Evaluating Plasmid Suitability for Molecular Cloning through restriction enzyme analysis. *Agrobacterium tumefaciens*

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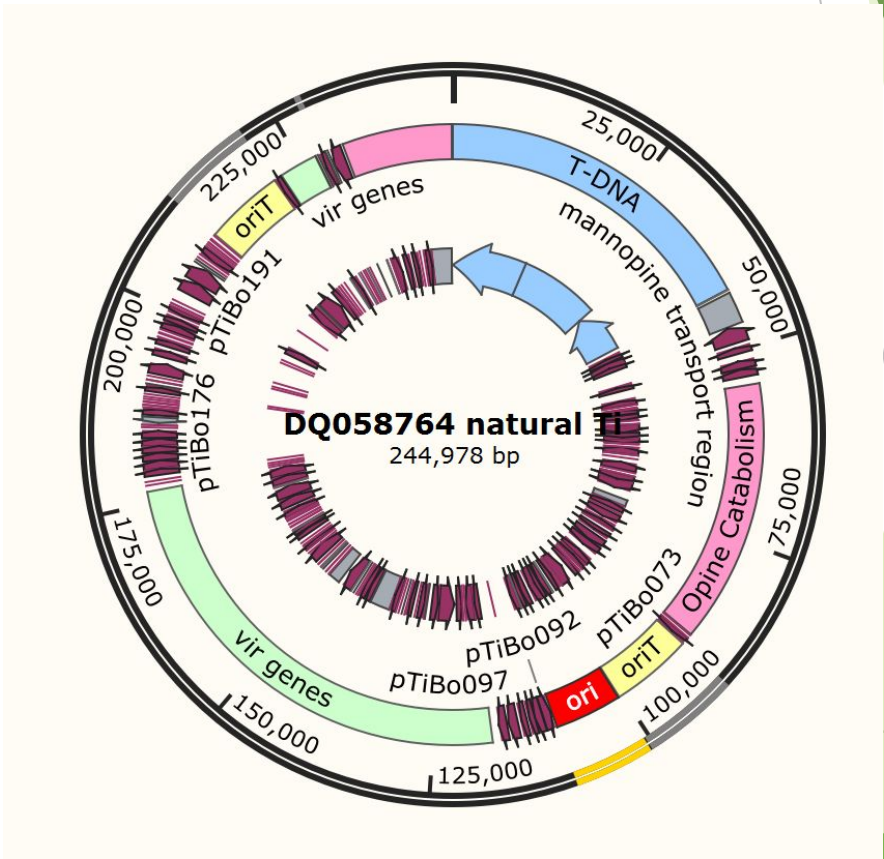
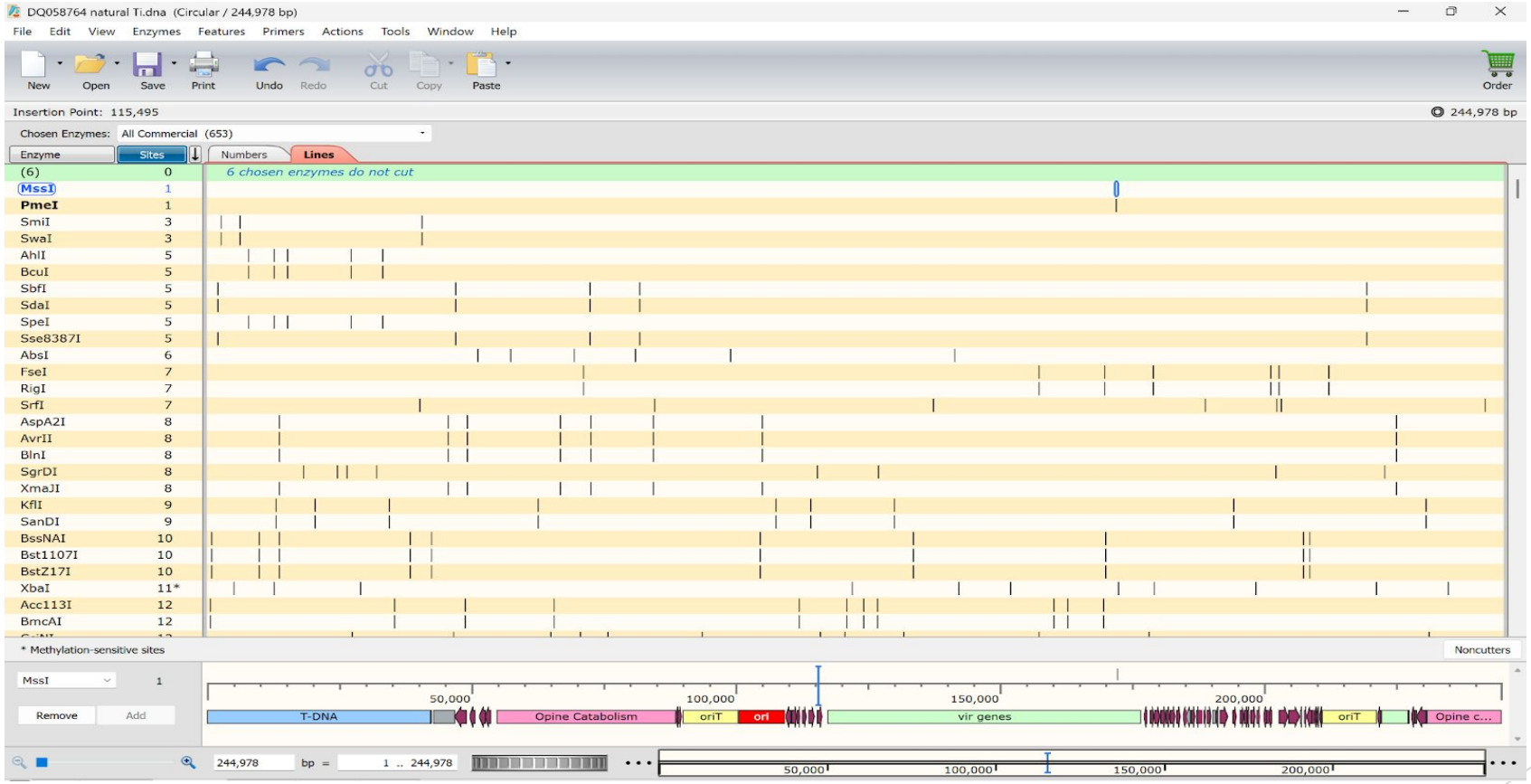
NC 010929.1

- pTiBo542
- Accession number**:** DQ058764.
- Size: 244,978 bp.
- Origin of replication (ori): repABC operons (a three-gene operon).
- T-DNA with oncogenes.
- Vir genes – present.
- No selectable markers and MCS.

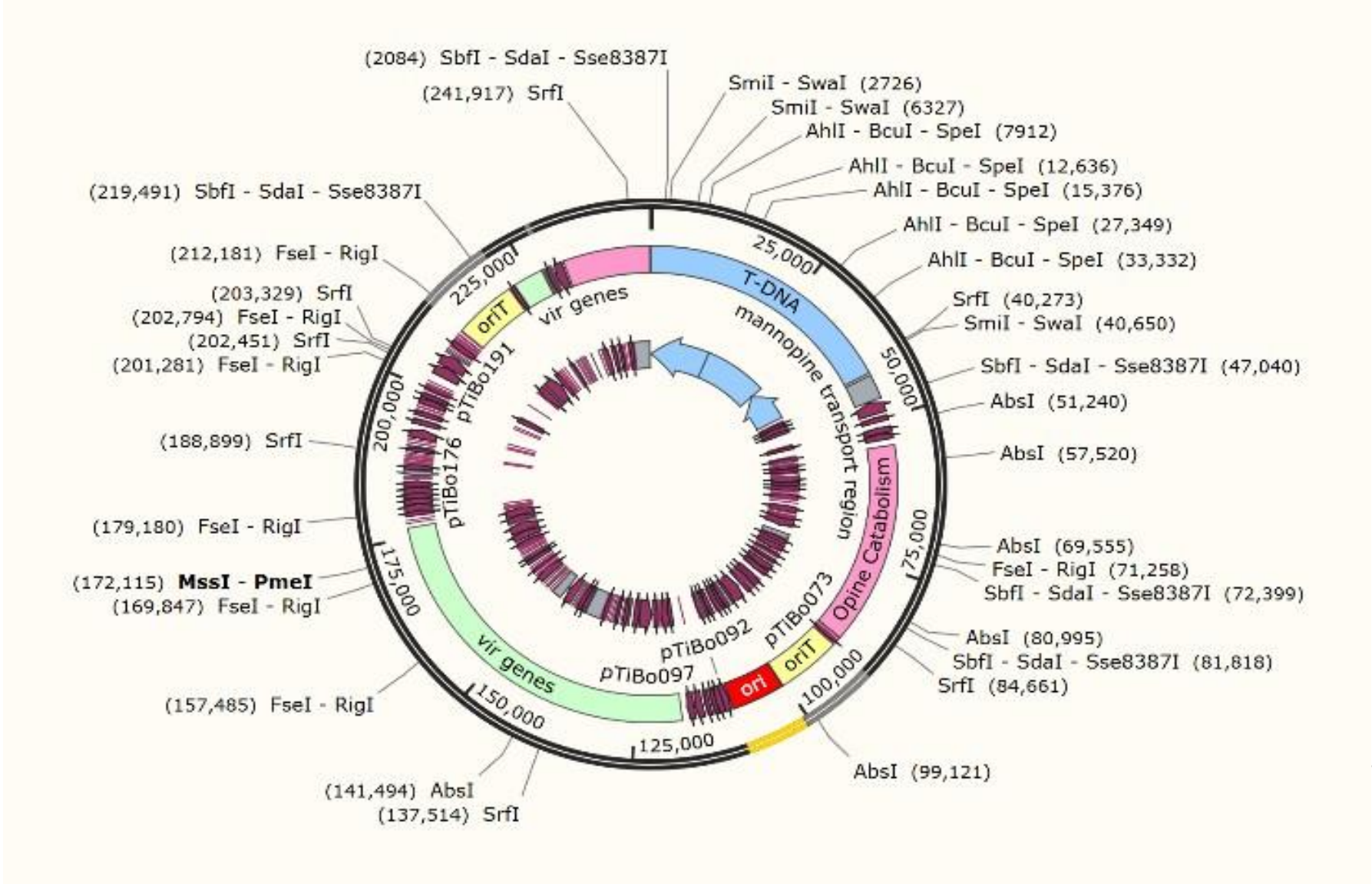


Restriction Map: Virtual restriction digestion performed with SnapGene

- 1 cutters: Mss1, Pme1
- 2 cutters: (Nil)
- 3 cutters: Smi1, Swa1
- Rest of the restriction enzymes cut at multiple sites

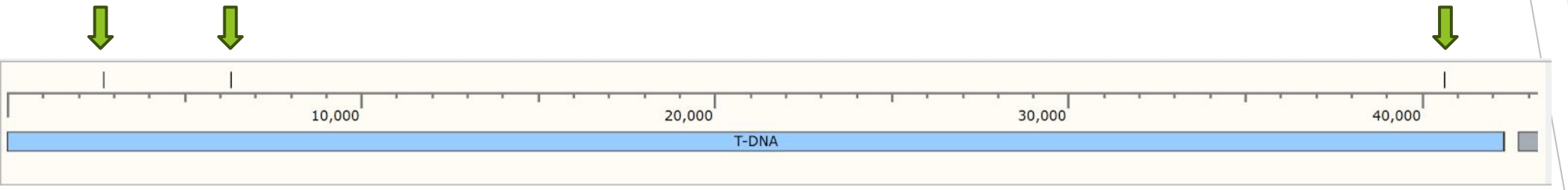


Restriction map from Snapgene:



Final Cloning Sites:

- Unique cutters: Mss1, Pme1(Restriction site within vir gene – cannot be used).
- Restriction enzymes SmiI and SwaI produced three cuts within the T-DNA region itself (at ~2,726 bp, ~6,327 bp, and ~40,650 bp). These sites can be exploited for inserting the gene of interest.

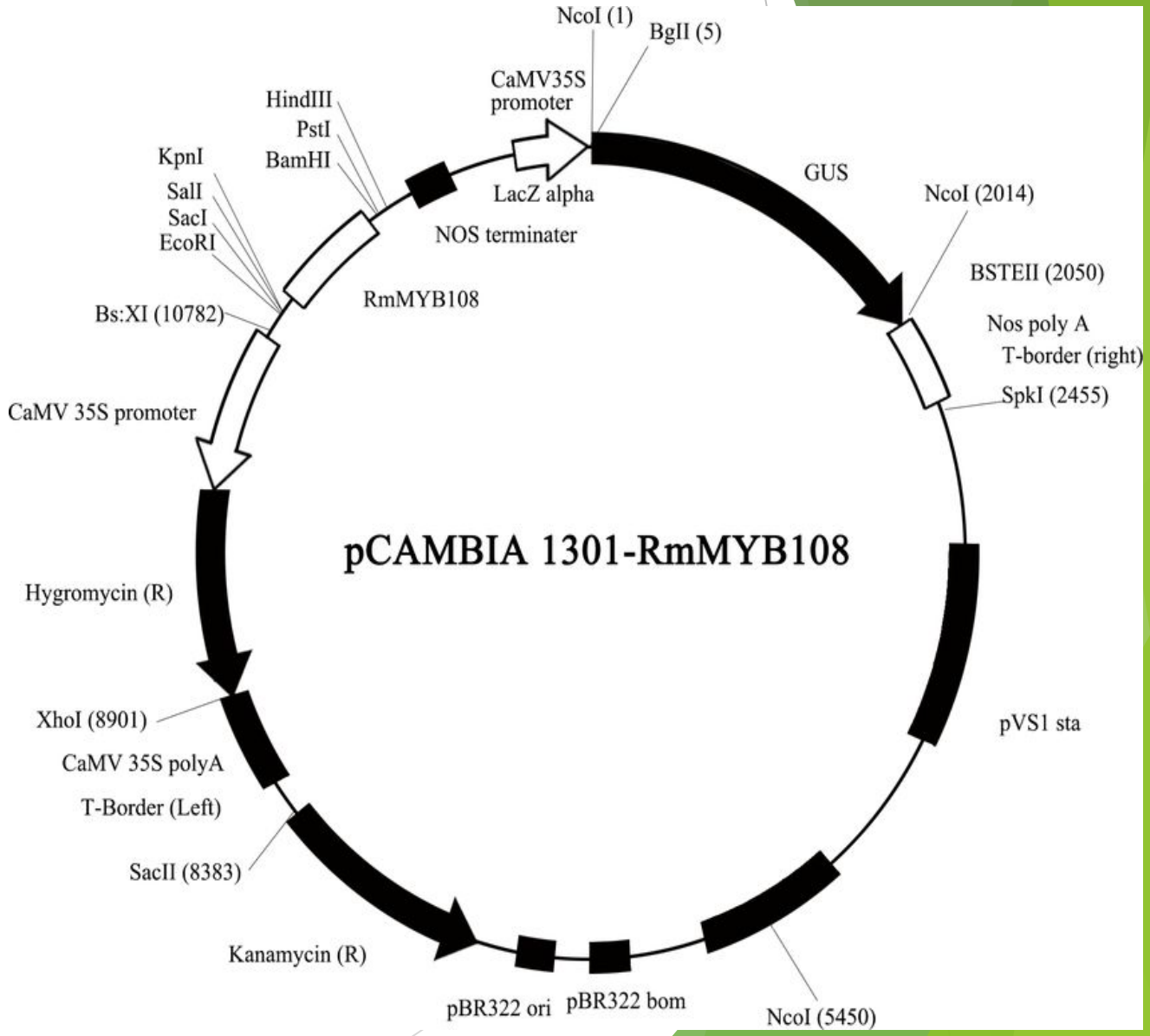


List: UnMethylated Lane

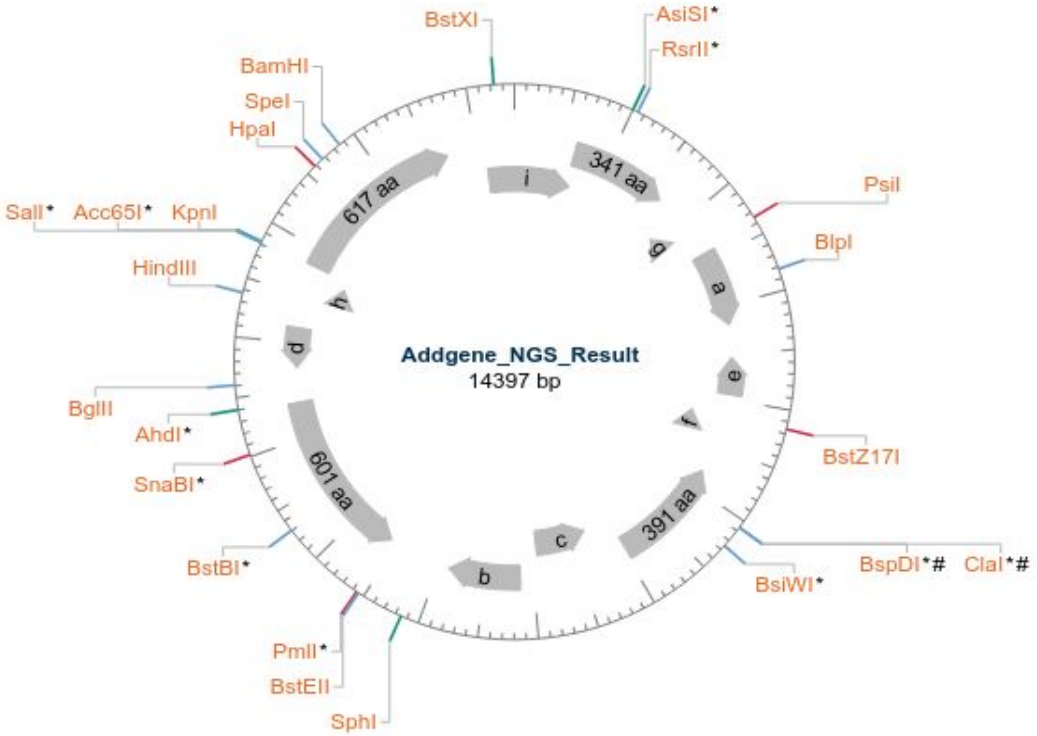
#	Ends	Coordinates	Length (bp)	Affected by Methylation
1	SwaI - SwaI	40651-2726	207054	
2	SwaI - SwaI	6328-40650	34323	
3	SwaI - SwaI	2727-6327	3601	

pCAMBIA 1301-RmMYB108

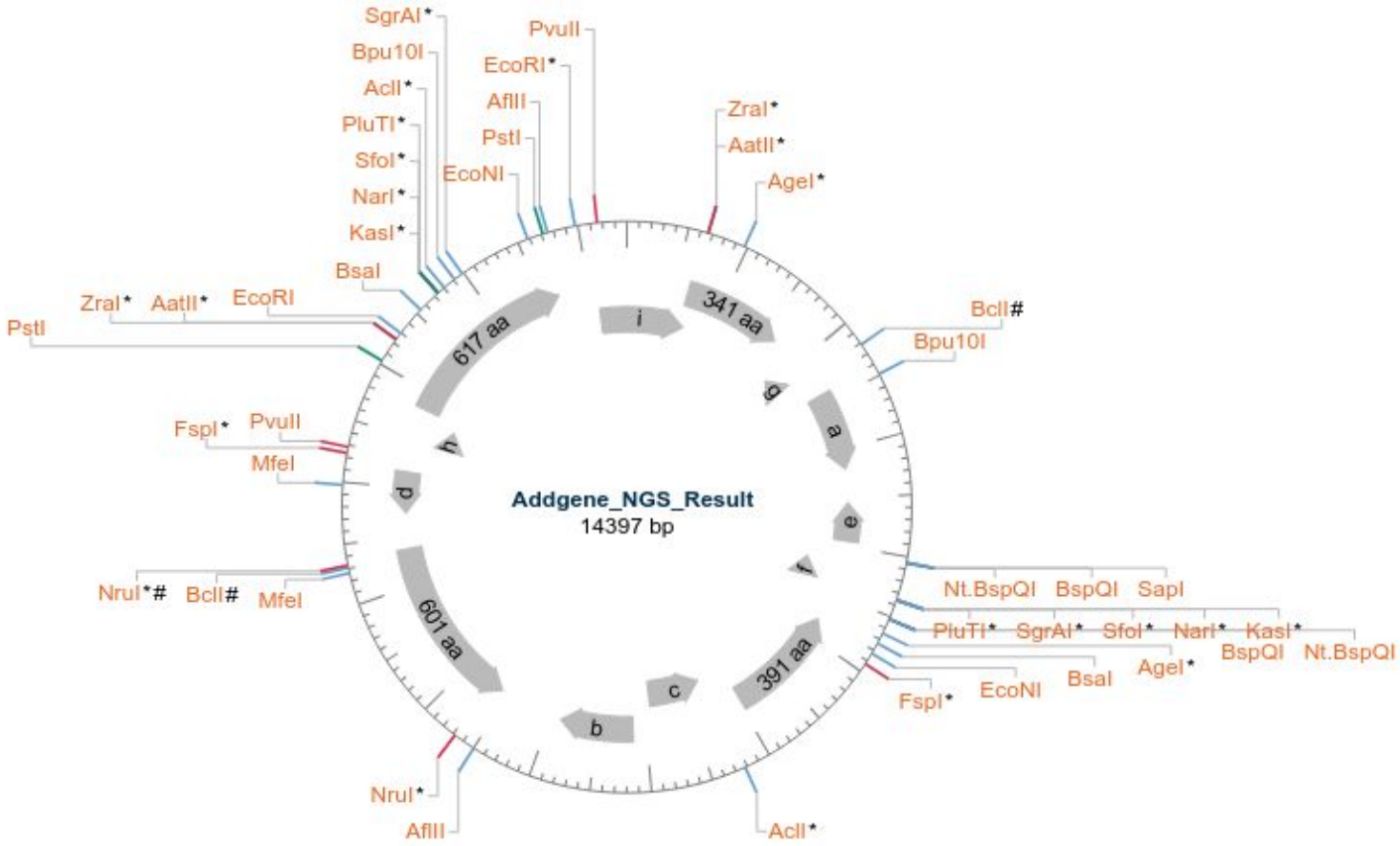
- pCAMBIA 1301-RmMYB108
- Accession number : #173180
- an engineered binary vector derived from the pCAMBIA 1301
- Size: 14397 bp. After ligation, approximately 11-12 kb
- Origin of replication (ori): pVS1 (for *Agrobacterium tumefaciens*), pBR322 ori (for *E. coli*).
- The T-DNA includes the CaMV 35S promoter, GUS reporter, lacZ alpha, NOS terminator and foreign DNA(e.g., RmMYB108)
- Vir genes not present.
- Selectable markers and MCS:
  - Kanamycin resistance for bacterial selection.
  - Hygromycin resistance (hpt gene) for plant selection.
  - Multiple cloning site (MCS) within the T-DNA, allowing insertion of genes like RmMYB108.



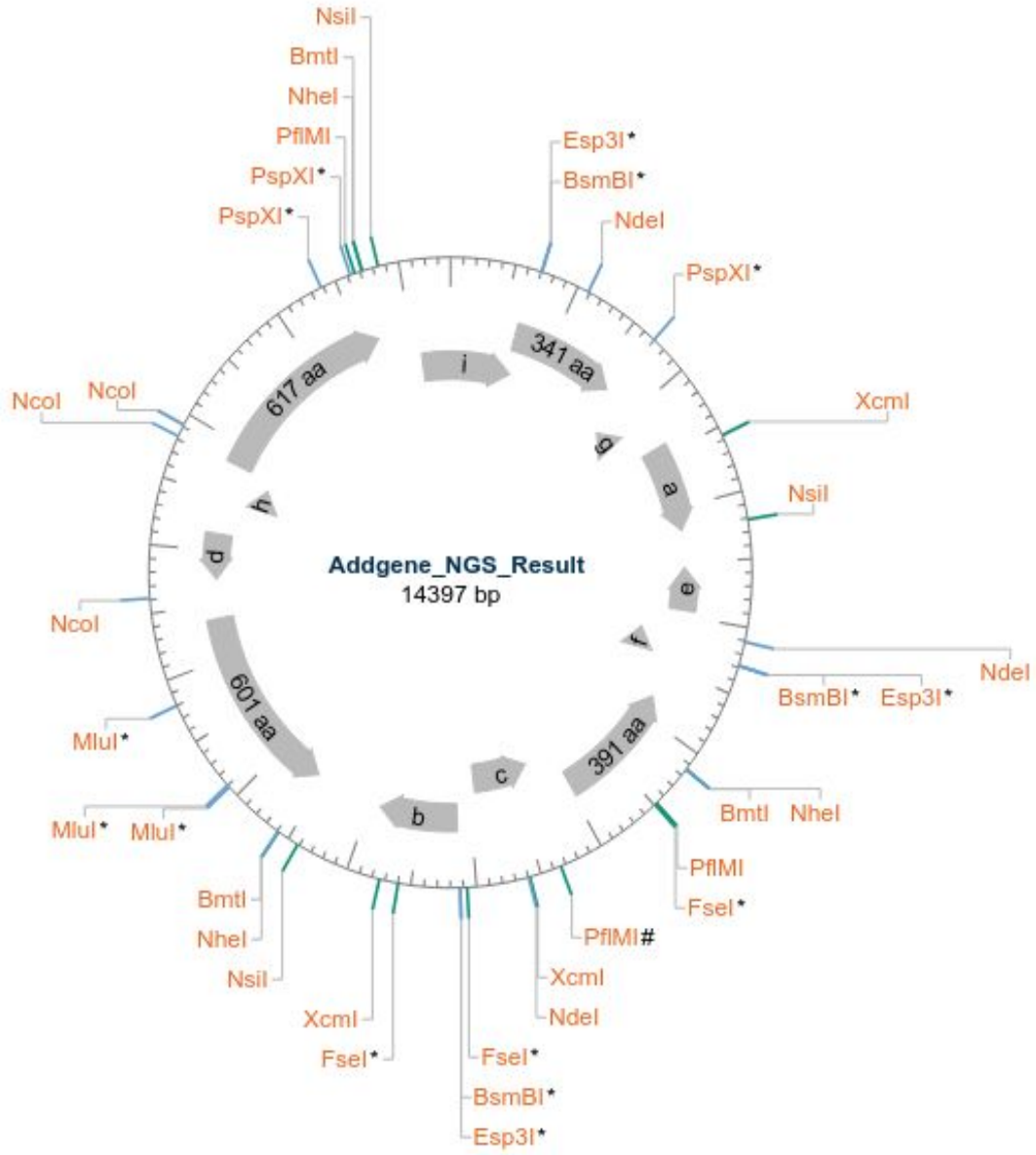
Restriction Map:



1 cutters: Several

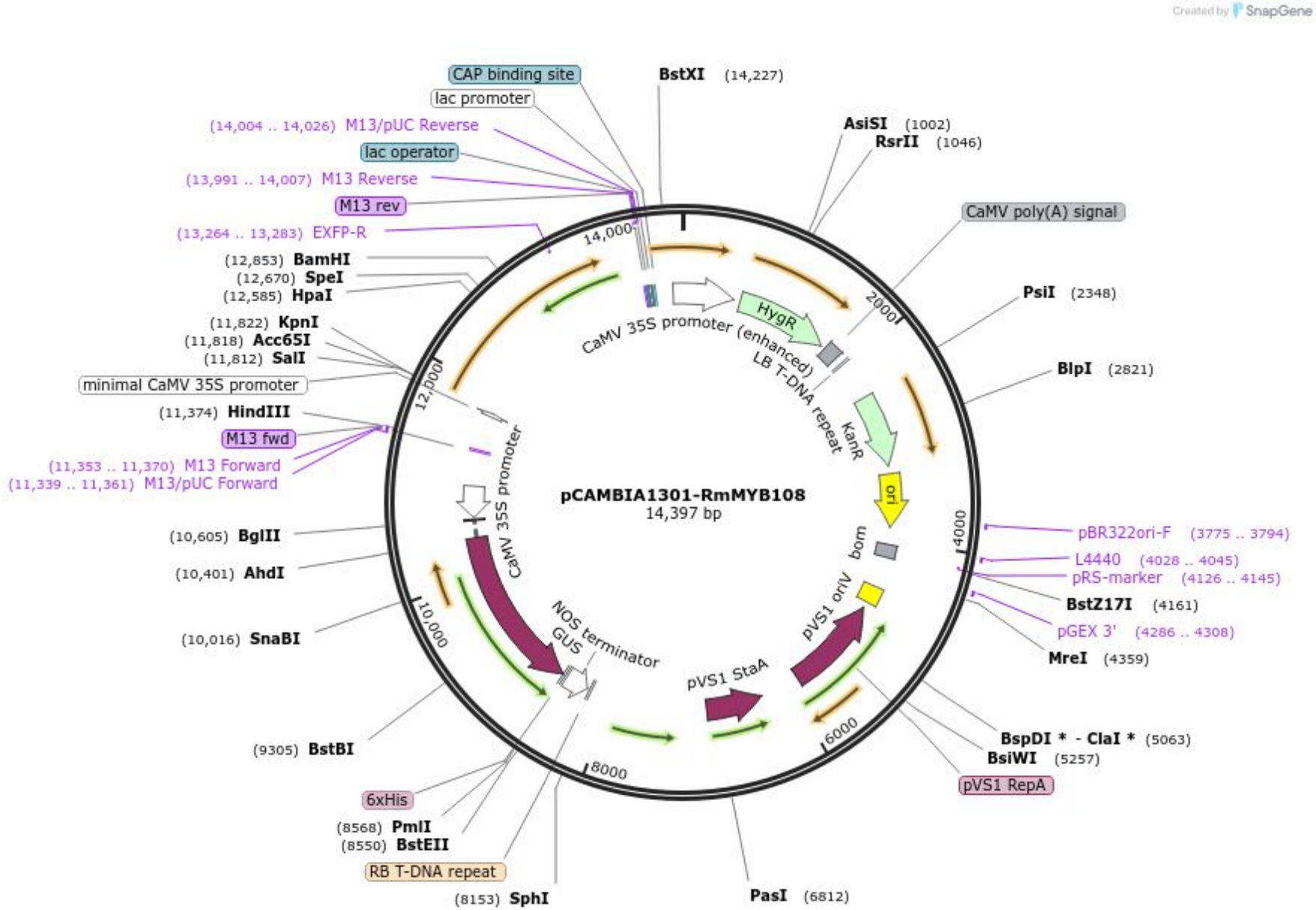


2 cutters: Several



3 cutters: Several

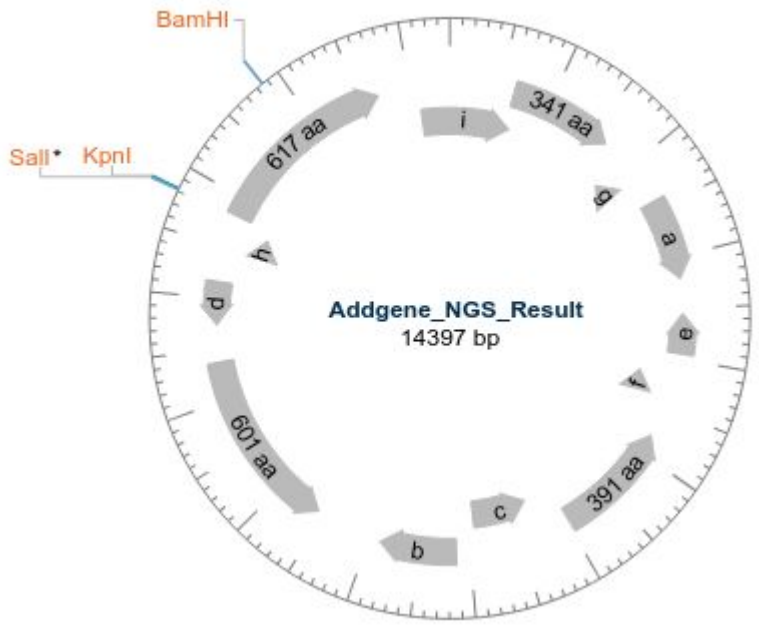
Restriction map from Snapgene:



Final Cloning Sites:

- Unique single cutter enzymes such as KpnI (11822 bp) or SalI (11812 bp) in the left flank of the RmMYB108 gene

- BamHI (12853 bp) to the right flank of the RmMYB108 gene, were identified as unique cutters within the T-DNA region. These sites are suitable for inserting a gene of interest, as they are located in the MCS or linker regions between the CaMV 35S promoter and RmMYB108/GUS, avoiding disruption of essential elements like LB/RB.



CUSTOM DIGEST

Features	pTiBo542	pCAMBIA1301-RmMYB108
Origin of Replication (ORI)	Naturally occurring plasmid in *Agrobacterium tumefaciens* (>200 kb), unstable in *E. coli*, repABC-type ori	Engineered plasmid derived from Ti plasmid backbone, pVS1 ori (for stability in *Agrobacterium*) + pBR322(for replication of *E.coli*)
Function	Causes crown gall disease in plants (pathogenic role)	Designed for plant transformation and transgene expression
T-DNA and T-DNA borders	Has oncogene and opine genes, naturally present LB and RB	Simplified T-DNA borders flanking cloning region with transgene(RmMYB108), Engineered LB and RB for precise transfer
Selectable markers	Natural resistance traits; lacks standard laboratory markers	Hygromycin resistance (hpt gene)for plants; Kanamycin resistance (nptII) for bacteria
Reporter gene	None	gusA(beta-glucuronidase,histochemical reporter)
Vir genes	Present and scattered	Absent
Restriction enzymes/ Multiple cloning site(MCS)	Limited, scattered sites; no defined MCS; cutting may disrupt essential genes	Engineered Multiple Cloning Site (MCS) with unique restriction sites (EcoRI, BamHI, HindIII, XbaI, etc.)
Promoters	Natural bacterial/ plant promoters controlling oncogenes and opine genes.	CaMV 35s (strong constitutive plant promoter)
Cloning suitability	Poor (very large, no MCS/ selectable marker, unstable for lab cloning)	Excellent (compact, defined MCS, selectable markers, strong promoters)

Conclusion:

- pTiBo542 represents the natural pathogenic mechanism of *Agrobacterium tumefaciens*, with large T-DNA regions, vir genes, and opine metabolism functions; it lacks the essential features (MCS, selectable markers, compact size) required for practical molecular cloning.
- pCAMBIA1301-RmMYB108 is optimized for laboratory use, containing defined left and right borders, strong promoters, a reporter gene (gusA), and selectable markers that facilitate efficient cloning and transgene expression in plants.
- Thus, pTiBo542 is valuable for understanding the biological basis of plant transformation, whereas pCAMBIA1301-RmMYB108 is the plasmid of choice for practical genetic engineering applications.