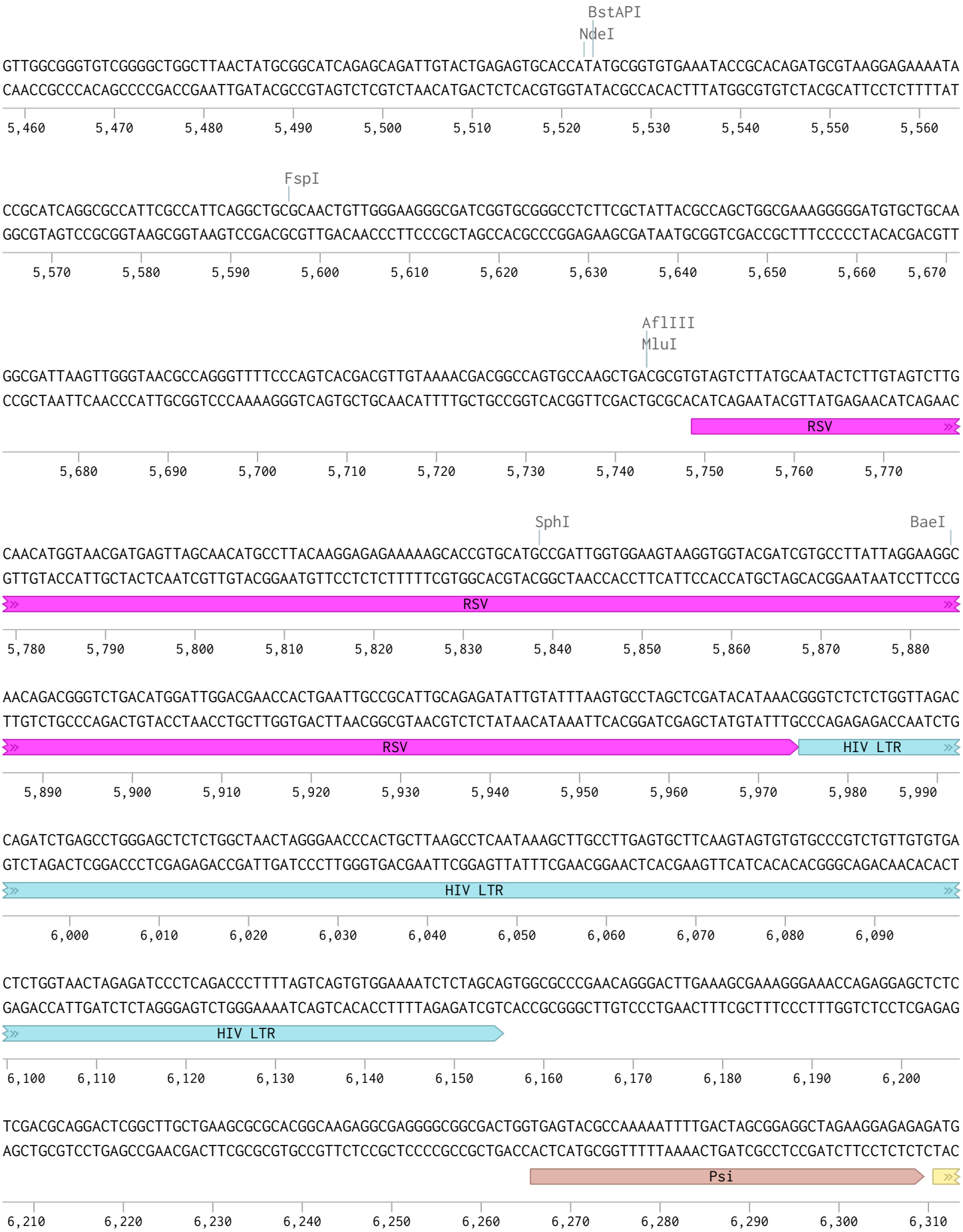
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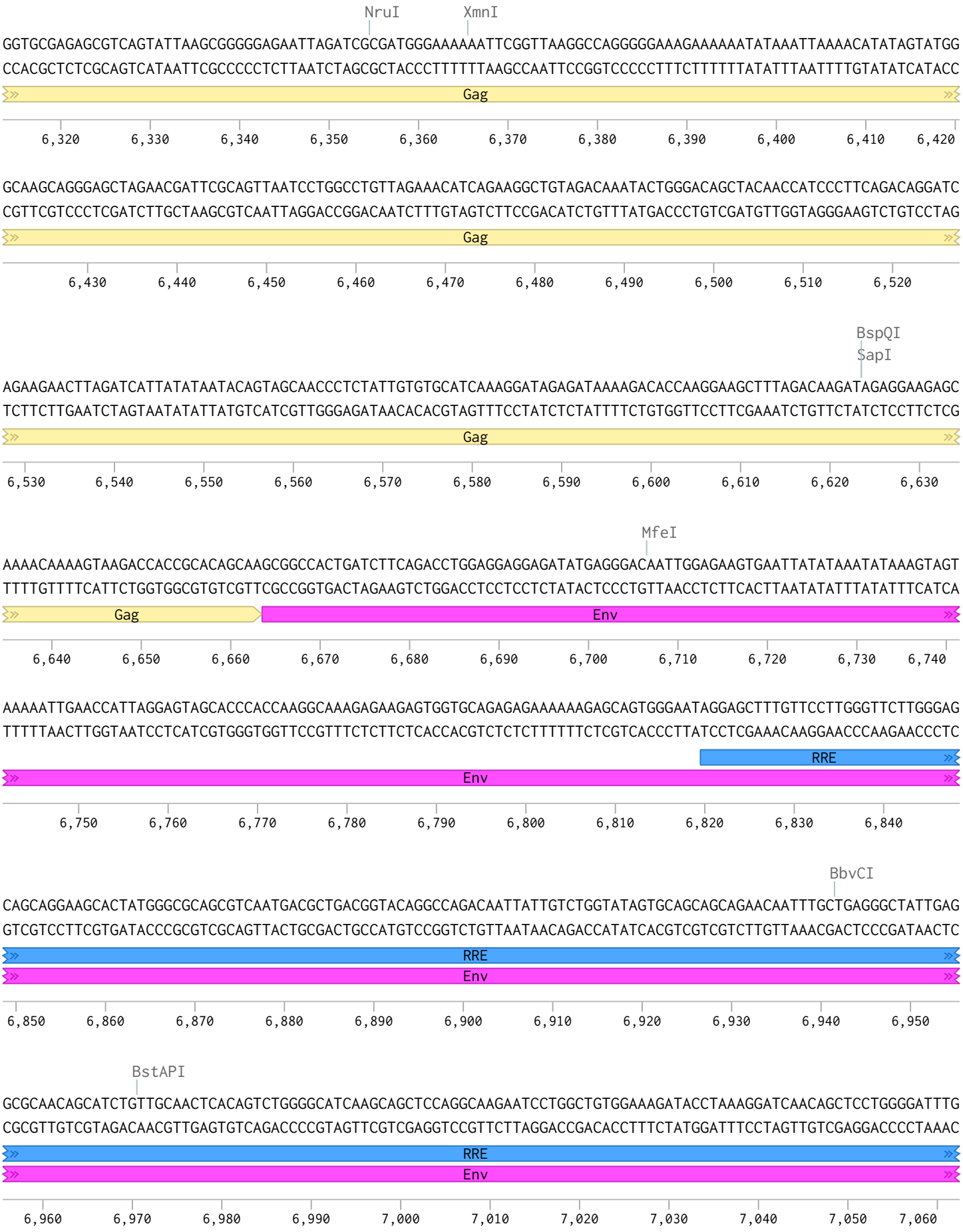
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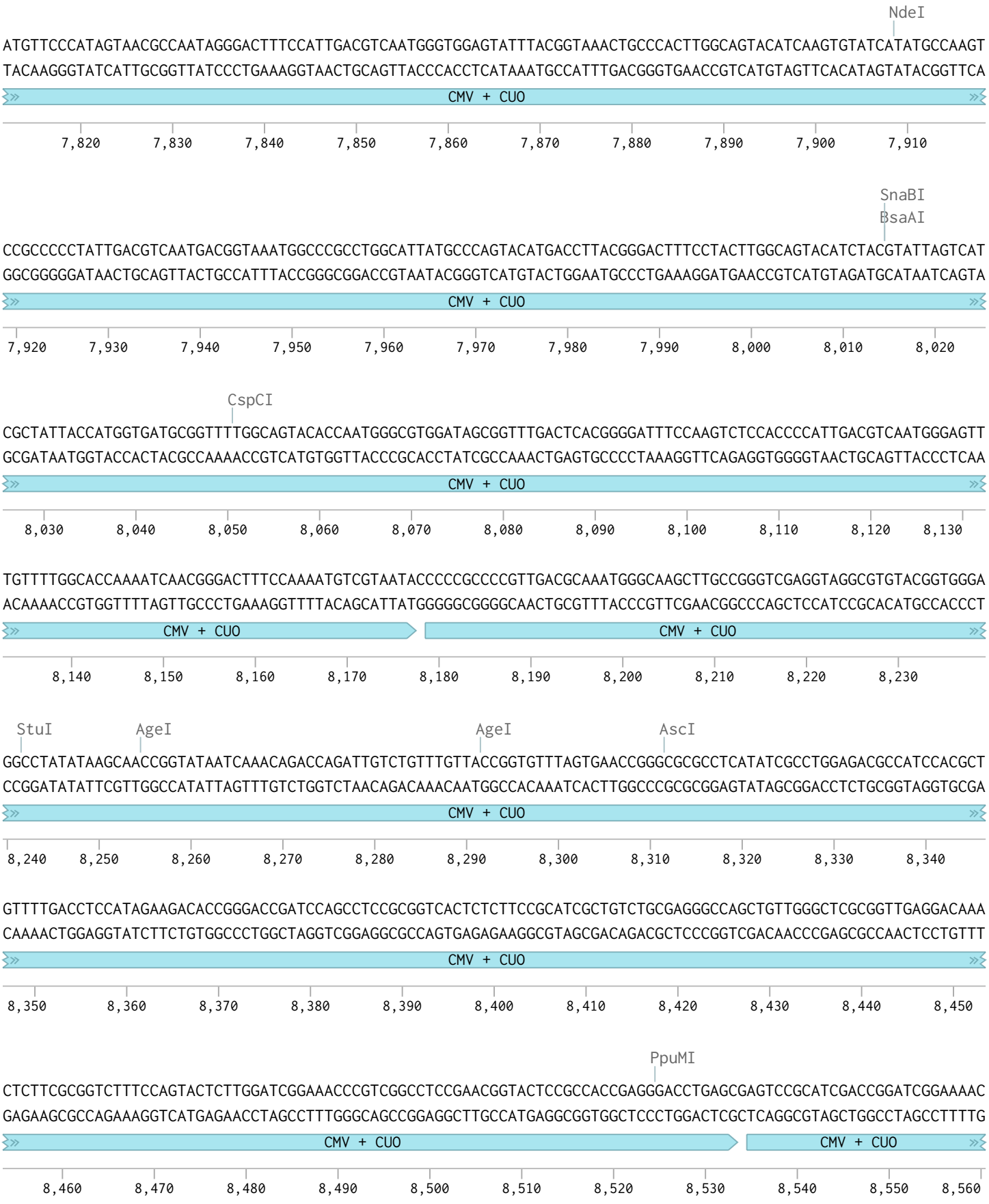
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CMV + CUO
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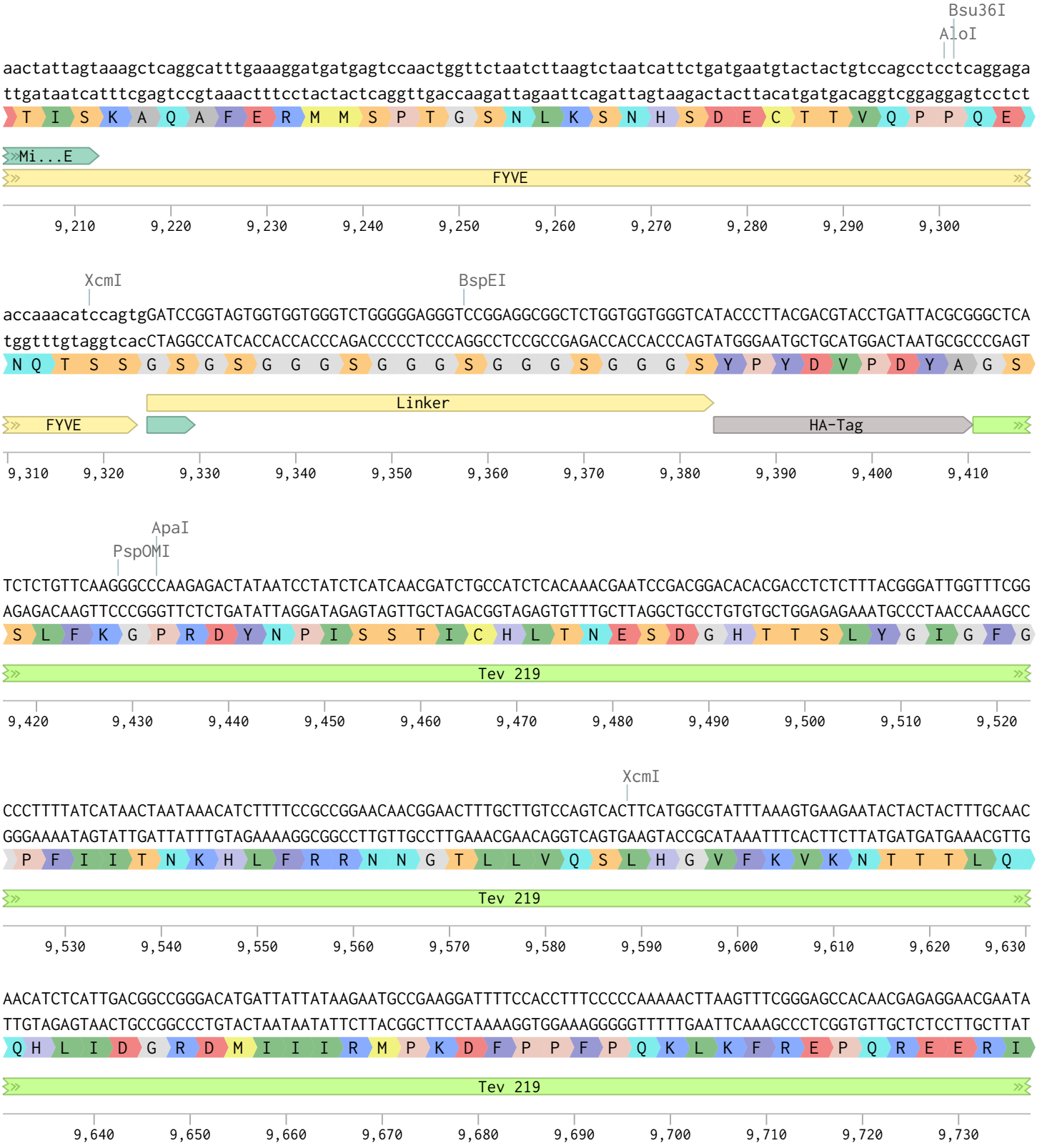
PaeR7I
XhoI
TliI
BlpI
CTCTCGAGAAAGGCGTCTAACCAGTCACAGTCGCAAGGTAGGCTGAGCACCGTGGCGGGCGGCAGCGGGTGGCGGTCGGGGTTGTTTCTGGCGGAGGTGCTGCTGAT
GAGAGCTCTTTCCGCAGATTGGTCAGTGTCAGCGTTCCATCCGACTCGTGGCACCGCCCGCCGTCGCCCACCGCCAGCCCCAACAAAGACCGCCTCCACGACGACTA
CMV + CUO
8,570
8,580
8,590
8,600
8,610
8,620
8,630
8,640
8,650
8,660
GATGTAATTAAAGTAGGCGGTCTTGAGACGGCGGATGGTCGAGGTGAGGTGTGGCAGGCTTGAGATCCAGCTGTTGGGGTGAGTACTCCCTCTCAAAAGCGGGCATT
CTACATTAATTTCATCCGCCAGAACTCTGCCGCCTACCAGCTCCACTCCACACCGTCCGAACTCTAGGTCGACAACCCCACTCATGAGGGAGAGTTTTCGCCCGTAA
CMV + CUO
CMV + CUO
8,670
8,680
8,690
8,700
8,710
8,720
8,730
8,740
8,750
8,760
8,770
MscI
AleI
ACTTCTGCGCTAAGATTGTCAGTTTCCAAAAACGAGGAGGATTTGATATTCACCTGGCCCGATCTGGCCATACACTTGAGTGACAATGACATCCACTTTGCCTTTCT
TGAAGACGCGATTCTAACAGTCAAAGGTTTTTGCTCCTCCTAAACTATAAGTGGACCGGGCTAGACCGGTATGTGAACTCACTGTTACTGTAGGTGAAACGGAAAGA
CMV + CUO
8,780
8,790
8,800
8,810
8,820
8,830
8,840
8,850
8,860
8,870
8,880
BmtI
NheI
BarI
CTCCACAGGTGTCCACTCCCAGGTCCAAGTTTGGTCTAGAGCTAGCGCCACCATGggtggatctagtttcgtaactgcaaatgaagattctgtacctgaaaacactt
GAGGTGTCCACAGGTGAGGGTCCAGGTTCAAACCAGATCTCGATCGCGGTGGTACcccacctagatcaaagcattgacgtttacttctaagacatggacttttgtgaa
M G G S S F V T A N E D S V P E N T
CMV + CUO
FYVE
8,890
8,900
8,910
8,920
8,930
8,940
8,950
8,960
8,970
8,980
gcaaagaaggcttggttttgggccagaaacagcctacttgggttcctgattcagaagctccaaactgtatgaactgccaagtcaaatttacttttaccaaacggcga
cgtttcttccgaaccaaaacccggtctttgtcggatgaacccaaggactaagtcttcgaggtttgacatacttgacggttcagtttaaatgaaaatggtttgccgct
C K E G L V L G Q K Q P T W V P D S E A P N C M N C Q V K F T F T K R R
Minimal FYVE
FYVE
8,990
9,000
9,010
9,020
9,030
9,040
9,050
9,060
9,070
9,080
9,090
caccattgccgagcatgtgggaaagtattttgtggtgtctgttgtaataggaagtgtaaactgcaatatctagaaaaggaagcaagagtatgtgtagtctgctatga
gtggtaacggctcgtacaccctttcataaaacaccacagacaacattatccttcacatttgacgttatagatcttttccttcgttctcatacacatcagacgatact
H H C R A C G K V F C G V C C N R K C K L Q Y L E K E A R V C V V C Y E
Minimal FYVE
FYVE
9,100
9,110
9,120
9,130
9,140
9,150
9,160
9,170
9,180
9,190
9,200


Bsu36I
AloI
aactattagtaaagctcaggcatttgaaaggatgatgagtccaactggttctaatcttaagtctaatcattctgatgaatgtactactgtccagcctcctcaggaga
ttgataatcatttcgagtccgtaaactttcctactactcaggttgaccaagattagaattcagattagtaagactacttacatgatgacaggtcggaggagtcctct
T I S K A Q A F E R M M S P T G S N L K S N H S D E C T T V Q P P Q E
Mi...E
FYVE
9,210 9,220 9,230 9,240 9,250 9,260 9,270 9,280 9,290 9,300
XcmI
BspEI
accaaacatccagtgGATCCGGTAGTGGTGGTGGGTCTGGGGGAGGGTCCGGAGGCGGCTCTGGTGGTGGGTCATACCCTTACGACGTACCTGATTACGCGGGCTCA
tggtttgtaggtcacCTAGGCCATCACCACCACCCAGACCCCCTCCCAGGCCTCCGCCGAGACCACCACCCAGTATGGGAATGCTGCATGGACTAATGCGCCCGAGT
N Q T S S G S G S G G G S G G G S G G G S G G G S Y P Y D V P D Y A G S
Linker
FYVE
HA-Tag
9,310 9,320 9,330 9,340 9,350 9,360 9,370 9,380 9,390 9,400 9,410
ApaI
PspOMI
TCTCTGTTCAAGGGCCCAAGAGACTATAATCCTATCTCATCAACGATCTGCCATCTCACAAACGAATCCGACGGACACACGACCTCTCTTTACGGGATTGGTTTCGG
AGAGACAAGTTCCCGGGTTCTCTGATATTAGGATAGAGTAGTTGCTAGACGGTAGAGTGTTTGCTTAGGCTGCCTGTGTGCTGGAGAGAAATGCCCTAACCAAAGCC
S L F K G P R D Y N P I S S T I C H L T N E S D G H T T S L Y G I G F G
Tev 219
9,420 9,430 9,440 9,450 9,460 9,470 9,480 9,490 9,500 9,510 9,520
XcmI
CCCTTTTATCATAACTAATAAACATCTTTTCCGCCGGAACAACGGAACTTTGCTTGTCCAGTCACTTCATGGCGTATTTAAAGTGAAGAATACTACTACTTTGCAAC
GGGAAAATAGTATTGATTATTTGTAGAAAAGGCGGCCTTGTTGCCTTGAAACGAACAGGTCAGTGAAGTACCGCATAAATTTCACTTCTTATGATGATGAAACGTTG
P F I I T N K H L F R R N N G T L L V Q S L H G V F K V K N T T T L Q
Tev 219
9,530 9,540 9,550 9,560 9,570 9,580 9,590 9,600 9,610 9,620 9,630
AACATCTCATTGACGGCCGGGACATGATTATTATAAGAATGCCGAAGGATTTTCCACCTTTCCCCCAAAAACTTAAGTTTCGGGAGCCACAACGAGAGGAACGAATA
TTGTAGAGTAACTGCCGGCCCTGTACTAATAATATTCTTACGGCTTCCTAAAAGGTGGAAAGGGGGTTTTTGAATTCAAAGCCCTCGGTGTTGCTCTCCTTGCTTAT
Q H L I D G R D M I I I R M P K D F P P F P Q K L K F R E P Q R E E R I
Tev 219
9,640 9,650 9,660 9,670 9,680 9,690 9,700 9,710 9,720 9,730


BsrGI
TGCCTGGTAACAACAAATTTTCAAACTAAATCTATGTCTTCAATGGTGAGTGACACATCTTGTACATTTCCCTCTAGTGACGGGATTTTCTGGAAGCATTGGATACA
ACGGACCATTGTTGTTTAAAAGTTTGATTTAGATACAGAAGTTACCACTCACTGTGTAGAACATGTAAAGGGAGATCACTGCCCTAAAAGACCTTCGTAACCTATGT
C L V T T N F Q T K S M S S M V S D T S C T F P S S D G I F W K H W I Q
Tev 219
9,740 9,750 9,760 9,770 9,780 9,790 9,800 9,810 9,820 9,830 9,840
GACTAAGGACGGGCAGTGCGGATCACCACTCGTGTCCACACGCGATGGATTCATTGTCGGGATTCATAGTGCTTCTAACTTCACCAACACCAATAATTATTTTACTT
CTGATTCCTGCCCGTCACGCCTAGTGGTGAGCACAGGTGTGCGCTACCTAAGTAACAGCCCTAAGTATCACGAAGATTGAAGTGGTTGTGGTTATTAATAAAATGAA
T K D G Q C G S P L V S T R D G F I V G I H S A S N F T N T N N Y F T
Tev 219
9,850 9,860 9,870 9,880 9,890 9,900 9,910 9,920 9,930 9,940 9,950
Tth111I
AloI
PflFI
CTGTGCCAAAAAACTTCATGGAGCTGCTTACTAGCCAAGAAGCCCAACAGTGGGTGAGTGGGTGGCGCCTGAACGCCGACTCCGTCTTGTGGGGGGGTCACAAGGTC
GACACGGTTTTTTGAAGTACCTCGACGAATGATCGGTTCTTCGGGTTGTCACCCACTCACCCACCGCGGACTTGCGGCTGAGGCAGAACACCCCCCCAGTGTTCCAG
S V P K N F M E L L T S Q E A Q Q W V S G W R L N A D S V L W G G H K V
Tev 219
9,960 9,970 9,980 9,990 10,000 10,010 10,020 10,030 10,040 10,050
TTTATGGTATAA
AAATACCATATT
F M V *
Tev 219
10,060 10,070