innovate achieve lead

Improving C3 Plant productivity By Using Cyanobacterial Bicarbonate Transporters

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Food security

Population growth- Crop yields need to nearly double over the next 35 years.

Improving photosynthesis: genetic engineering strategies

Increased photosynthetic yield

&

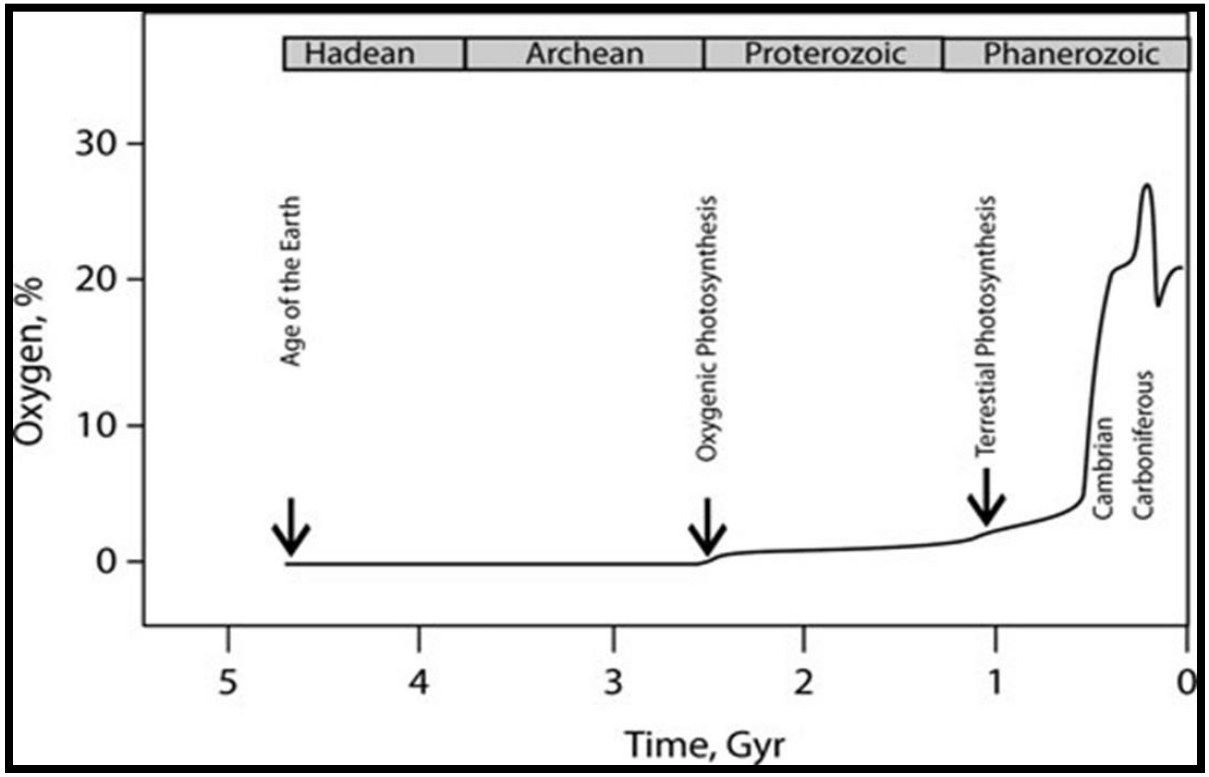
Water-use efficiency (WUE)

CO_2 concentrating mechanism (CCM)

Cyanobacteria based approaches

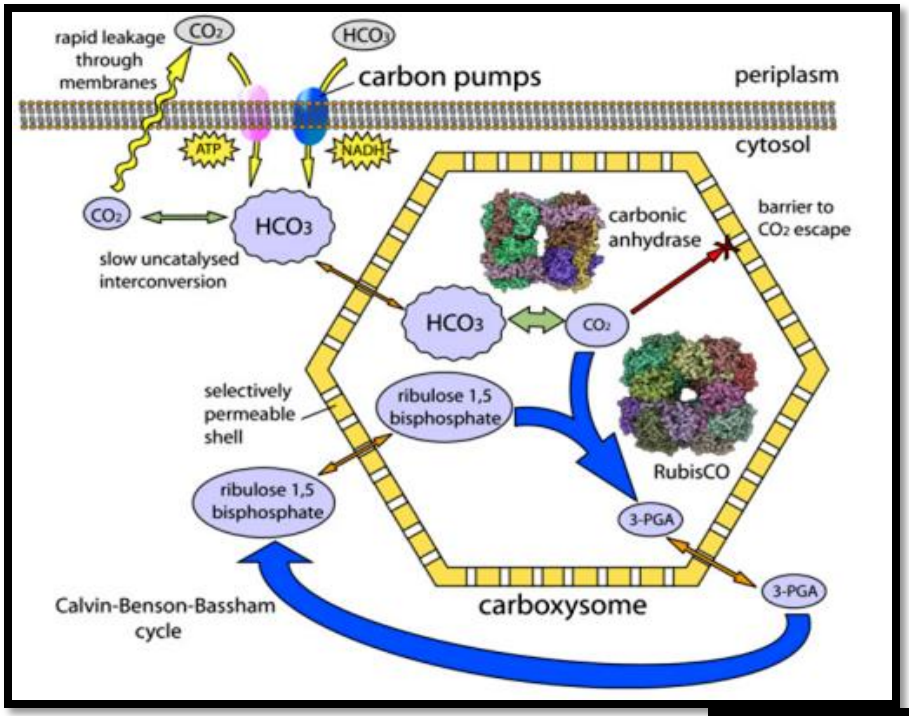
- ✓ Carboxysomes
- ✓ Ci transporters

RubisCO and Great Oxidation event

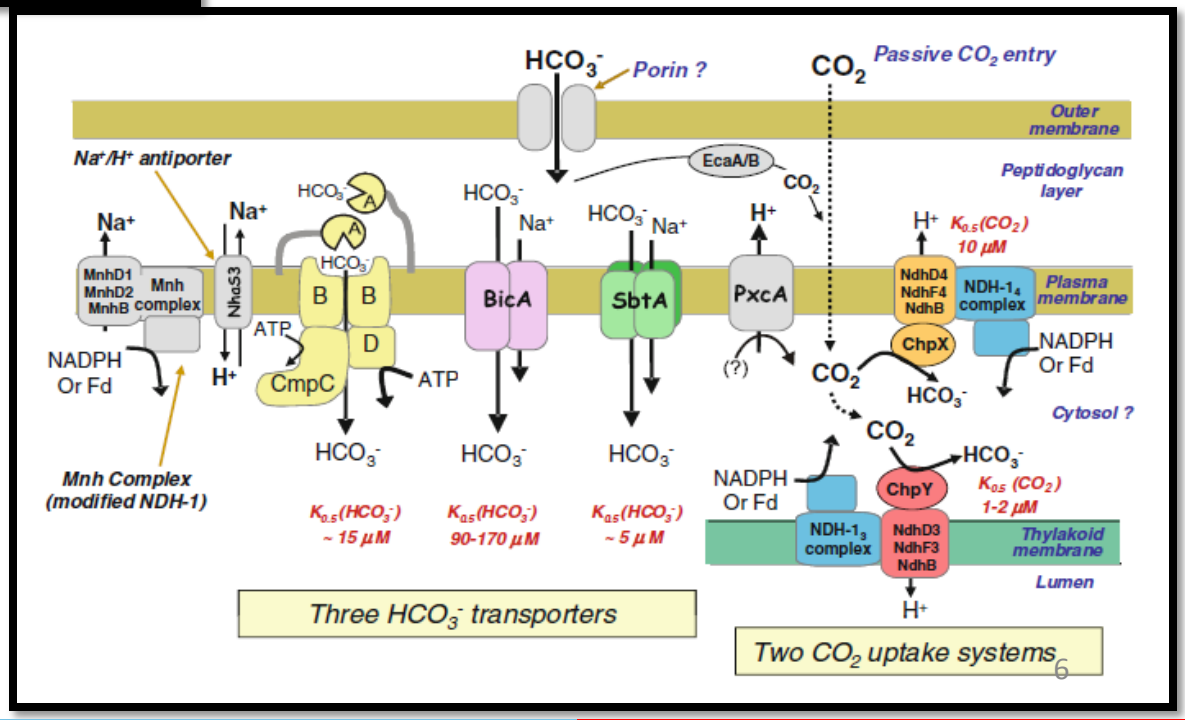


Evolution of oxygenic photosynthesis (GOE) in cyanobacteria ~2.5 billion years ago

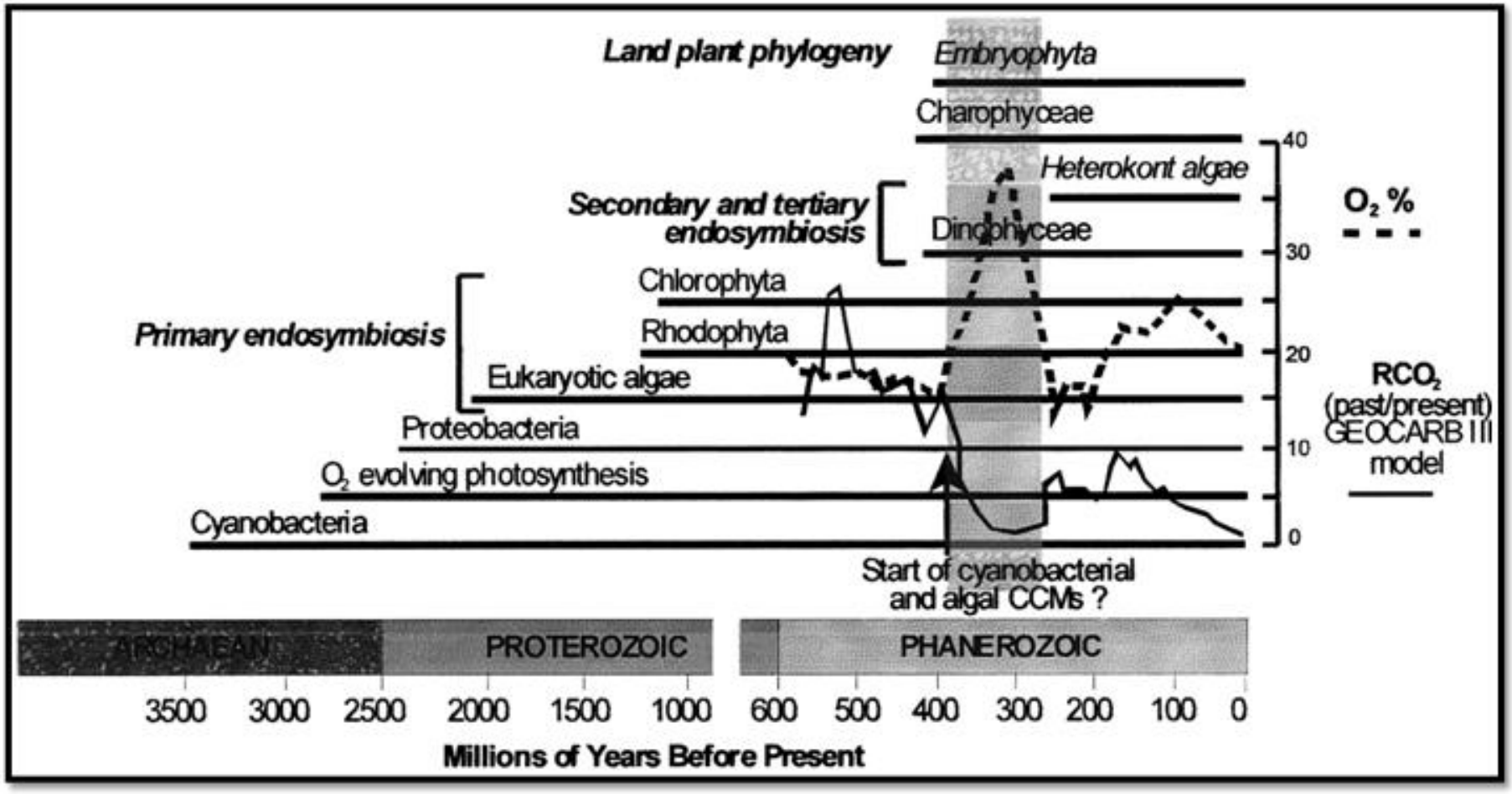
Carboxysomes



Ci transporters

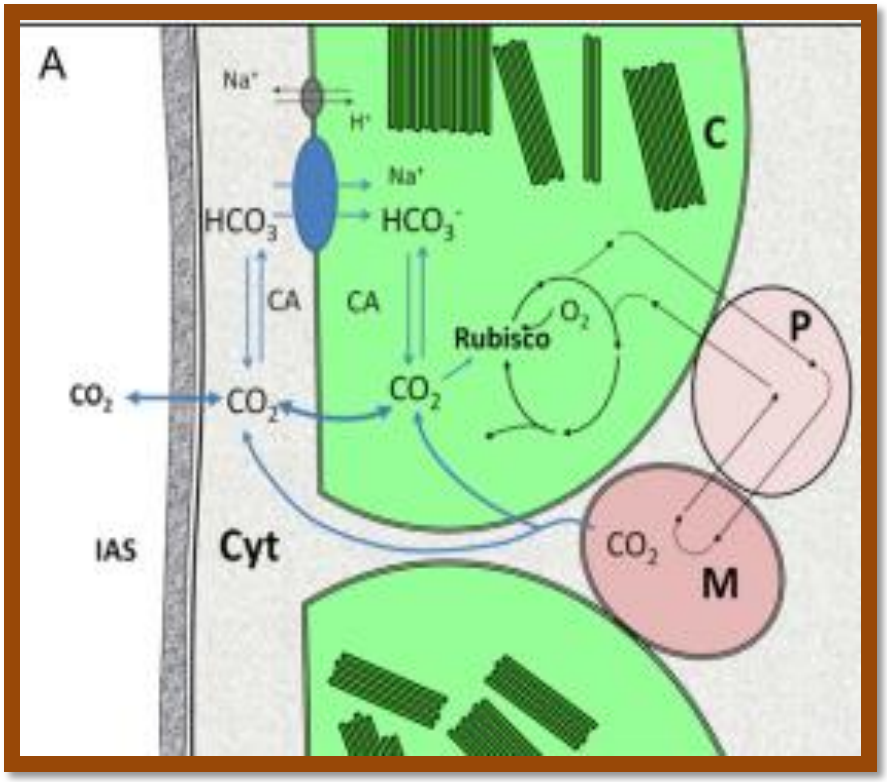


The evolution of photosynthetic cyanobacteria, algae, higher plants, and chemoautotrophic proteobacteria over the past 3.5 billion years.



Badger M.R. and Price G.D. 2003

Contd…..



Price G. D. *et al.* 2011

Concept of incorporating cyanobacterial bicarbonate transporters to chloroplast inner envelope to check the effect on plant photosynthesis

Problems

1. Correct targeting to chloroplast envelope
2. Uncertainty about the post translational activation of transporter protein.

Advantages

1. Possibly 5% to 15% improvement in photosynthetic CO_2 fixation.
2. Energetically efficient process.
3. Increased water and nitrogen use efficiency.

Phases in engineering a cyanobacterial CCM into C_3 chloroplasts

Phase 1a. **Transferring active HCO_3^- pumps to the chloroplast envelope.**

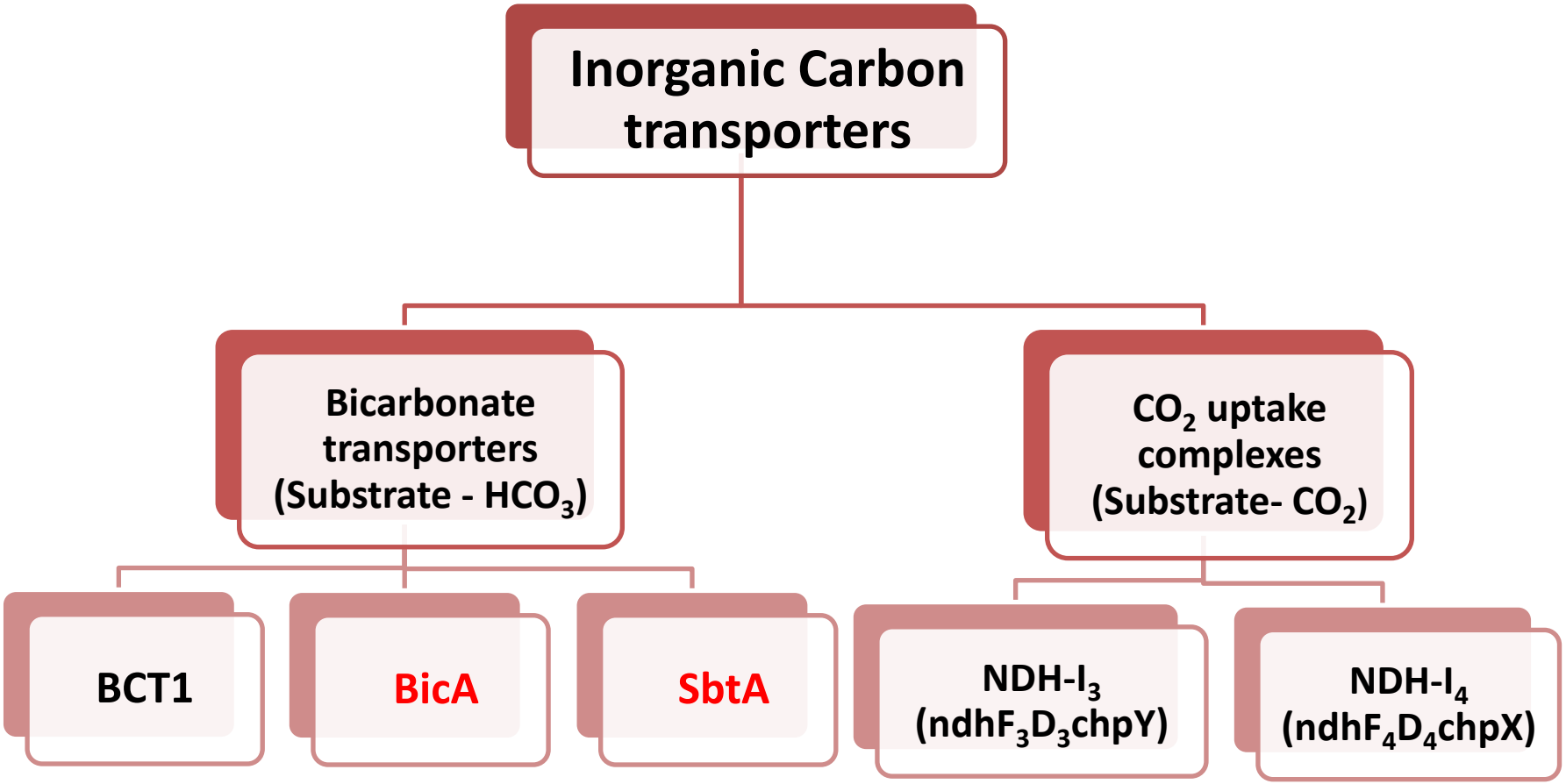
Phase 1b. **Building a functional cyanobacterial carboxysome in the chloroplast stroma.**

Phase 2. **Combining the traits from phase 1a and 1b.**

Phase 3. **Eliminating carbonic anhydrase from the stroma.**

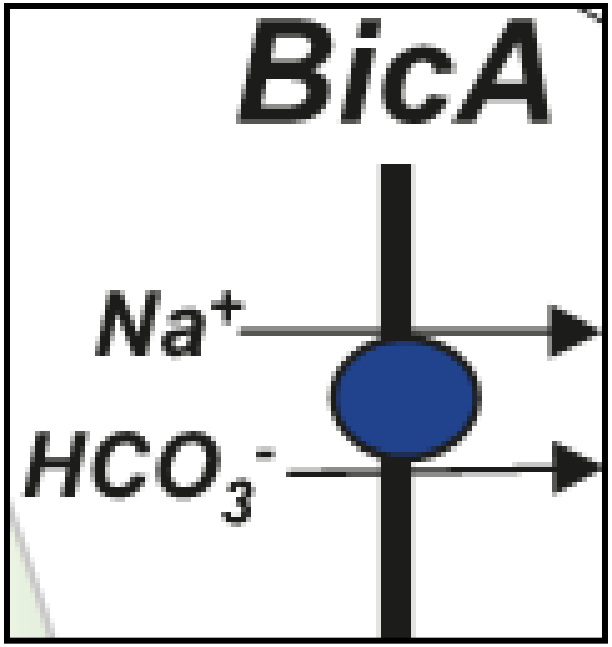
Phase 4. **Building a functional NDH-1 CO_2 uptake complex in the thylakoid membranes**

Types of *Ci* Transporters



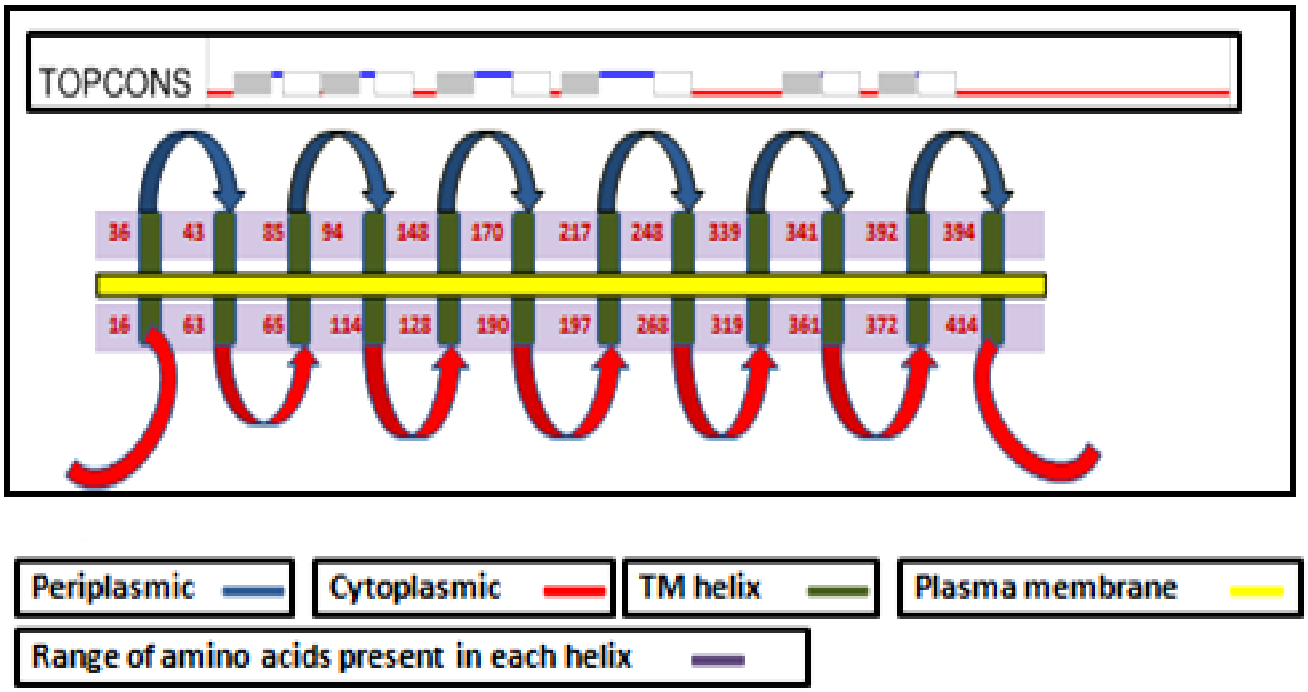
BicA transporter

- **BicA belongs to SulP/SLC26 family**
- **BicA is a low/medium affinity bicarbonate transporter**
- **Cyanobacterial BicA family shows the relation to the Arabidopsis SulP sulphate transporter family ,the human SLC26A family and a few of bacterial BicA-like proteins**



Price G.D. and Howitt S. M. 2011

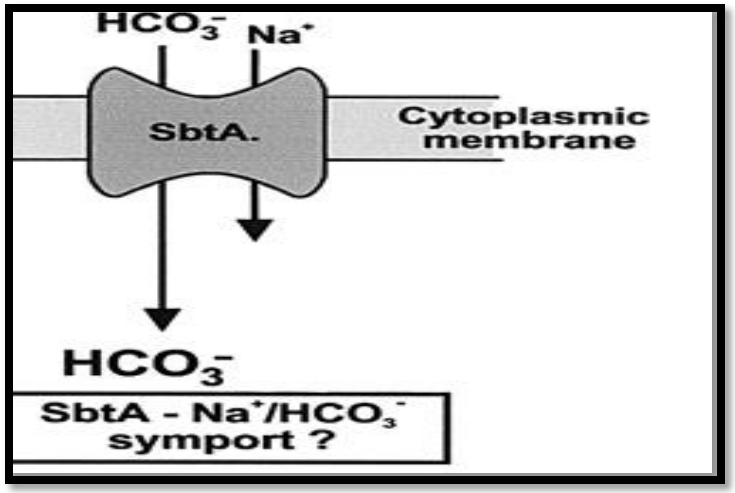
Contd…..



- **12 transmembrane helices, both termini cytoplasmic**
- **Size(gene)- 1.701 kb, 566 amino acids, Mol.wt.-59.6 kD**

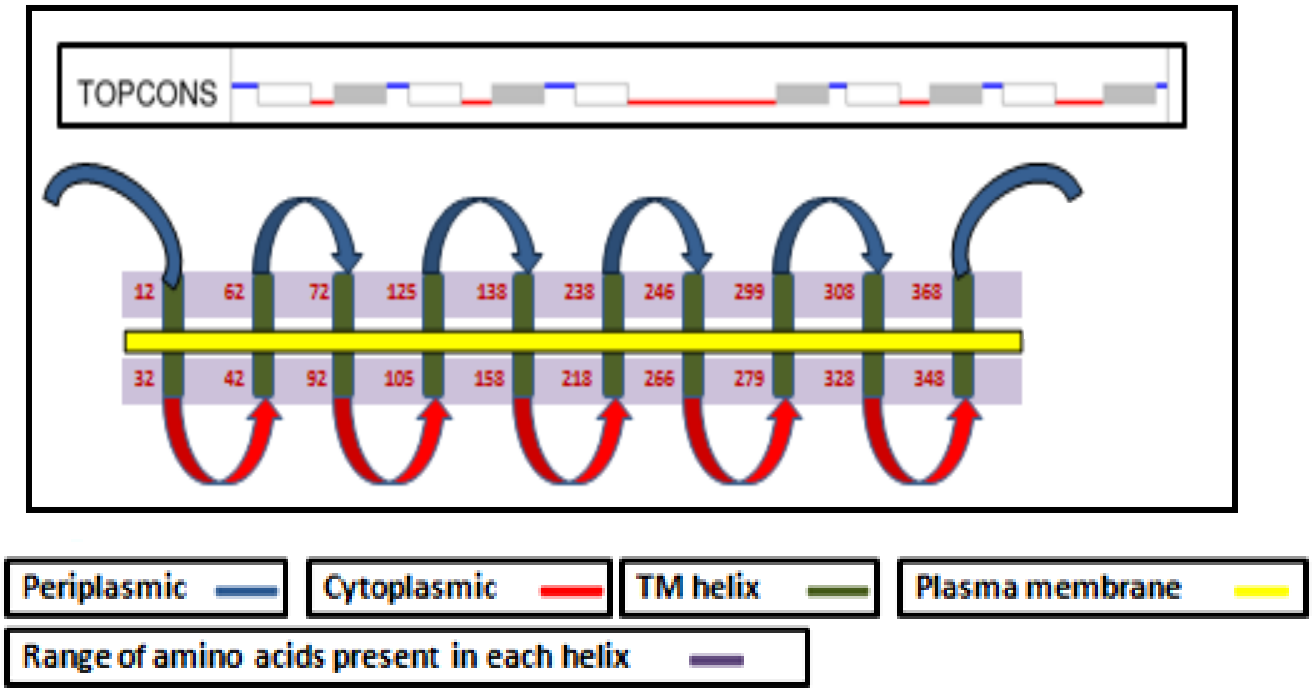
SbtA transporter

- **SbtA belongs to Sodium solute symporter (SSS) family**
- **SbtA is a high affinity bicarbonate transporter**
- **95-member family of SbtA transporter has related homologs within a large number of β -cyanobacteria and a number of α-cyanobacteria and proteobacteria**



Price G.D., 2013

Contd…..



- **10 transmembrane helices, both termini periplasmic**
- **5+5 structure**
- **Size(gene)-1.12kb, 374 amino acids, Mol.wt.-39.7 kD**

BicA and SbtA transporters, as most suitable candidates

- **In cytosol of a leaf cell approximately 250 µM HCO_3^- is present and this is maintained by cytosolic carbonic anhydrase activity**
- **Uptake affinities for sodium dependent bicarbonate transporter**
  - **(i) SbtA : 5-15 µM**
  - **(ii) BicA : 90-170 µM**
- **SbtA and BicA require about 1 mM Na^+, while leaf cytosol contains 1-3 mM Na^+ so this will form a sufficient gradient across chloroplast envelope leads to energization of transporter**
- ***Arabidopsis thaliana* proteome analysis have shown that several Na^+/H^+ antiporters are present on chloroplast envelope**

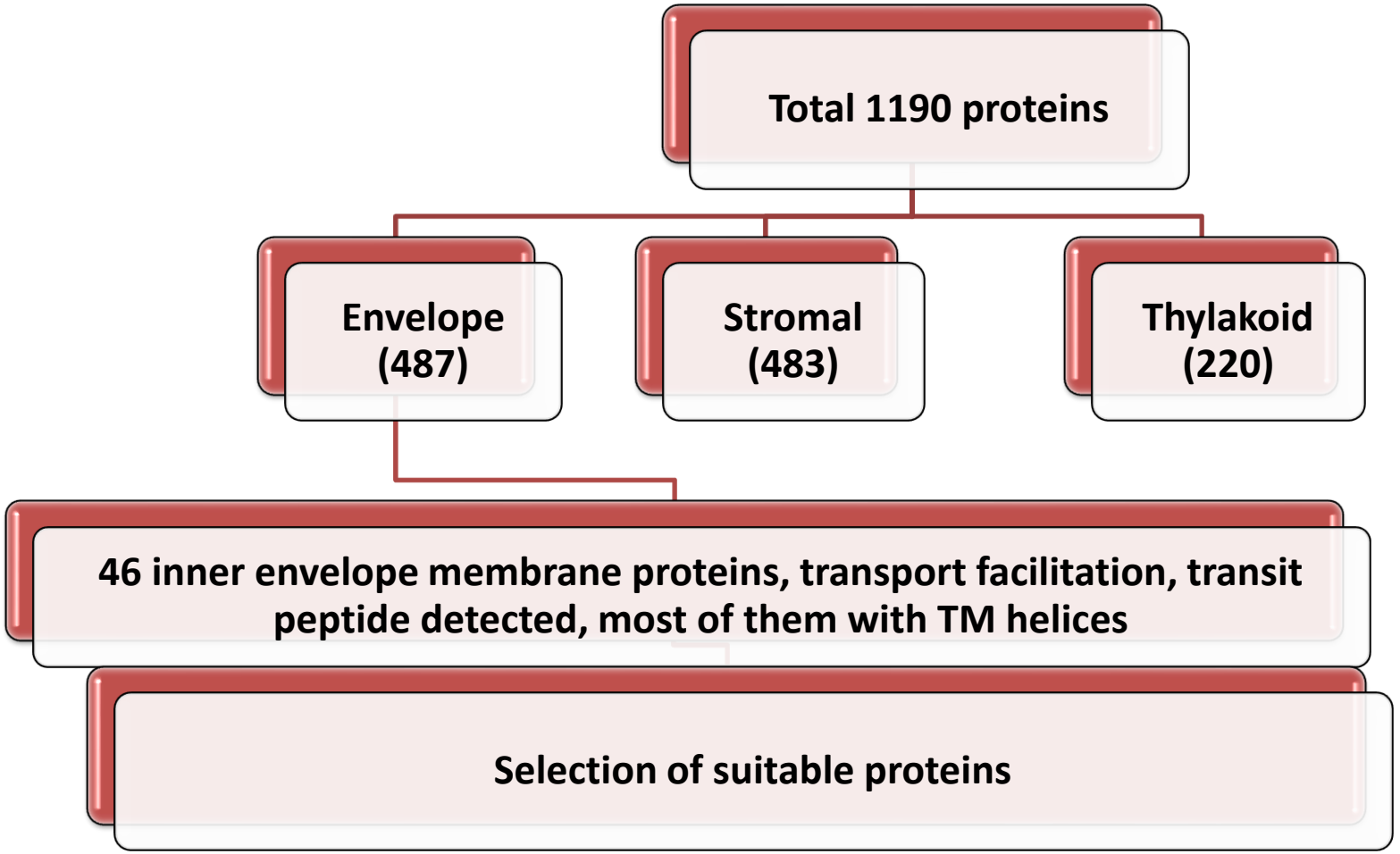
Objectives

- **Analysis of *bicA*/*sbtA* and screening of transit peptide elements**

- **Synthesis of fusion construct of *bicA*/*sbtA* transporter gene with a transit peptide element and its transformation into C_3 host plant/s followed by reporter gene assay**

- **Evaluation of successful transformants for increased growth rate/biomass yield**

Screening of suitable transit peptide elements



Categorization of *Arabidopsis thaliana* proteins to find out transit peptide elements from suitable tentative candidates

List of various bioinformatics tools, online databases and software used for analysis

1. Localization in inner envelope

2. Transport facilitation

- **AT_chloro database** (http://grenoble.prabi.fr/at_chloro)**:** Maintains information for all proteins that have been identified from *Arabidopsis thaliana.*

3. Transit peptide prediction

- **ChloroP 1.1 server** (http://www.cbs.dtu.dk/services/ChloroP/)**:**Predicts chloroplast transit peptides.
- **TargetP 1.1 server** (http://www.cbs.dtu.dk/services/TargetP/): Predicts N- terminal presequence for chloroplastic, mitochondrial and secretory proteins.

4. Trans-membrane domain prediction

- **OCTOPUS** (http://octopus.cbr.su.se/)
- **TMHMM (**http://www.cbs.dtu.dk/services/TMHMM/)
- **TOPCONS (**http://topcons.cbr.su.se/)

List of protein candidates used as a source of TP (Transit Peptide) elements for *bicA* gene

Sr. No.	Name of Transit Peptide	Gene accession Number	Function of the Source protein of Transit Peptide	Length of Transit Peptide	No. of TM helices	Location of N- & C-terminus
1	TP1	AT1G70610	ATPase transporter	177 bp	6	Cytosolic
2	TP5	AT2G24820	Tic55, protein precursor import	144 bp	2	Cytosolic
3	TP9	AT5G17520	Maltose transporter RCP1	141 bp	10	Cytosolic
4	TP10	AT5G22830	Mg transporter	186 bp	2	Cytosolic

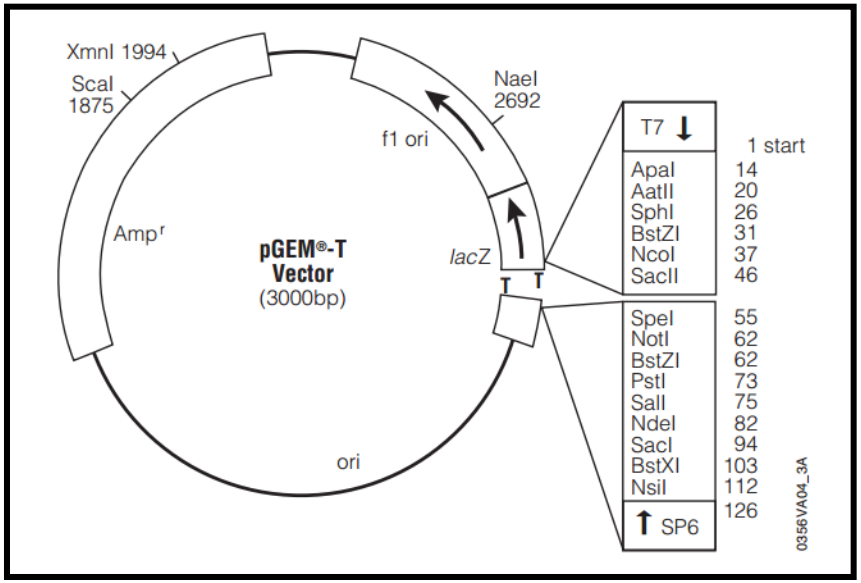
List of protein candidates used as a source of TP (Transit Peptide) elements for *sbtA* gene

Sr. No.	Name of Transit peptide	Gene accession Number	Function of the Source protein of Transit Peptide	Length of Transit Peptide	No. of TM helices	Location of N- & C-terminus
1	TP3	AT3G56160	Transporter Na/X	141 bp	7	Periplasmic
2	TP4	AT2G02590	Transporter multidrug	234 bp	6	Periplasmic

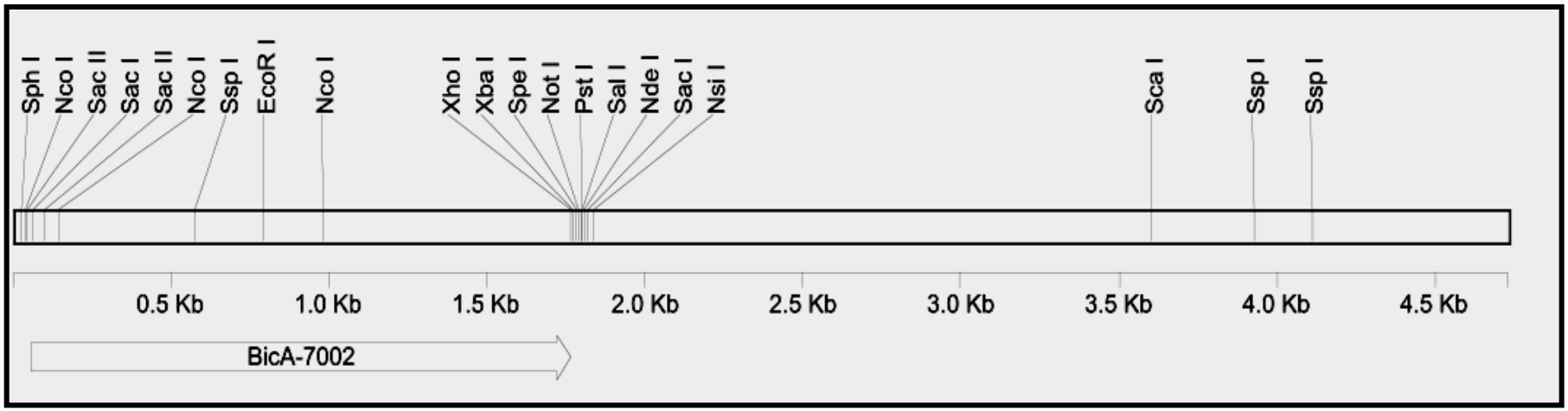
Preparation of *bicA* gene

bicA gene construct (~4.7kb)

- ***bicA* (*Synechococcus* sp. PCC 7002)**
- **Cloned in pGEM®-T vector**
- **pGEM®-T vector (3 kb)+*bicA* gene (1.701 kb)**
- **Procured as a kind gift from Dr. Price's lab**



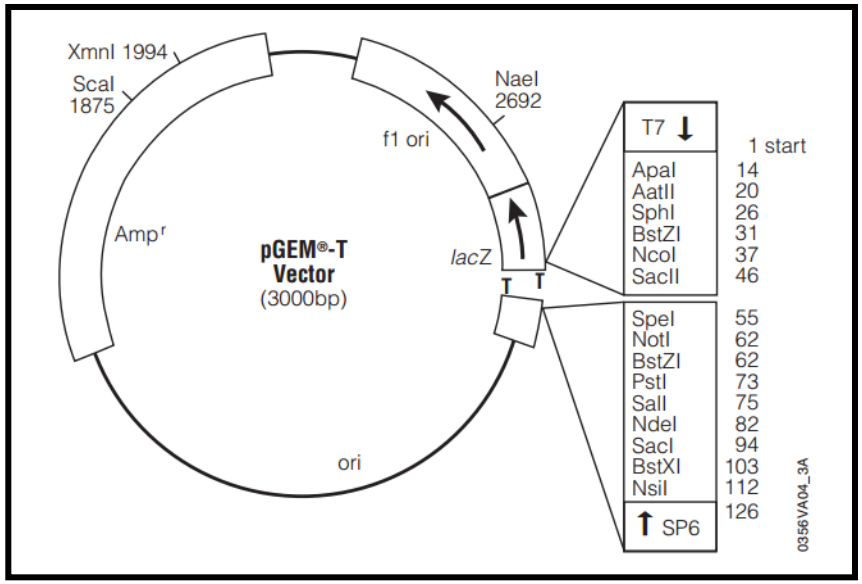
pGEM®-T vector map



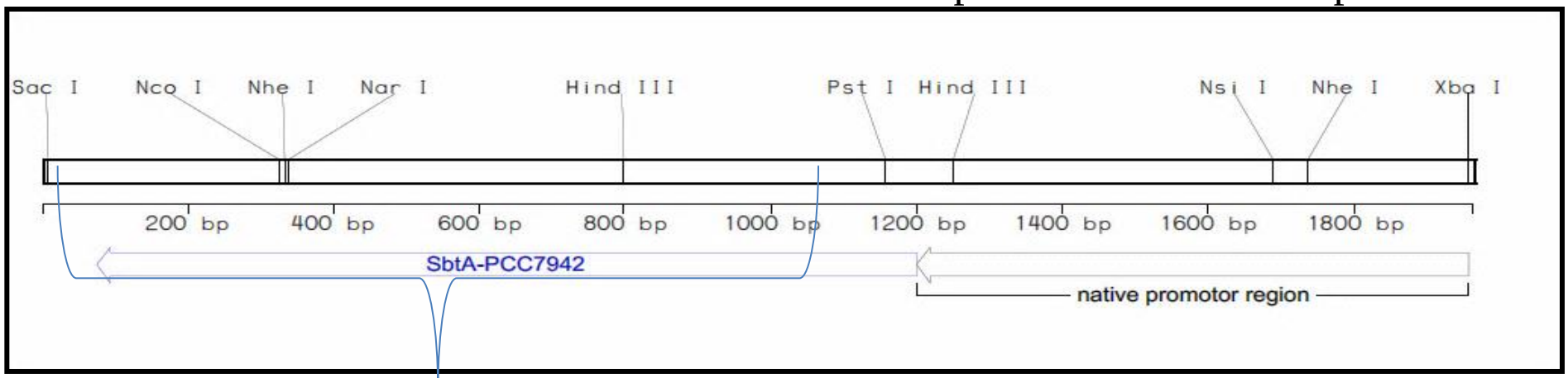
Preparation of *sbtA* gene

sbtA gene construct (~4.12 kb)

- ***sbtA* (*Synechococcus elongatus* PCC 7942)**
- **Cloned in pGEM®-T vector**
- **pGEM®-T vector (3 kb)+ *sbtA* gene (1.12kb)**
- **Procured as a kind gift from Dr. Price's lab**



pGEM®-T vector map



~1.12kb

Cloning strategy for *bicA* gene

***bicA* gene was taken out from pGEM®-T vector using *SacI* & *XbaI* restriction enzymes**

TP elements were amplified by primers which include restriction enzyme sites of *NdeI* and *SacI* at 5' and 3' end respectively

TP element and *bicA* gene were cloned into pCold IV vector B/W *NdeI* and *XbaI* sites

Sequencing of fused segment (TP+*bicA* gene) to confirm cloning

Fused segment was cloned into plant expression vector pRI 101-AN

Analysis of transformants

Cloning strategy for *sbtA* gene

***sbtA* gene was taken out from pGEM®-T vector using PCR with primers having *KpnI* & *BamHI* restriction enzymes**

TP elements were amplified by primers which include restriction enzyme sites of *NdeI* and *Kpn*I at 5' and 3' end respectively

TP element and *sbtA* gene were cloned into pCold I vector B/W *NdeI* and *BamHI* sites

Sequencing of fused segment (TP+*sbtA* gene) to confirm cloning

Fused segment was cloned into plant expression vector pRI 101-AN

Analysis of transformants

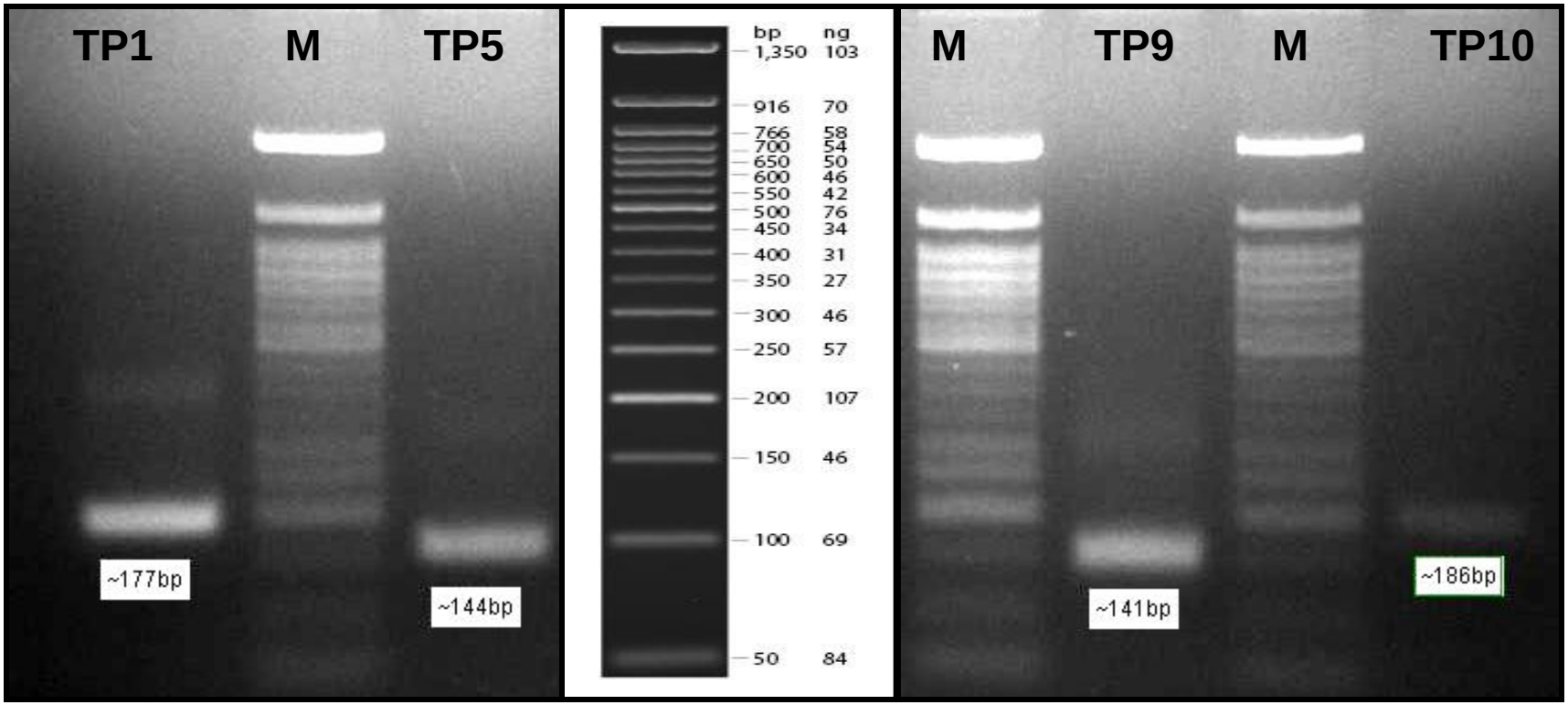
Primer sequences used for amplification of TP elements for *bicA* gene

S.N.	Primer name	Primer sequence (5' to 3')	Primer length
1	BicA -TP1-NdeI-F	CGA ACA CAT ATG GCT CAG CAA GTA CTC	27
2	BicA -TP1-SacI-R	CAC CGT GAG CTC ATT AAT CGA AGG C	25
3	BicA -TP5-NdeI-F	GCG TCC CAT ATG GCT GTT CCA TTT CTA	27
4	BicA -TP5-SacI-R	ATA GAG CTC GCG GAG GAG ACG TAG	24
5	BicA -TP9-NdeI-F	CGC CAT ATG GAA GGT AAA GCC ATC	24
6	BicA -TP9-SacI-R	ATA GAG CTC AGA GAC AGC ACC ACC	24
7	BicA -TP10-NdeI-F	GGC CCA CAT ATG GCG TTA ACT CC	23
8	BicA -TP10-SacI-R	GGC GAG CTC TTT CGA TCT CGA TAA AAC	27

Primer sequences used for amplification of TP elements for *sbtA* gene

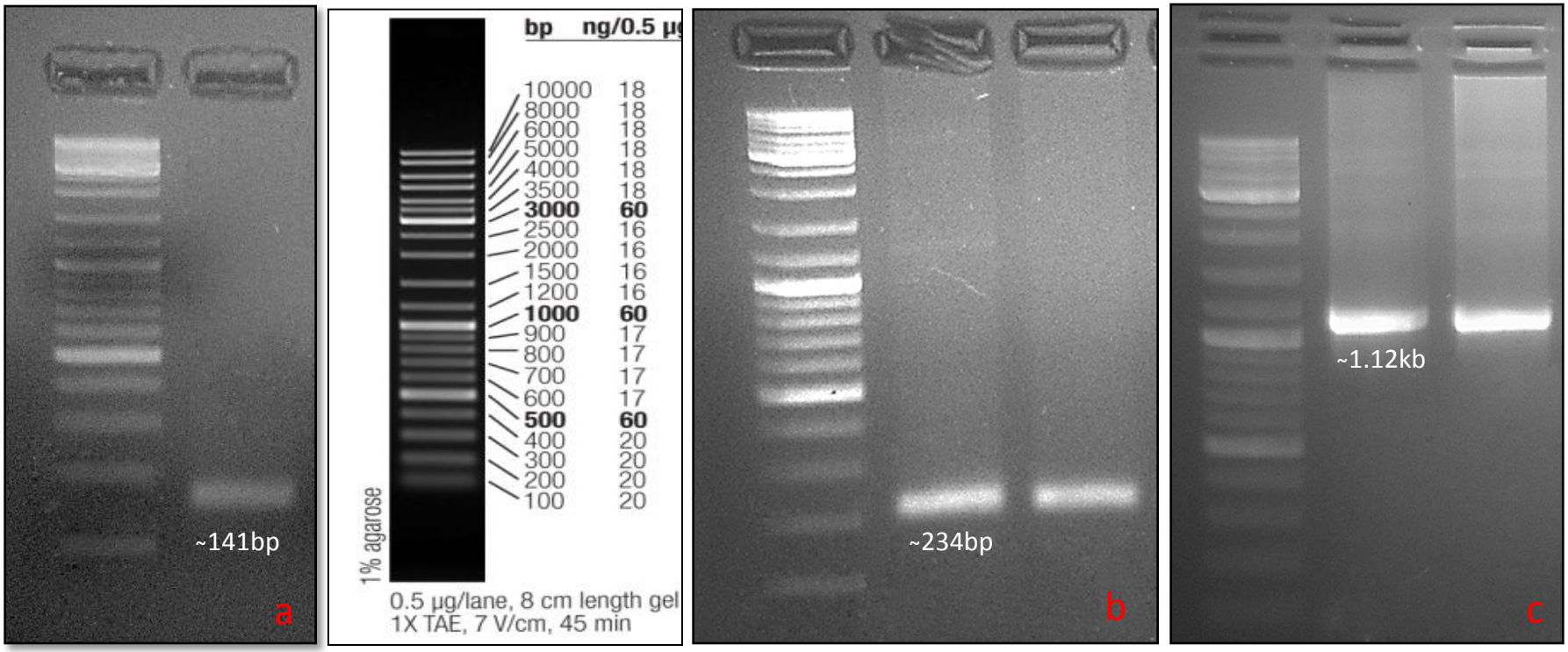
S.N.	Primer name	Primer sequences (5' to 3')	Primer length
1	SbtA - KpnI-F	AGAGGTACCCGTGGATTTCTTG	22
2	SbtA –BamHI-R	TAAGGATCCGCCGCCAATGG	20
3	SbtA –TP3-NdeI-F	ACTCATATGGCGATAGCTAGTA	22
4	SbtA –TP3-KpnI-R	TTGGGTACCTCTAATGGATCTG	22
5	SbtA –TP4-NdeI-F	ATACATATGGCGATTTCTACA	21
6	SbtA –TP4-KpnI-R	TTCGGTACCTTTGGTGTTT	19

PCR amplification of TP 1, 5, 9 and 10



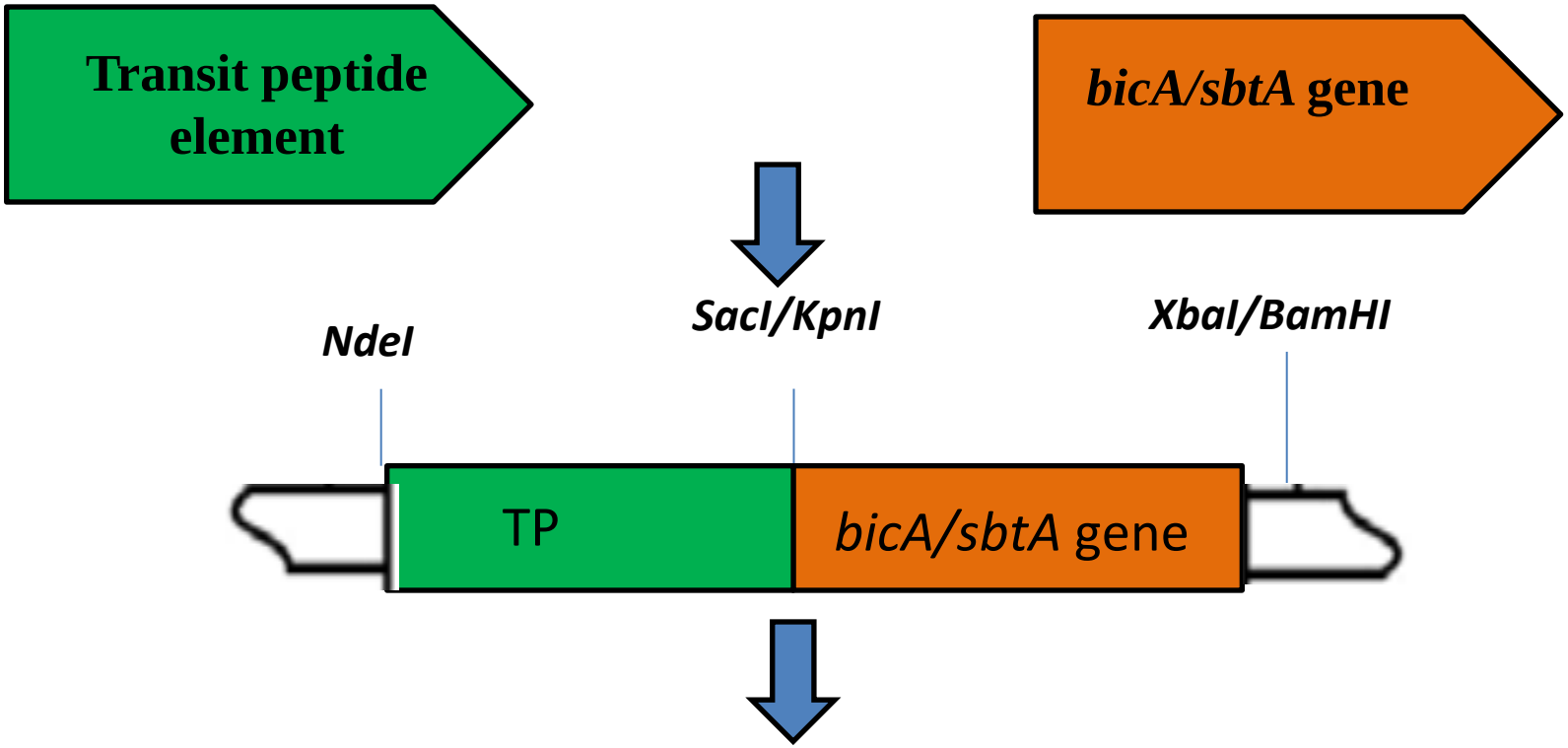
a. PCR product of TP1 (product size ~ 177 bp)
b. PCR product of TP5 (product size ~ 144 bp)
c. PCR product of TP9 (product size ~ 141 bp)
d. PCR product of TP10 (product size ~ 186 bp)

PCR amplification of TP3,TP4 and *sbtA*



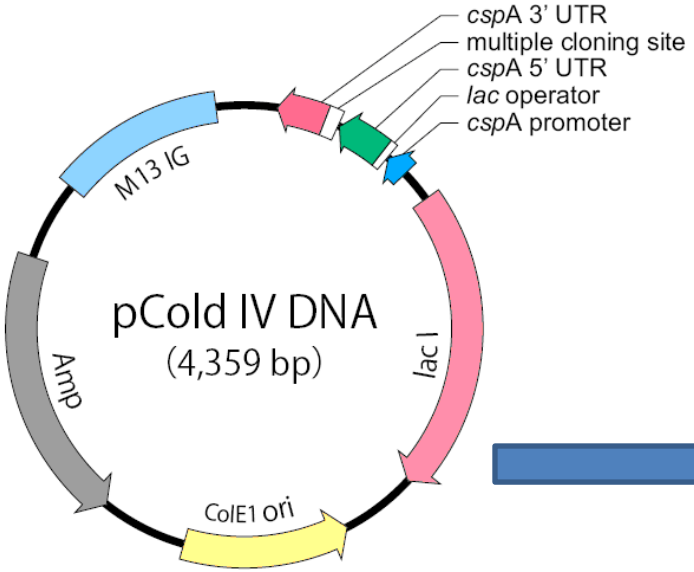
a. PCR product of TP3 (product size ~ 141 bp)
b. PCR product of TP4 (product size ~ 234 bp)
c. PCR of *sbtA* (product size ~ 1.12 kb)

Synthesis of fusion construct of TP elements and *bicA/sbtA* gene



Fusion constructs containing different transit peptide elements with *bicA/sbtA* genes were generated

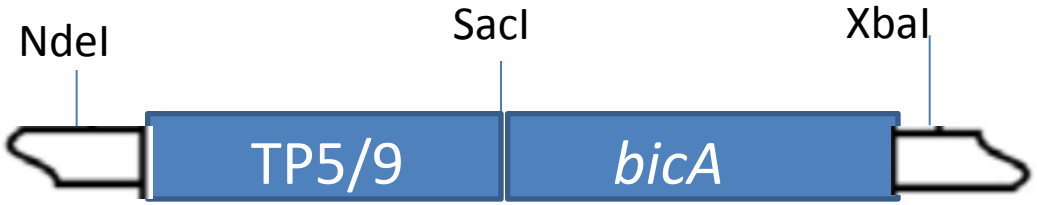
Cloning of TP element and *bicA* gene into pCold IV vector between *NdeI* and *XbaI* sites

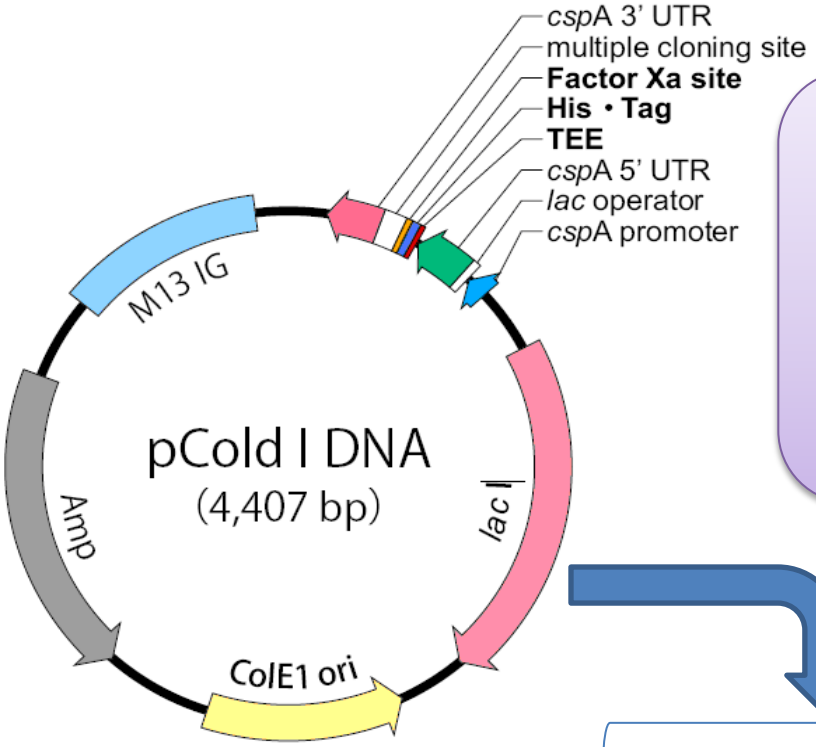


Cloning sites in pCold IV

Nde I	*Sac* I	*Kpn* I	*Xho* I	*Bam*H I	*Eco*R I	*Hind* III	*Sal* I	*Pst* I	*Xba* I
CATATG	GAGCTC	GGTACC	CTCGAG	GGATCC	GAATTC	AAGCTT	GTCGAC	CTGCAG	TCTAGA
His Met	Glu Leu	Gly Thr	Leu Glu	Gly Ser	Glu Phe	Lys Leu	Val Asp	Leu Gln	Ser Arg

TP5/9 along with *bicA* was cloned between *NdeI* and *XbaI* sites



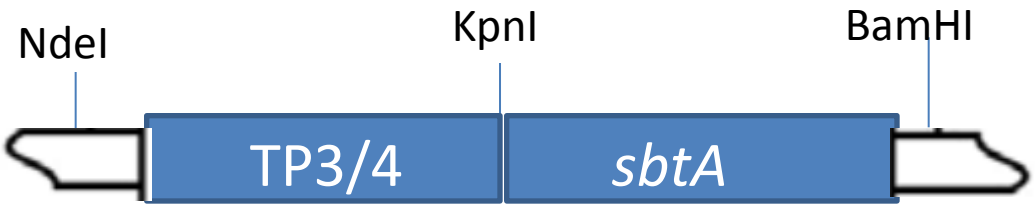


Cloning of TP element and *sbtA* gene into pCold I vector between *NdeI* and *BamHI* sites

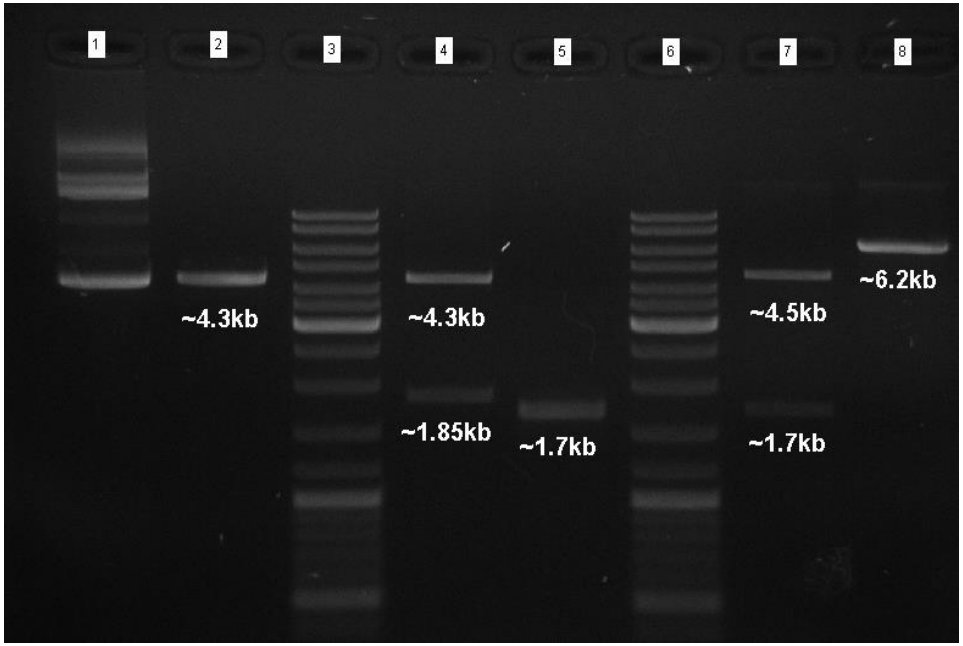
Cloning sites in pCold I

Nde I	*Sac* I	*Kpn* I	*Xho* I	*Bam*H I	*Eco*R I	*Hind* III	*Sal* I	*Pst* I	*Xba* I
CATATG	GAGCTC	GGTACC	CTCGAG	GGATCC	GAATTC	AAGCTT	GTCGAC	CTGCAG	TCTAG
His Met	Glu Leu	Gly Thr	Leu Glu	Gly Ser	Glu Phe	Lys Leu	Val Asp	Leu Gln	Ser Ar

TP3/4 along with *sbtA* was cloned between *NdeI* and *BamHI* sites

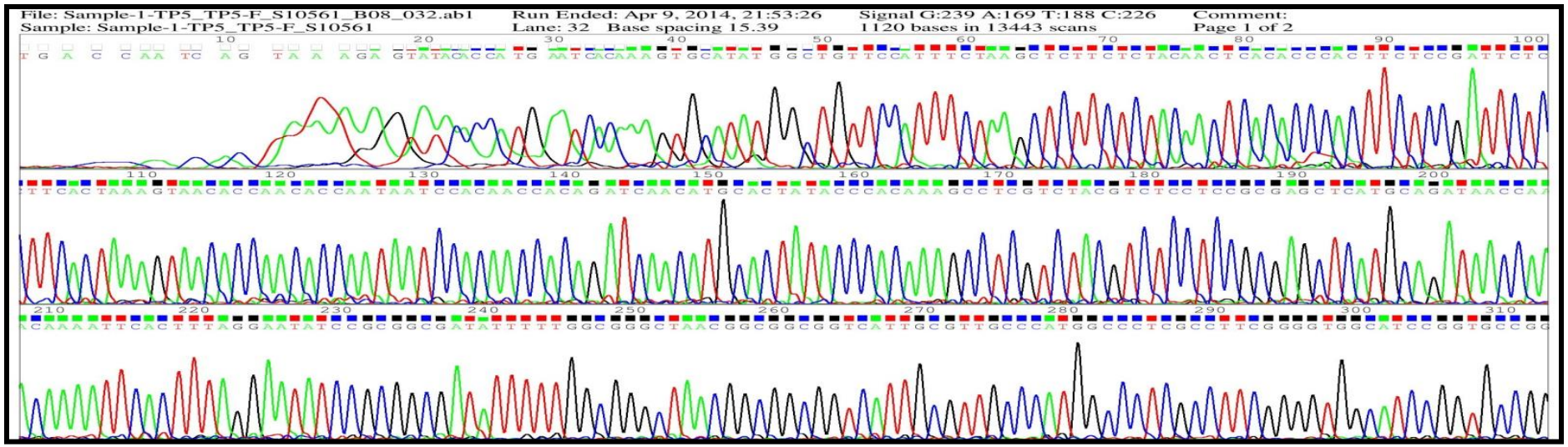


Clone confirmation (TP5+*bicA*+pCold IV) by restriction analysis

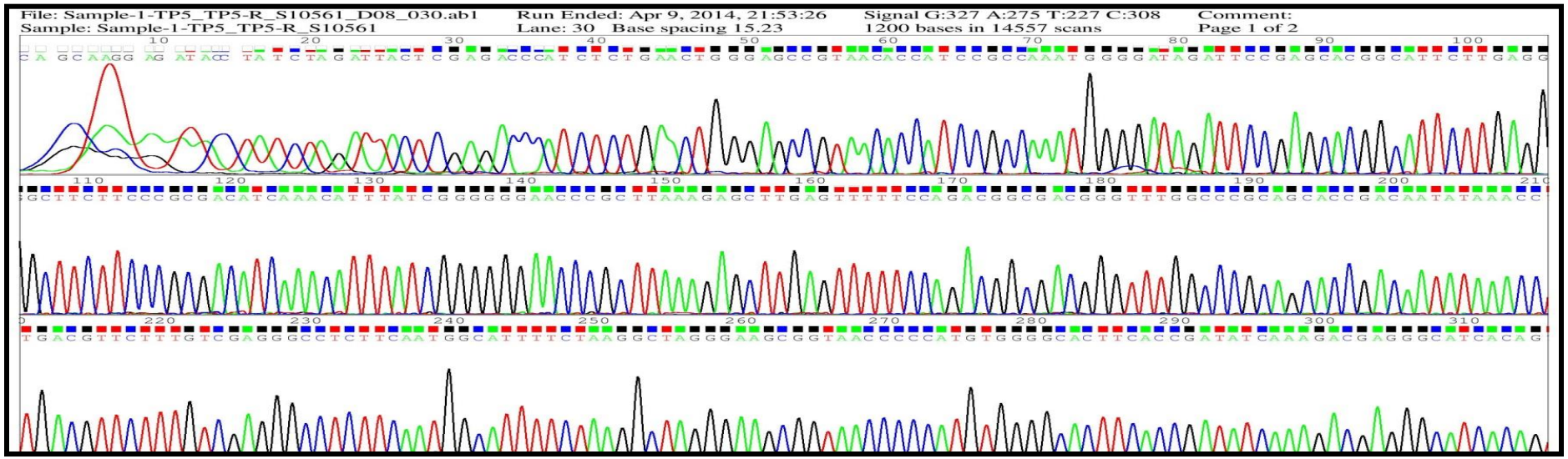


Lane 1: Uncut pCold IV plasmid
Lane 2 : Double digestion of pCold IV plasmid with *NdeI* and *XbaI* restriction enzymes
Lane 3 and 6: 100bp tp 10kb DNA ladder
Lane 4 : Double digestion of transformed/ recombinant pCold IV plasmid with *NdeI* and *XbaI* restriction enzymes
Lane 5: *bicA* gene double digested with *SacI*+*XbaI* restriction enzymes
Lane 7: Double digestion of transformed/ recombinant pCold IV plasmid with *SacI*+*XbaI* restriction enzymes
Lane 8: Digestion of transformed/ recombinant pCold IV plasmid with *SacI* enzyme

A

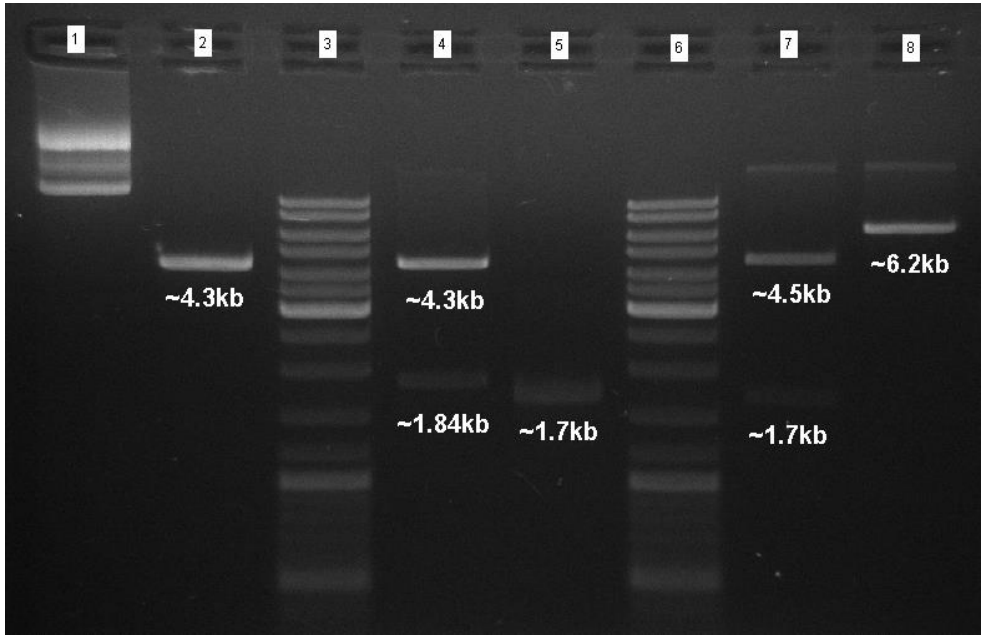


B



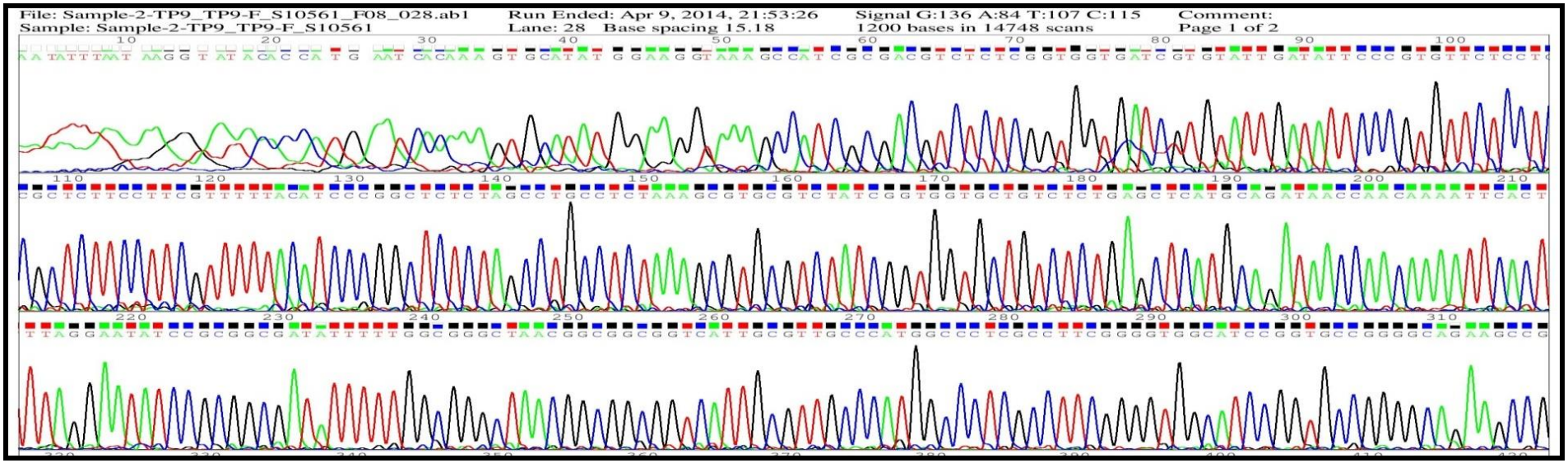
A. Chromatogram of forward sequencing B. Chromatogram of reverse sequencing

Clone confirmation (TP9+*bicA*+pCold IV) by restriction analysis

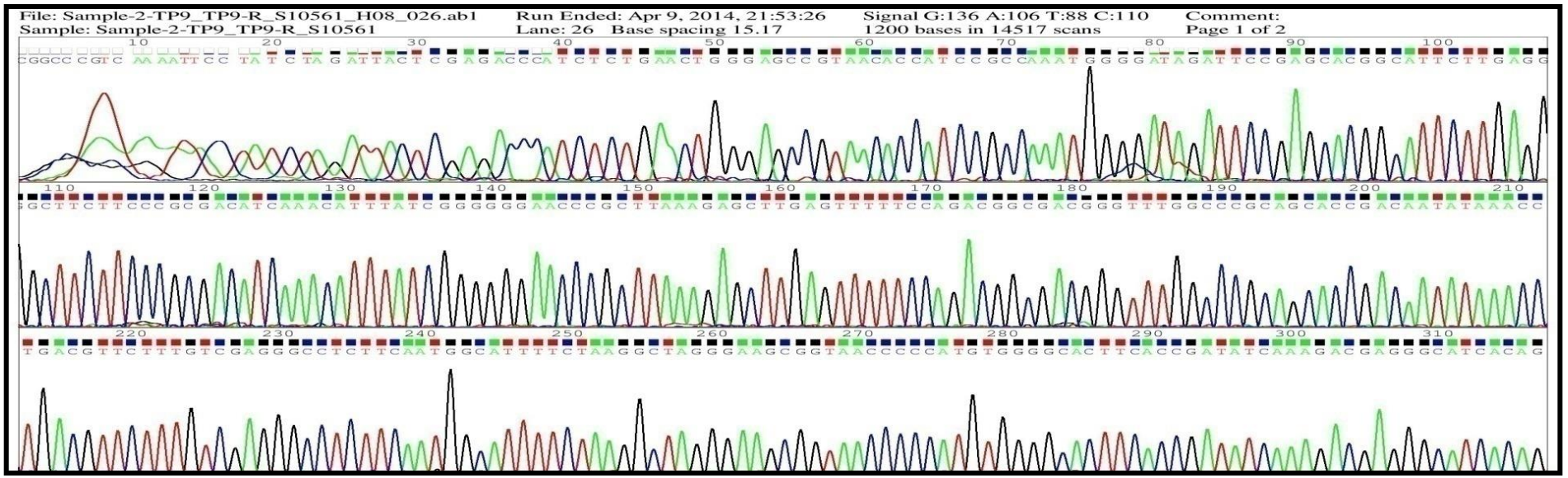


Lane 1: Uncut pCold IV plasmid
Lane 2 : Double digestion of pCold IV plasmid with *NdeI* and *XbaI* restriction enzymes
Lane 3 and 6: 100bp tp 10kb DNA ladder
Lane 4 : Double digestion of transformed/ recombinant pCold IV plasmid with *NdeI* and *XbaI* restriction enzymes
Lane 5: *bicA* gene double digested with *SacI*+*XbaI* restriction enzymes
Lane 7: Double digestion of transformed/ recombinant pCold IV plasmid with *SacI*+*XbaI* restriction enzymes
Lane 8: Digestion of transformed/ recombinant pCold IV plasmid with *SacI* enzyme

A

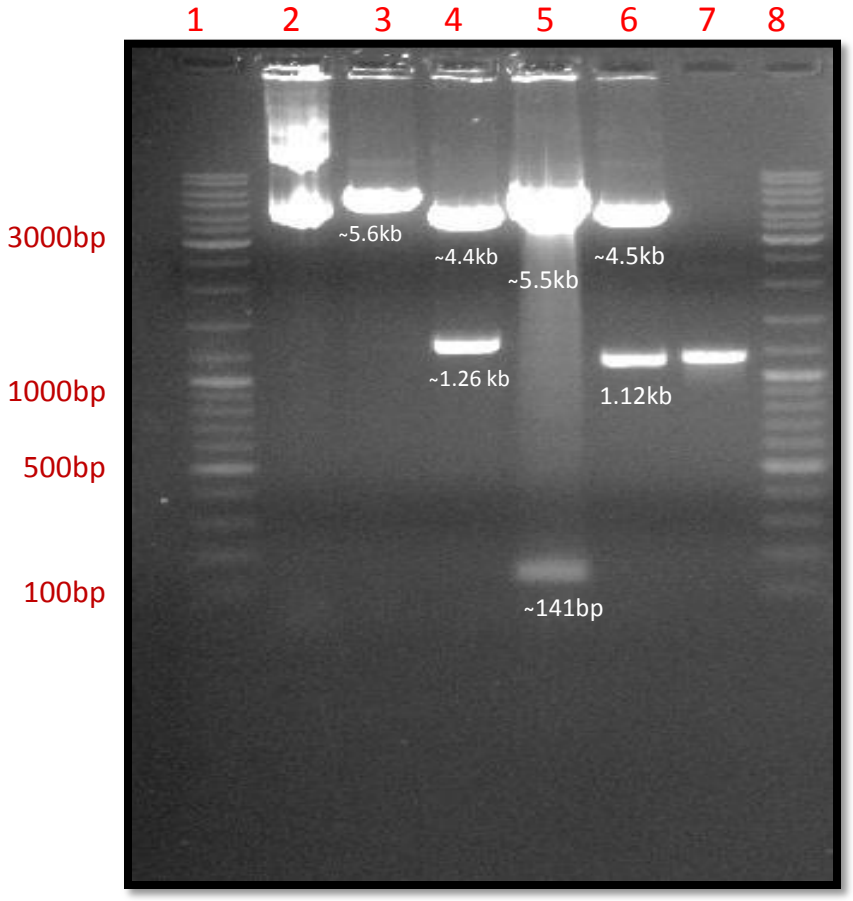


B



A. Chromatogram of forward sequencing B. Chromatogram of reverse sequencing

Clone (TP3 + *sbtA* + pCold I) confirmation by restriction analysis



Lane 1 - Marker(SM0331)

Lane 2 – pCold I + TP3+ *sbtA* plasmid

Lane 3 – pCold I + TP3+ *sbtA* plasmid digested with *BamHI*

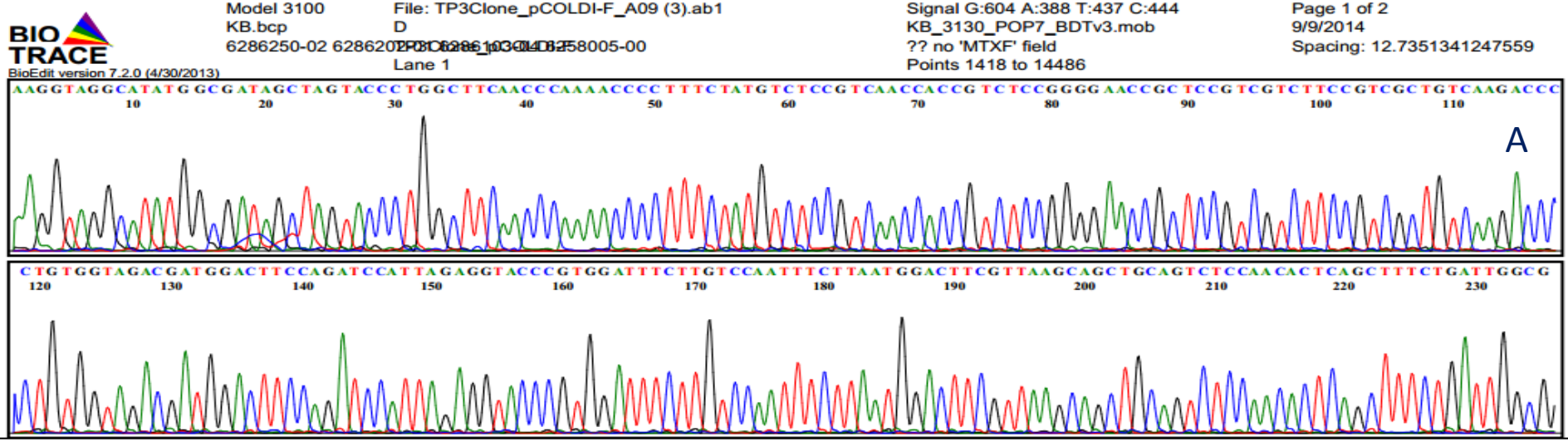
Lane 4 – pCold I + TP3+ *sbtA* double digested with *NdeI* and *BamHI*

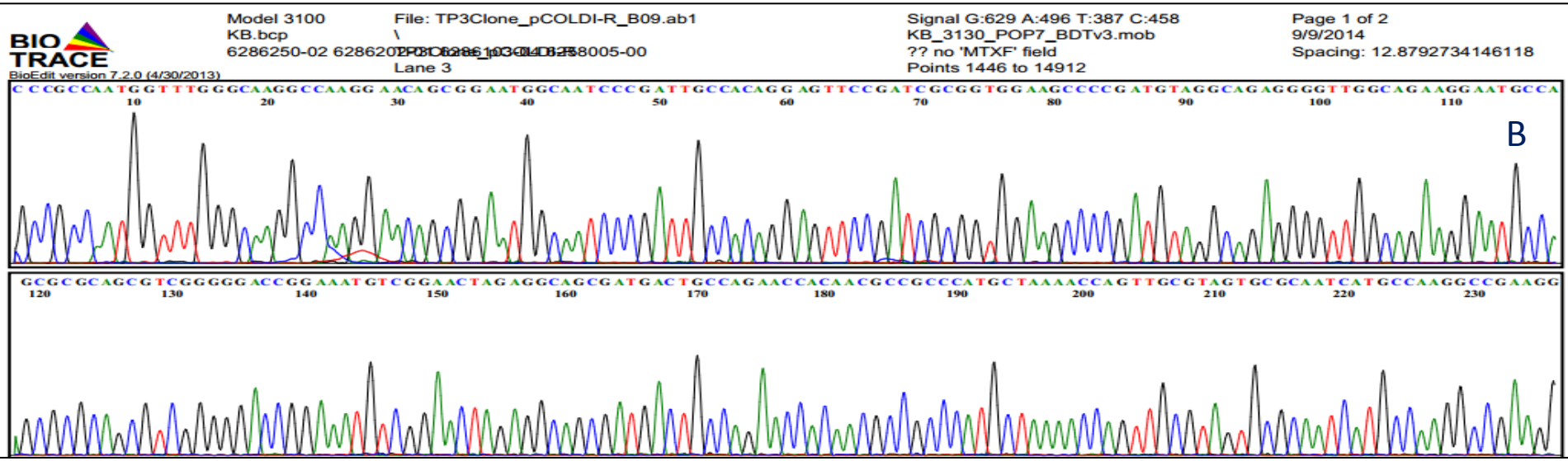
Lane 5 – pCold I+ TP3+ *sbtA* double digested with *NdeI* and *KpnI*

Lane 6 – pCold I + TP3+ *sbtA* double digested with *KpnI* and *BamHI*

Lane 7– PCR elute of *sbtA*

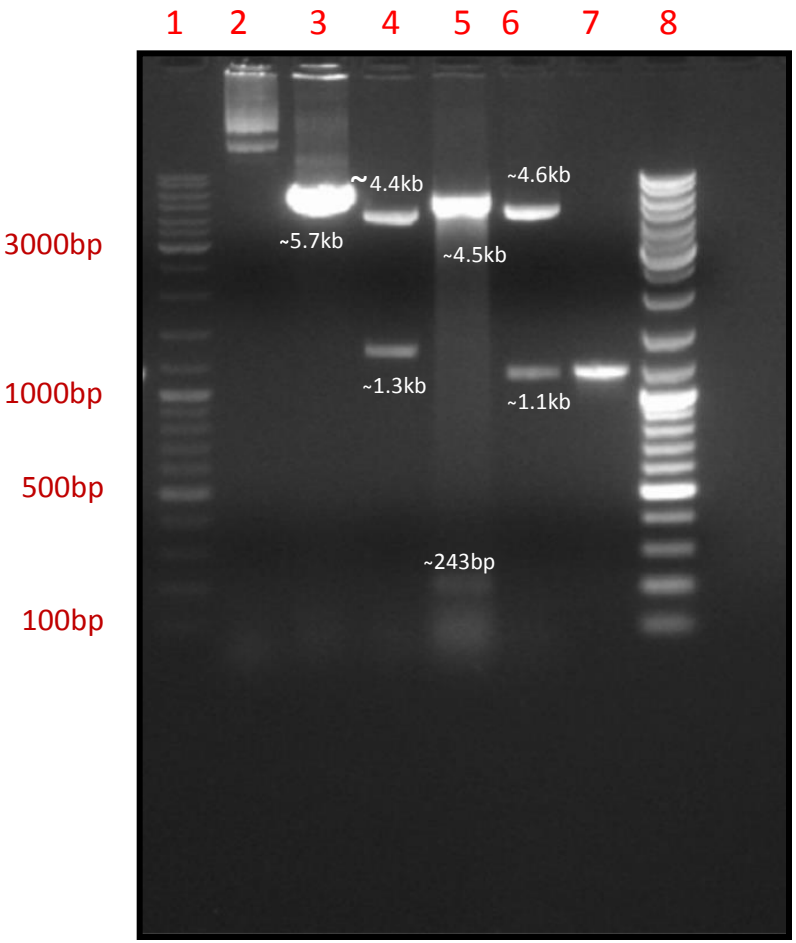
Lane 8 – Marker (SM0331)



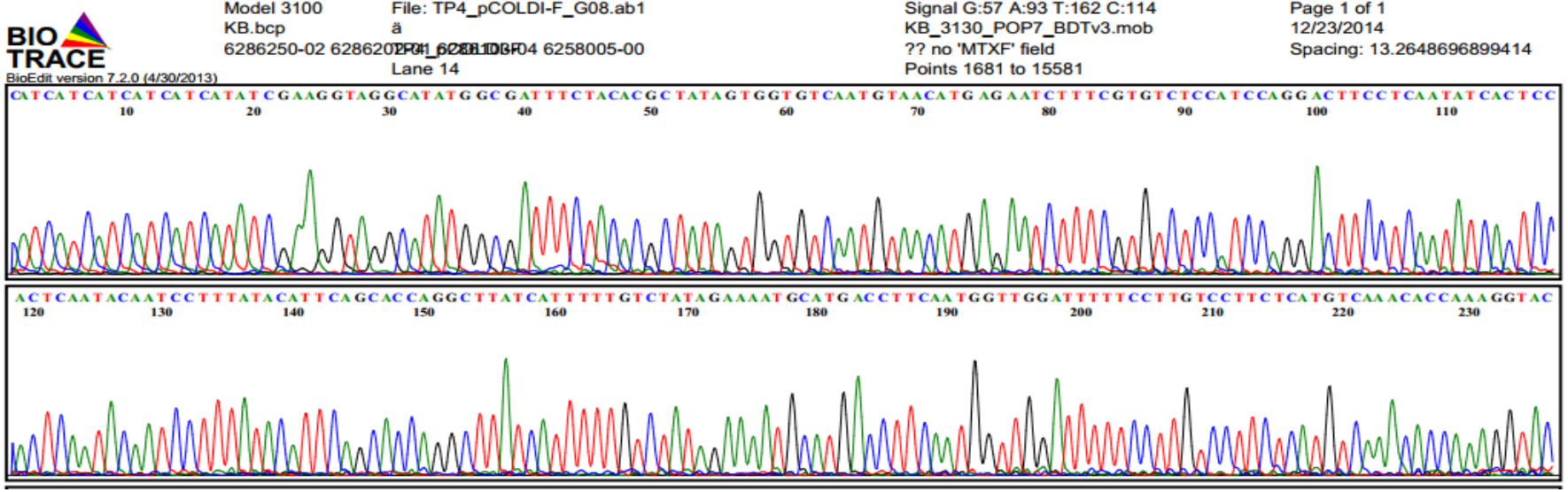


A. Chromatogram of forward sequencing B. Chromatogram of reverse sequencing

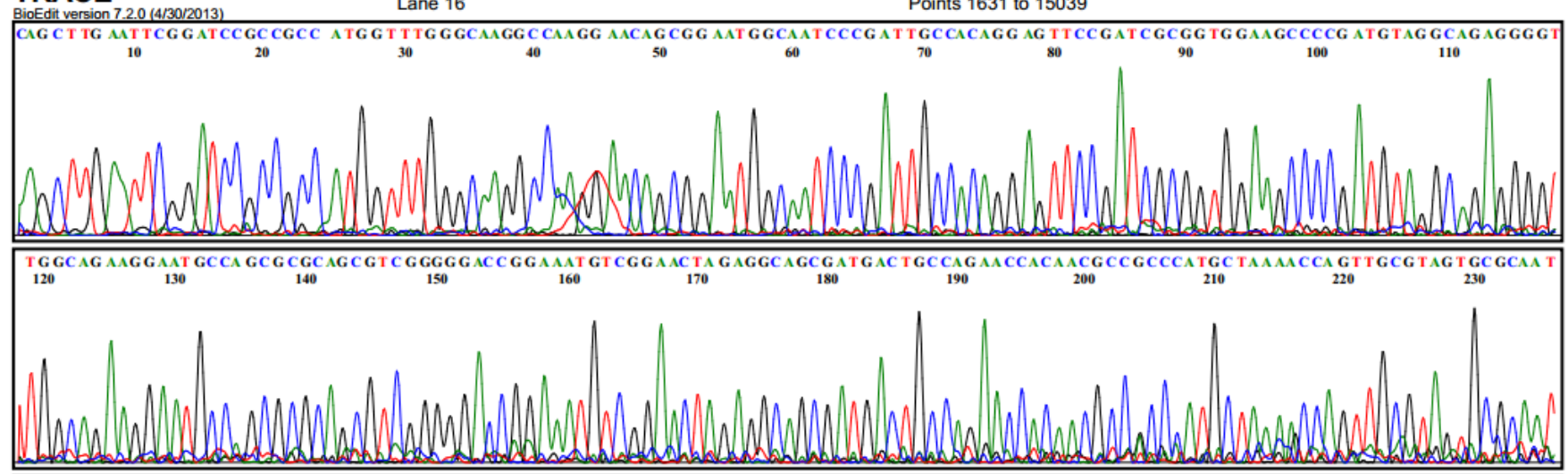
Clone (TP4 + *sbtA* + pCold I) confirmation by restriction analysis



Lane 1 - Marker (SM0331)
Lane 2 – pCold I+ TP4+ *sbtA* plasmid uncut
Lane 3 – pCold I + TP4+ *sbtA* plasmid digested with *BamHI*
Lane 4 – pCold I + TP4 + *sbtA* double digested with *NdeI* and *BamHI*
Lane 5 – pCold I + TP4+ *sbtA* double digested with *NdeI* and *KpnI*
Lane 6 – pCold I + TP4+ *sbtA* double digested with *KpnI* and *BamHI*
Lane 7 – PCR elute of *sbtA*
Lane 8 - Marker (SM0331)



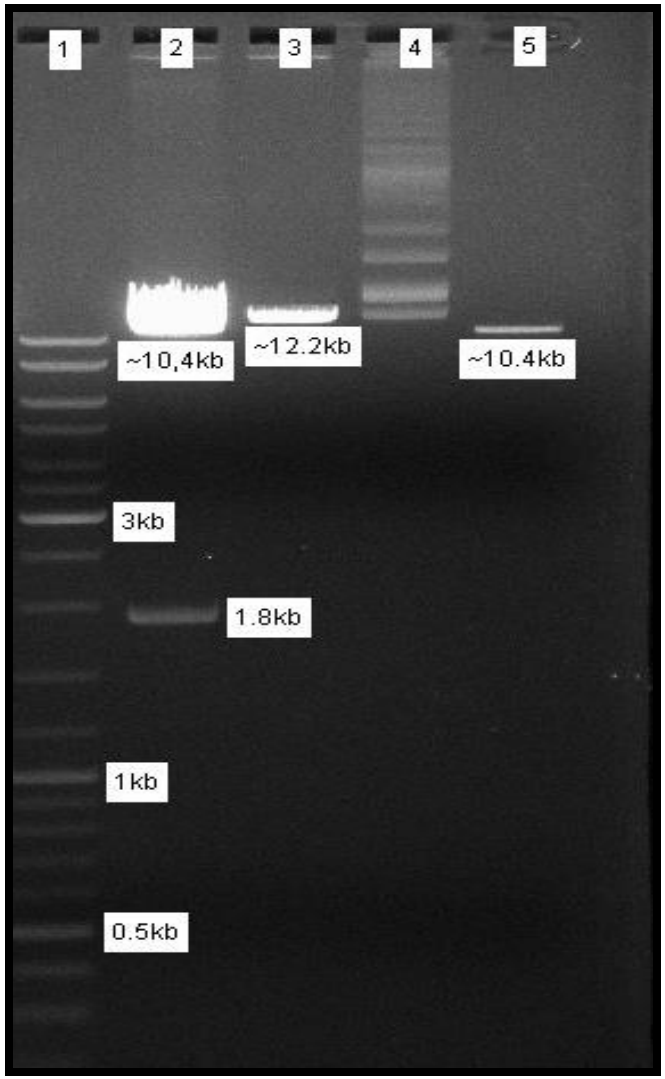
A



B

A. Chromatogram of forward sequencing B. Chromatogram of reverse sequencing

Clone confirmation (*gus* + pRI 101-AN) by restriction analysis



Lane 1: SM0331 DNA ladder (100bp to 10kb)

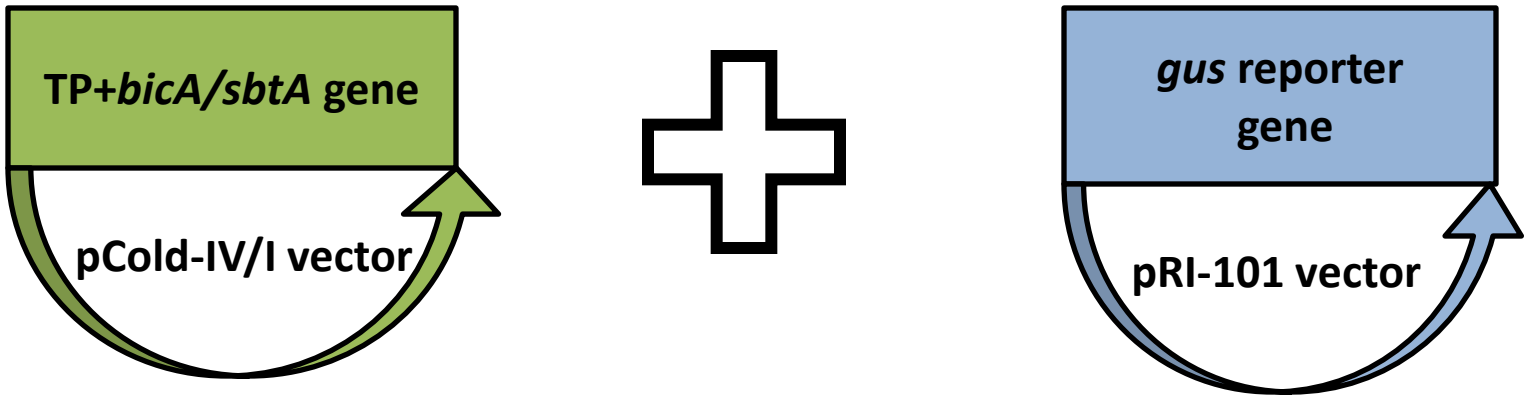
Lane 2: Double digestion of transformed/ recombinant pRI 101-AN plasmid with *BamHI* and *SacI* enzymes

Lane 3: Digestion of transformed/ recombinant pRI 101-AN with *BamHI* enzyme

Lane 4: Transformed/ recombinant pRI 101-AN plasmid uncut

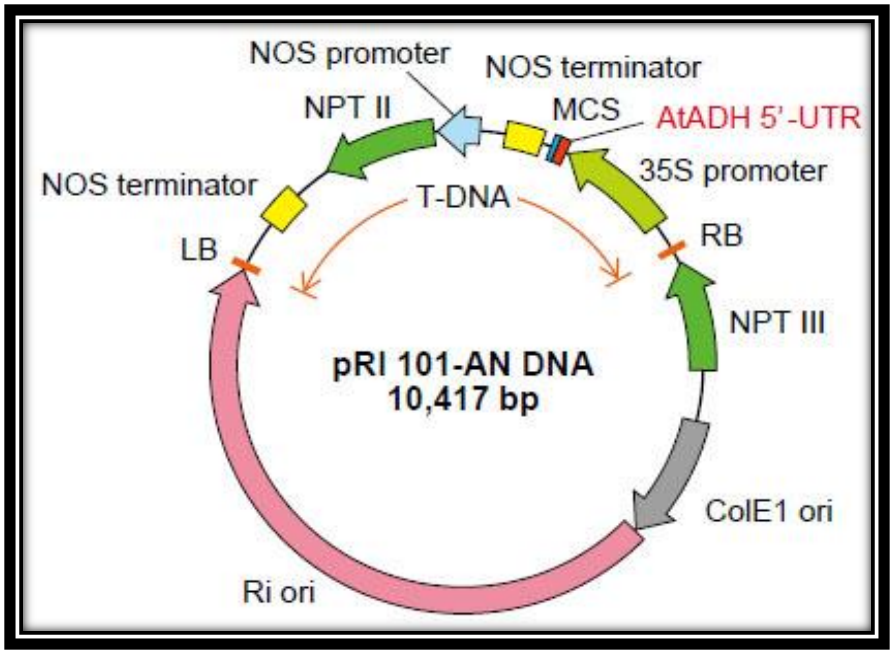
Lane 5: Double digestion pRI 101-AN plasmid with *BamHI* and *SacI* enzymes

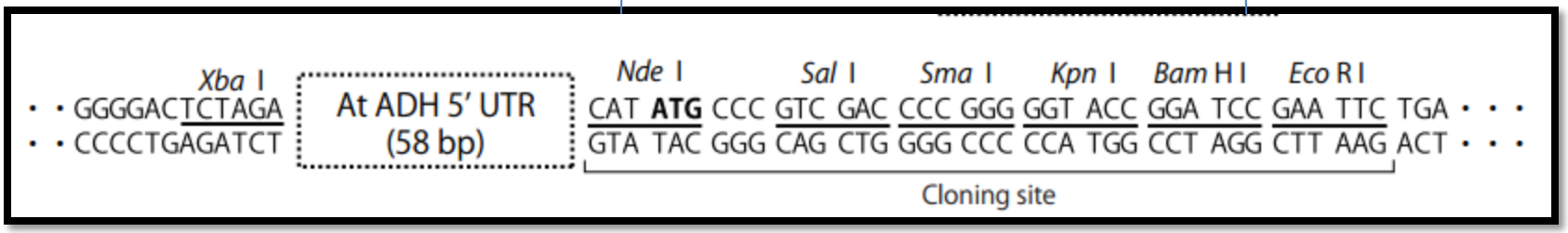
Contd…..



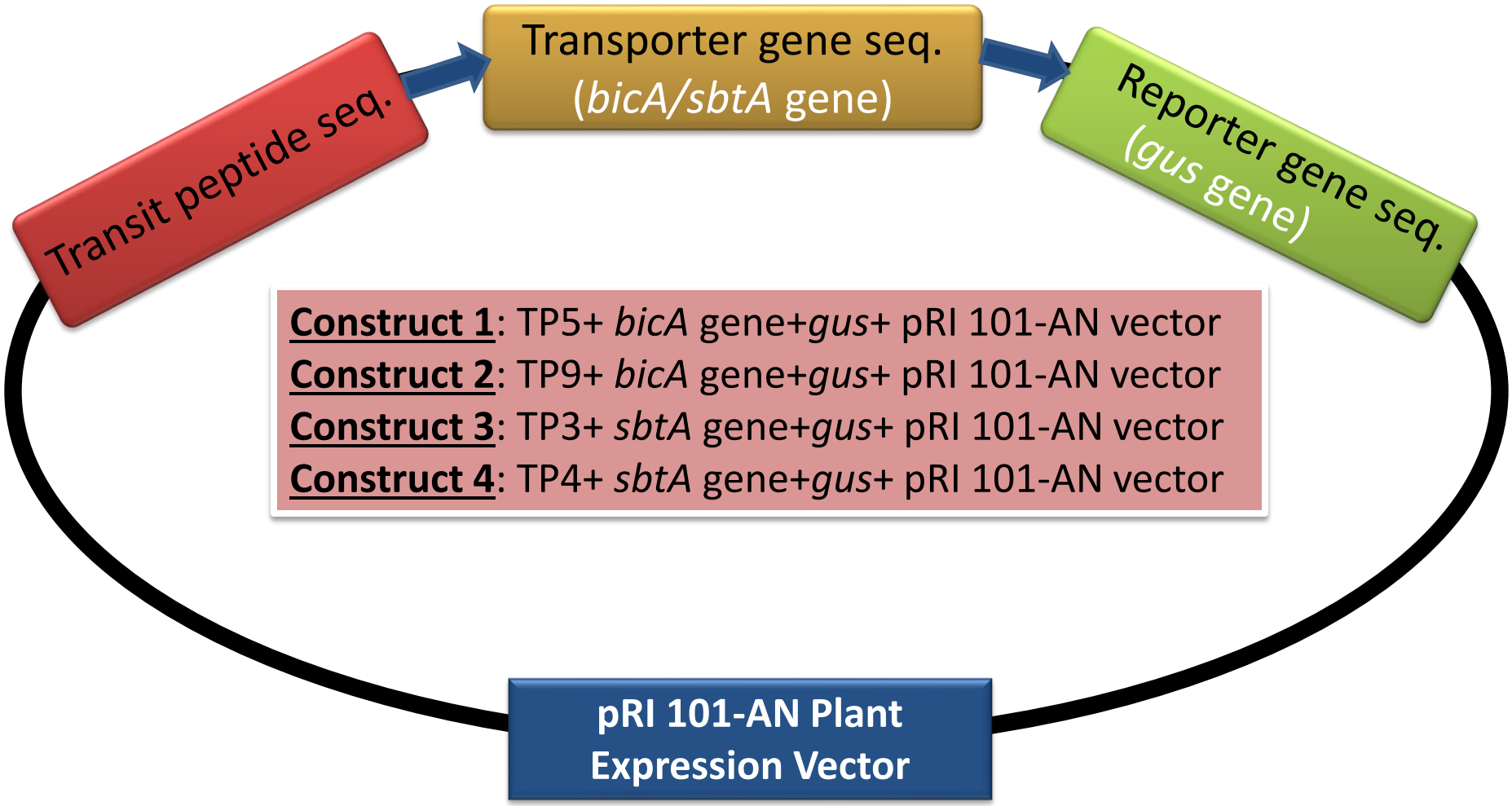
Fused genes (TP+*bicA/sbtA* gene) were cloned into *gus* reporter gene containing pRI 101-AN plant expression vector by sticky end cloning

Cloning of fusion constructs in plant expression vector (pRI 101-AN DNA)

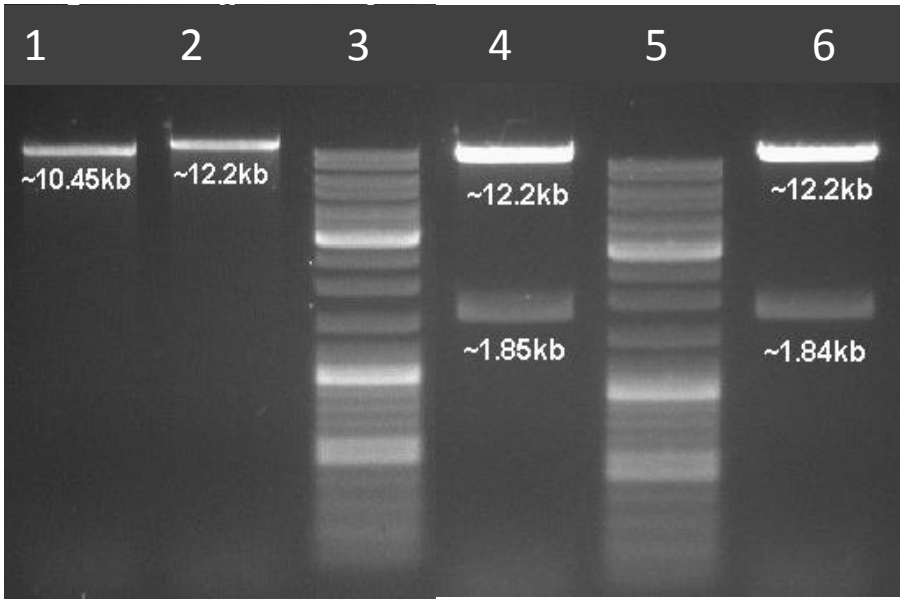




Fusion Construct preparation



Clone (pRI 101 + *bicA*+TP5 /TP9) confirmation by restriction analysis



Lane 1: Digestion of pRI 101 vector with *BamHI* enzyme

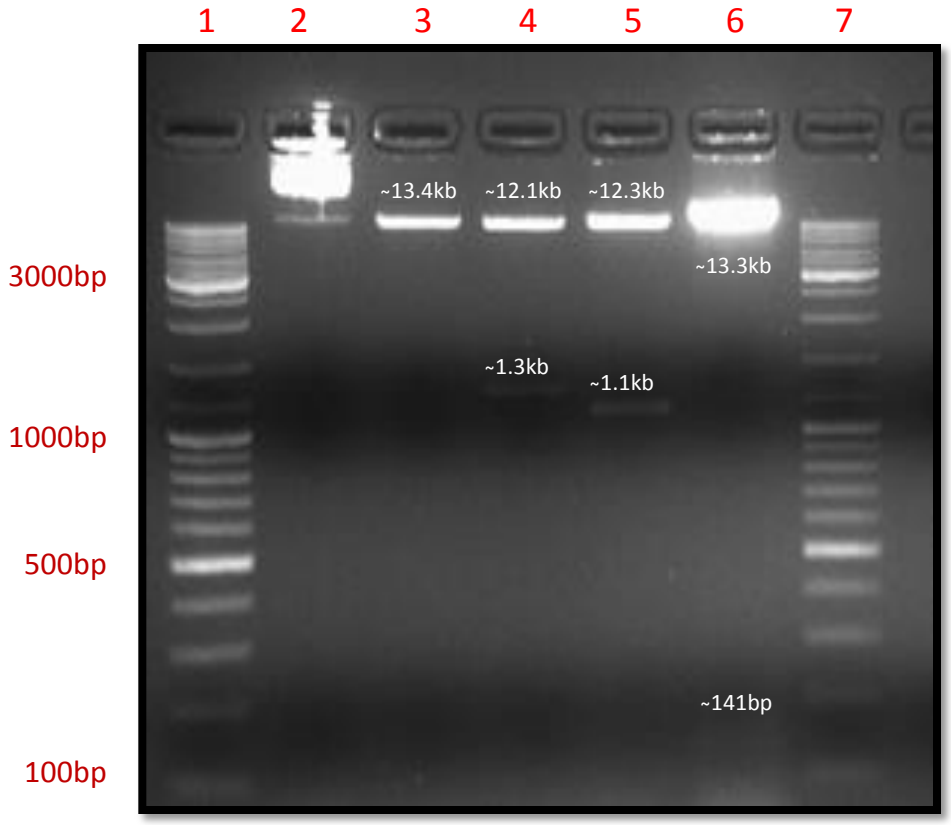
Lane 2: Digestion of "*gus* gene+ pRI 101" with *BamHI* enzyme

Lane 3 & 5 : SM0331 DNA ladder (100bp to 10kb)

Lane 4: Double digestion of "TP5+*bicA*+ *gus* gene+ pRI 101" with *NdeI*+*BamHI* enzymes

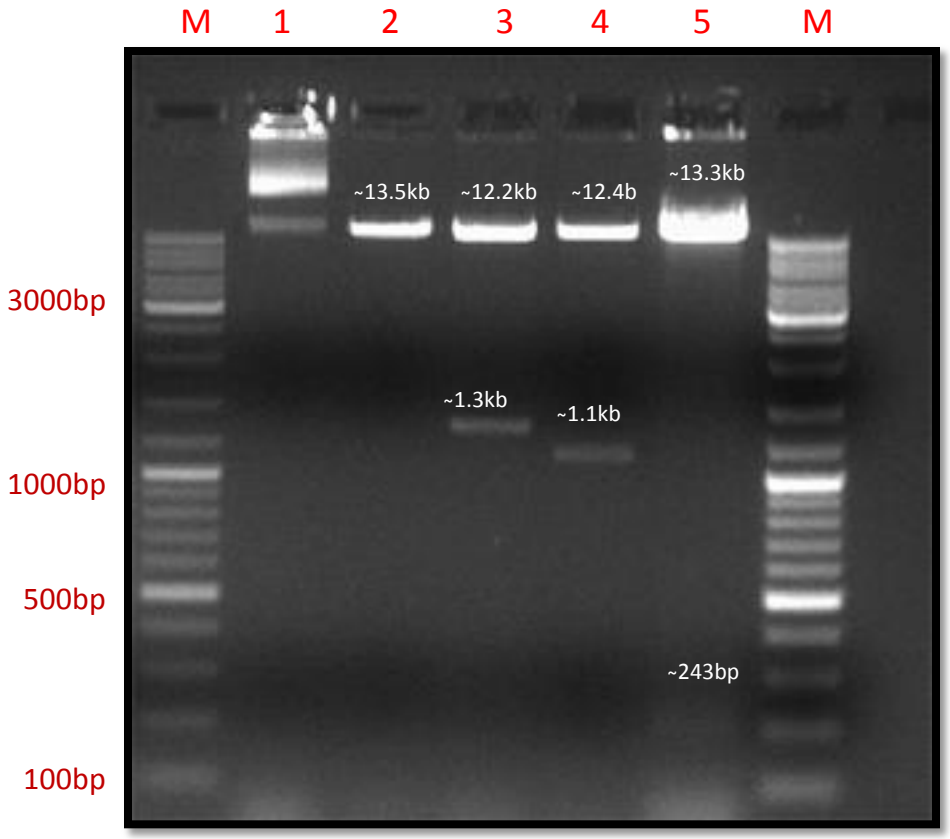
Lane 6: Double digestion of "TP9+*bicA*+ *gus* gene+ pRI 101" with *NdeI*+*BamHI* enzymes

Clone (pRI 101 + *sbtA*+TP3) confirmation by restriction analysis



Lane 1 - Marker (SM0331)
Lane 2 - pRI101+ TP3+ *sbtA* plasmid uncut
Lane 3 - pRI101+ TP3+ *sbtA* plasmid digested with *BamHI*
Lane 4 - pRI101+ TP3+ *sbtA* double digested with *NdeI* and *BamHI*
Lane 5 - pRI101+ TP3+ *sbtA* double digested with *KpnI* and *BamHI*
Lane 6 - pRI101+ TP3+ *sbtA* double digested with *NdeI* and *KpnI*
Lane 7 -Marker(SM0331)

Clone (pRI 101 + *sbtA*+TP4) confirmation by restriction analysis



M - Marker(SM0331)
Lane 1 – pRI101+ TP4+ *sbtA* plasmid uncut
Lane 2 – pRI101+ TP4+ *sbtA* plasmid digested with *BamHI*
Lane 3 – pRI101+ TP4+ *sbtA* double digested with *NdeI* and *BamHI*
Lane 4 – pRI101+ TP4+ *sbtA* double digested with *KpnI* and *BamHI*
Lane 5 –pRI101+ TP4+ *sbtA* double digested with *NdeI* and *KpnI*
M - Marker(SM0331)

Electroporation

- Recombinant pRI 101-AN constructs were transformed into *Agrobacterium tumefaciens* GV3101/LBA4404 strain by electroporation



Electroporation conditions:
Program- Agr (For *Agrobacterium tumefaciens*)
Cuvette size- 0.1mm
Voltage- 2.20kv
Pulses- 5 pulses (each with 5ms)

- *Agrobacterium* mediated plant transformation was performed using below mentioned methods:

1. Co-culture method
2. Floral-dip method

Plant transformation using bicA/sbtA construct

Particle bombardment | **Agro-infection** | **Floral dip**



PDS-1000/He system







Explant : *Nicotiana tabacum* leaf
Constructs:
bicA+TP5, bicA+TP9
sbtA+TP3, sbtA+TP4

Explant : *Nicotiana tabacum* leaf and callus
Constructs:
bicA+TP5, bicA+TP9
sbtA+TP3, sbtA+TP4

Explant : *Arabidopsis thaliana* floral buds
Constructs:
bicA+TP5, bicA+TP9
sbtA+TP3, sbtA+TP4

Protocol of fluorometric GUS assay

Tobacco leaves were bombarded with recombinant plasmids of bicA and sbtA

↓

Bombarded leaves were incubated for 48 hrs (16/8hr photoperiod) at 25°C

↓

Leaves were crushes in liq.N_2 and suspended in extraction buffer

↓

Centrifuged at 13,000 rpm for 20 min at 4°C, 90 µl supernatant was added to 10 µl pre-warmed assay buffer, incubated at 55°C for 20 mins

↓

20 µl methanol was added and incubated at 37°C for 2 hrs followed by addition of 900 µl carbonate stop buffer

↓

Measured the fluorescence in Spectroflurometer Fluoroskan Ascent (Ex. 365nm, Em. 455nm), Specific GUS activity was calculated and plotted on graph

Contd…..

MUG (non-fluorescent) $\xrightarrow{GUS}$

glucuronic acid + 4MU (fluorescent)

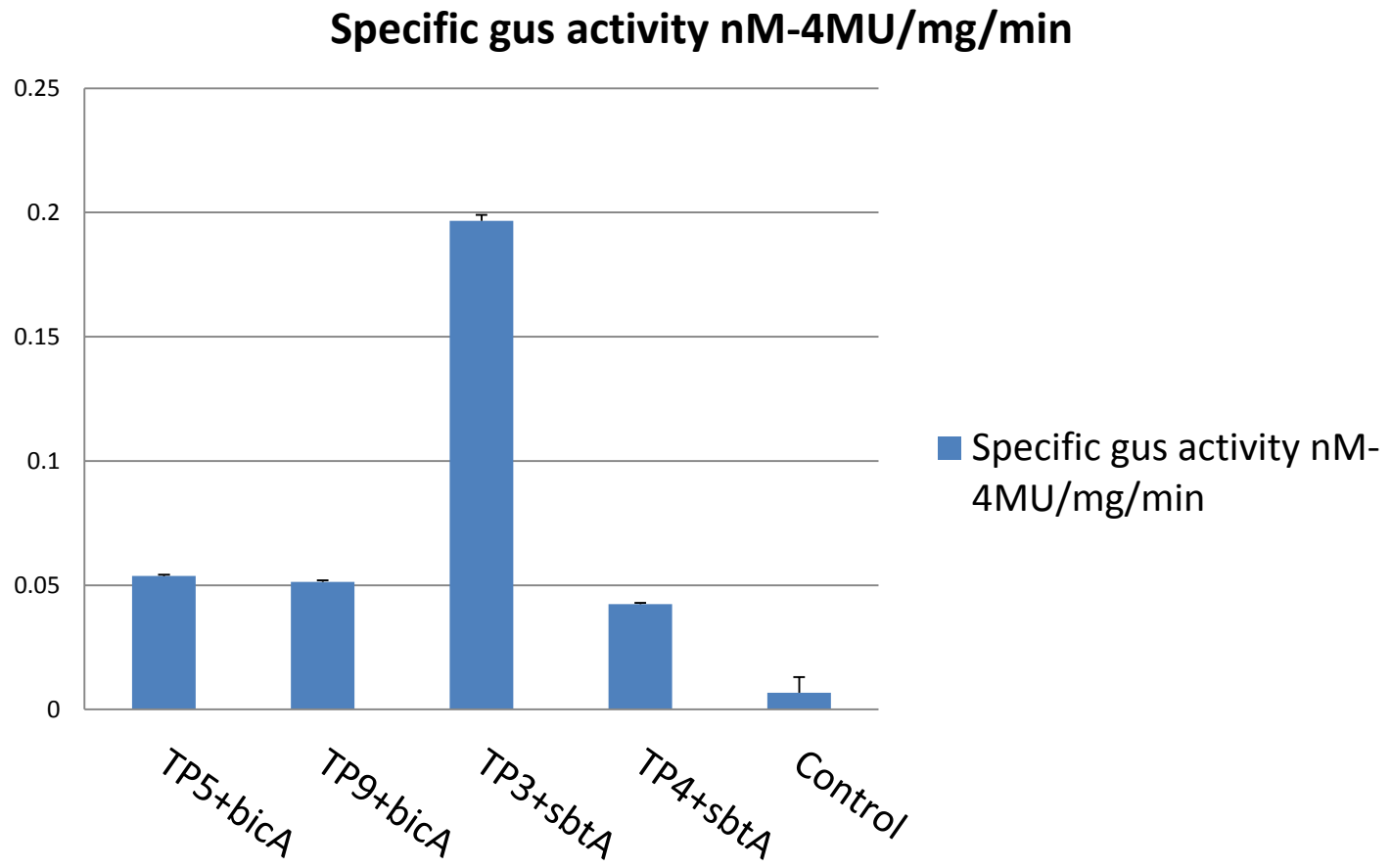
Gus specific activity:

no. of nano moles of MU/mg of protein/minute = **0.07 X/Y**

X=rfu of each construct

Y=total protein concentration for each construct

GUS fluorometric assay for bicA/sbtA constructs

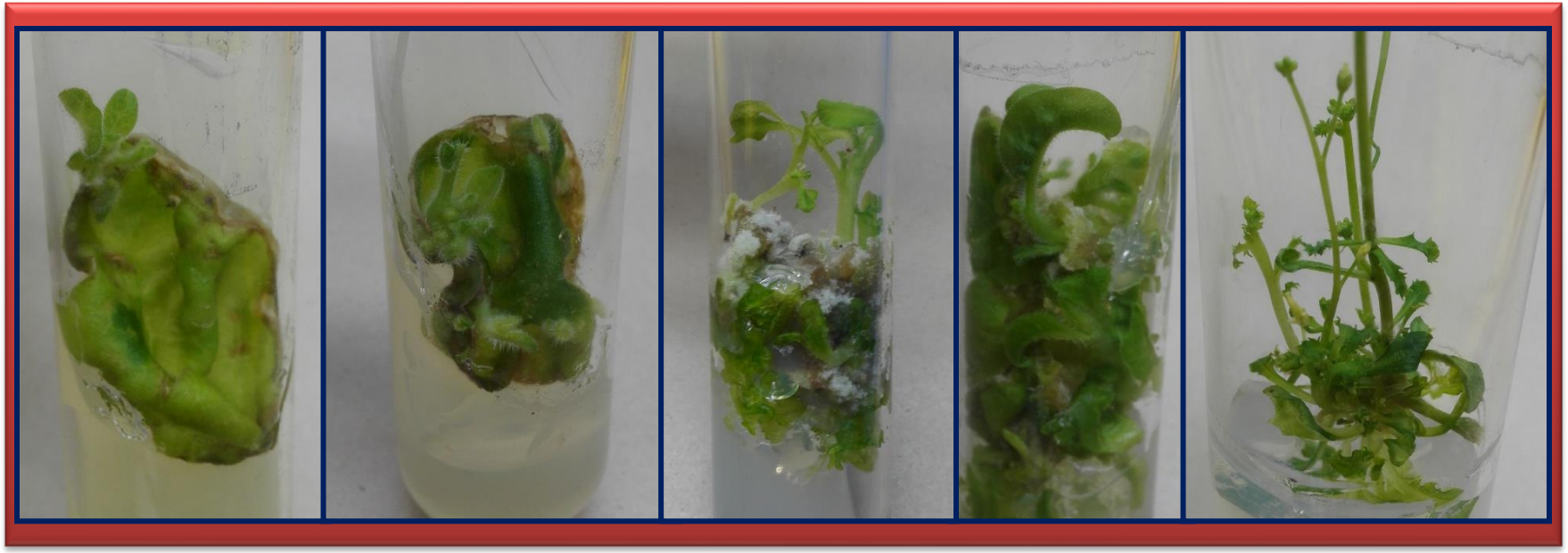


Agrobacterium mediated gene transformation of *Nicotiana tabacum* using leaf explants



A. Potted plant of *Nicotiana tabacum*
B. Leaf explant
C. Co-culture of explants with Agrobacterium containing gene of interest
D. Inoculation of the leaf discs on to co-cultivation media MS + BAP (2.0 mg/L) + NAA (0.2 mg/L)
E. Transfer of the explants to the regeneration medium(MS + BAP (2.0 mg/L) + NAA (0.2 mg/L), Kanamycin (50 mg/L) and Cefotaxime (250 mg/L)

Regeneration of shoots in Agro-infected tobacco leaves with Agro GV3101 (pRI 101-AN+ TP3/TP4+ *sbtA*) construct



Media composition: MS + BAP (2.0 mg/L) + NAA (0.2 mg/L), Kanamycin 50 mg/L and Cefotaxime 250 mg/L

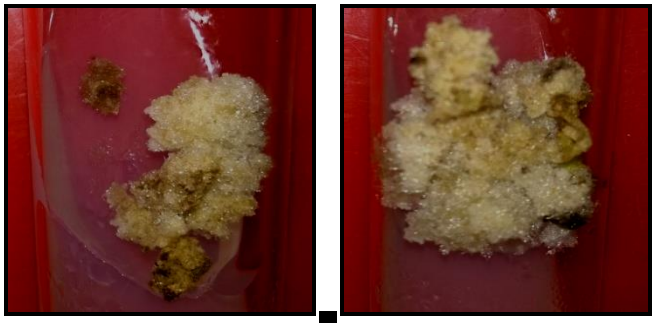
Agrobacterium mediated plant transformation using callus tissue as explant (pRI 101-AN+ TP9+ *bicA)*



[A]

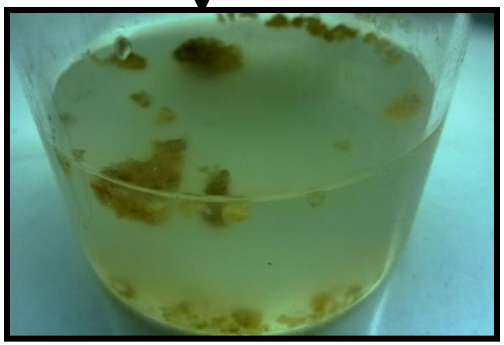
Callus induction from leaf

2,4-D 4mg/lit



[B]

Agroinfection



[C]

Regeneration



[D]

A. *Nicotiana tabacum* plant
B. 4-5 week old callus obtained from leaf explants of *Nicotiana tabacum*
C. Callus tissue dipped in *Agrobacterium* culture for agroinfection
D. Culturing of agroinfected callus on regeneration media (MS+2,4-D,4 mg/lit, kanamycin 50 mg/lit, cefotaxime 75 mg/lit)

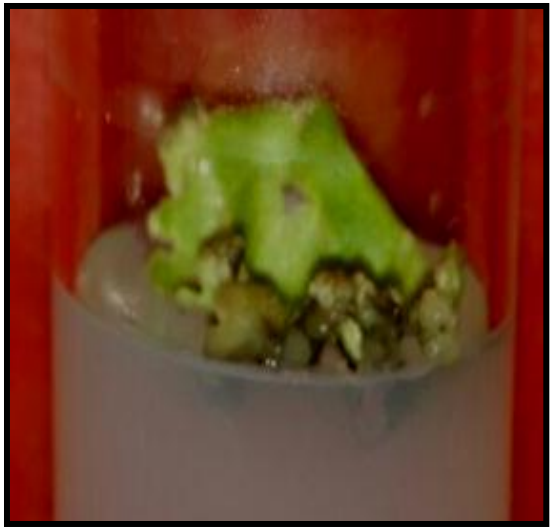
Agrobacterium mediated plant transformation using leaf tissue as explants (pRI 101-AN+ TP5+ *bicA)*



[A]



[B]



[C]

A. *Nicotiana tabacum* plant.
B. Leaf explants of *Nicotiana tabacum* dipped in *Agrobacterium* culture for agroinfection.
C. Culturing of agroinfected leaves on regeneration media (MS+2,4-D, 4 mg/L, kanamycin 50 mg/L, cefotaxime 75 mg/L)

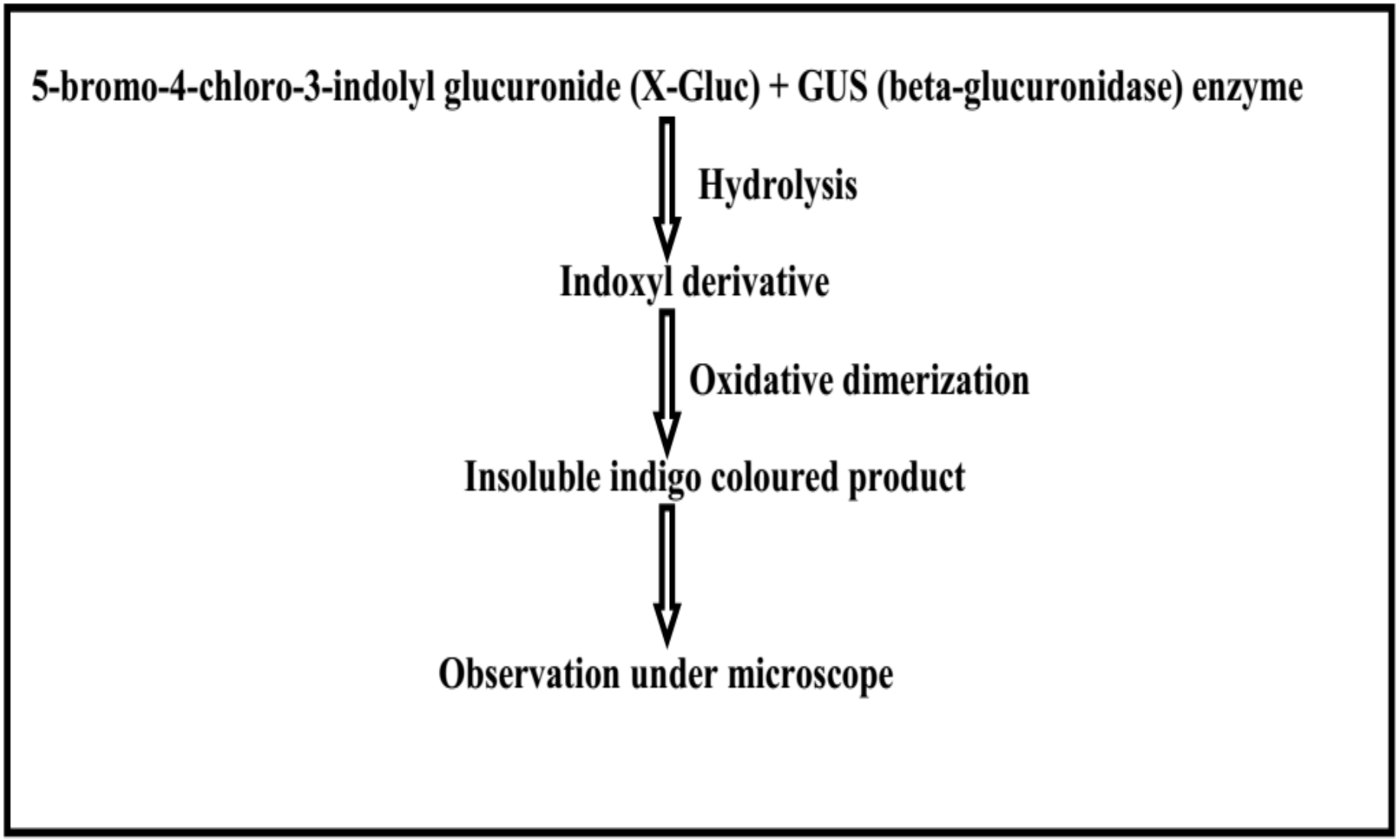
Analysis of transformants by GUS histochemical assay

1. • A small amount of fresh callus tissue (From test and experimental control both) into a centrifuge tube, immersed into 500 µl of staining solution

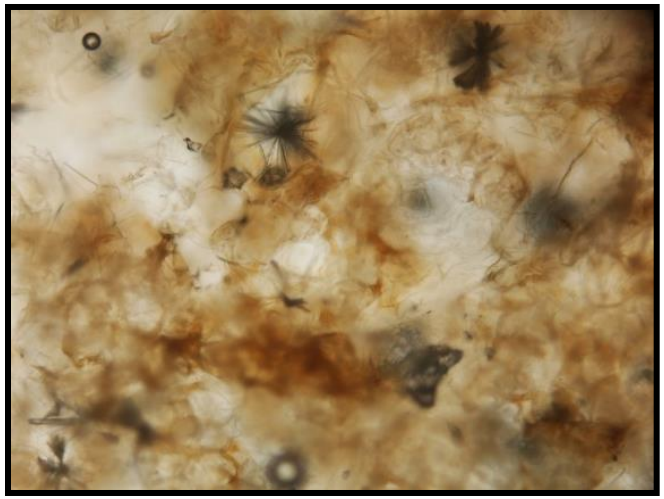
2. • Incubated sample at 37°C for 12-18 hrs. (If there is excessive staining then washed the solution with 50% ethanol until tissue cleared)

3. • A small section of callus tissue (both test and control); fixed on a slide with a drop of 100% glycerol and observed under microscope and compared the results with experimental control

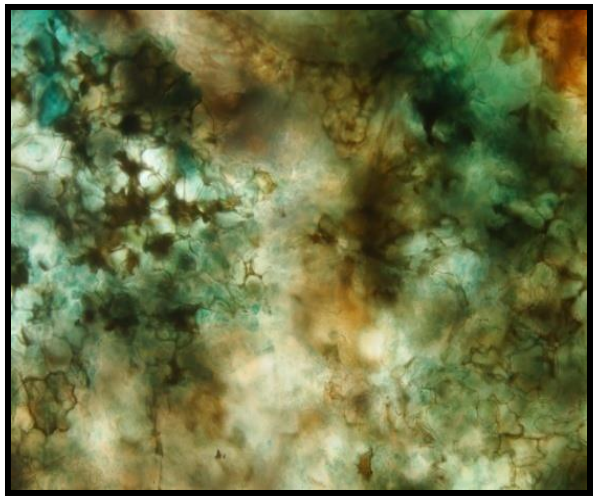
Contd…..


5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) + GUS (beta-glucuronidase) enzyme
Hydrolysis
Indoxyl derivative
Oxidative dimerization
Insoluble indigo coloured product
Observation under microscope

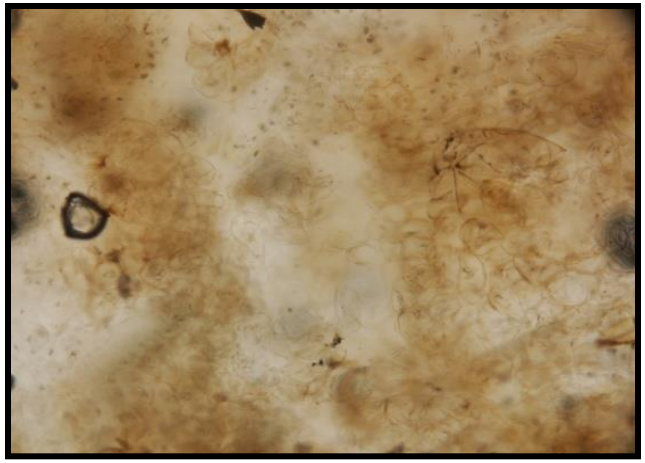
Results of GUS staining assay



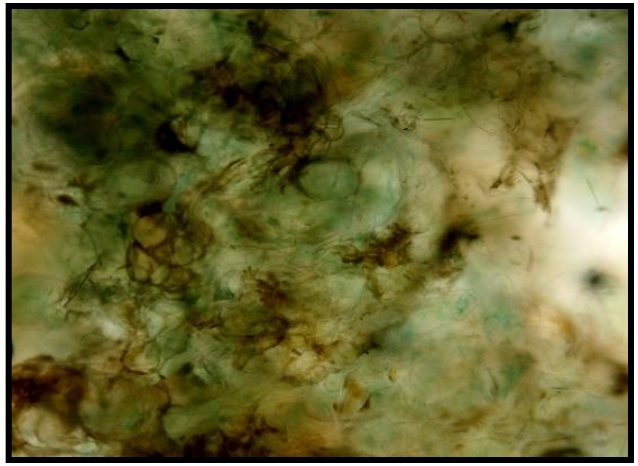
[A] Negative control



[B] TP5+BicA construct



[C] Negative control



[D] TP9+BicA construct

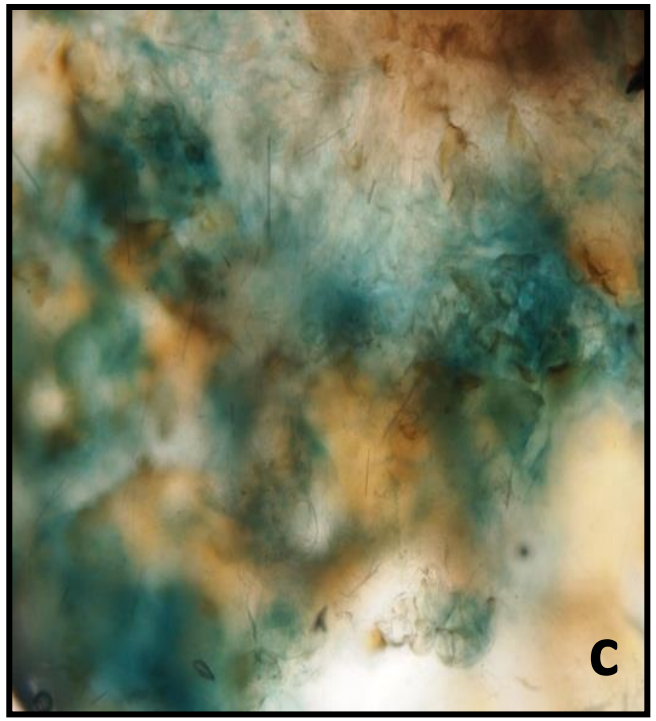
Negative control – Callus which has not been infected with *Agrobacterium*

Construct – Callus which has been infected with *Agrobacterium* containing recombinant *(TP5/9+bicA+gus)* pRI 101 construct

Contd…..







a) **Control Callus (Untransformed)**

b) **Transformed callus sbtA +TP4 construct**

c) **Transformed callus sbtA + TP3 construct**

Floral dip method


[1]


[2]

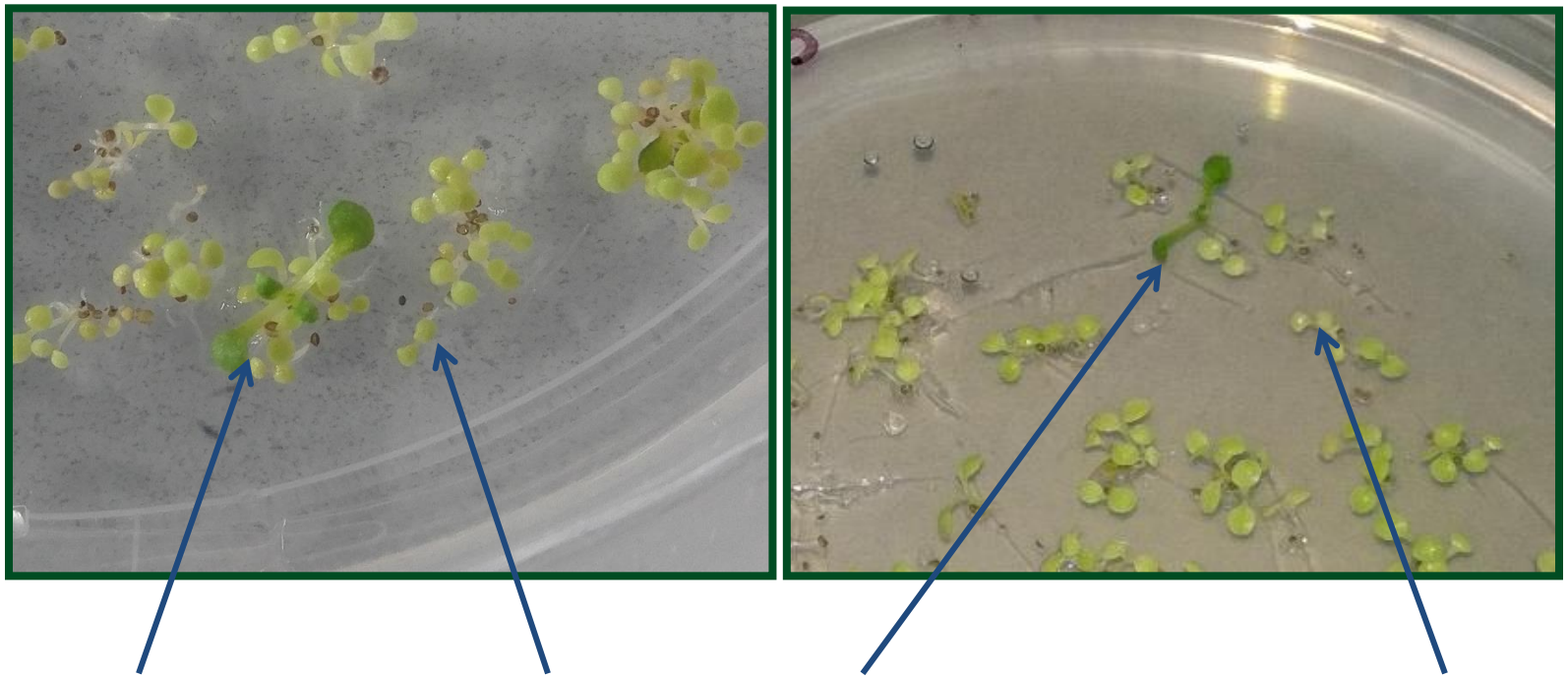

[3]


[4]

1. *Arabidopsis thaliana* plant
2. Dipping of *Arabidopsis* flowers in to *Agrobacterium* culture (20-30 seconds)
3. Plant covered in plastic bag, after floral dip (To maintain humidity for 12 hrs)
4 Plant after 3 weeks of floral dip (Showing silique development)

Selection of transgenic plants

Screening of the seeds for positive transformants on ½ MS media containing kanamycin



Transformed Untransformed Transformed Untransformed

SbtA **BicA**

T1 plants transformed with bicA/ sbtA fusion constructs





T_2 generation would be raised using T_1 seeds for selection of stable transformants

Future Plans

- **Analysis of transformants (generated via *Agrobacterium* mediated method) by reporter gene assay**

- **Screening of T_1 seeds (generated via floral dip method) and further generation of T_2 plants**

- **Southern blot hybridization and qPCR assays would be performed to check the transgene expression**

References

1. **Price G.D. (2011). Inorganic carbon transporters of the cyanobacterial CO_2 concentrating mechanism. *Photosynthesis Research.* 109:47-57.**
2. **Price *et al*. (2011), The prospects of using cyanobacterial bicarbonate transporters to improve leaf photosynthesis. *Molecular plant physiology.*155:20-26.**
3. **Price G.D. *et al.* (2013) The cyanoacterial CCM as a source of genes for improving phtosynthetic CO_2 fixation in crop species. *Journal of Experimental Botany. 64:753-768*.**
4. **Price, G. D. & Howitt, S. M. (2011) The cyanobacterial bicarbonate transporter BicA: its physiological role and the implications of structural similarities with human SLC26 transporters .*Biochemistry & Cell Biology*. 89:178-188.**
5. **Price, G. D. , Shelden, M. C & Howitt, S. M. (2011) Membrane topology of the cyanobacterial bicarbonate transporter, SbtA, and identification of potential regulatory loops. *Molecular Membrane Biology.* 28:265-275.**
6. **Shelden, M. C., Howitt, S. M., & Price, G. D. (2010). Membrane topology of the cyanobacterial bicarbonate transporter, BicA, a member of the SulP (SLC26A) family. *Molecular Membrane Biology. 27*:12-23.**

Thank You