

SUPPLEMENTARY MATERIALS

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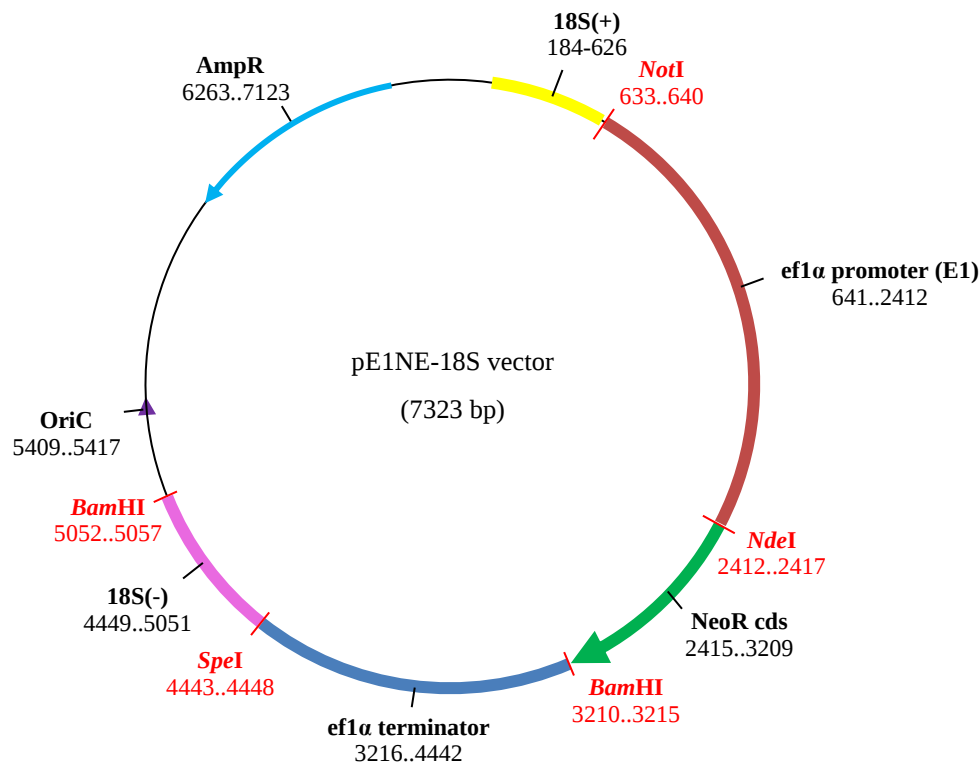
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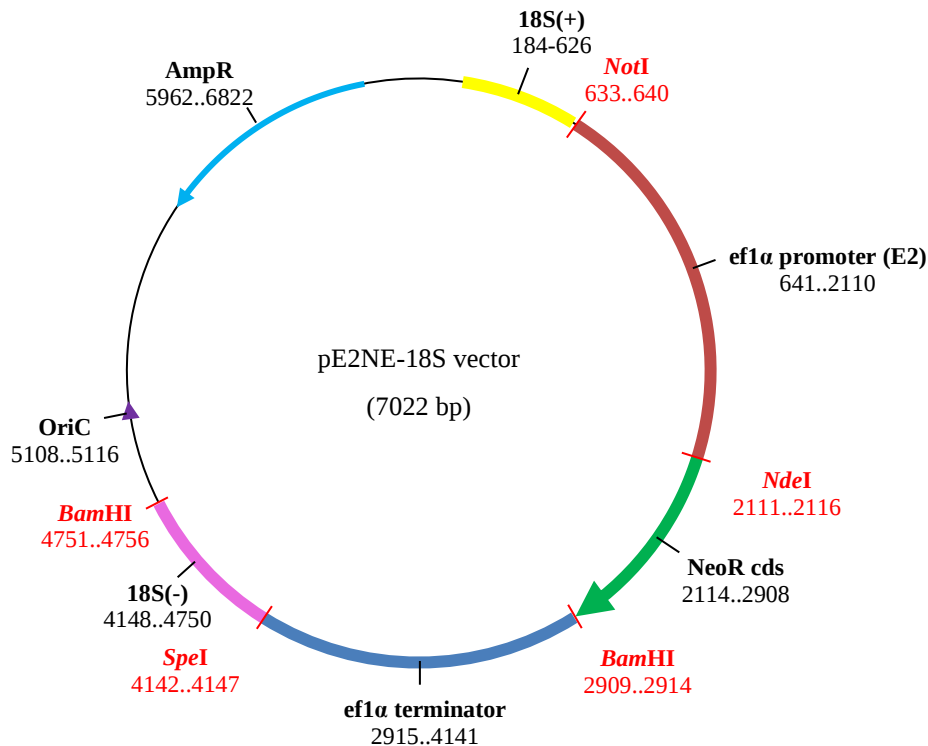
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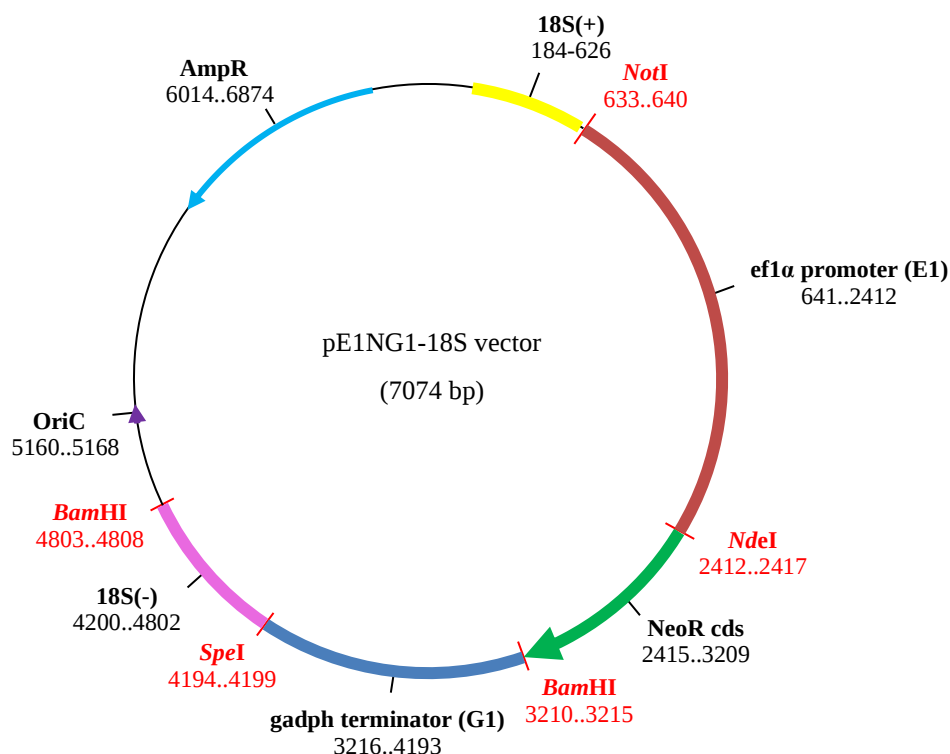
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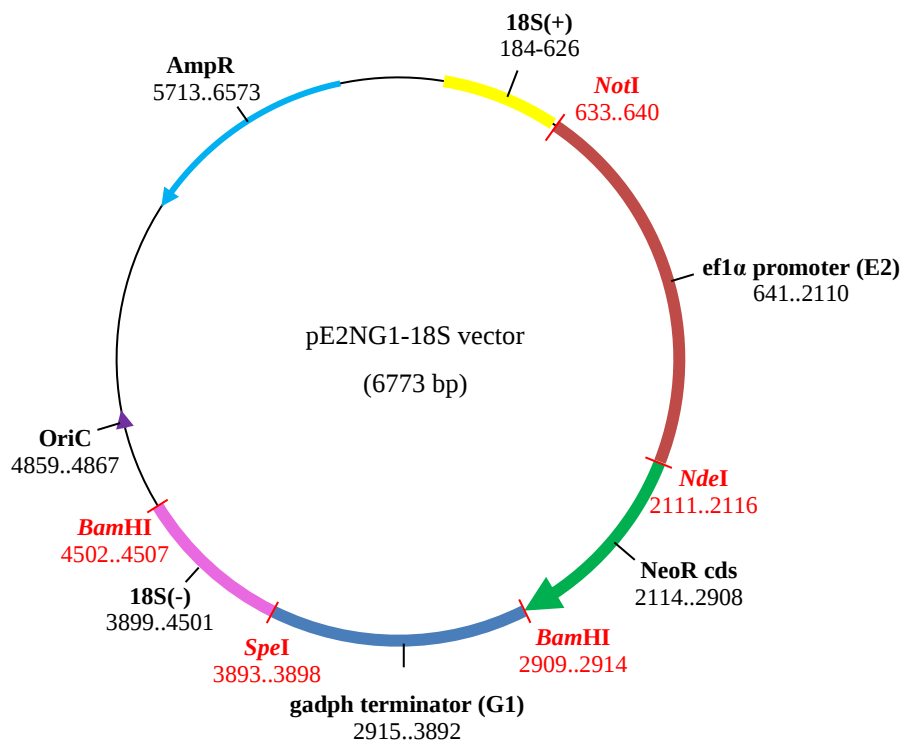
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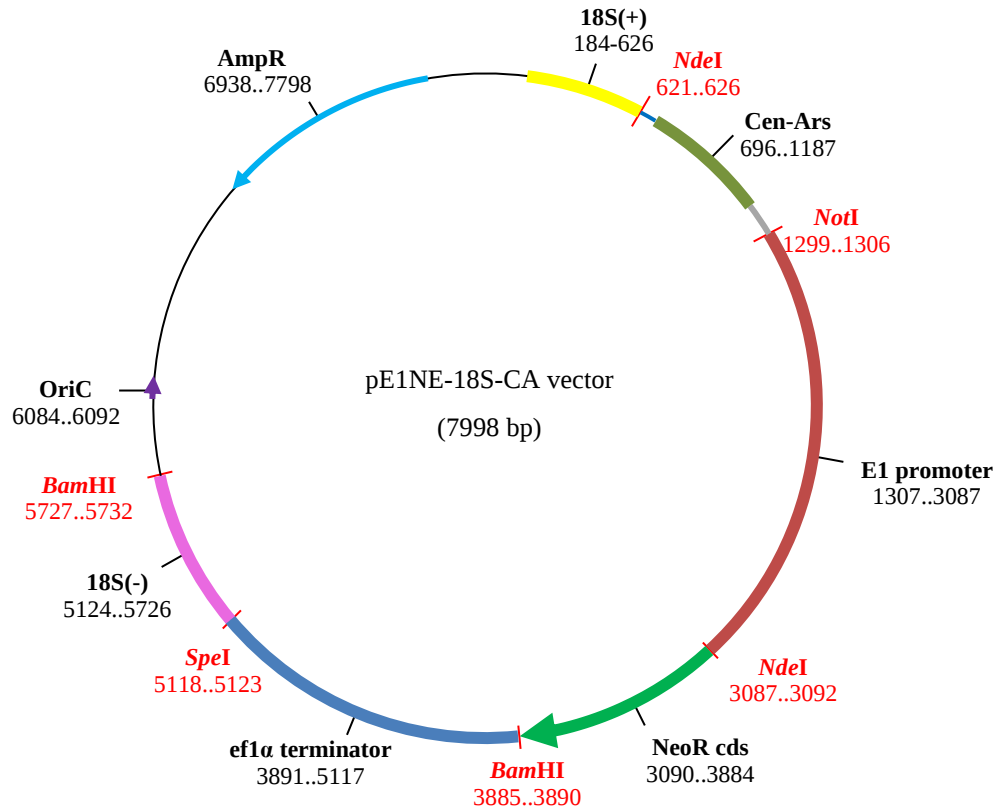
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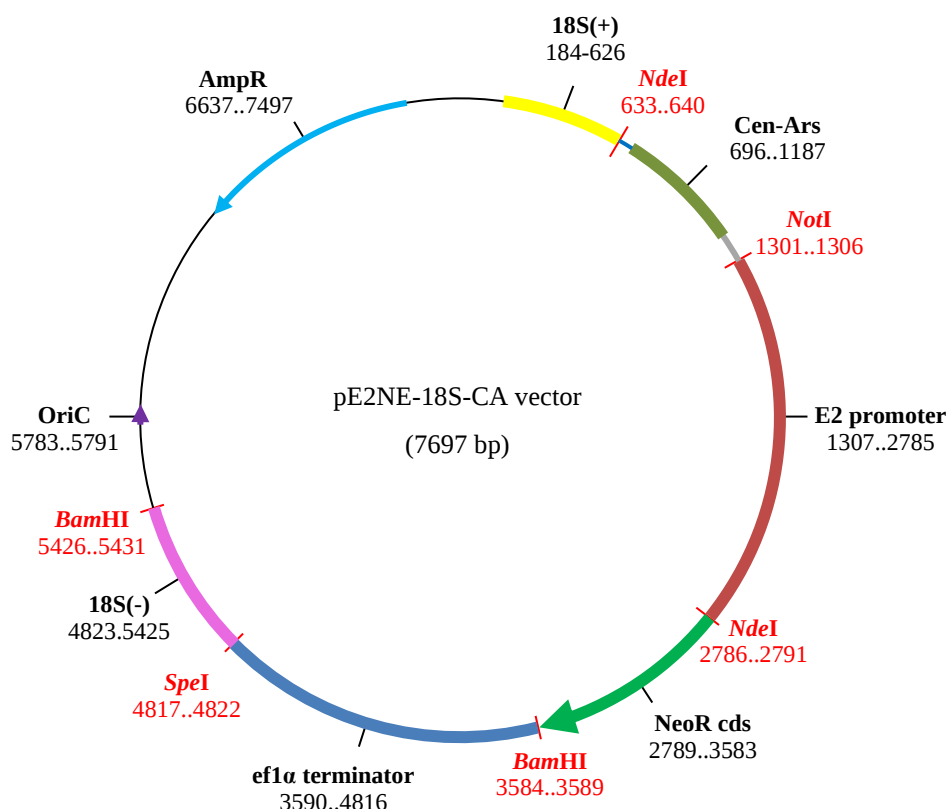
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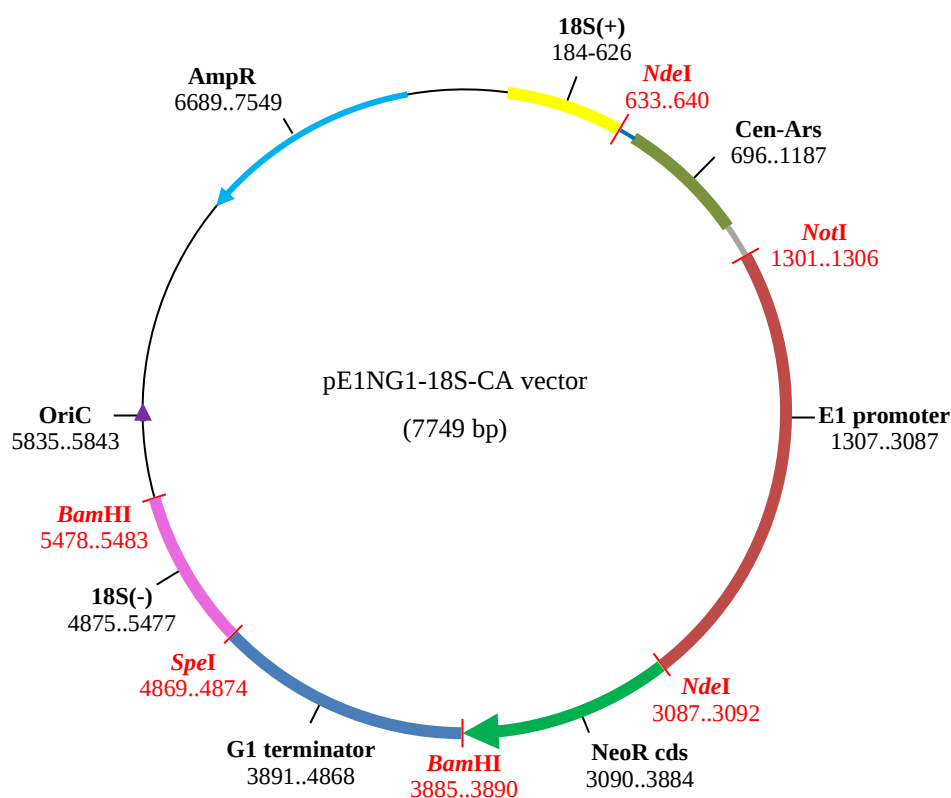
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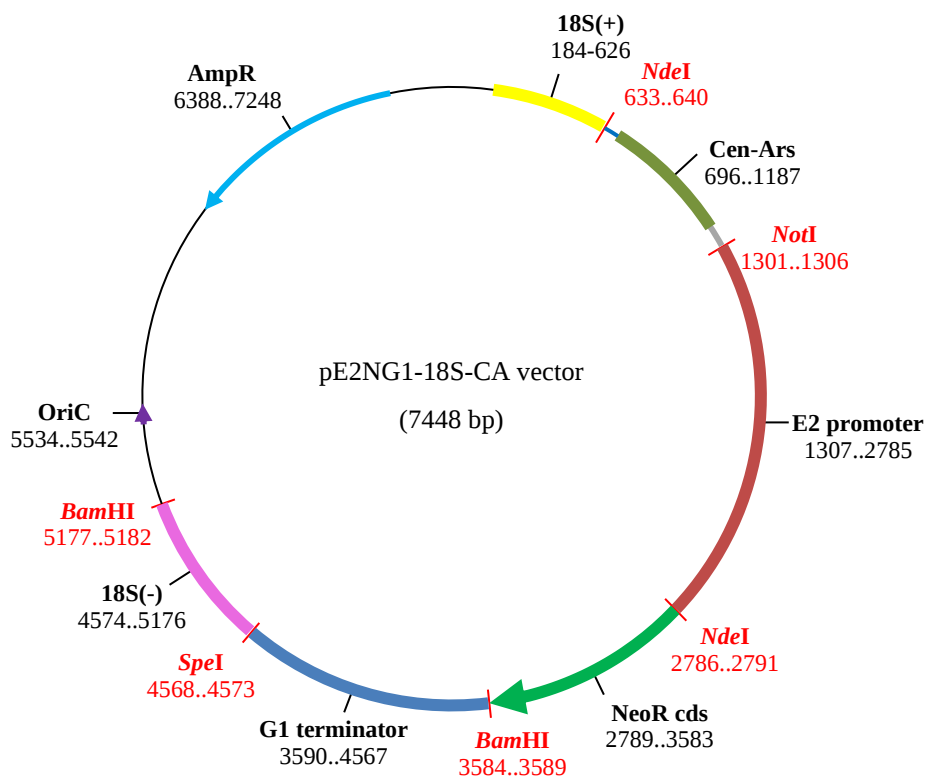
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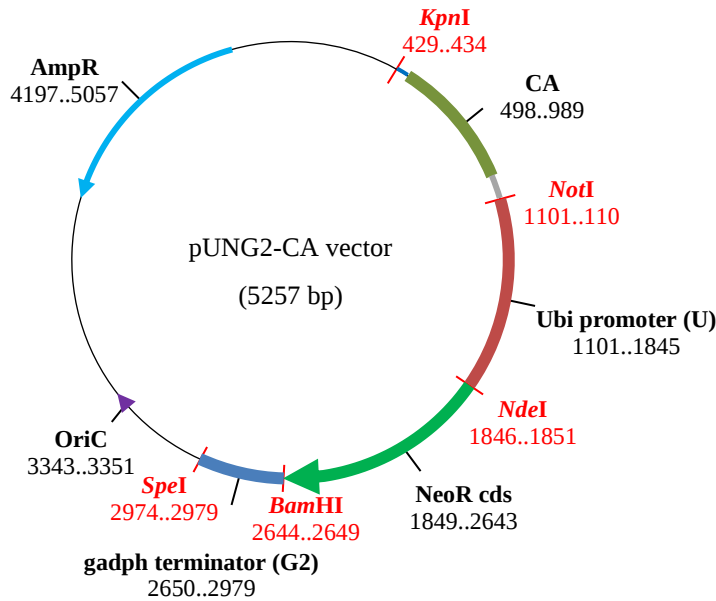
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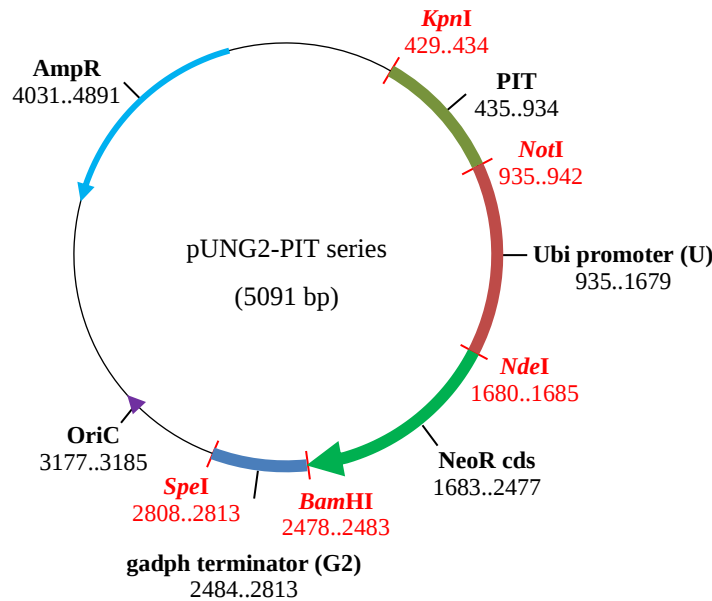
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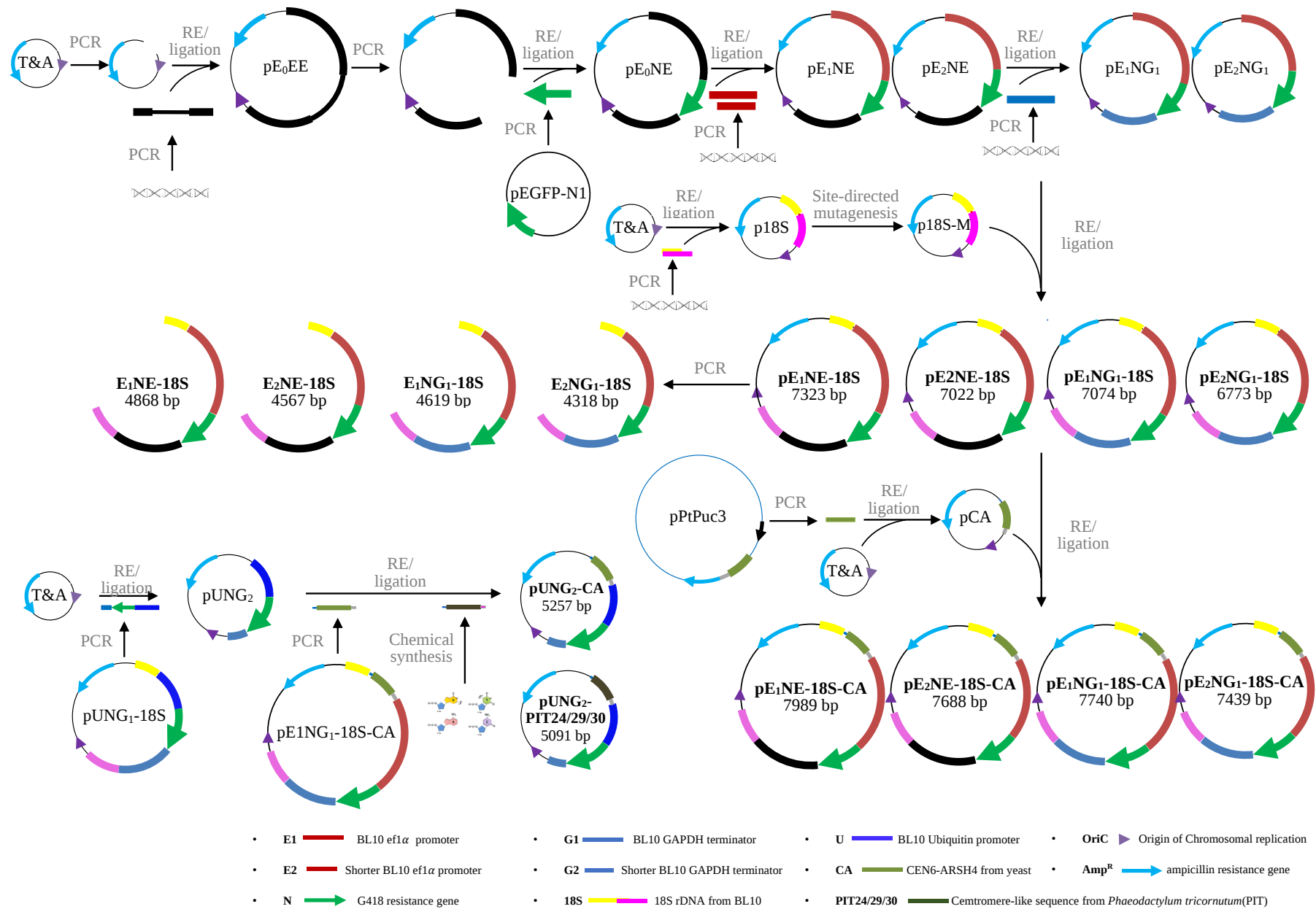
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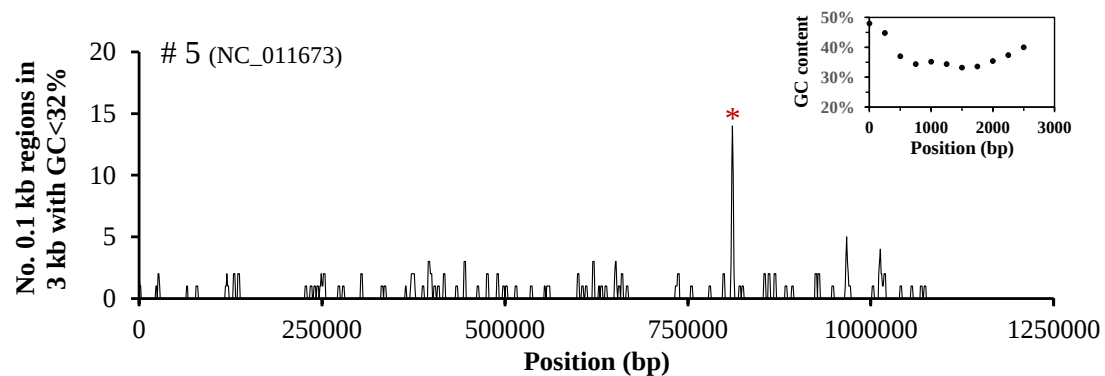
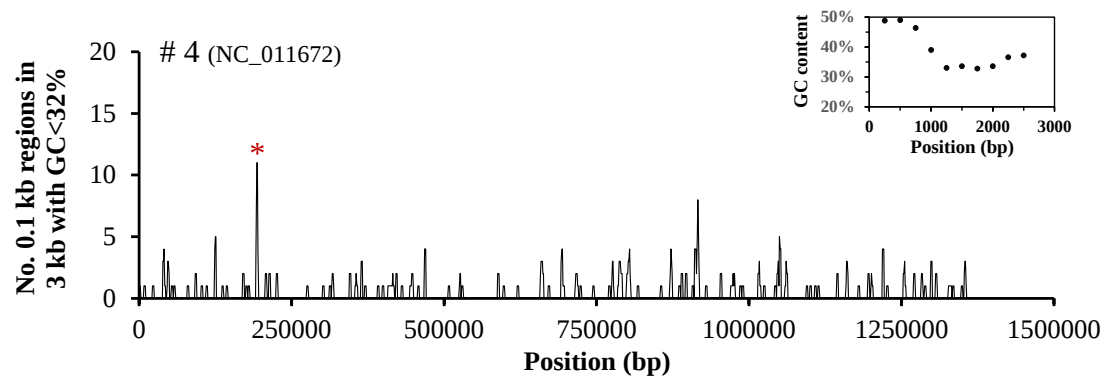
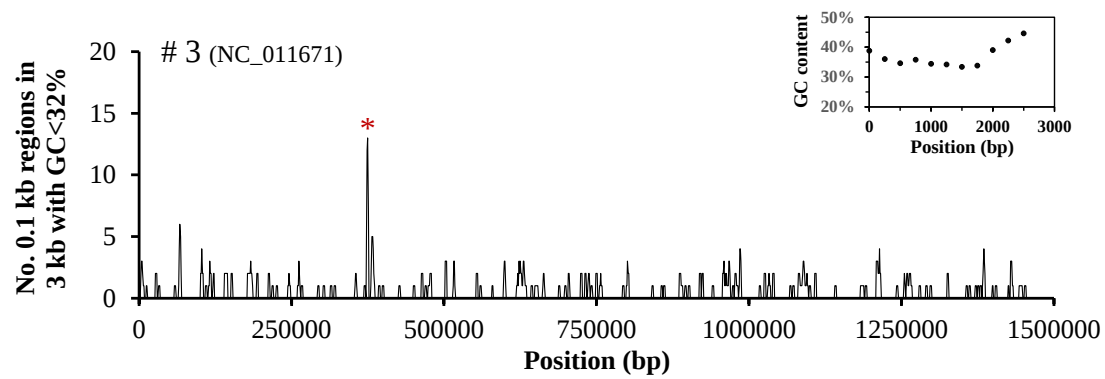
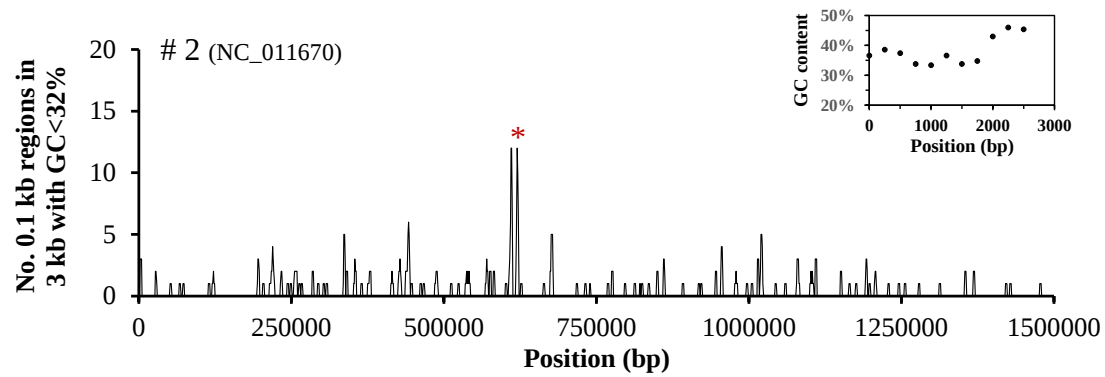
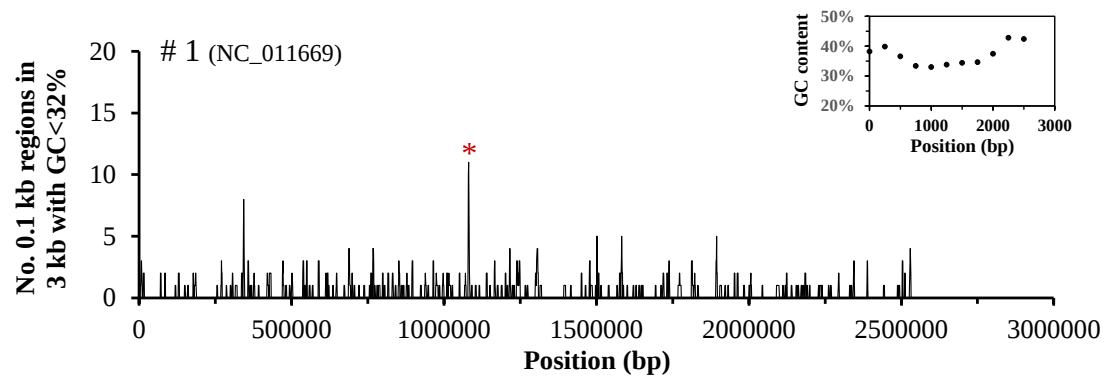
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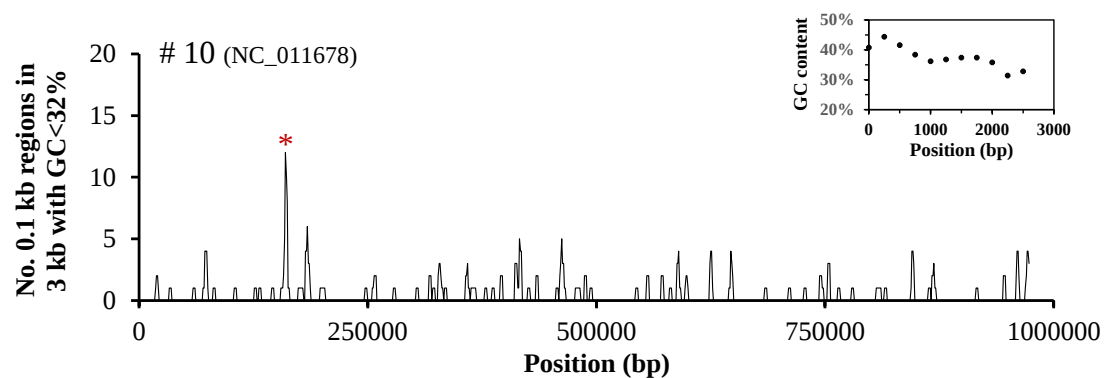
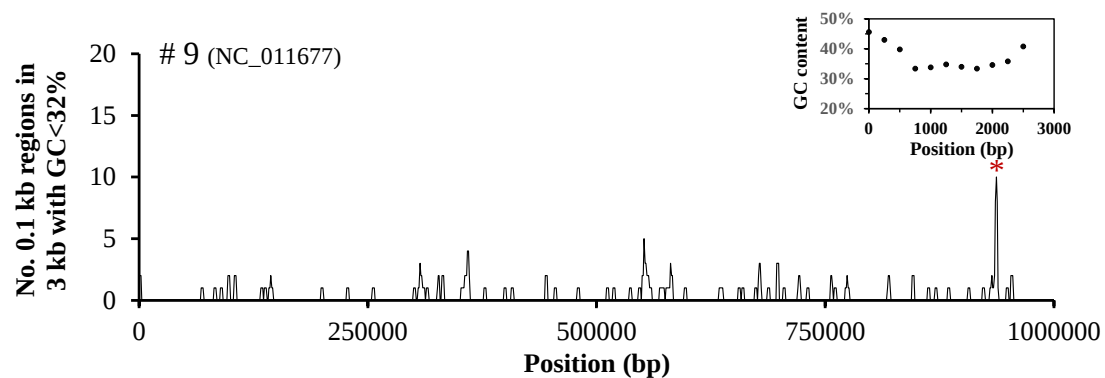
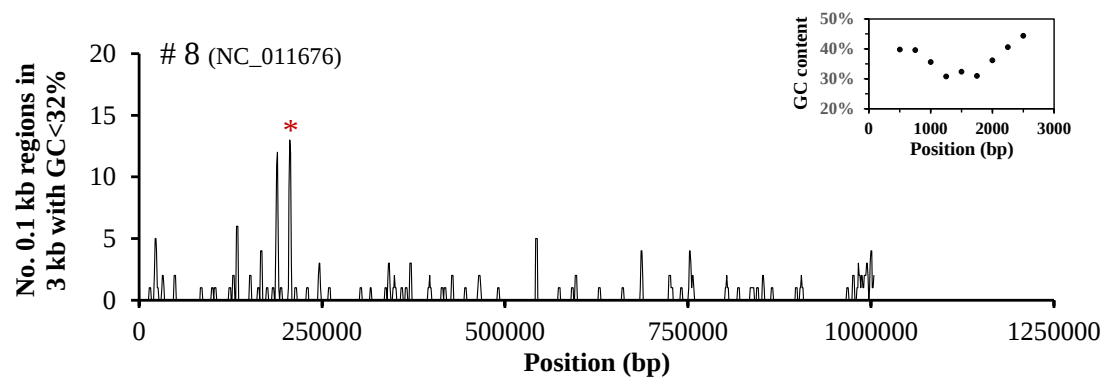
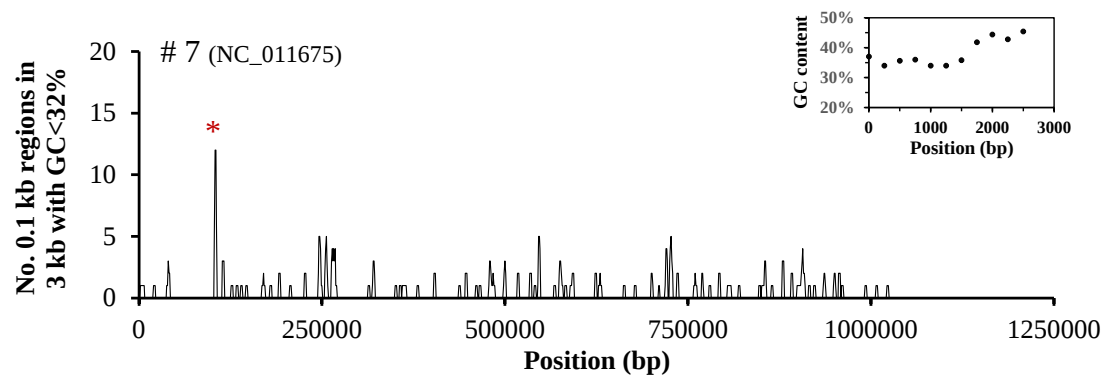
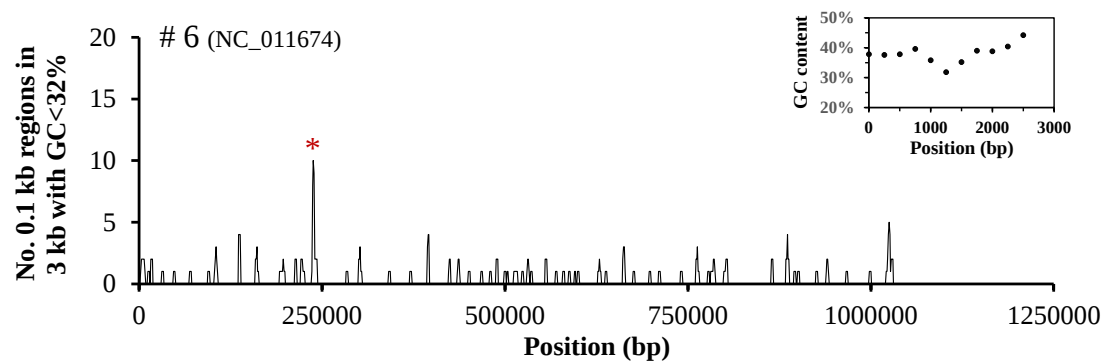
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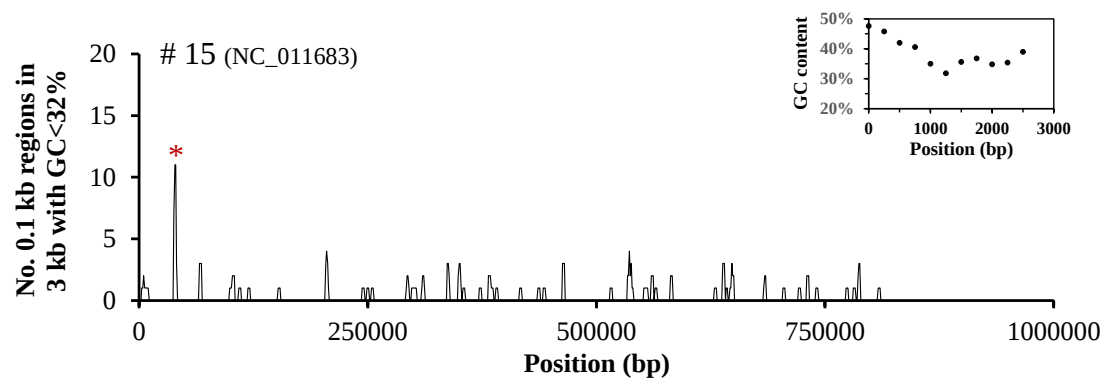
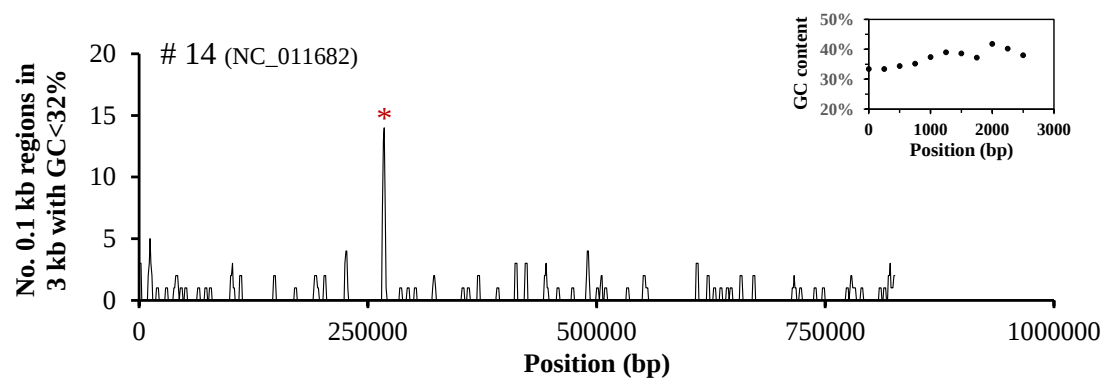
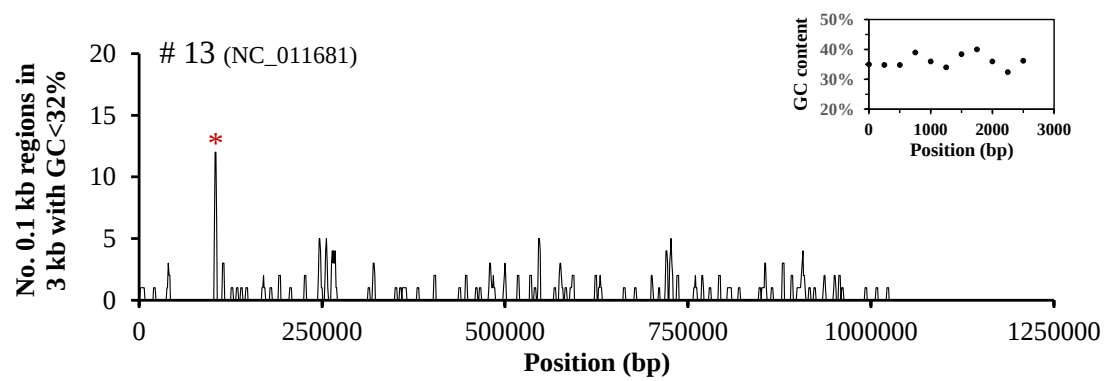
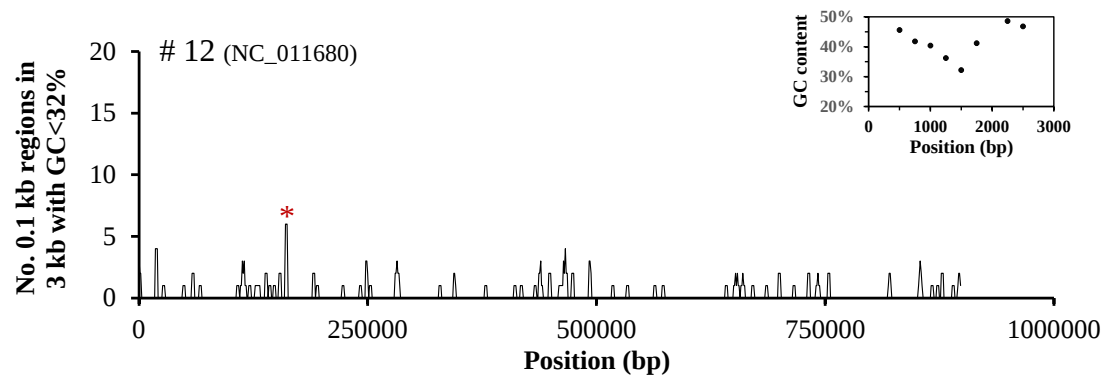
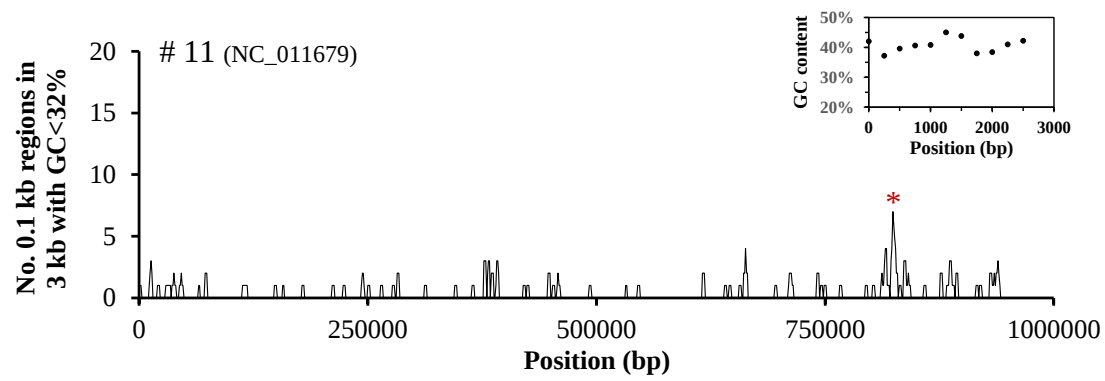
Supplemental Fig. S1 Maps and sequences of circular vectors utilized in this study.

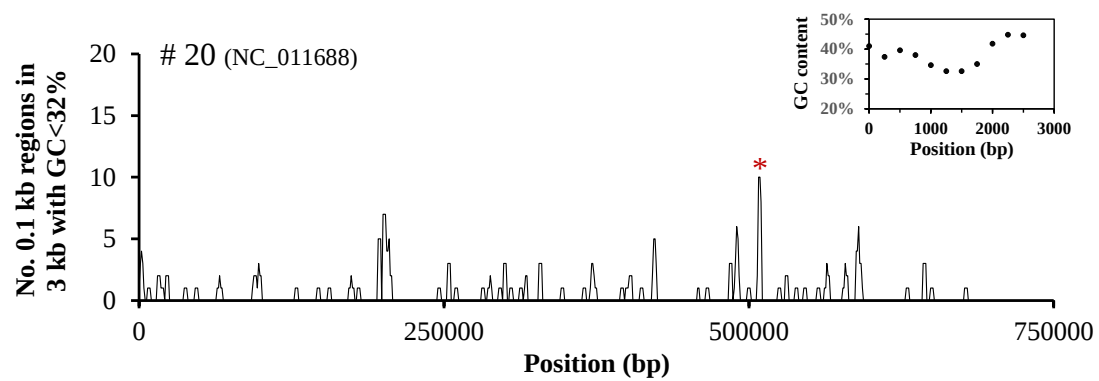
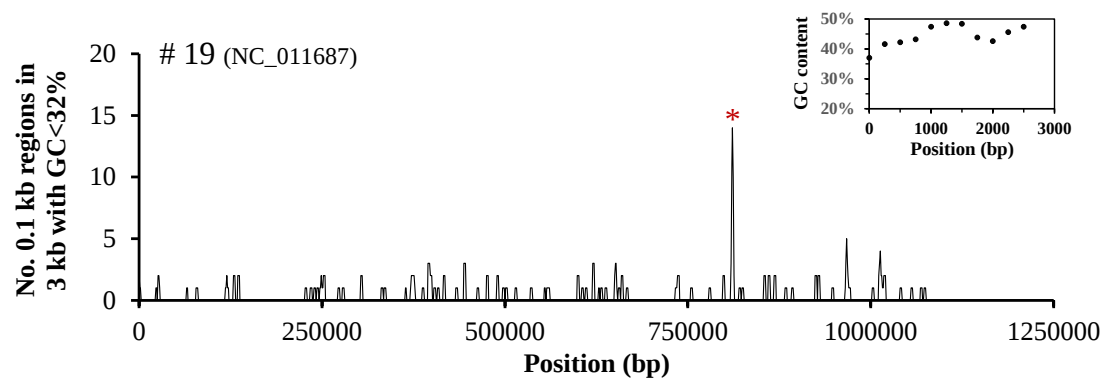
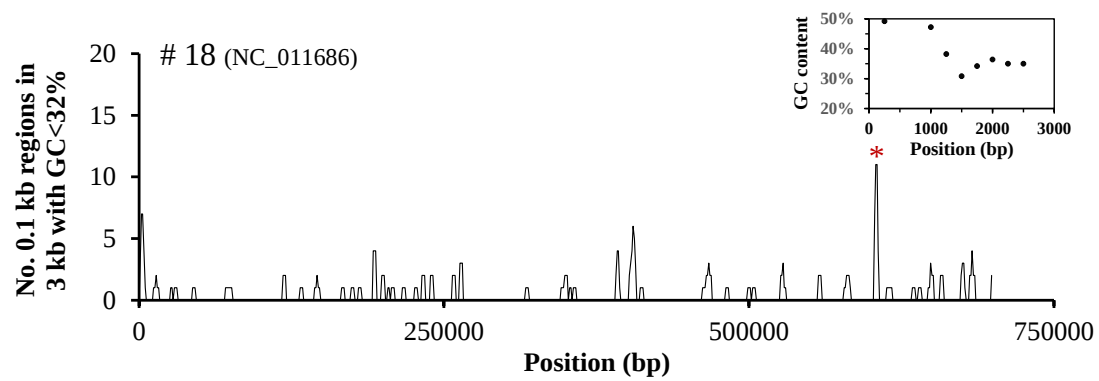
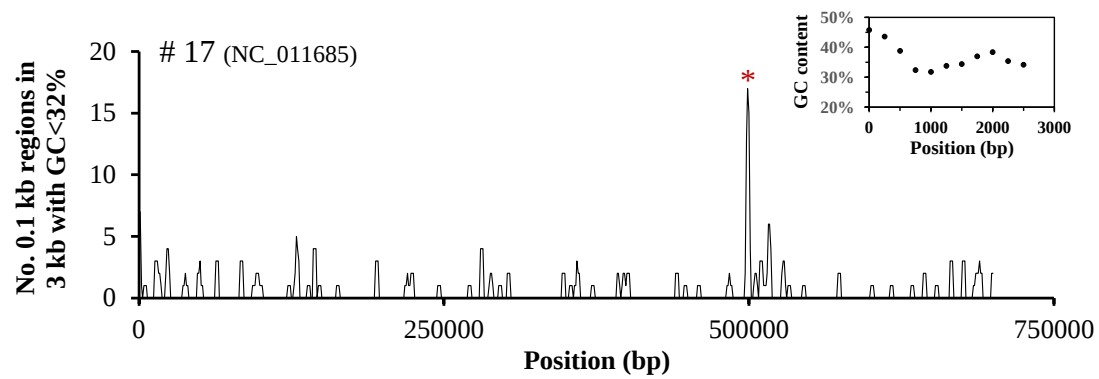
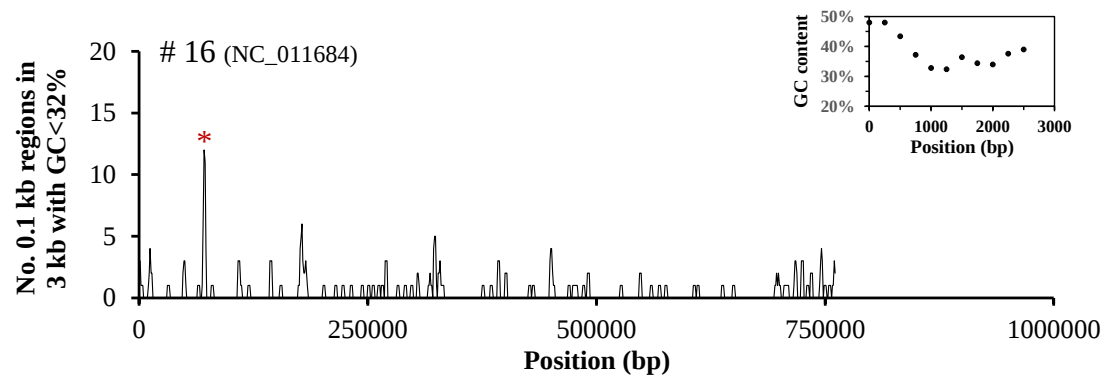


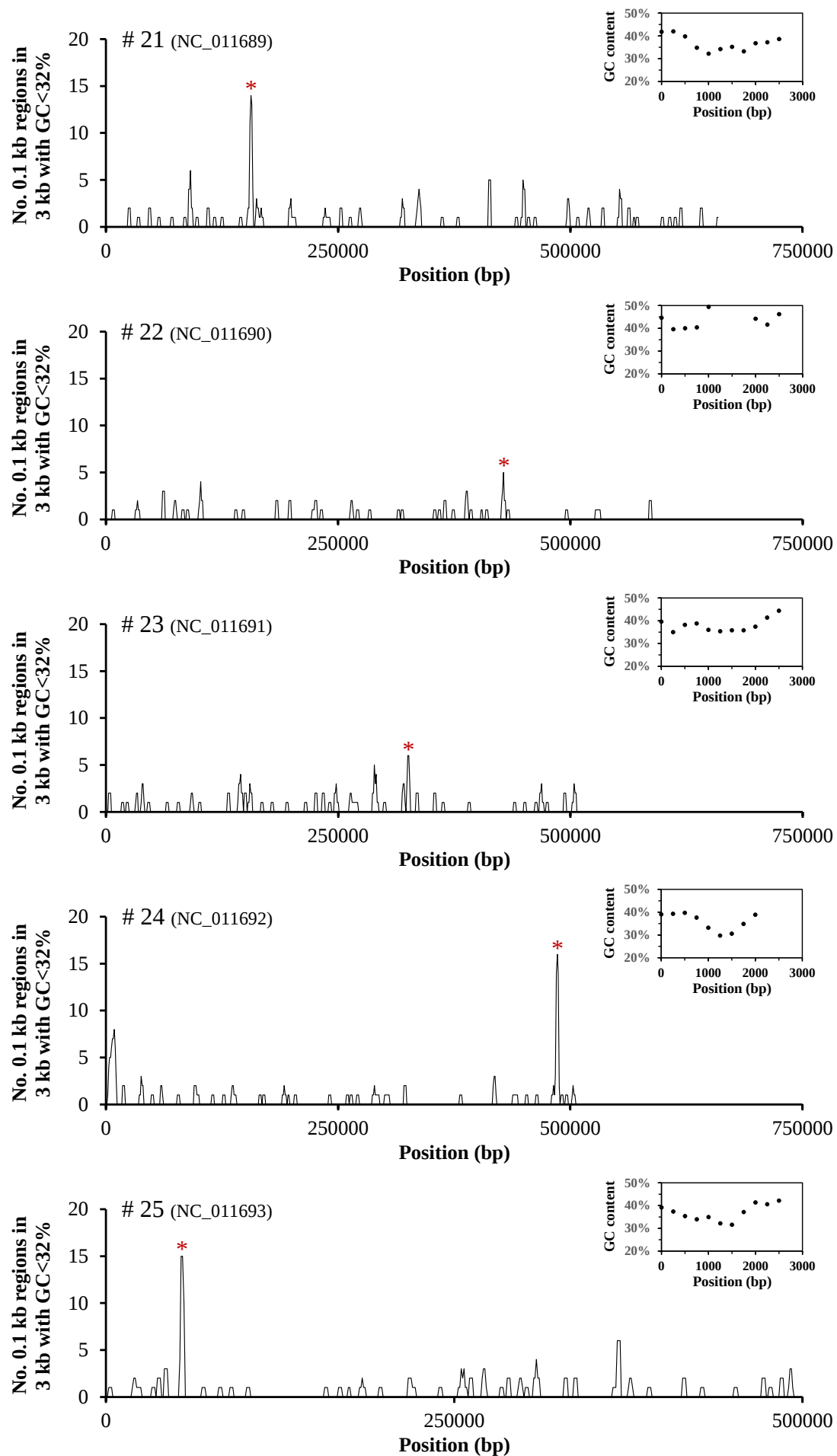
Supplemental Fig. S2 Flow chart illustrating plasmid construction process. See Materials and Methods for details.

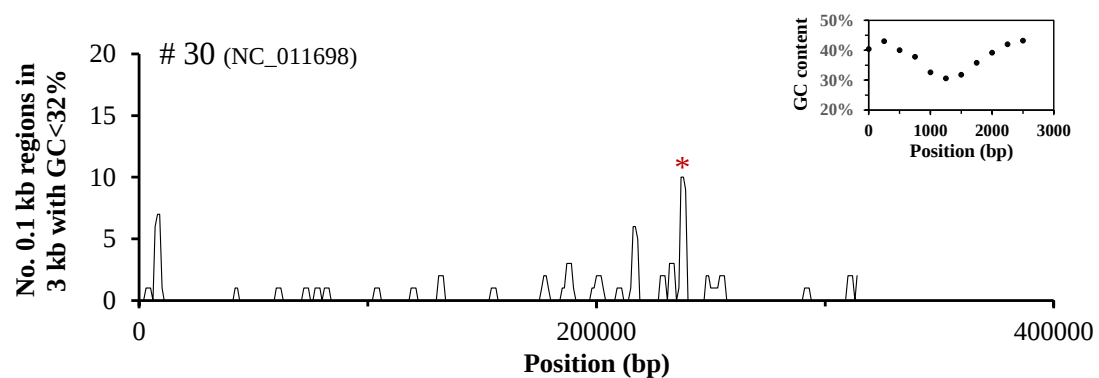
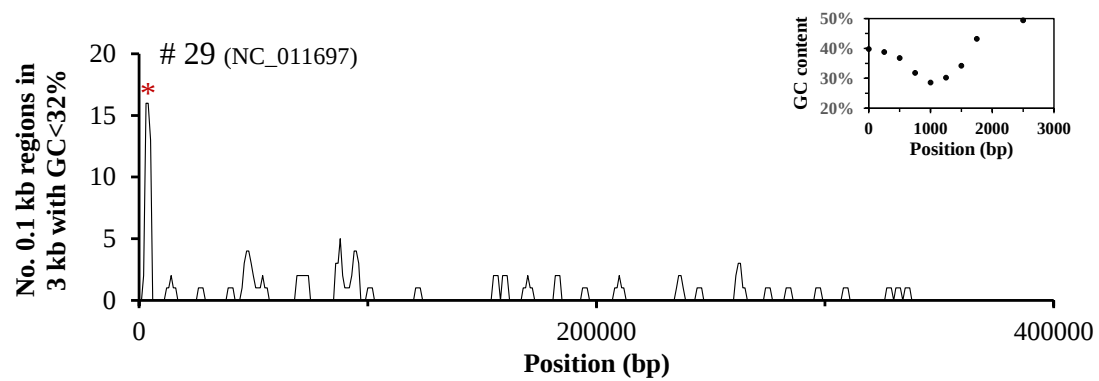
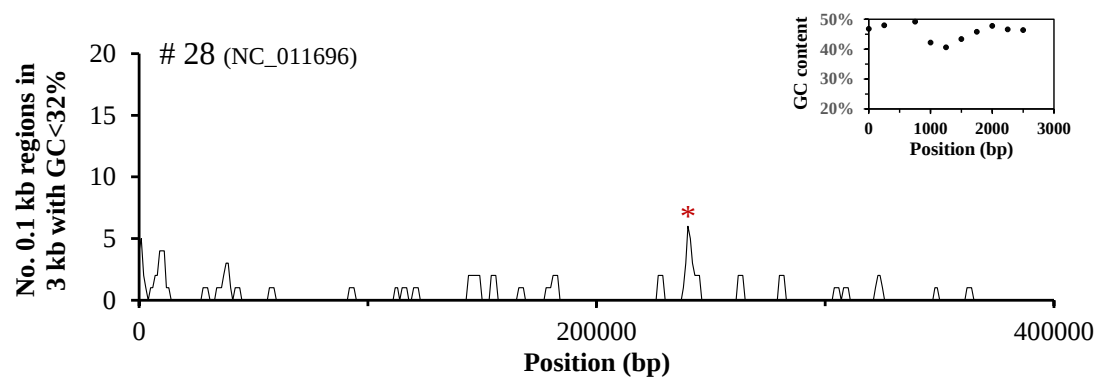
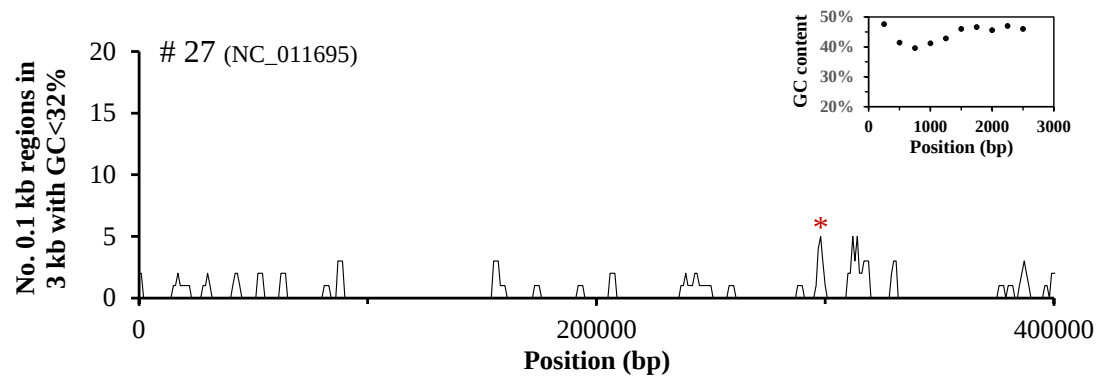
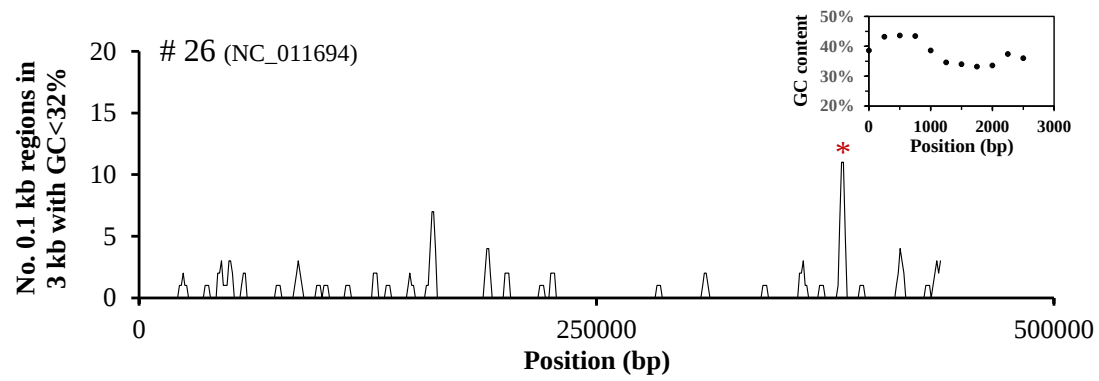


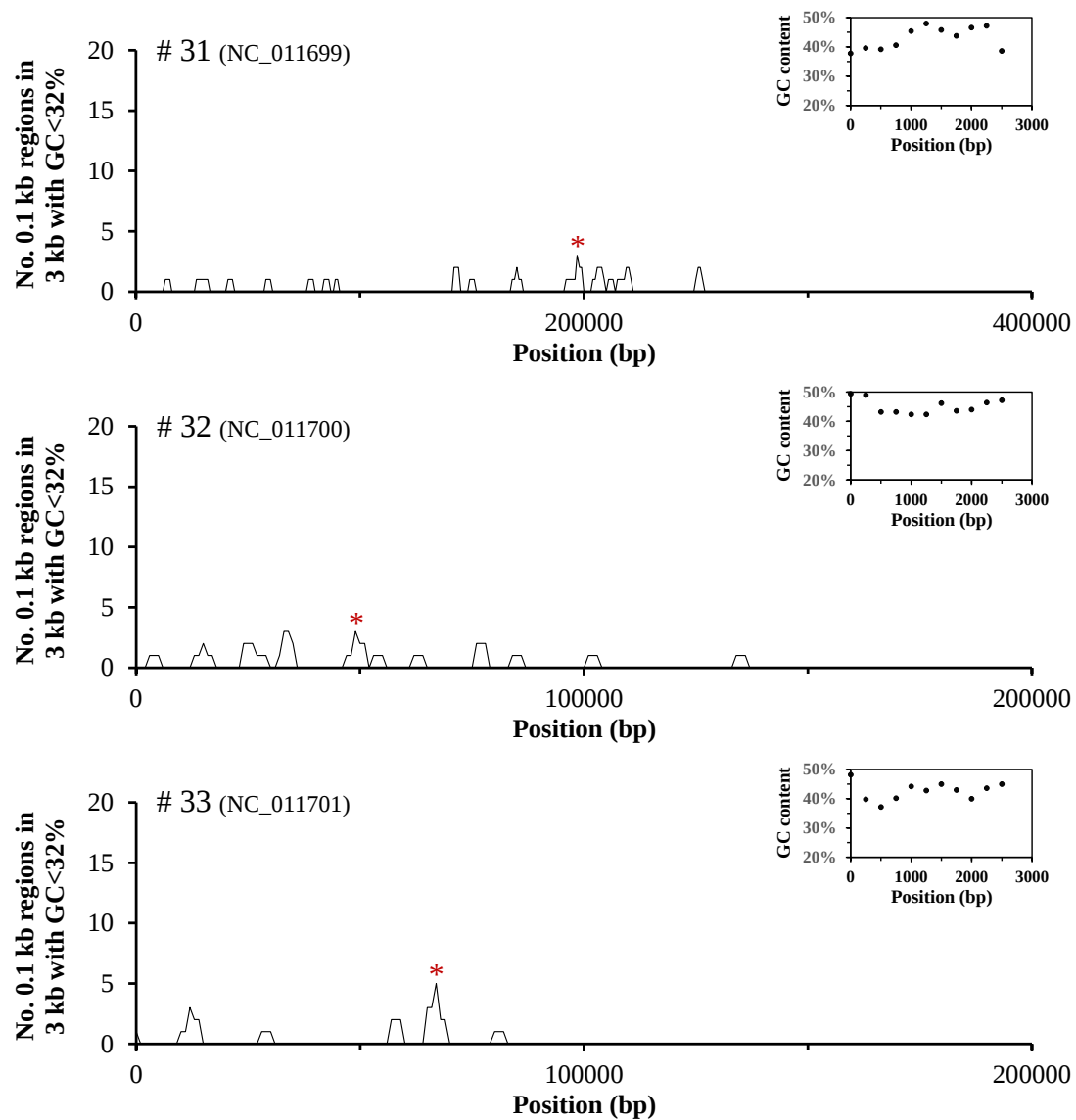




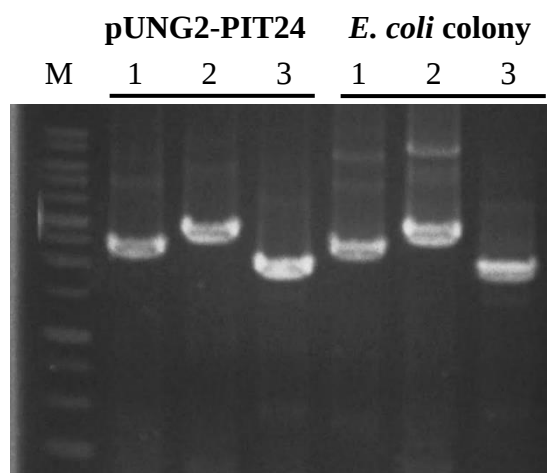








Supplemental Fig. S3 Predicted positions of centromeres on each chromosome of *P. tricornutum*. Chromosomal sequences of *P. tricornutum* CCAP 1055/1 were obtained from the NCBI dataset GCA_000150955.2. The analysis began with the identification of 100-bp regions (with a 50-bp overlap) within a 3-kb sliding window (advancing in 1-kb steps) with a GC content below 32%. The 3-kb region containing the highest number of such 100-bp windows was selected for further analysis. Within this region, the 500-bp sequence with the lowest GC content was identified.



Supplemental Fig. S4 Results of colony PCR performed on transformed *E. coli* obtained from the rescue assay. The pUNG-PIT24 vector was used as a control group in the colony PCR comparison.

Supplemental Table S1 a PCR conditions. **b** primer sequences used in PCR analysis.

a)

Purpose	Primer set	PCR mixer		PCR condition		
		Component	Vol (μL)	Step	Time (m)	Temp (°C)
Preparing circular DNA constructs	ef1-(-1772)- <i>NotI</i> (F) & ef1-2229- <i>SpeI</i> (R)	DNA(100~500mg/L)	1	Initial denaturation	2.0	95
	T&A-495- <i>SpeI</i> (F) & T&A-170- <i>NotI</i> (R)	F primer (10 μM)	5	Denaturation	1.0	95
	N1-2629- <i>NdeI</i> (F) & N1-3423- <i>BamHI</i> (R)	R primer (10 μM)	5	Annealing	0.30	55~60
	E1EE-2783- <i>BamHI</i> (F) & E1EE-1780- <i>NdeI</i> (R)	dNTP mix (10mM)	1	Extension	2.0-8.0	72
	E1NE-1- <i>NotI</i> (F) & E1NE-1478- <i>NdeI</i> (R)	Pfu 10X buffer	5	Final extension	5.0	72
	gadh-t-1- <i>BamHI</i> (F) & gadph-t-978- <i>SpeI</i> (R)	Pfu DNA polymerase	0.5			
	18S-124- <i>NdeI</i> (F) & 18S-566- <i>NdeI</i> (R)	H ₂ O	32.5			
	18S-NKNS-934 (F) & 18S- <i>BamHI</i> -1536 (R)					
	γT&A-457- <i>BamHI</i> (F) & γT&A-182- <i>NdeI</i> (R)					
	Puc3-2479- <i>NdeI</i> (F) & Puc3-3144- <i>NotI</i> (R)					
	E1NG1-18S-633- <i>KpnI</i> (F) & E1NG1-18S-1298- <i>NotI</i> (R)					
	ubi-(-762)- <i>NotI</i> (F) & ubi-(-1)- <i>NdeI</i> (R)					
	UNG1-18S-641- <i>NotI</i> (F) & UNG1-18S-2505- <i>SpeI</i> (R)					
Preparation of linear DNA constructs	18S(+) (F) & 18S(-) (R)	DNA(50~100mg/L)	1	Initial denaturation	3.0	95
	E (F) & G2 (R)	F primer (10 μM)	1.25	Denaturation	0.15	95
	U (F) & G2 (R)	R primer (10 μM)	1.25	Annealing	0.15	55
		PCR Mix	12.5	Extension	3.0-5.0	72
		H ₂ O	9	Final extension	10.0	72
Checking centromeric plasmid in BL10	pE2NG1-18S-CA-2218 (F)+ pE2NG1-18S-CA-4376 (R)	DNA (50~100mg/L)	1	Initial denaturation	1.0	94
	pE2NG1-18S-CA-5210 (F)+ pE2NG1-18S-CA-6397 (R)	F primer (10 μM)	1	Denaturation	0.5	98
	pE2NG1-18S-CA-3453 (F)+ pE2NG1-18S-CA-3128 (R)	R primer (10 μM)	1	Annealing	0.5	55
		PCR Mix	25	Extension	0.2-1.3	72
		H ₂ O	22	Final extension	3.0	72
	UNG2-CA-151 (F) & UNG2-CA-3476 (R)					
	UNG2-CA-2513 (F) & UNG2-CA-4435 (R)					
	UNG2-CA-3809 (F) & UNG2-CA-731 (R)					
	UNG2-PIT24-151 (F) & UNG2-PIT24-3310 (R)					
	UNG2-PIT24-3643 (F) & UNG2-PIT24-3310 (R)					
	UNG2-PIT24-2347 (F) & UNG2-PIT24-4269 (R)					
	D-ef1α (F)					
	D-ef1α (R)					

Checking	neo-qPCR (F) &	cDNA (100 mg/L)	1	Initial denaturation	3.0	95
expression	neo-qPCR (R)	F primer (10 µM)	0.4	Denaturation	} 40 cycles	95
level of	ef1a-qPCR (F) &	R primer (10 µM)	0.4	Anneal + Ext		60
neo in	ef1a-qPCR (R)	Fast SYBR Green	10	Melt curve anal.	0.15	95
BL10		Master Mix (2X)			1.0	60
(qRT-PCR)		H ₂ O	8.2		0.15	95
Colony PCR	UNG2-PIT24-1005 (F) &	<i>E. coli</i> cells	1	Initial denaturation	1.0	94
(Rescue	UNG2-PIT24-3310 (R)	F primer (10 µM)	1	Denaturation	} 32 cycles	98
assay)	UNG2-PIT24-2347 (F) &	R primer (10 µM)	1	Annealing		55
	UNG2-PIT24-4269 (R)	PCR Mix	25	Extension		72
	UNG2-PIT24-3643 (F) &	H ₂ O	22	Final extension	3.0	72
	UNG2-PIT24-1280 (R)					

b)

Usage	Primer name	Primer sequence (5'-3')
Preparation of circular DNA constructs	ef1-(-1772)- <i>NotI</i> (F)	AAGCAGCGGCCGCGGAAAGCACCCCGAAGAAAAAG
	ef1-2229- <i>SpeI</i> (R)	ACTAGTCACAGGCATTGGCAGGTTT
	T&A-495- <i>SpeI</i> (F)	TAAGCAACTAGTGGCGTAATCATGGTCATAGC
	T&A-170- <i>NotI</i> (R)	TGCTTAAGCGGCCGCTACAATCTGCTCTGATGCCG
	N1-2629- <i>NdeI</i> (F)	GCATATGATTGAACAAGATGG
	N1-3423- <i>BamHI</i> (R)	GGATCCTTAGAAGAAGCTCGTCAAGAAGGC
	E1EE-2783- <i>BamHI</i> (F)	TAAGCAGGATCCGTTGGTTTGACCTCTTATACTTGATC
	E1EE-1780- <i>NdeI</i> (R)	TGCTTACATATGTTCTTGATGAAATCACGGTGA
	E1NE-1- <i>NotI</i> (F)	ATAAGAATGCGGCCGCTAGCTAGCT
	E1NE-1478- <i>NdeI</i> (R)	CGCCGGAATTCCATATGTTCTTGATGAA
	gadph-t-1- <i>BamHI</i> (F)	CGCGGATCCATGTACCCAATA
	gadph-t-978- <i>SpeI</i> (R)	GCGGACTAGTACAAACTTGAAGC
	18S-124- <i>NdeI</i> (F)	CCGGAATTCCATATGATATGGATACC
	18S-566- <i>NdeI</i> (R)	GGAATTCATATGCTTCTGGAGC
	18S-NKNS-934 (F)	CATATGGGTACCGCGGCCGCACTAGTCTGGGGATCG
	18S- <i>BamHI</i> -1536 (R)	CGCGGATCCTGCAAAAATCTAGCCCCAGCACG
	yT&A-457- <i>BamHI</i> (F)	TTATACGCGGATCCCTCTAGAGTC
	yT&A-182- <i>NdeI</i> (R)	GCGGAATTCCATATGGTGCACCTCTCAG
	Puc3-2479- <i>NdeI</i> (F)	CATATGGGGCCCGTTGCACTCACTCCGCTTTGGTTTCA
	Puc3-3144- <i>NotI</i> (R)	GCGGCCGCGTGATAATGCCAATCGCTAAGAAAAAAG
	E1NG1-18S-633- <i>KpnI</i> (F)	GGTACCGTTGCAGTCACTCCGCTTTG
	E1NG1-18S-1298- <i>NotI</i> (R)	GCGGCCGCGTGATAATGCCAATCGCTAA
	ubi-(-762)- <i>NotI</i> (F)	ATTTATAGCGGCCGCGCACAGTTACTCCGAGTC
	ubi-(-1)- <i>NdeI</i> (R)	GGGCCCACATATGGTTGTTTGCTTATCTA
	UNG1-18S-641- <i>NotI</i> (F)	GCGGCCGCGGGAGACTTTGAAAGGCACAG
	UNG1-18S-2505- <i>SpeI</i> (R)	ACTAGTTTGTGAATGAAAA
Preparation of linear DNA constructs	18S(+) (F)	CCTGCAGTAATTCTGAAATAATACATGCTG
	18S(-) (R)	TGCAAAAATCTAGCCCCAGCACG
	E (F)	CTAGCTAGCTCATGCTCCTTTCCCG
	U (F)	GGGAGACTTTGAAAGGCACA
	G2 (R)	ACTAGTTTGTGAATGAAAAGAGA
Confirming centromeric plasmid in BL10	pE2NG1-18S-CA-2218 (F)	GTGCCACAGCAGACCAGTAA
	pE2NG1-18S-CA-4376 (R)	TGCAAATGTTGACGATGAGC
	pE2NG1-18S-CA-5210 (F)	AGCTTGCGTAATCATGGTC
	pE2NG1-18S-CA-6397 (R)	GCATTGGTAACTGTCAGACCAA
	pE2NG1-18S-CA-3453 (F)	CGTTGGCTACCCGTGATATT
	pE2NG1-18S-CA-3128 (R)	ATACTTTCTCGGCAGGAGCA
	UNG2-CA-151 (F)	CGGCATCAGAGCAGATTGTA
	UNG2-CA-3476 (R)	TTTTTGTGATGCTCGTCAGG
	UNG2-CA-2513 (F)	CGTTGGCTACCCGTGATATT
	UNG2-CA-4435 (R)	GGATGGAGGCGGATAAAGTT
	UNG2-CA-3809 (F)	GCAGAGCGAGGTATGTAGGC
	UNG2-CA-731 (R)	CACAGGATTTTCGTGTGTGG
	UNG2-PIT24-1005 (F)	ATCCTGGCAGACATGTGGAA
	UNG2-PIT24-3310 (R)	TTTTTGTGATGCTCGTCAGG
	UNG2-PIT24-2347 (F)	CGTTGGCTACCCGTGATATT
	UNG2-PIT24-4269 (R)	GGATGGAGGCGGATAAAGTT
	UNG2-PIT24-3643 (F)	GCAGAGCGAGGTATGTAGGC
	UNG2-PIT24-1280 (R)	CGTCTGTTCTCTCTGCCTCT
	UNG2-PIT24-151 (F)	CGGCATCAGAGCAGATTGTA

	D-efl α (F)	CTTGCTTGCCGTATGCTC
	D-efl α (R)	GGTGGTGGTGGACTTGC
Checking expression level of <i>neo</i> in BL10	neo-363 (F)	GCGGCTGCATACGCTTGATCC
	neo-457 (R)	CAAGACCGGCTTCCATCCGAG
	efl α -616 (F)	GACATCAAGCGCGGTAAC
	efl α - 786 (R)	GATCTCGGCGAACTTGC

Supplemental Table S2 Formula of culture media**M3 medium**

Ingredients	Unit
Peptone	0.5 g
Yeast extract	1.0 g
Glucose	2.0 g
Nutrient solution	500 µL
Vitamin solution	50 µL
Agar (if needed)	8 g
Sea salt	32 g
Distilled water	to 1 L

Nutrient solution

Ingredients	Unit
Ferric chloride hexahydrate	1.3 g
Manganese chloride tetrahydrate	0.36 g
Boric acid	33.6 g
EDTA	45.0 g
Sodium dihydrogen phosphate dihydrate	20.0 g
Sodium nitrate	100.0 g
Distilled water	to 1 L

Vitamin solution

Ingredients	Unit
Vitamin B1	2.0 g
Vitamin B12	0.1 g
Vitamin H	0.1 g
Distilled water	to 1 L

H3 medium

Ingredients	Unit
Peptone	1.0 g
Yeast extract	2.0 g
Glucose	4.0 g
Seawater 28‰	to 1 L

LB medium

Ingredients	Unit
LB broth	25.0 g
Agar (if needed)	15 g
Distilled water	to 1 L

GYSS medium

Ingredients	Unit
Glucose	45.0 g
Yeast extract	4.5 g
Sodium sulfate	9.0 g
Seawater 1‰	to 1 L

2X GYSS medium

Ingredients	Unit
Glucose	90.0 g
Yeast extract	9.0 g
Sodium sulfate	9.0 g
Seawater 1‰	to 1 L