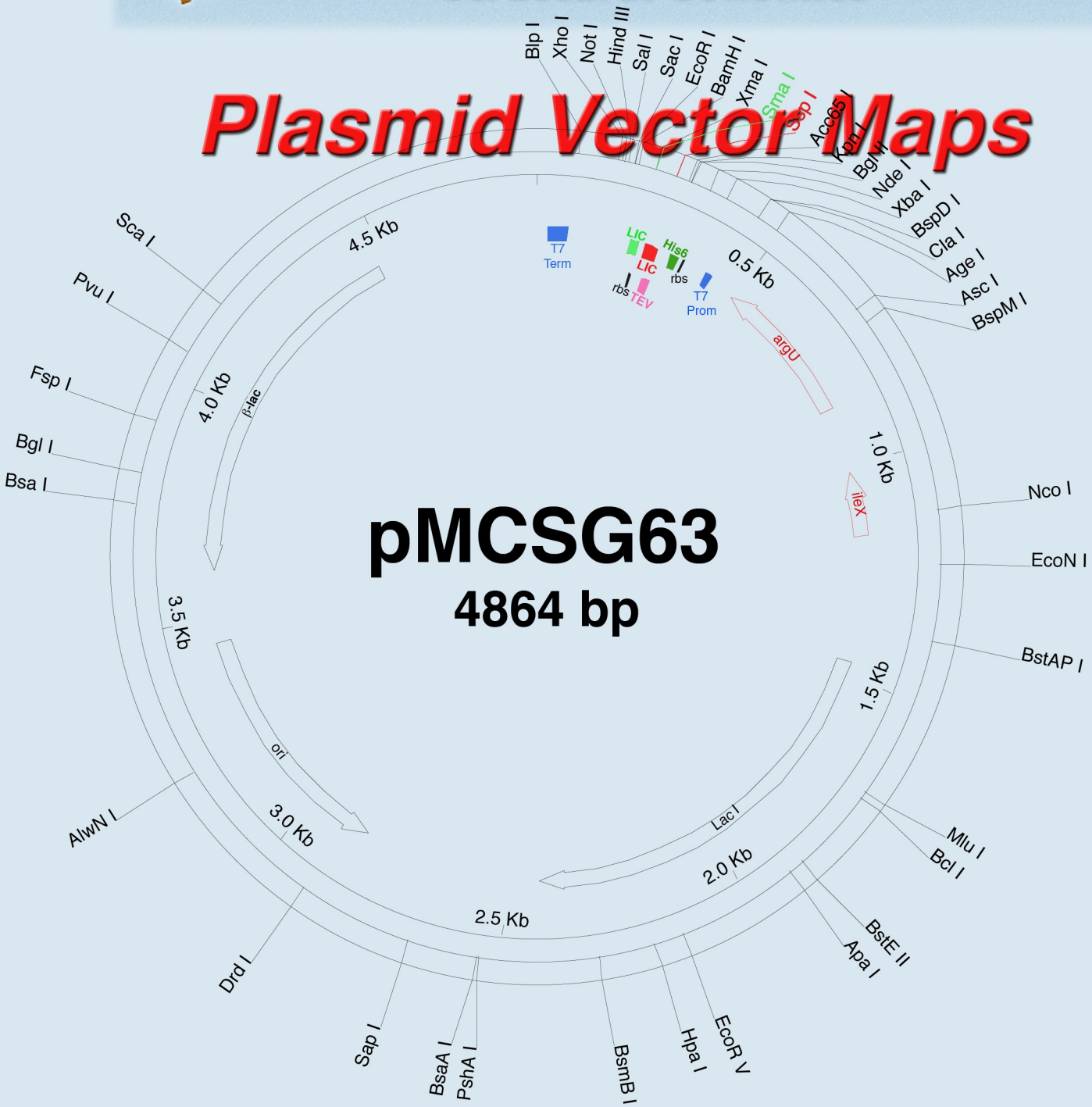
Midwest Center for Structural Genomics

PSI PSI:BIOLOGY

Plasmid Vector Maps



Name	base vector	antibiotic(s)	tag&sites	lab	tag MW	plasmid kb	purpose	construction	sites used	other DNA	comments
(pET30Xa/LIC	pET30a	kan	N-His-Thrmb-S-Xa-LICb-C	Novagen			parental	see pMCSG1)			**LICb** refers to LIC based on BseRI site
(pET21a	pET	amp	N-T7tag-C	Novagen			parental	see pMCSG3)			
pMCSG1	pET30Xa/LIC	kan	N-His-Xa-LICb-C	MD			- thromb-Stag		NdeI-BglII		
pMCSG3	pET21a	amp	N-His-Xa-LICb-C	FC			make ampR	pMCSG1 frag	SphI-XhoI		Into pET21a backbone with Xba-Xho from pMCSG1. Amp R
pMCSG6	pMCSG3	amp	N-His-**TEV**-LICb-C	MD							TEV protease site. **SspI sites eliminated**.
pMCSG7	pMCSG6	amp	N-His-TEV-**LICs**-C	MD	2755	5286	production	pMCSG6+oligos	Stols, et al. (2002)		**LICs refers to LIC based on SspI site. Base vector.**
pMCSG8	pMCSG7	amp	N-His-**Sloop**-TEV-LICs-C	MD	4399	5340	solubility	pMCSG7+oligos	BlgII-KpnI		Sloop in leader to improve solubility, reduce toxicity
pMCSG9	pMCSG7	amp	N-His-**MBP**-TEV-LICs-C	MD	43713	6405	solubility	pMCSG7+PCR	KpnI	pRK739	MBP in leader to improve solubility, give affinity tag
pMCSG10	pMCSG7	amp	N-His-**GST**-TEV-LICs-C	MD	29046	5961	solubility	pMCSG7+PCR	BglII	pGEX-2TK	GST in leader to improve solubility, give affinity tag, give detection method
pMCSG11	pACYCDuet	Cm	N-His-TEV-LICs-C	MD	2755	4079	coexpression	PCR of pMCSG7	NdeI-XhoI	pACYCDuet-1	for expression of multiple proteins by use with other vectors
pMCSG12	pACYCDuet	Cm	N-His-Sloop-TEV-LICs-C	MD	4399	4133	coexpression	PCR of pMCSG8	NdeI-XhoI	pACYCDuet-1	"
pMCSG13	pACYCDuet	Cm	N-His-MBP-TEV-LICs-C	MD	43713	5198	coexpression	frag of pMCSG9	NdeI-XhoI	pACYCDuet-1	"
pMCSG14	pACYCDuet	Cm	N-His-GST-TEV-LICs-C	MD	29046	4754	coexpression	frag of pMCSG10	NdeI-XhoI	pACYCDuet-1	"
pMCSG15	pMCSG7	amp	N-LIC-**GS**-TEV-**AviTag-C**	BK	4595	5355	phage display	Scholle, et al.			C-terminal biotinylation when expressed with BirA
pMCSG16	pMCSG7	amp	N-His-**AviTag-GS**-TEV-LIC-C	BK	3290	5355	phage display	Scholle, et al.			N-terminal biotinylation when expressed with BirA
pMCSG17	pMCSG7	amp	N-**Stag**-TEV-LICs-C	MD	3760	5316	coexpression	oligo linker	NdeI-BglII	pMCSG7	Stag leader, **without his tag**, for coexprn/detection of complexes
pMCSG18	pMCSG7	amp	N-His-TEV-LICs-**GFP**-C	MD/WE	2755	6009	screening	pMCSG7+PCR	BamHi-SspI	pQBIT7-GFP	GFP+leader=30021 Da. Readthroughs (PCR w/o stop) -> 30K+target
pMCSG19	pMCSG7	amp	N-**MBP-TVMV**-His-TEV-LICs-C	MD	45050/2711	6441	salvaging	pMCSG7+PCR	NdeI-BglII	pRK1035	for in vivo cleavage of MBP by TVMV protease
pMCSG19B	pMCSG7	amp	N-**MBP-TVMV**-His-TEV-LICs-C TVMV protease	MD/WE	45050/2711	7468	salvaging	pMCSG7+PCR	NdeI-BglII	pRK1035	for in vivo cleavage of MBP by TVMV protease
pMCSG19C	pMCSG7	amp	N-**MBP-TVMV**-His-TEV-LICs-C TVMV protease	MD/WE	45050/2711	7468	salvaging	pMCSG7+PCR	NdeI-BglII	pRK1035	for in vivo cleavage of MBP by TVMV protease
pMCSG20	pMCSG17	amp	N-**Stag-GST**-TEV-LICs-C	MD/LS	30051	5991	coexpression	frag of pMCSG10	BglII	pMCSG10+17	17 with GST, no **his tag**, for coexprn/detection of complexes
pMCSG21	pCDFDuet	spec	N-His-TEV-LICs-C	MD/WE	2755	3852	coexpression	PCR of pMCSG7	NdeI-XhoI	pMCSG7	for expression of multiple proteins by use with other vectors
pMCSG22	pMCSG21	spec	N-His-Sloop-TEV-LICs-C	MD/WE	4399	3906	coexpression	frag of pMCSG12	NcoI-XhoI	pMCSG12	"
pMCSG23	pMCSG21	spec	N-His-MBP-TEV-LICs-C	MD/WE	43713	4971	coexpression	frag of pMCSG13	NdeI-XhoI	pMCSG13	"
pMCSG24	pMCSG21	spec	N-His-GST-TEV-LICs-C	MD/WE	29046	4527	coexpression	frag of pMCSG14	NdeI-XhoI	pMCSG14	"
pMCSG25	pMCSG7	amp	N-Protease-His-TEV-LICs-C	MD/WE		5664	solubility	PCR of Shewanella	Nde		*Shewanella oneidensis* self cleaving protease
pMCSG26	pMCSG7	amp	N-LIC-His-C	WE		5226	solubility	synth oligos	Bam-Xba		COOH-terminal His tag
pMCSG27	pMCSG26	amp	N-LIC-His10-TVMV-MBP-C	WE		6362	solubility				COOH-terminal His tag
pMCSG28	pMCSG26	amp	N-LIC-TEV-His-C	WE		5253	solubility				COOH-terminal His tag
pMCSG29	pMCSG27	amp	N-LIC-TEV-His10-TVMV-MBP-C	WE		6389	solubility				COOH-terminal His tag
pMCSG30	pMCSG7	amp	N-His-TEV-MPB-LIC2-SacB-LIC2-C	WE-GB	42557	8314	MBP fusion	MBP PCR	LIC		non-cleavable MBP with linker
pMCSG31	pASKIBA3+	amp	N-TetP-TVMVprotease-T7-MBP-TVMV-His6-TEV-LIC-C	WE		5822	solubility				inducible TVMV protease
pMCSG32	pMCSG28	amp	N-MBP-TVMV-LIC-TEV-His-C	WE		6418	solubility	MBP PCR	XbaI-XmaI		NH4-terminal MBP + COOH-terminal His tag
pMCSG33	pMCSG28	amp	N-His10-MBP-TEV-LIC-His-C	WE		6457	sol/capture	9His10 PCR	XbaI-XmaI		NH4-terminal MBP + NH4 & COOH-terminal His tags
pMCSG34	pMCSG32	amp	N-MBP-TVMV-LIC-TEV-His-C TVMV protease	WE		7445	solubility	ligation pRK1037	SphI		protease cds in clockwise orientation
pMCSG34B	pMCSG32	amp	N-MBP-TVMV-LIC-TEV-His-C TVMV protease	WE		7445	solubility	ligation pRK1037	SphI		protease cds in anti-clockwise orientation
pMCSG35	pMCSG28	amp	N-His10-MBP-TEV-LIC-TEV-His6-C TVMV protease	WE		7484	solubility	ligation pRK1037	XbaI/XmaI; SphI		protease cds in clockwise orientation
pMCSG35B	pMCSG28	amp	N-His10-MBP-TEV-LIC-TEV-His6-C TVMV protease	WE		7484	solubility	ligation pRK1037	XbaI/XmaI; SphI		protease cds in anti-clockwise orientation
~~pMCSG36~~	~~pMCSG26~~	~~amp~~	~~N-LIC-C~~	~~WE~~		~~5224~~	~~coexpression~~		~~XbaI/BamHI~~		~~MCSG7 LIC site in MCSG26 w/o His tag~~
pMCSG37	pMCSG9	amp	N-His10-MBP-His6-TEV-LIC-C	WE		6426	solubility		Acc65I/XbaI		MCSG9His10 with His6 at C-term of MBP
pMCSG38	pMCSG7	amp	N-His6-TEV-LIC-C TVMV protease	WE		6313	coexpression		SphI		protease cds in anti-clockwise orientation
pMCSG38C	pMCSG7	amp	N-His6-TEV-LIC-C TVMV protease	WE		6313	coexpression		SphI		protease cds in clockwise orientation
pMCSG39	pMCSG7	amp	N-MBP-TVMV-His10-TEV-LIc-C	WE		6453	salvaging	MCSG19+His4			pMCSG19 with 10 His residues
pMCSG40	pMCSG19	amp	N-osmY-TVMV-His6-TEV-LIC-C dsbA	WE		6796	secretion	MCSG19+osmY			N-terminal His-tag
pMCSG41	pMCSG28	amp	N-LIC-TEV-His6-C TVMV protease	WE		6280	salvaging	MCSG28+TVMV	SphI		C-terminal His-tag for artificial operons with TVMV site linker

Name	base vector	antibiotic(s)	tag&sites	lab	tag MW	plasmid kb	purpose	construction	sites used	other DNA	comments
pMCSG42	pMCSG32	amp	N-MBP-TVMV-LIC-TEV-His6-C	WE		7445	salvaging	MCSG32+TVMV	SphI		C-terminal His-tag for artificial operons with TVMV site linker
pMCSG43	pMCSG32	amp	N-osmY-TVMV-LIC-TEV-His6-C dsbA	WE		6773	secretion	MCSG32+osmY			C-terminal His-tag
pMCSG44				RJ							
pMCSG45				RJ							
pMCSG46	pMCSG7	amp	N-HN6-NusA-TEV-LIC-C	RJ		6759	solubility	MCSG7+NusA			NusA cds replacing MBP for solubility
pMCSG47	pMCSG7	amp		RJ							
pMCSG48	pMCSG7	amp	N-His8-NusA-TEV-LIC-C	RJ		6741	solubility	MCSG7+NusA			NusA cds replacing MBP for solubility
pMCSG49	pMCSG7	amp	N-His-TEV-**LICs**-C	WE		4278	production	MCSG7 digested	PshAI BasAI		MCSG7 digested with PshAI & BsaAI removing 1 kb
pMCSG50	pMCSG60	amp	N-His6-biotin-TEV-LICs-BirA-C	WE		6347	biotinylation				15 aa biotinylation signal WEtween His6 & LIC; BirA in 2nd LIC site
pMCSG51	pMCSG50	amp	N-His6-biotin-TEV-LICs-BirA-C	WE		5346	biotinylation	pMCSG50 digested	PshAI Tth111I		MCSG50 digested with PshAI & Tth111I removing 1 kb
pMCSG52	pMCSG7	amp	N-His-TEV-LIC-C argU-ileX	WE		5809	production				MCSG7: argU tRNA in SgrAI site; ileX tRNA in SphI site
pMCSG53	pMCSG52	amp	N-His-TEV-LIC-C argU-ileX	WE		4808	production		PshAI Tth111I		MCSG52 digested with PshAI & Tth111I removing 1 kb
pMCSG54	pMCSG49	amp	N-His-FLAG-TEV-LIC-C	WE		4302	imm. assays	pMCSG49+tag	Bgl II + Kpn I		
pMCSG55	pMCSG49	amp	N-His-HA-TEV-LIC-C	WE		4305	imm. assay	pMCSG49+tag	Bgl II + Kpn I		
pMCSG56	pMCSG49	amp	N-His-HA-HA-TEV-LIC-C	WE		4332	imm. assay	pMCSG49+tag	Bgl II + Kpn I		
pMCSG57	pMCSG49	amp	N-His-STRII-TEV-LIC-C	WE		4302	imm. assay	pMCSG49+tag	Bgl II + Kpn I		
pMCSG58	pMCSG53	amp	N-LIC-His-C argU-ileX	WE		4748	expression	pMCSG53+26	Bgl I + Xba I		truncated C-terminal (26) vector plus rare tRNAs
pMCSG59	pMCSG53	amp	N-LIC-TEV-His-C argU-ileX	WE		4775	expression	pMCSG53+28	Bgl I + Xba I		truncated C-terminal (28) vector plus rare tRNAs
pMCSG60	pMCSG7	amp	N-His6-TEV-LIC1-LIC2-C	WE		5342	coexpression	MCSG7+LIC26			oligo of MCSG26 LIC site + rbs added to MCSG7
pMCSG61	pMCSG30	amp	N-His6-TEV-MBP-LIC-C	RJ		6422	solubility				MBP fusion; no LIC restriction site (PCR propagation)
pMCSG62	pMCSG53	amp	N-His6-biotin-TEV-LIC-BirA-C argU-ileX	WE		5869	biotinylation	pMCSG53+51	Bgl I + Xba I		
pMCSG63	pMCSG53	amp	N-His6-TEV-LIC1-LIC2-C argU-ileX	WE		4864	coexpression	pMCSG53+60	Bgl I + Xba I		
pMCSG64	pMCSG60	amp	N-His-FLAG-TEV-LIC1-LIC2-C	WE		5366	imm. assays	pMCSG60+tag	Bgl II + Kpn I		
pMCSG65	pMCSG60	amp	N-His-HA-TEV-LIC1-LIC2-C	WE		5369	imm. assays	pMCSG60+tag	Bgl II + Kpn I		
pMCSG66	pMCSG60	amp	N-His-HA-HA-TEV-LIC1-LIC2-C	WE		5396	imm. assays	pMCSG60+tag	Bgl II + Kpn I		
pMCSG67	pMCSG60	amp	N-His-STRII-TEV-LIC1-LIC2-C	WE		5366	imm. assays	pMCSG60+tag	Bgl II + Kpn I		
pMCSG68	pMCSG53	amp	N-His-STRII-TEV-LIC-C argU-ileX	WE		4832	imm. assays	pMCSG53+57	Bgl I + Xba I		
pMCSG69	pMCSG53	amp	N-MBP-TVMV-His-TEV-LIC-C argU-ileX	WE		5963	expression	pMCSG53+19	Bgl I + Xba I		
pMCSG70	pMCSG53	amp	N-LIC-TEV-His10-TVMV-MBP-C argU-ileX	WE		5911	expression	pMCSG53+29	Bgl I + Xba I		
pMCSG71	pMCSG53	amp	N-MBP-TVMV-LIC-TEV-His-C argU-ileX	WE		5940	expression	pMCSG53+32	Bgl I + Xba I		
pMCSG72	pMCSG53	amp	N-NusA-TVMV-His6-TEV-LIC argU-ileX	RJ							
pMCSG73	pMCSG53	amp	N-NusA-TVMV-His6-STRII-TEV-LIC argU-ileX	RJ							
pMCSG74	pMCSG53	amp	N-NusA-TVMV-STRII-His6-TEV-LIC argU-ileX	RJ							
pMCSG75	pMCSG62	amp	N-LIC-TEV-biotin-His6-BirA-C argU-ileX	WE		5876	expression	MCSG62+LIC28	Xba I + Ssp I		MCSG28 LIC plus biotin-binding sequence replaces Ssp I-LIC
pMCentr1	pDONR/Zeo	zeocin	**attL1**-TEV-LICs-**attL2**	MD	*no exprn*	*2115*	to Gateway	Oligos + BP rxn	att sites		Bridge to Gateway vectors (LIC gives ENTR vector)
pMCentr2	pDONR221	kan	**attL1**-TEV-LICs-**attL2**	MD/WE	*no exprn*	*2586*	to Gateway	Oligos + BP rxn	att sites		Bridge to Gateway vectors (LIC gives ENTR vector)
pMCentr3	pDONR221	kan	**attL1**-TVMV-LICs-**attL2**	WE	*no exprn*	*2586*	to Gateway	Oligos + BP rxn	att sites		Bridge to Gateway vectors (LIC gives ENTR vector)
pMCdest19	pMCSG19	amp, Cm	**attR1**-ccdB-CmR-**attR2**	MD/WE	45050/2711	*8081*	for Gateway LR	PCR of pDEST17	KpnI-HindIII	pDEST17	Gateway: Expression only after LR reaction with ENTR vector

pMCSG26

COOH-terminal His Tag constructed from pMCSG

LIC Region:

```
    Xba I                                   rbs                G   H   H   H   H   H   H   ·
5' tCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA gtc tct ccc GGG CAC CAC CAT CAT CAT CAT TAA CGg atc c 3'
3' aga tcT TTA TTA AAA CAA ATT GAA ATT CTT CCT CAG AGA GGG ccc gtg gtg gtA GTA GTA GTA ATT GCC TAG g 5'
                                                     Sma I                                BamH I
```

Vector: digest with Sma I and treat with T4 polymerase and dATP

PCR Products: treat with T4 polymerase and dTTP

Forward Primer: GTC TCT CCC **ATG** . . . (ATG is start codon for the gene of interest followed by gene-specific sequences)

Reverse Primer: TG GTG GTG CCC AXX . . . (followed by gene-specific sequence [reverse complement of final codons]) **Note:** If the final codon of your gene ends in "U" then you only need the extra Gly residue; otherwise a second spacer codon is needed. We normally use Ala (GCT) [see below].

Options for Reverse Primer:

codons for additional spacer amino acids can be added
additional His codons could be added although the Gly residue will separate the new codons from the original six His residues
a TEV (or TVMV) cleavage site can be added if desired

```
reverse primer (Gly-Ala):        TG GTG GTG CCC AGC ...
                               ≤ H   H   H   G   A

reverse primer (Gly-Ala-TEV):    TG GTG GTG CCC AGC GGA TTG GAA GTA CAG GTT CTC ...
                               ≤ H   H   H   G   A   S   Q   F   Y   L   N   E
```

pMCSG27

COOH-terminal His Tag with COOH-terminal MBP and TVMV recognition site; LIC cloning site identical to MCSG26

LIC Region:

```
                                                                                                                   TVMV site              MBP->
    Xba I                                      rbs                G   H   H   H   H   H   H   H   H   H   H   E   T   V   R   F   Q   S   K   I   E
5' tCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA gtc tct ccc GGG CAC CAC CAT CAT CAT CAT CAC CAC CAT CAC GAA ACC GTG CGT TTC CAG TCT AAA ATC GAA 3'
3' aga tcT TTA TTA AAA CAA ATT GAA ATT CTT CCT CAG AGA GGG ccc gtg gtg gtA GTA GTA GTA GTG GTG GTA GTG CTT TGG CAC GCA AAG GTC AGA TTT TAG CTT 5'
                                                         Sma I
```

Vector: digest with Sma I and treat with T4 polymerase and dATP

PCR Products: treat with T4 polymerase and dTTP

Forward Primer: GTC TCT CCC **ATG** . . . (ATG is start codon for the gene of interest followed by gene-specific sequences)

Reverse Primer: TG GTG GTG CCC AXX . . . (followed by gene-specific sequence [reverse complement of final codons]) **Note:** If the final codon of your gene ends in “U” then you only need the extra Gly residue; otherwise a second spacer codon is needed. We normally use Ala (GCT) [see below].

Options for Reverse Primer:

codons for additional spacer amino acids can be added
a TEV (or other) cleavage site can be added if desired

```
reverse primer (Gly-Ala):        TG GTG GTG CCC AGC ...
                               ≤ H   H   H   G   A

reverse primer (Gly-Ala-TEV):    TG GTG GTG CCC AGC GGA TTG GAA GTA CAG GTT CTC ...
                               ≤ H   H   H   G   A   S   Q   F   Y   L   N   E
```

pMCSG28

COOH-terminal His Tag with TEV site (constructed from pMCSG26)
forward primer identical to pMCSG26; reverse primer specific for pMCSG28 (and pMCSG29)

LIC Region:

```
                                                                                  TEV site
    Xba I                                   rbs                 G   E   N   L   Y   F   Q   S   A   G   H   H   H   H   H   H   ·
5' tCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA gtc tct ccc GGG GAG AAC CTG TAC TTC CAA TCC GCC GGG CAC CAC CAT CAT CAT CAT TAA CGg atc c 3'
3' aga tcT TTA TTA AAA CAA ATT GAA ATT CTT CCT CAG AGA GGG ccc ctc ttg gAC ATG AAG GTT AGG CGG CCC GTG GTG GTA GTA GTA GTA ATT GCC TAG g 5'
                                                      Sma I                                                                  BamH I
```

Vector: digest with Sma I and treat with T4 polymerase and dATP

PCR Products: treat with T4 polymerase and dTTP

Forward Primer: GTC TCT CCC **ATG** . . . (ATG is start codon for the gene of interest followed by gene-specific sequences)

Reverse Primer: G GTT CTC CCC AXX . . . (followed by gene-specific sequence [reverse complement of final codons])

Note: If the final codon of your gene ends in "U" then you only need the extra Gly residue; otherwise a second spacer codon is needed. We normally use Ala (GCT) [see below].

```
reverse primer (Gly-Ala):        G GTT CTC CCC AGC ...
                            ←  L   N   E   G   A
```

pMCSG29

COOH-terminal His Tag with TEV recognition site, COOH-terminal MBP and TVMV recognition site; LIC cloning site identical to MCSG28

LIC Region:

```
                                                                                  TEV site
    Xba I                                   rbs                 G   E   N   L   Y   F   Q   S   A   G   H   H   H   H   H   H   H   H   H   H
5'  tCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA gtc tct ccc GGG GAG AAC CTG TAC TTC CAA TCC GCC GGG CAC CAC CAT CAT CAT CAT CAC CAC CAT CAC
3'  aga tcT TTA TTA AAA CAA ATT GAA ATT CTT CCT CAG AGA GGG ccc ctc ttg gAC ATG AAG GTT AGG CGG CCC GTG GTG GTG GTA GTA GTA GTA GTG GTA GTG
                                                         Sma I

         TVMV site            MBP->
 E   T   V   R   F   Q   S   K   I   E   E
GAA ACC GTG CGT TTC CAG TCT AAA ATC GAA GAA 3'
CTT TGG CAC GCA AAG GTC AGA TTT TAG CTT CTT 5'
```

Vector: digest with Sma I and treat with T4 polymerase and dATP

PCR Products: treat with T4 polymerase and dTTP

Forward Primer: GTC TCT CCC **ATG** . . . (ATG is start codon for the gene of interest followed by gene-specific sequences)

Reverse Primer: TG GTG GTG CCC AXX . . . (followed by gene-specific sequence [reverse complement of final codons]) **Note:** If the final codon of your gene ends in "U" then you only need the extra Gly residue; otherwise a second spacer codon is needed. We normally use Ala (GCT) [see below].

Options for Reverse Primer:

codons for additional spacer amino acids can be added if desired

```
reverse primer (Gly-Ala):     G GTT CTC CCC AGC ...
                            ← L   N   E   G   A
```

pMCSG32

Amino-terminal MBP plus TVMV site from pMCSG19 with the TEV site and COOH-terminal His Tag from pMCSG28 (constructed from pMCSG28)
forward primer same as pMCSG26 with addition of two bases (AC) to frame-shift reading frame; reverse identical to pMCSG28 and pMCSG29

LIC Region:

```
                                        MBP
        S   G   R   Q   T   V   D   E   A   L   K   D   A   Q   T   N   S   I   T   S   L   Y   K   K
5' ... AGC GGT CGT CAG ACT GTC GAT GAA GCC CTG AAA GAC GCG CAG ACT AAT TCG ATC ACA AGT TTG TAC AAA AAA ... 3'
3' ... TCG CCA GCA GTC TGA CAG CTA CTT CGG GAC TTT CTG CGC GTC TGA TTA AGC TAG TGT TCA AAC ATG TTT TTT ... 5'

                      TVMV site                                 TEV site
        A   G   S   E   T   V   R   F   Q   S   L   S     G   E   N   L   Y   F   Q   S   A   G   H   H   H   H   H   H   ·
5' ... GCA GGC TCG GAA ACC GTG CGT TTC CAG Agt ctc tcc c GGG GAG AAC CTG TAC TTC CAA TCC GCC GGG CAC CAC CAT CAT CAT CAT TAA CGg atc c 3'
3' ... CGT CCG AGC CTT TGG CAC GCA AAG GTC TCA GAG AGG G ccc ctc ttg gAC ATG AAG GTT AGG CGG CCC GTG GTG GTA GTA GTA GTA ATT GCC TAG g 5'
                                                  Sma I                                                                  BamH I
```

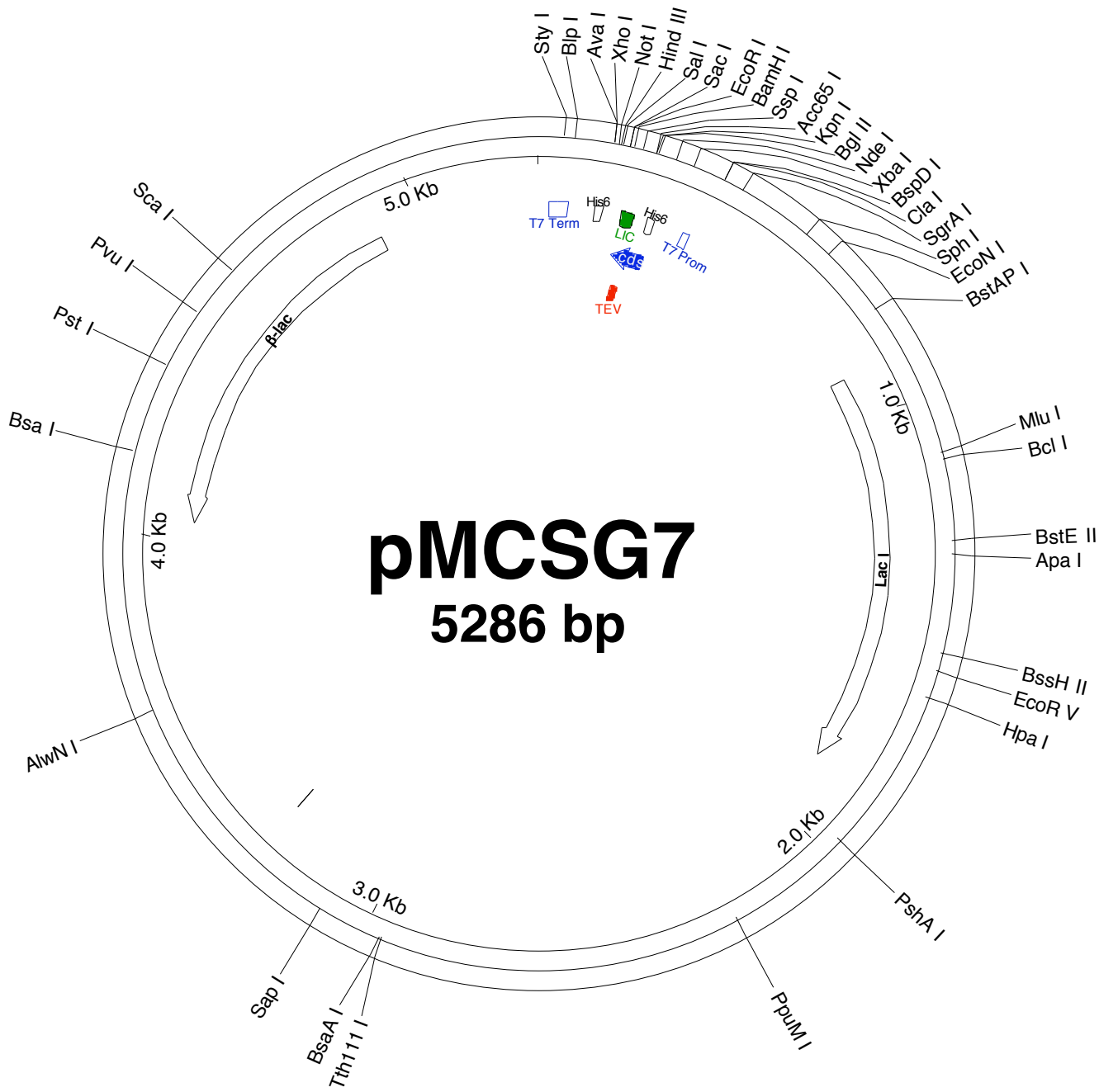
Vector: digest with Sma I and treat with T4 polymerase and dATP

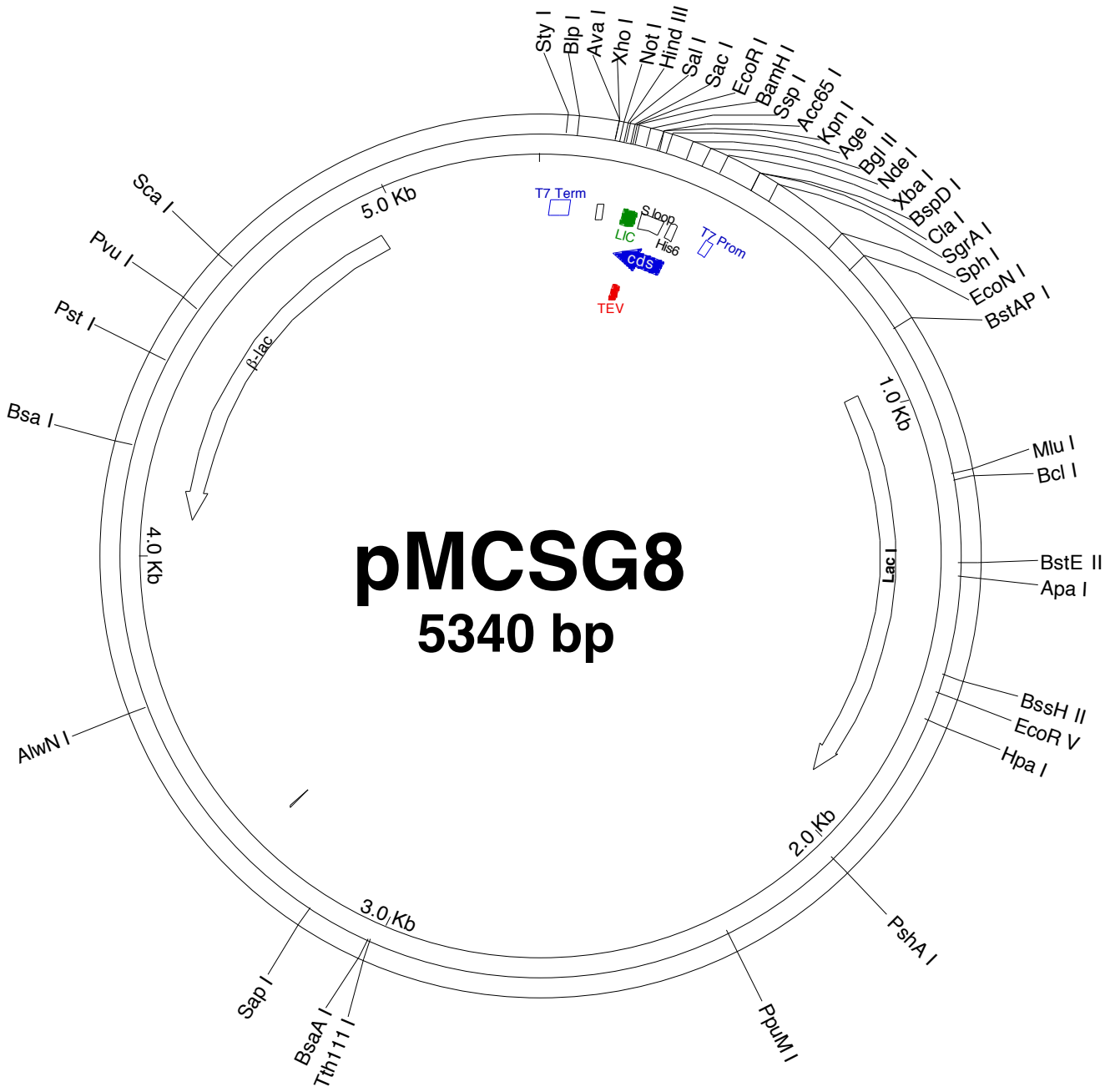
PCR Products: treat with T4 polymerase and dTTP

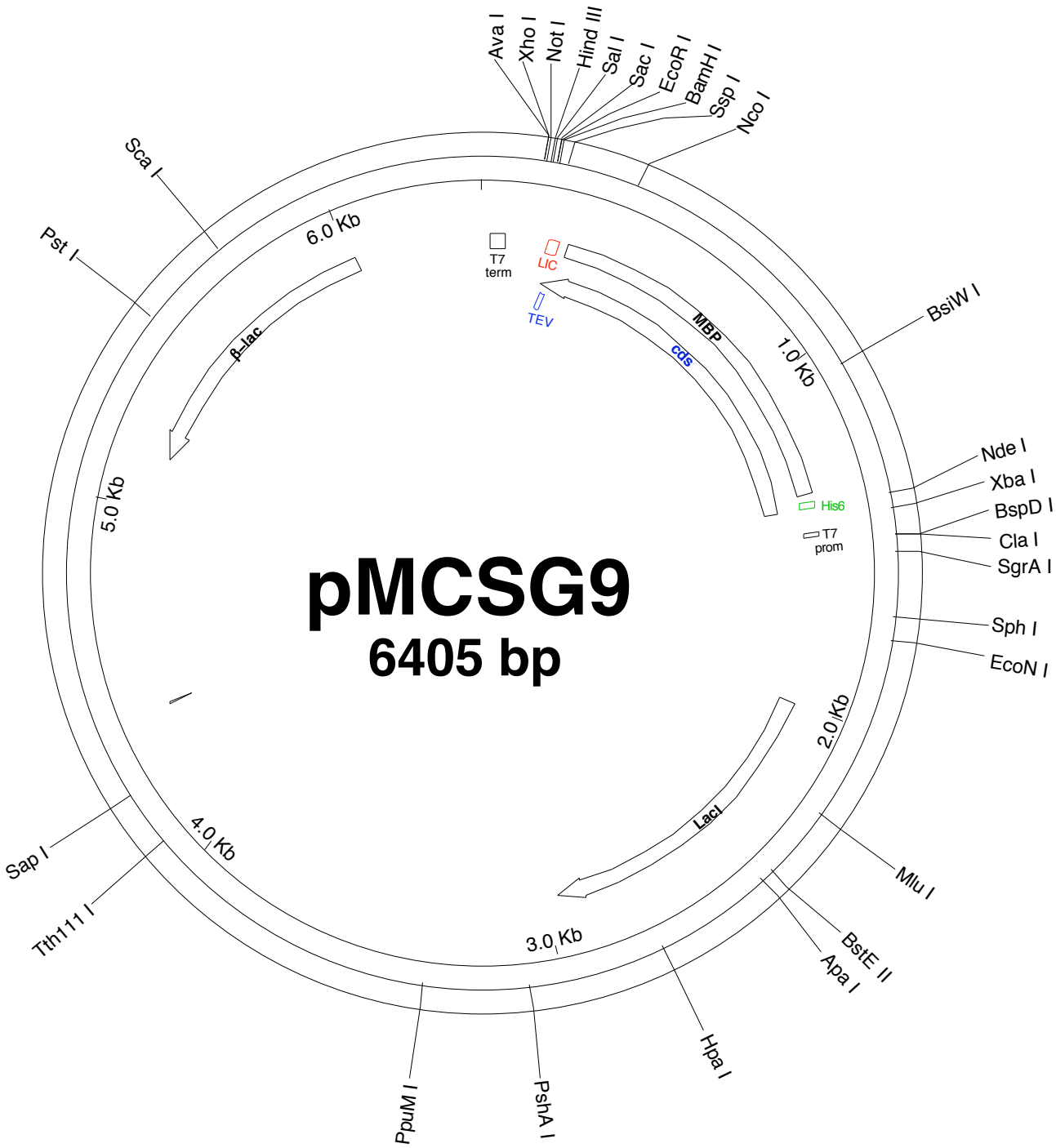
```
                                S   L   S   H   M
        Forward Primer:         GT CTC TCC CAC ATG . . .  (ATG is start codon for the gene of interest followed by gene-specific sequences)

        Reverse Primer:         G GTT CTC CCC AXX . . .   (followed by gene-specific sequence [reverse complement of final codons])
```

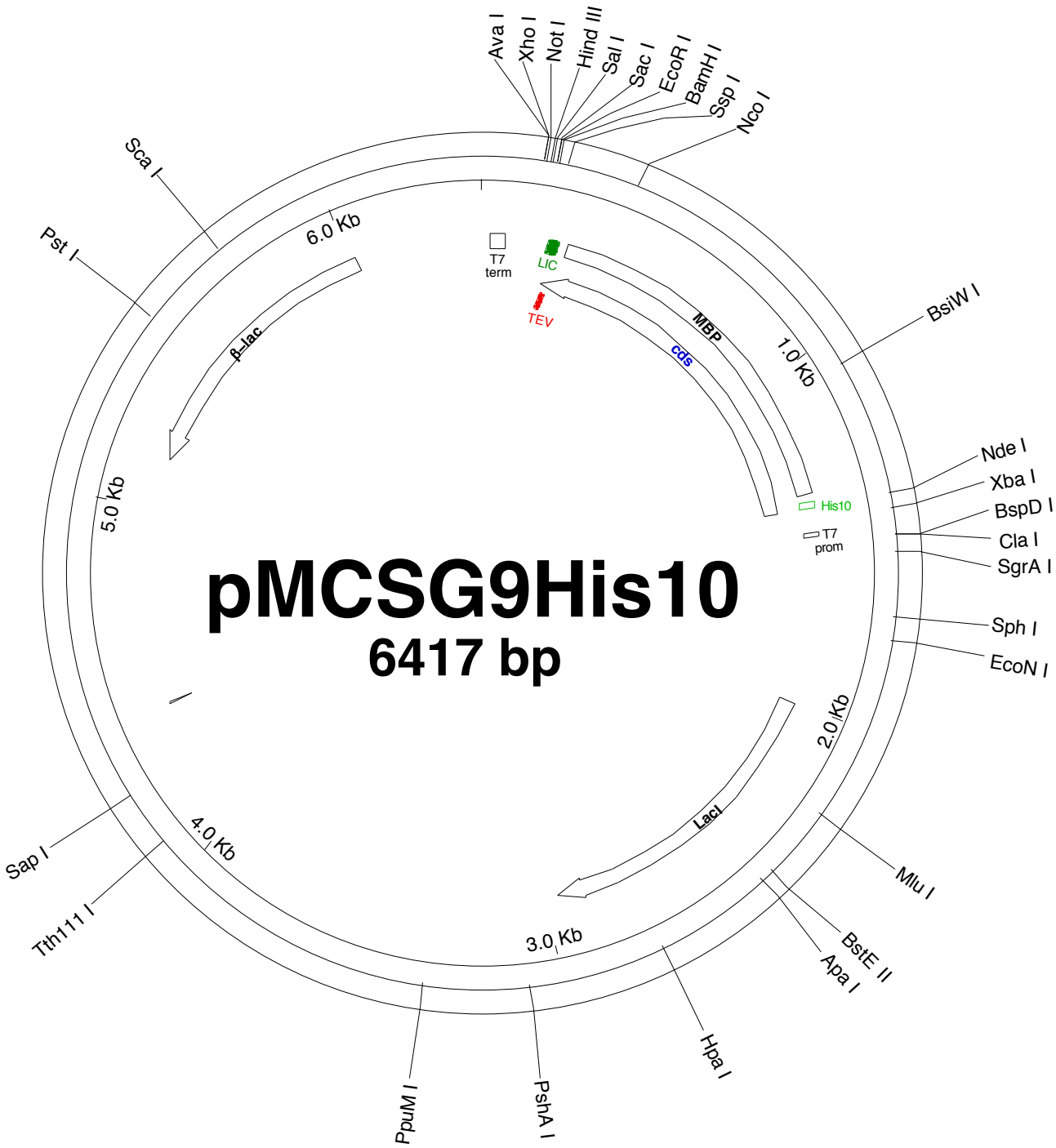
Note: If the final codon of your gene ends in "U" then you only need the extra Gly residue; otherwise a second spacer codon is needed. We normally use Ala (GCT) [see below].

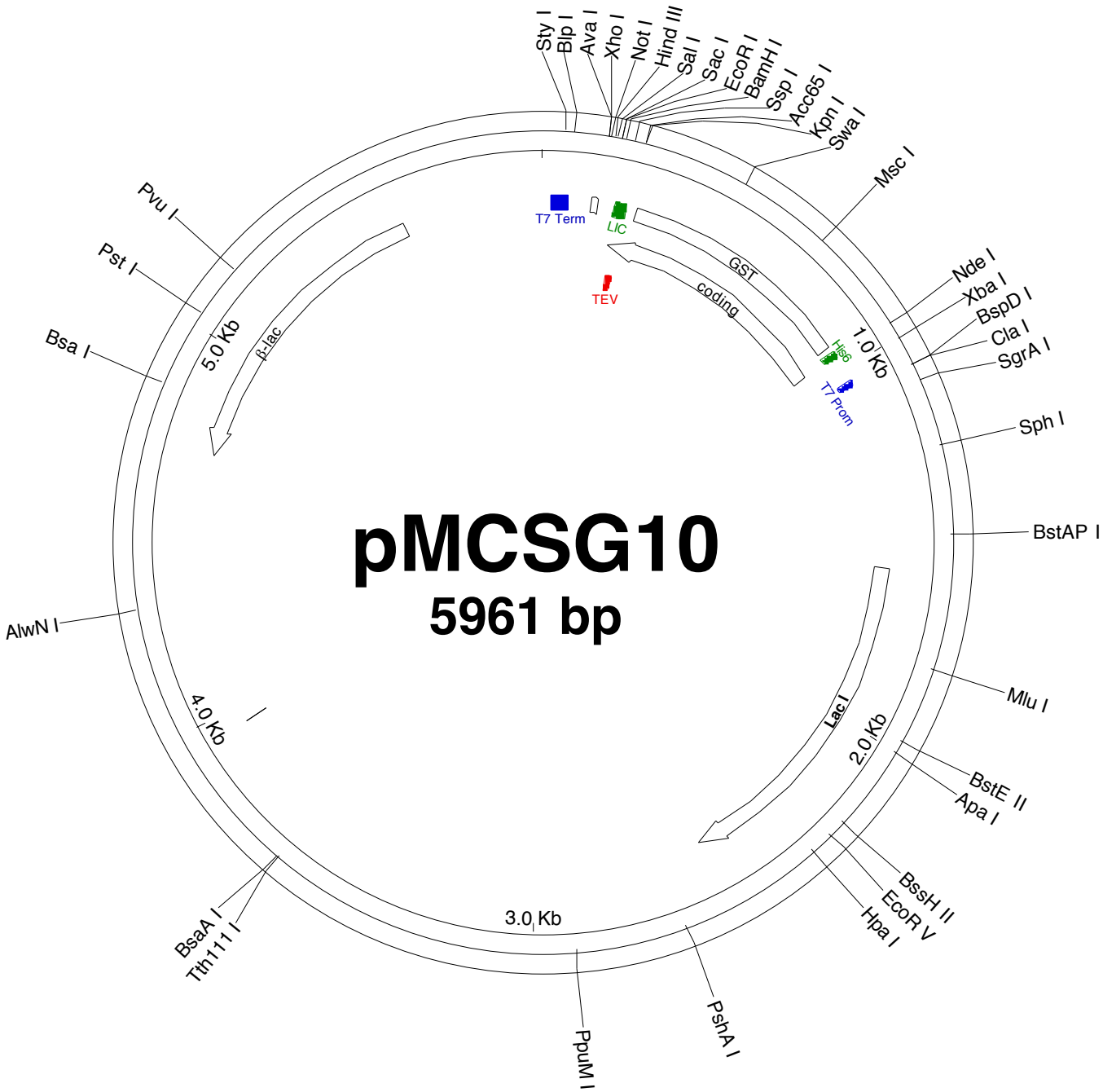
```
reverse primer (Gly-Ala):        G GTT CTC CCC AGC ...
                             ← L   N   E   G   A
```

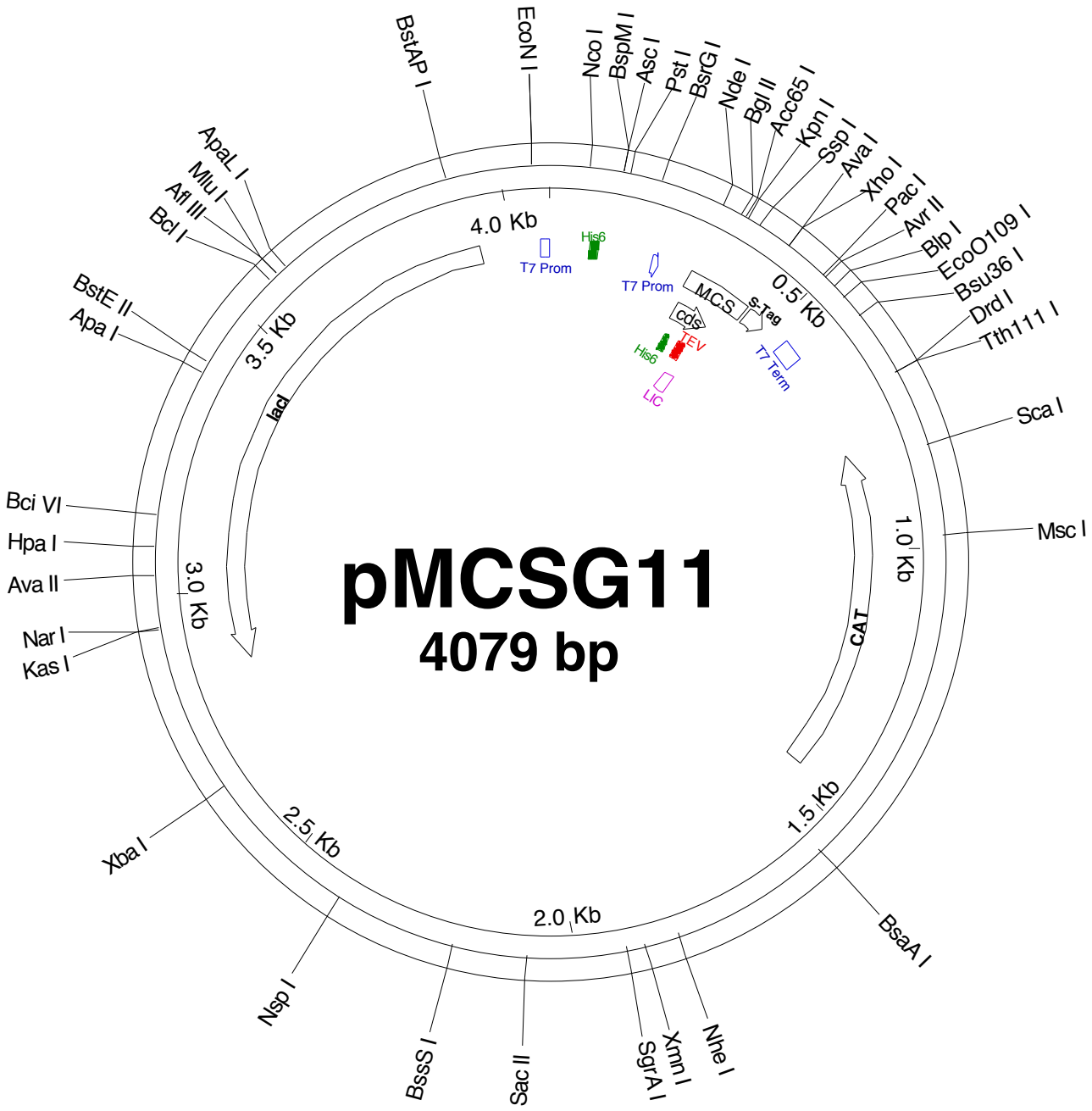

pMCSG7
5286 bp
Sty I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
AlwN I
Bsa I
Pst I
Pvu I
Sca I
5.0 Kb
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
β-lac
Lac I
T7 Term
His6
LIC
His6
T7 Prom
ccdB
TEV

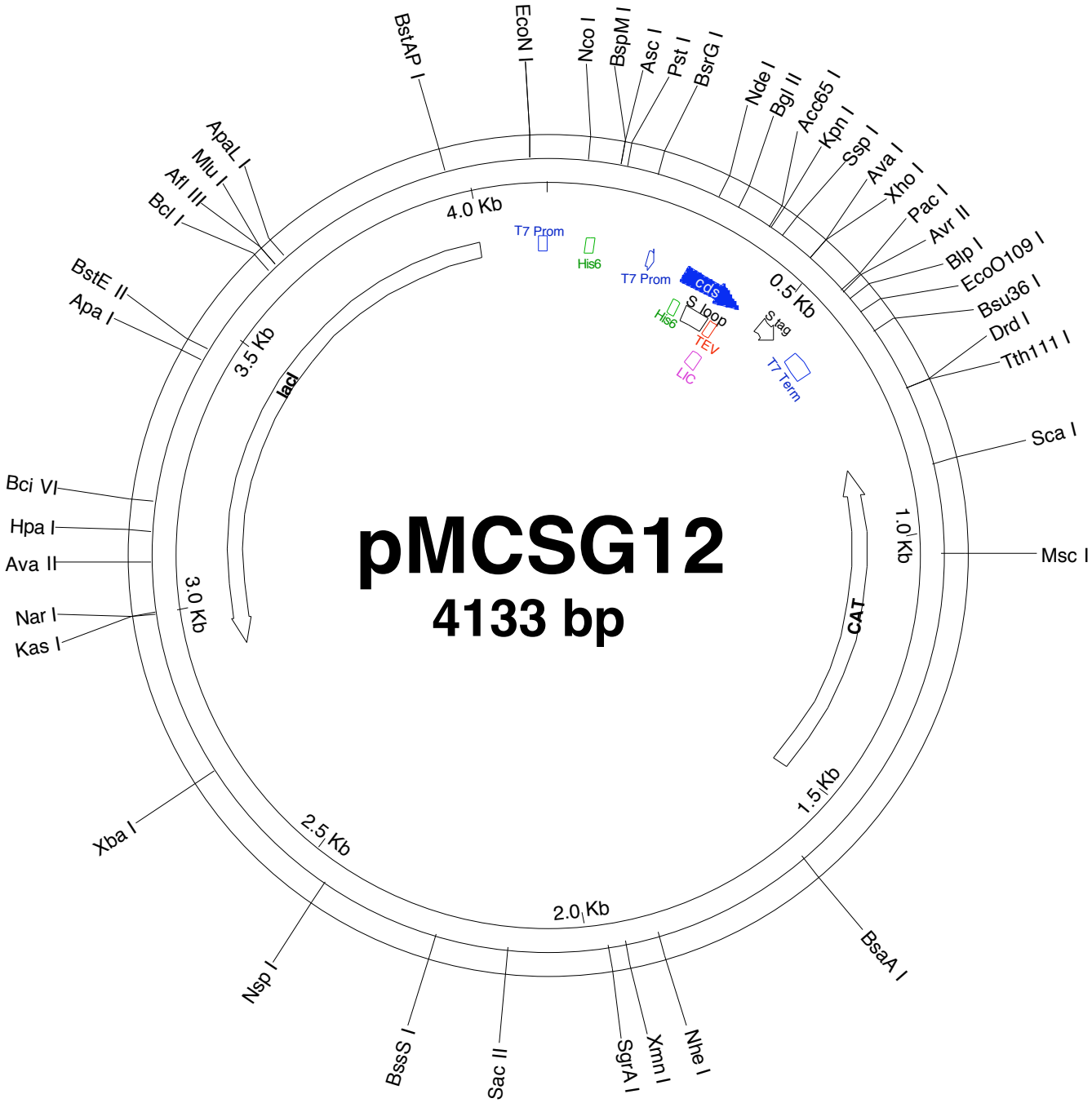

pMCSG8
5340 bp
Sty I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
Age I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
AlwN I
Bsa I
Pst I
Pvu I
Sca I
T7 Term
S.loop
LIC
His6
T7 Prom
cds
TEV
β-lac
Lac I
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb

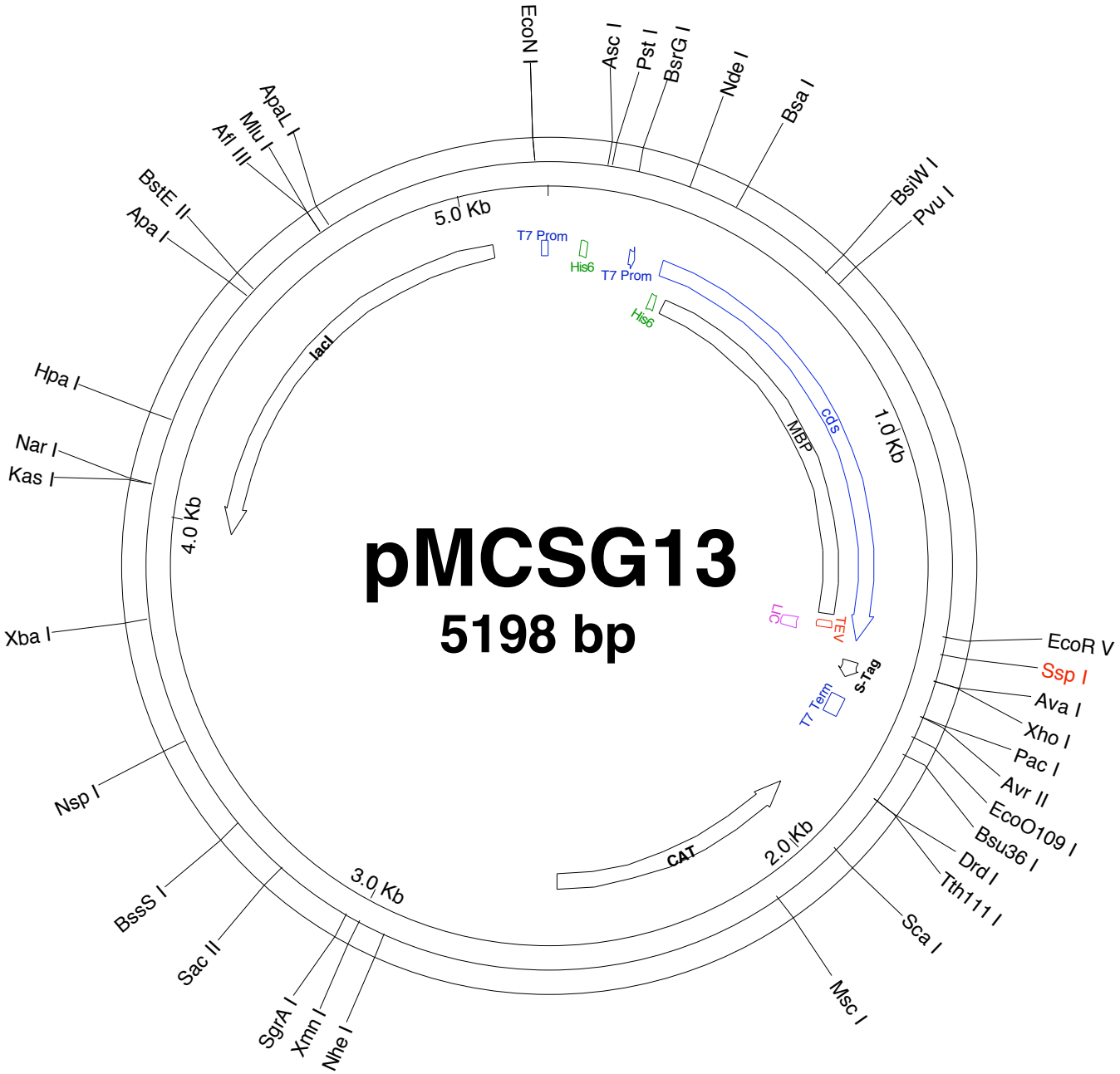

pMCSG9
6405 bp
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Nco I
BsiW I
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
Mlu I
BstE II
Apa I
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
Pst I
Sca I
T7
term
LIC
TEV
MBP
cds
His6
T7
prom
β-lac
LacI
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb

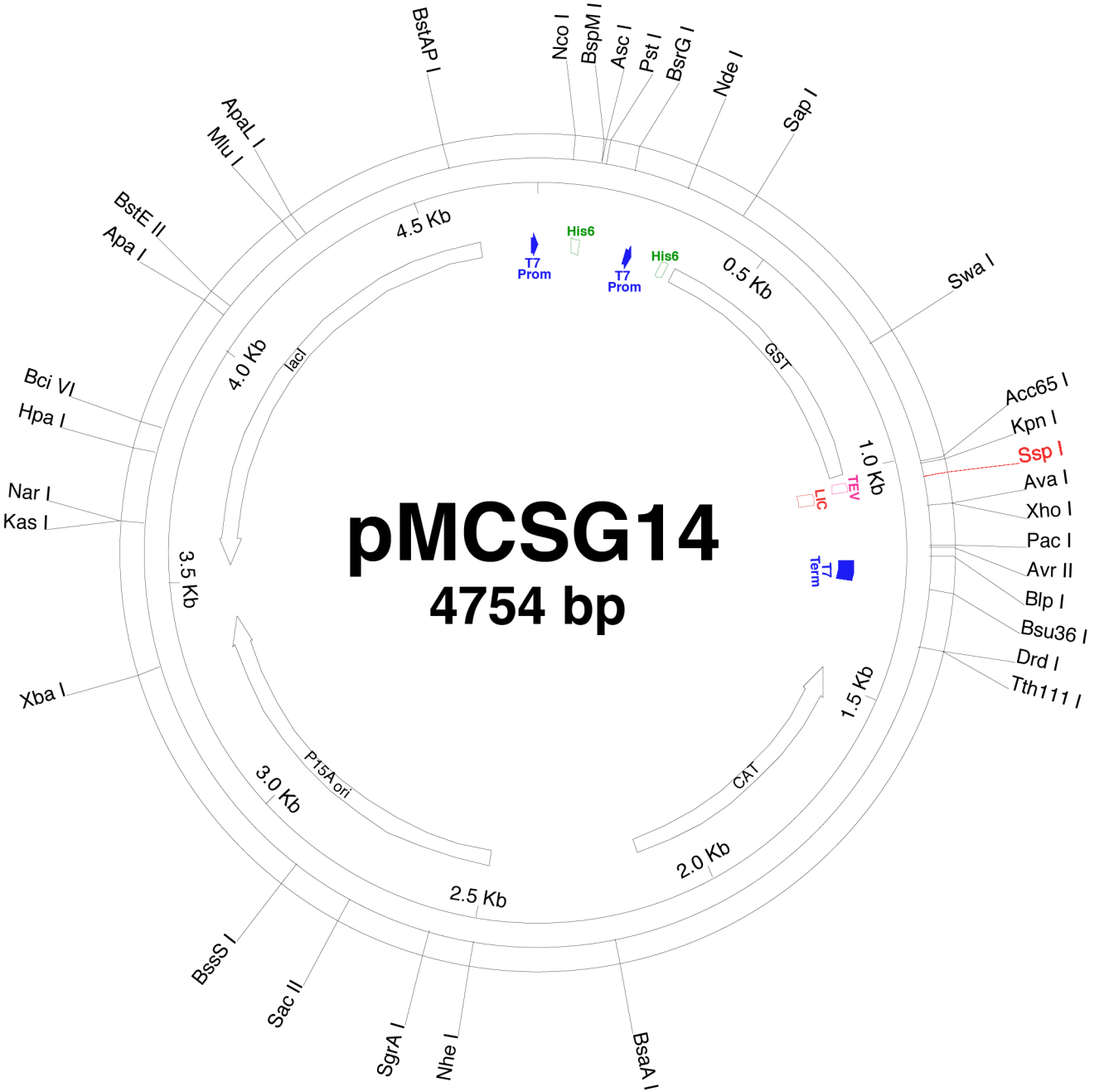

pMCSG9His10
6417 bp
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Nco I
BsiW I
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
Mlu I
BstE II
Apa I
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
Pst I
Sca I
T7
term
LIC
TEV
MBP
cds
His10
T7
prom
β-lac
LacI
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb

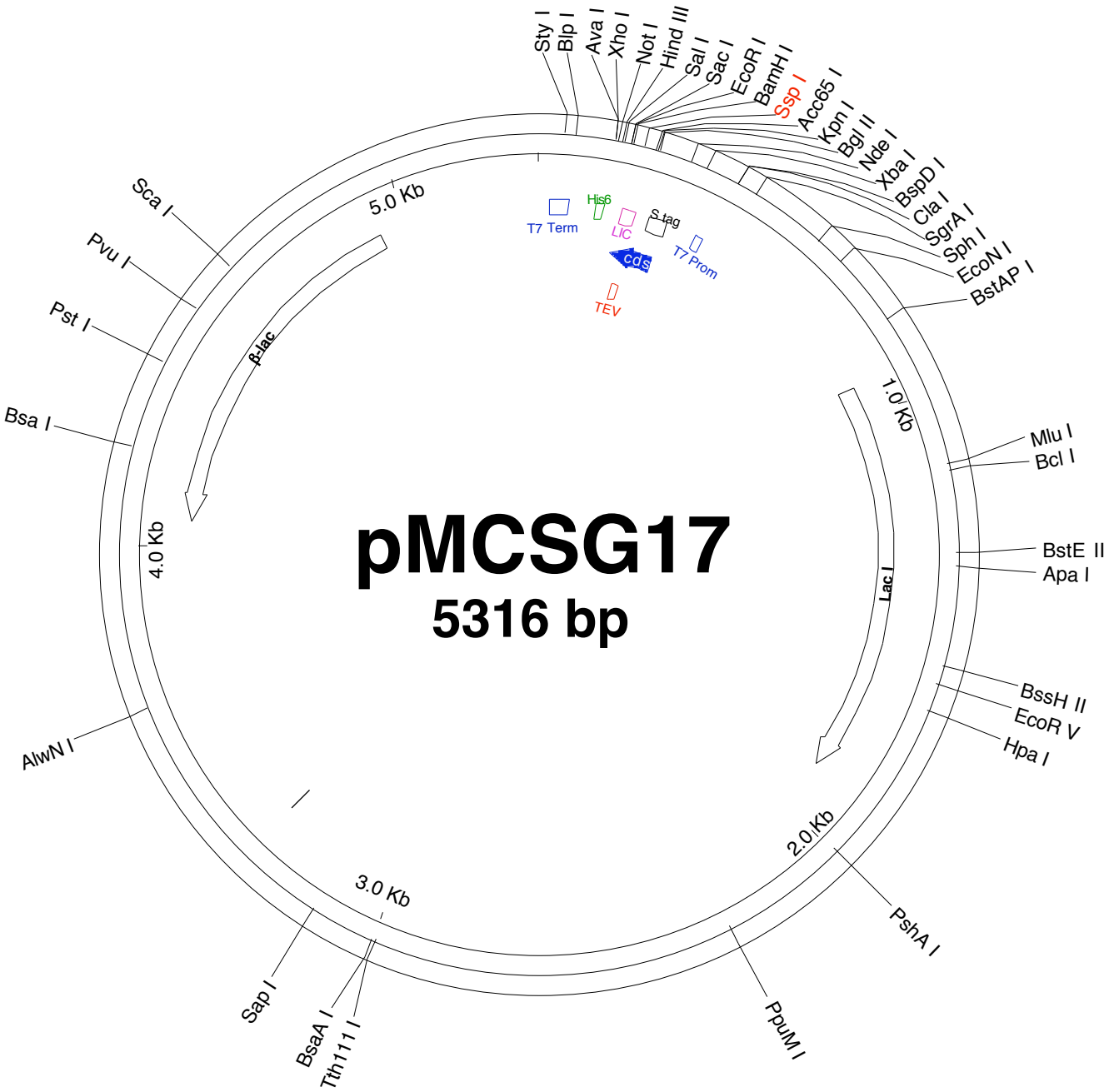

pMCSG10
5961 bp
Sty I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
Swa I
Msc I
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
BstAP I
Mlu I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
AlwN I
Bsa I
Pst I
Pvu I
T7 Term
LIC
TEV
GST
coding
His6
T7 Prom
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
β-lac
Lac I

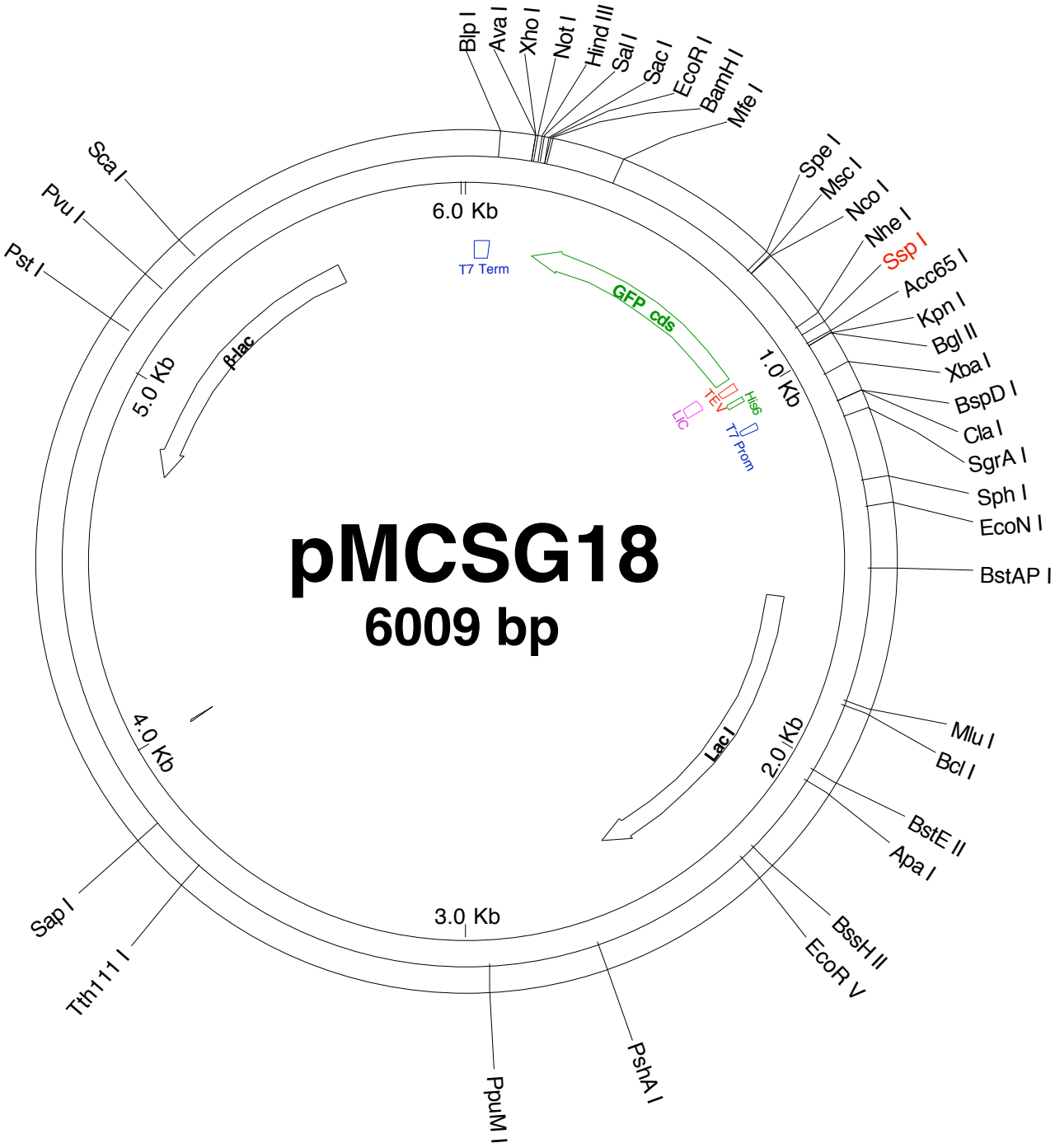

pMCSG11
4079 bp
4.0 Kb
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
T7 Prom
His6
T7 Prom
MCS
S-Tag
cds
TEV
His6
LIC
T7 Term
lacI
CAT
BstAP I
EcoN I
Nco I
BspM I
Asc I
Pst I
BsrG I
Nde I
Bgl II
Acc65 I
Kpn I
Ssp I
Ava I
Xho I
Pac I
Avr II
Blp I
EcoO109 I
Bsu36 I
Drd I
Tth111 I
Sca I
Msc I
BsaA I
Nhe I
Xmn I
Sgr A I
Sac II
BssS I
Nsp I
Xba I
Kas I
Nar I
Ava II
Hpa I
Bci VI
Apa I
BstE II
Bcl I
Afl III
Mlu I
ApaL I

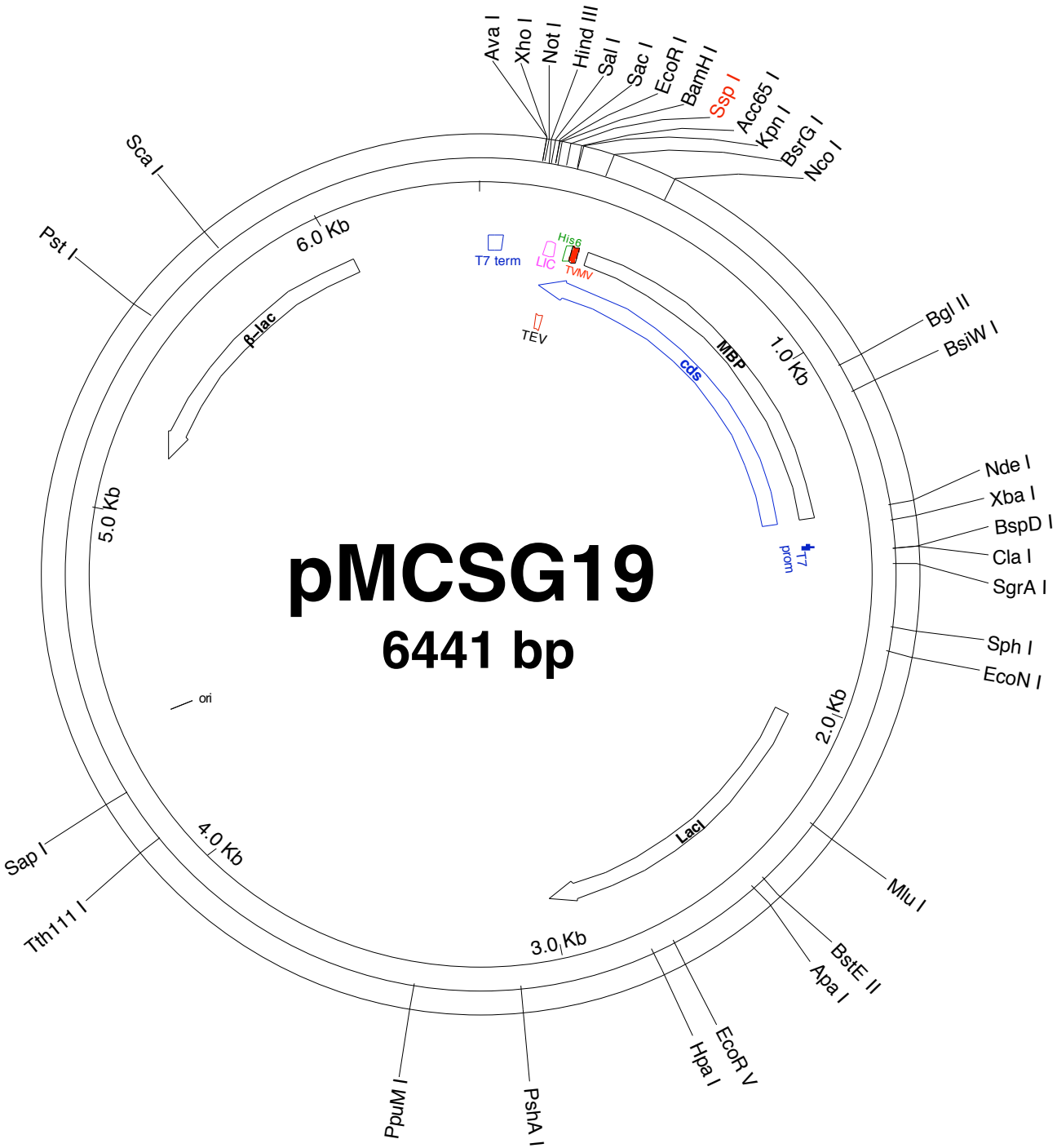

pMCSG12
4133 bp
BstAP I
EcoN I
Nco I
BspM I
Asc I
Pst I
BsrG I
Nde I
Bgl II
Acc65 I
Kpn I
Ssp I
Ava I
Xho I
Pac I
Avr II
Blp I
EcoO109 I
Bsu36 I
Drd I
Tth111 I
Sca I
Msc I
BsaA I
Nhe I
Xmn I
SgrA I
Sac II
BsssS I
Nsp I
Xba I
Bci VI
Hpa I
Ava II
Nar I
Kas I
BstE II
Apa I
Bcl I
Afl III
Mlu I
ApaL I
4.0 Kb
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
T7 Prom
His6
T7 Prom
ccdB
His6
S loop
TEV
LIC
S tag
T7 Term
CAT
lacI

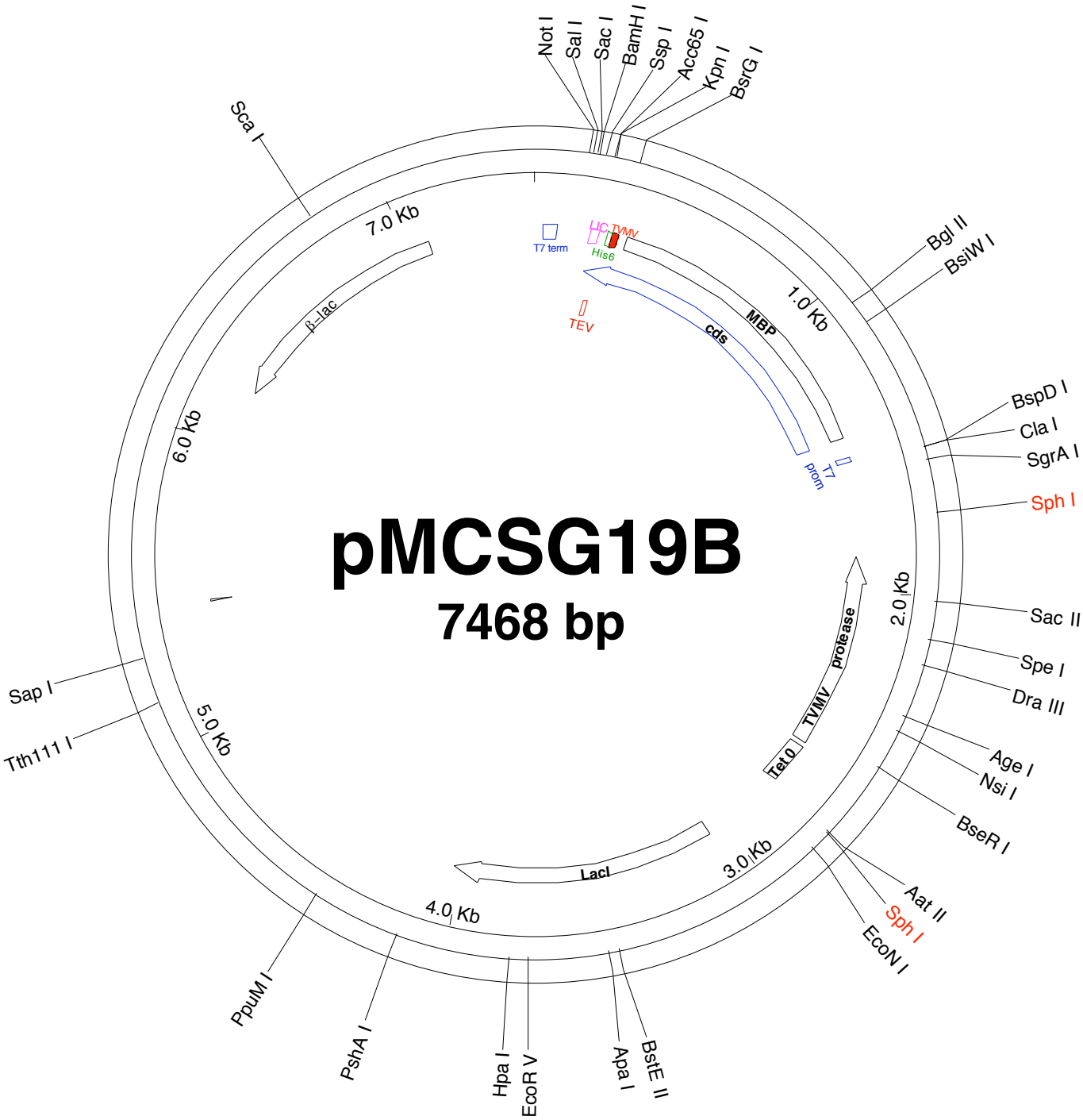

pMCSG13
5198 bp
5.0 Kb
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
T7 Prom
His6
T7 Prom
His6
cds
MBP
LIC
TEV
S-Tag
T7 Term
CAT
lacI
EcoN I
Asc I
Pst I
BsrG I
Nde I
Bsa I
BsiW I
Pvu I
EcoR V
Ssp I
Ava I
Xho I
Pac I
Avr II
EcoO109 I
Bsu36 I
Drd I
Tth111 I
Sca I
Msc I
Nhe I
Xmn I
SgrA I
Sac II
BssS I
Nsp I
Xba I
Kas I
Nar I
Hpa I
Apa I
BstE II
Afl III
Mlu I
ApaL I

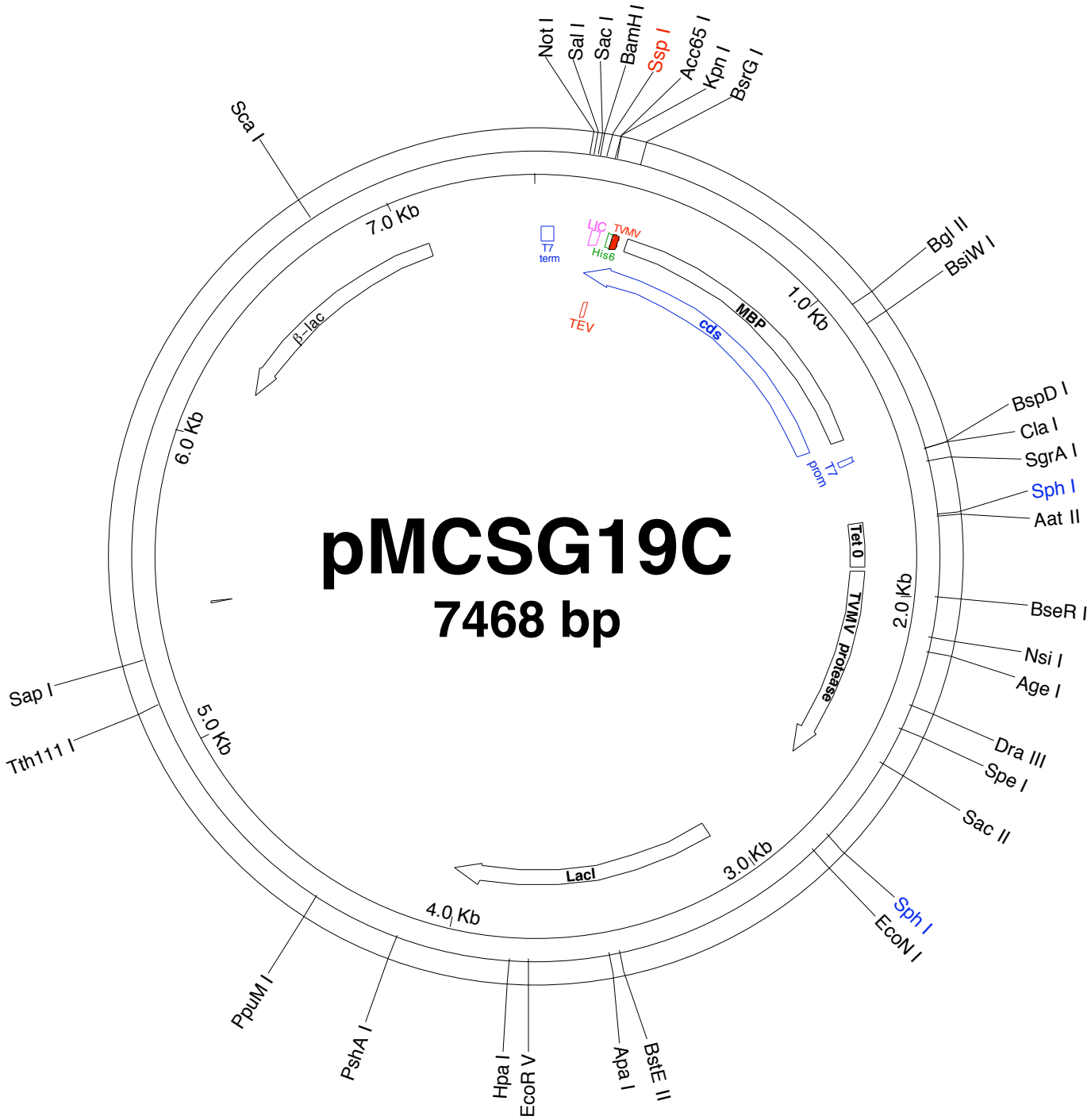

pMCSG14
4754 bp
BstAP I
Nco I
BspM I
Asc I
Pst I
BsrG I
Nde I
Sap I
Swa I
Acc65 I
Kpn I
Ssp I
Ava I
Xho I
Pac I
Avr II
Blp I
Bsu36 I
Drd I
Tth111 I
BsaA I
Nhe I
SgrA I
Sac II
BsssS I
Xba I
Nar I
Kas I
Bci VI
Hpa I
BstE II
Apa I
ApaL I
Mlu I
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
4.0 Kb
4.5 Kb
His6
His6
T7 Prom
T7 Prom
GST
LIC
TEV
T7 Term
CAT
P15A ori
lacI

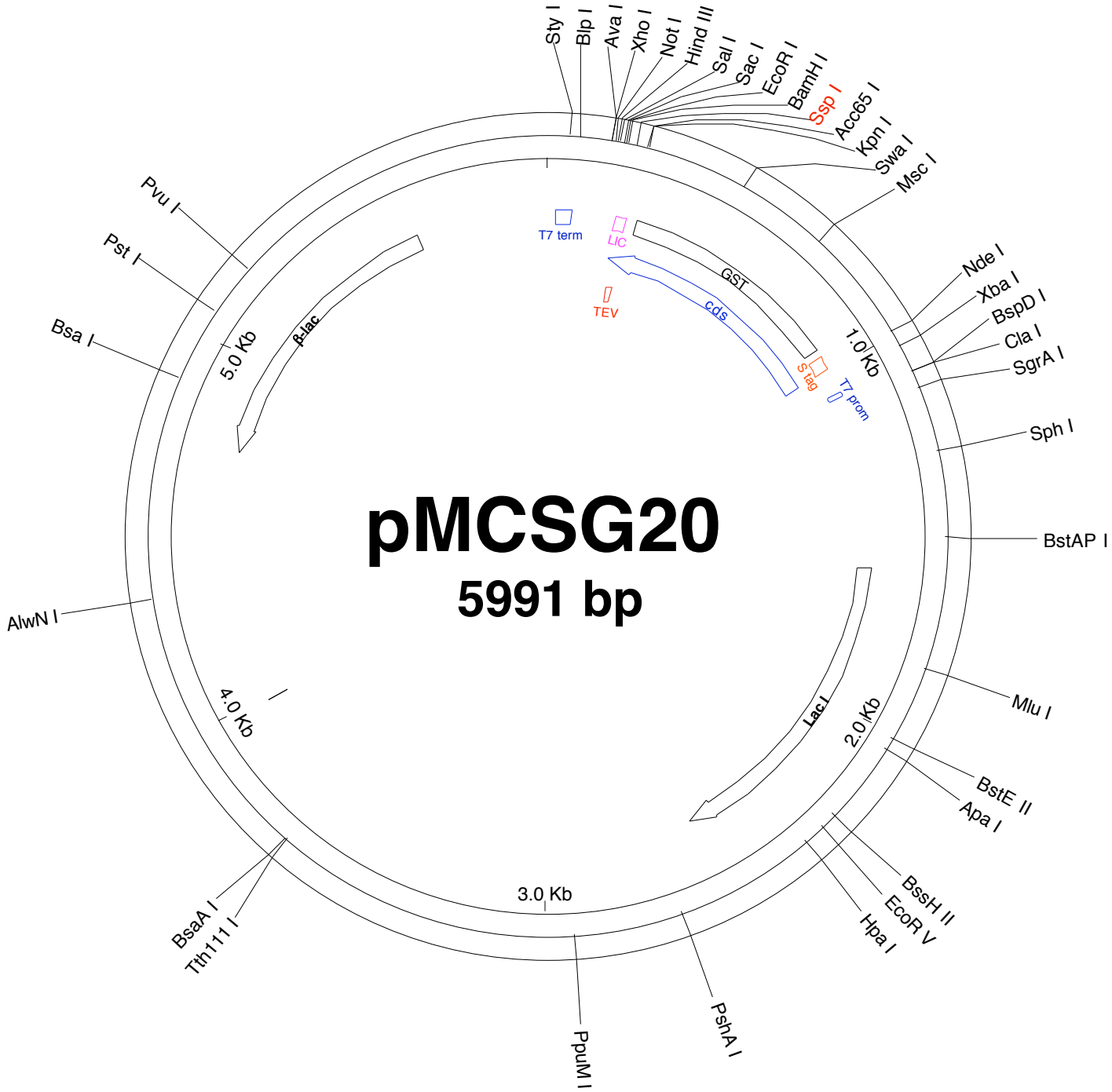

pMCSG17
5316 bp
Sty I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
2.0 Kb
1.0 Kb
Lac I
3.0 Kb
Sap I
BsaA I
Tth111 I
AlwN I
4.0 Kb
Bsa I
Pst I
Pvu I
Sca I
5.0 Kb
β-lac
T7 Term
His6
LIC
S tag
T7 Prom
cds
TEV

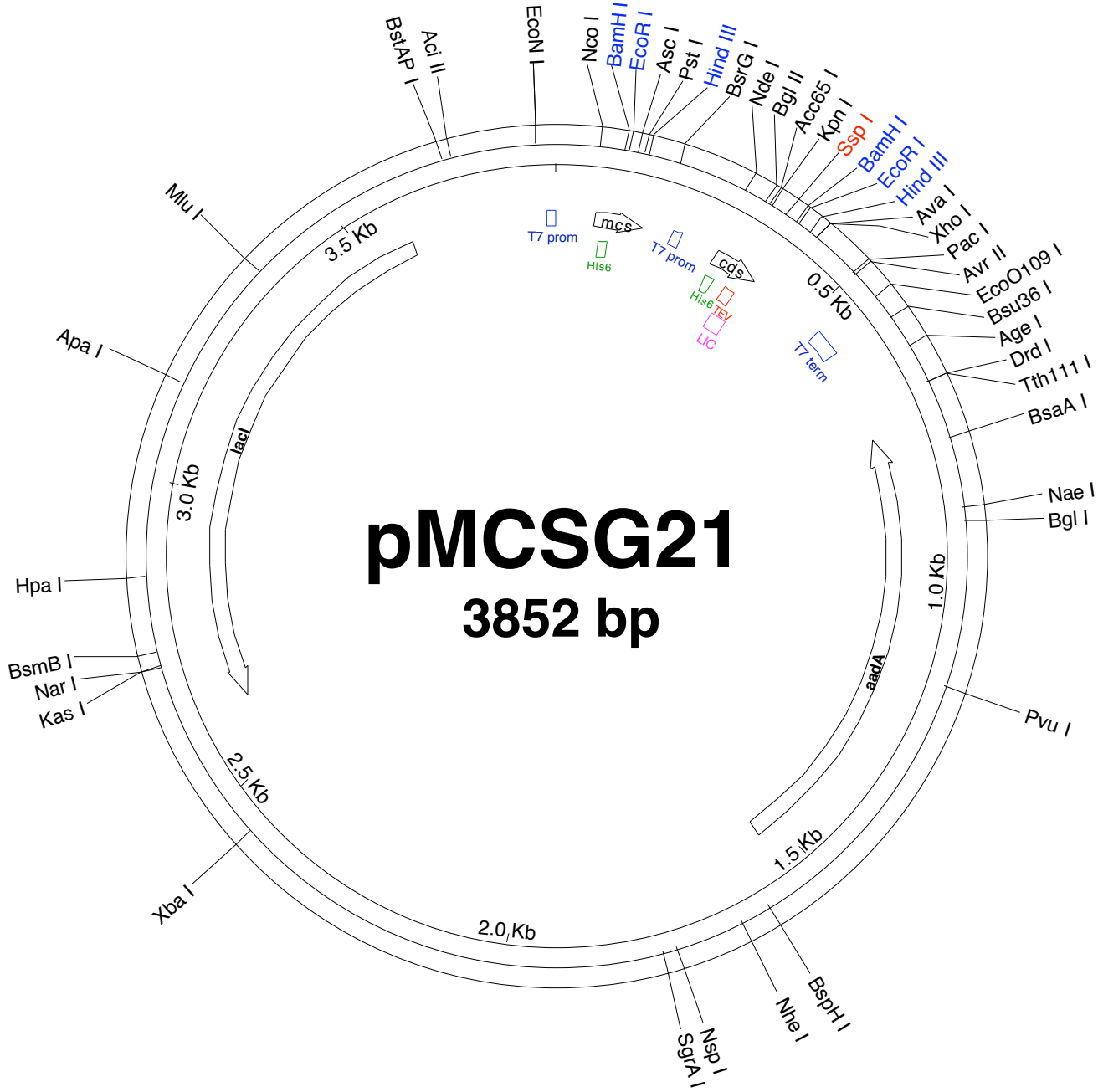

pMCSG18
6009 bp
6.0 Kb
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
T7 Term
GFP cds
TEV
His6
LIC
T7 Prom
β-lac
Lac I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Mfe I
Spe I
Msc I
Nco I
Nhe I
Ssp I
Acc65 I
Kpn I
Bgl II
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
PshA I
PpuM I
Tth111 I
Sap I
Pst I
Pvu I
Sca I

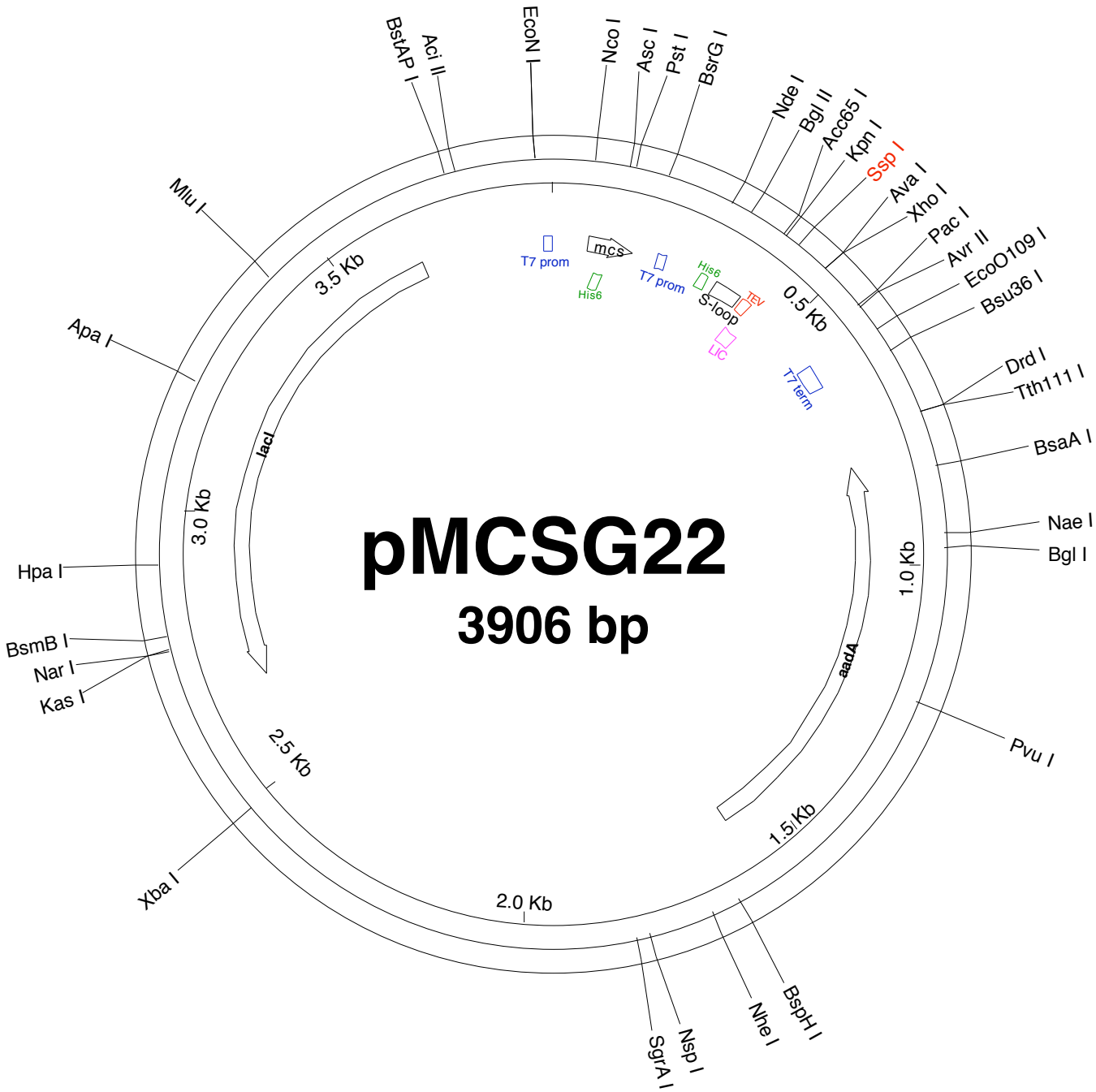

pMCSG19
6441 bp
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
BsrG I
Nco I
Bgl II
BsiW I
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
Mlu I
BstE II
Apa I
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
Pst I
Sca I
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb
T7 term
His6
LIC
TVMV
TEV
MBP
cds
T7 prom
β-lac
LacI
ori

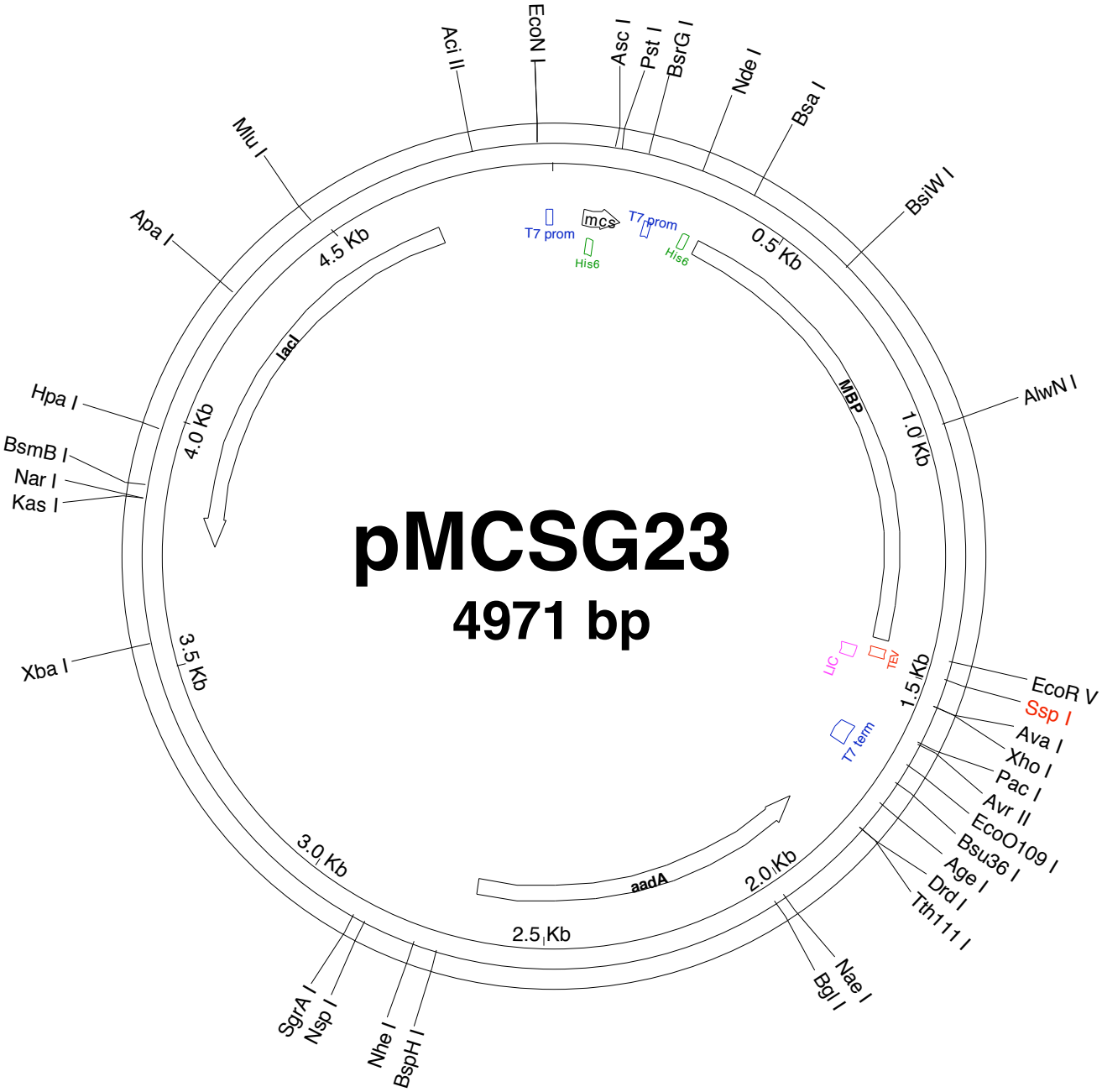

pMCSG19B
7468 bp
Not I
Sal I
Sac I
BamH I
Ssp I
Acc65 I
Kpn I
BsrG I
Bgl II
BsiW I
BspD I
Cla I
SgrA I
Sph I
Sac II
Spe I
Dra III
Age I
Nsi I
BseR I
Aat II
Sph I
EcoN I
BstE II
Apa I
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
Sca I
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb
7.0 Kb
T7 term
HC
TVMV
His6
TEV
cds
MBP
T7
prom
β-lac
LacI
Tet 0
TVMV
protease

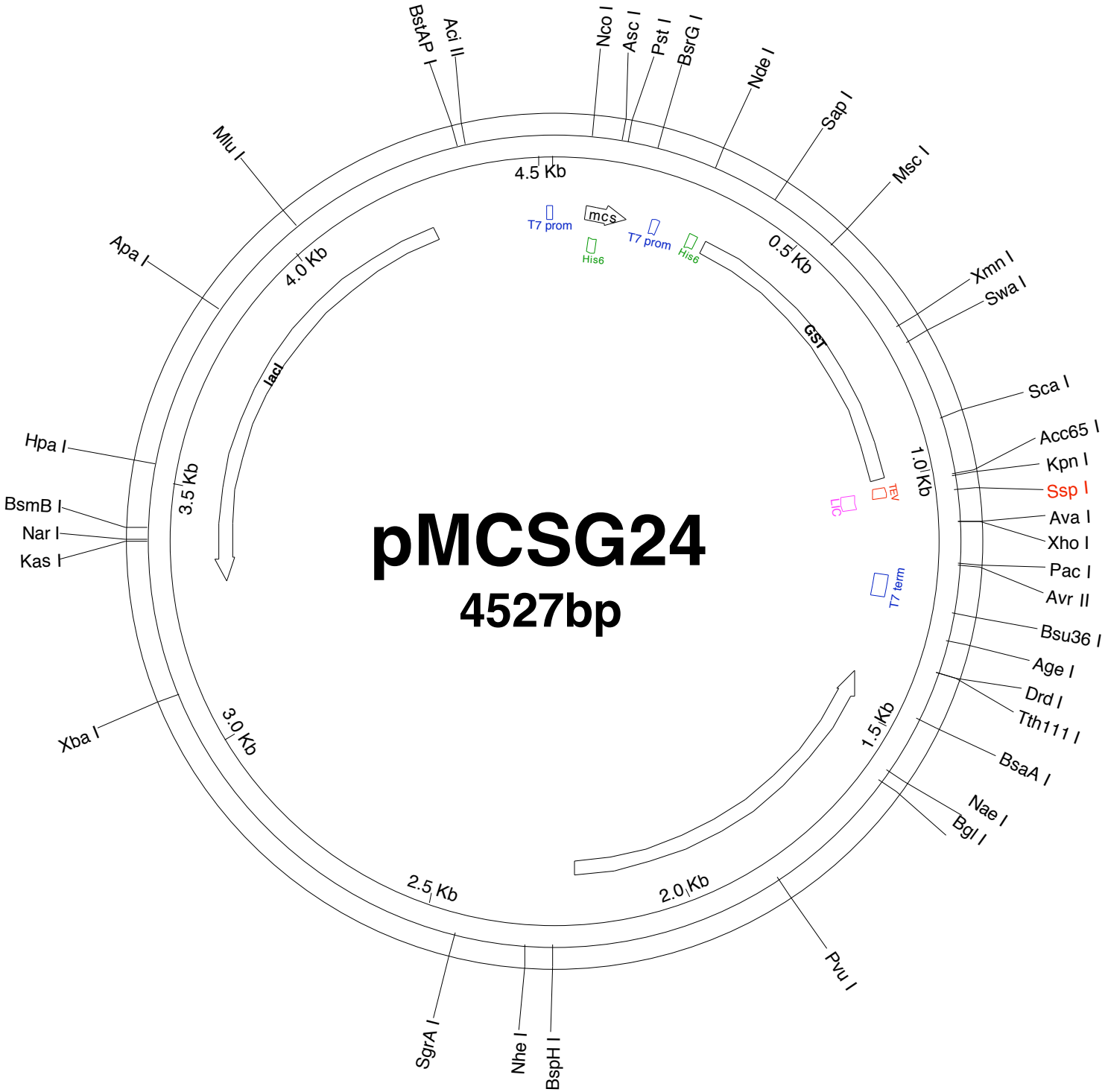

pMCSG19C
7468 bp
Not I
Sal I
Sac I
BamH I
Ssp I
Acc65 I
Kpn I
BsrG I
Bgl II
BsiW I
BspD I
Cla I
SgrA I
Sph I
Aat II
BseR I
Nsi I
Age I
Dra III
Spe I
Sac II
Sph I
EcoN I
BstE II
Apa I
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
Sca I
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb
7.0 Kb
MBP
cds
T7 prom
Tet 0
TVMV protease
LacI
β-lac
T7 term
His6
LIC
TVMV
TEV

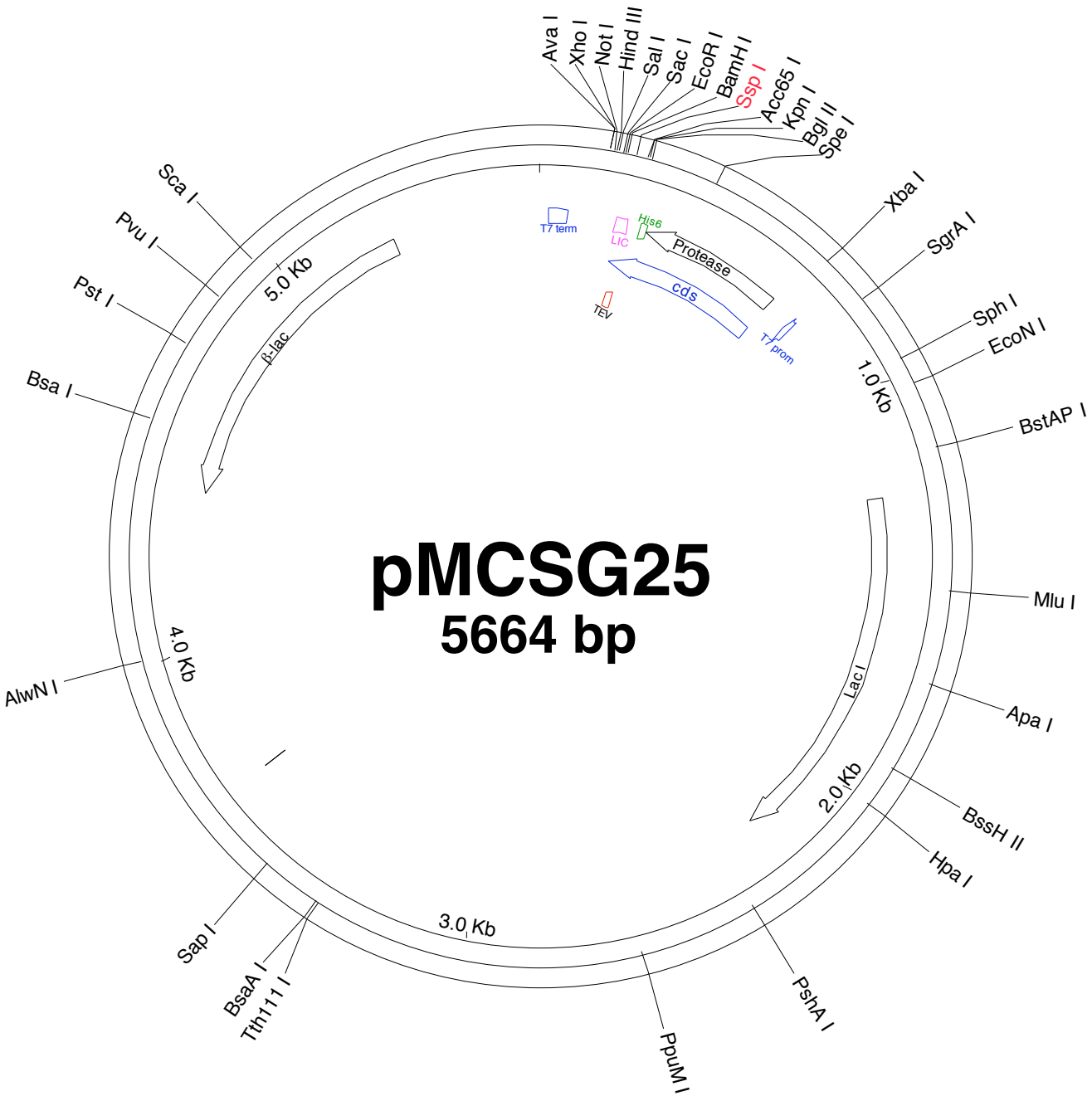

pMCSG20
5991 bp
Sty I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
Swa I
Msc I
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
BstAP I
Mlu I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
BsaA I
Tth111 I
AlwN I
Bsa I
Pst I
Pvu I
T7 term
LIC
TEV
GST
cds
S tag
T7 prom
β-lac
Lac I
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb

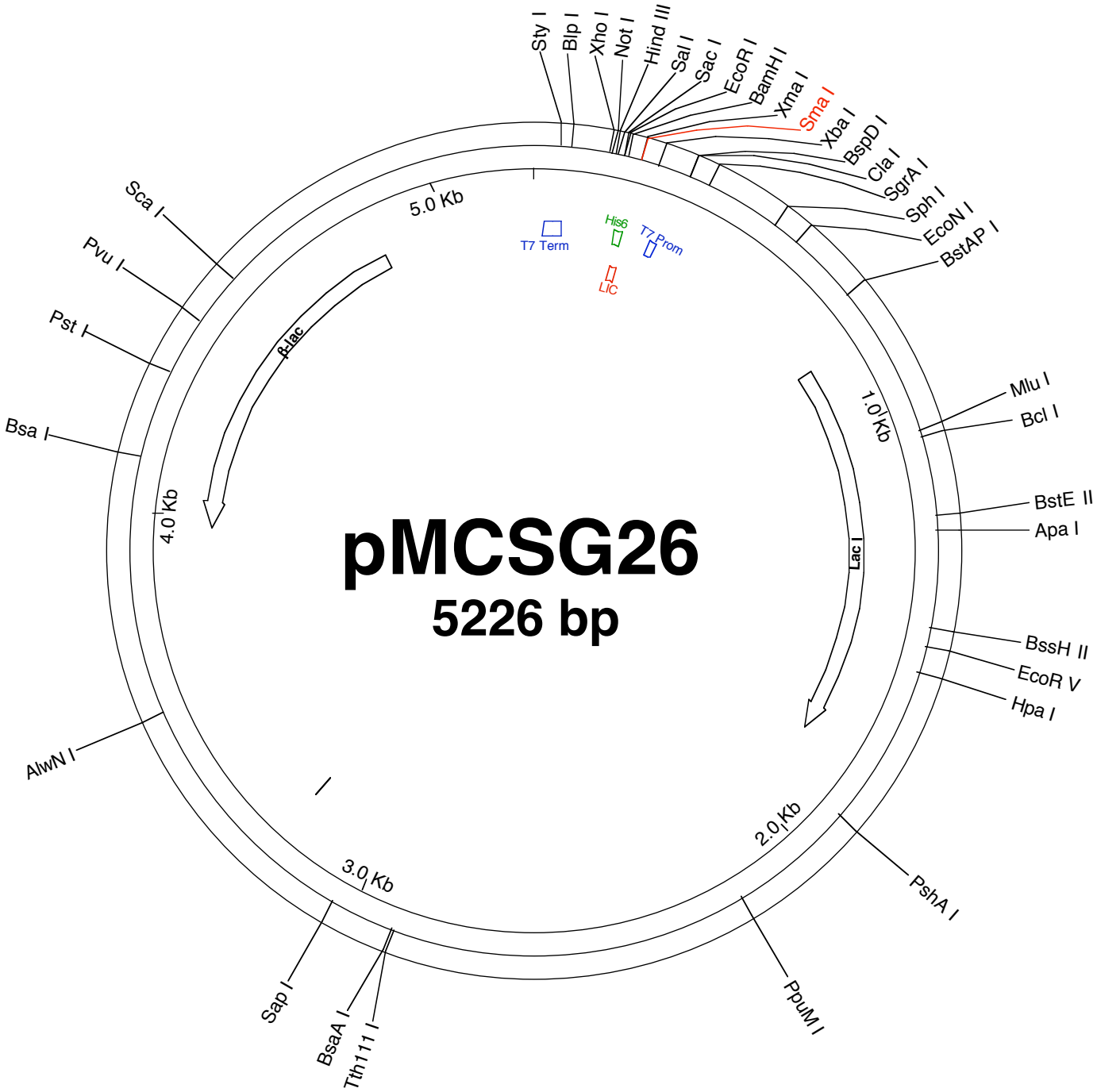

pMCSG21
3852 bp
EcoN I
Nco I
BamH I
EcoR I
Asc I
Pst I
Hind III
BsrG I
Nde I
Bgl II
Acc65 I
Kpn I
Ssp I
BamH I
EcoR I
Hind III
Ava I
Xho I
Pac I
Avr II
EcoO109 I
Bsu36 I
Age I
Drd I
Tth111 I
BsaA I
Nae I
Bgl I
Pvu I
BspH I
Nhe I
Nsp I
SgrA I
Xba I
Kas I
Nar I
BsmB I
Hpa I
Apa I
Mlu I
BstAP I
Aci II
T7 prom
mcs
His6
T7 prom
cds
His6
TEV
LIC
T7 term
lacI
aadA
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb

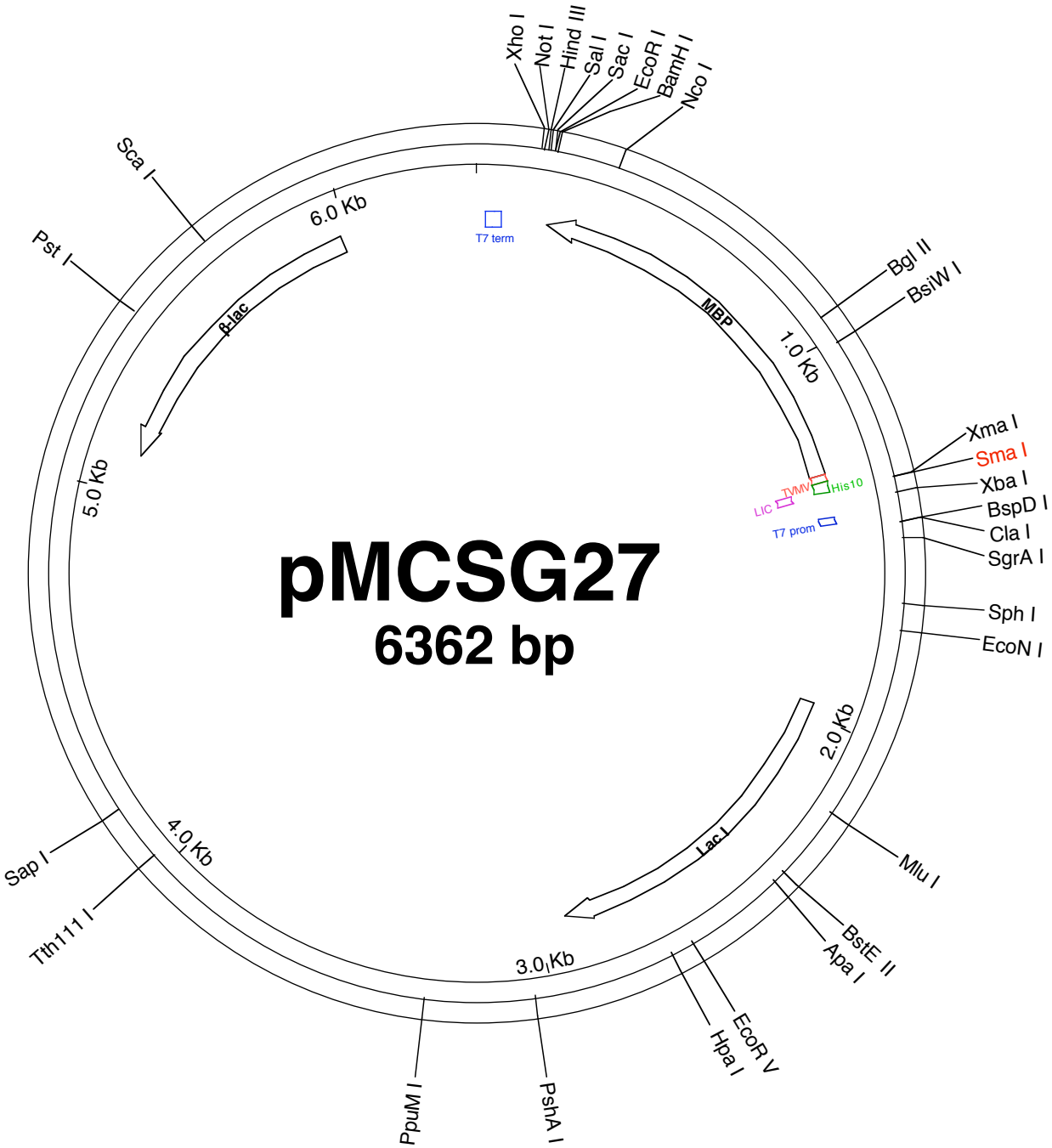

pMCSG22
3906 bp
BstAP I
Aci II
EcoN I
Nco I
Asc I
Pst I
BsrG I
Nde I
Bgl II
Acc65 I
Kpn I
Ssp I
Ava I
Xho I
Pac I
Avr II
EcoO109 I
Bsu36 I
Drd I
Tth111 I
BsaA I
Nae I
Bgl I
Pvu I
BspH I
Nhe I
Nsp I
SgrA I
2.0 Kb
Xba I
2.5 Kb
Kas I
Nar I
BsmB I
Hpa I
3.0 Kb
Apa I
Mlu I
3.5 Kb
lacI
T7 prom
mcs
His6
T7 prom
His6
S-loop
TEV
LC
0.5 Kb
T7 term
1.0 Kb
aadA
1.5 Kb

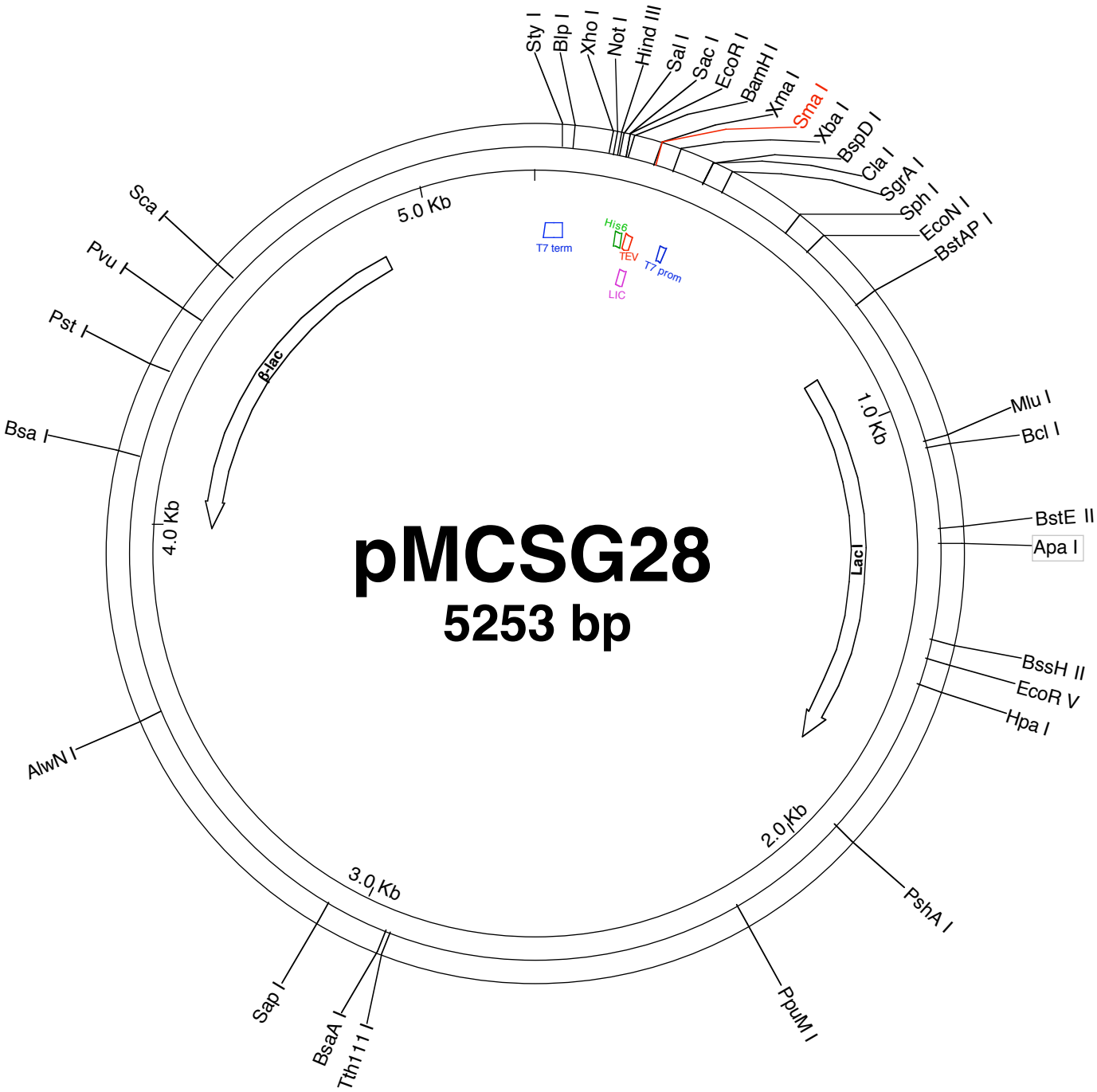

pMCSG23
4971 bp
Aci II
EcoN I
Asc I
Pst I
BsrG I
Nde I
Bsa I
BsiW I
AlwN I
EcoR V
Ssp I
Ava I
Xho I
Pac I
Avr II
EcoO109 I
Bsu36 I
Age I
Drd I
Tth111 I
Nae I
Bgl I
BspH I
Nhe I
Nsp I
SgrA I
Xba I
Kas I
Nar I
BsmB I
Hpa I
Apa I
Mlu I
4.5 Kb
4.0 Kb
3.5 Kb
3.0 Kb
2.5 Kb
2.0 Kb
1.5 Kb
1.0 Kb
0.5 Kb
lacI
MBP
aadA
T7 prom
mcs
T7 prom
His6
His6
LIC
TEV
T7 term

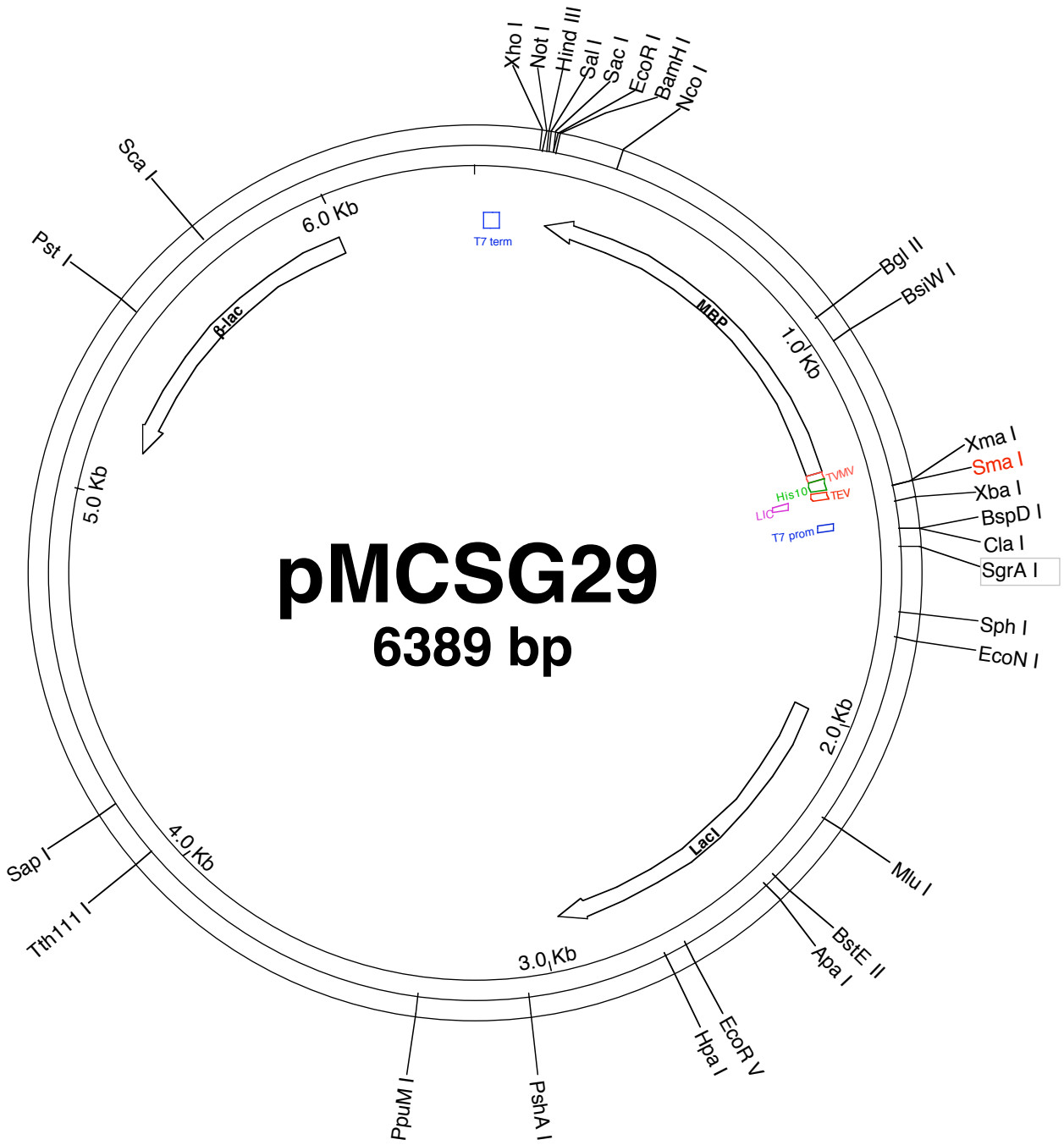

pMCSG24
4527bp
BstAP I
Aci II
Nco I
Asc I
Pst I
BsrG I
Nde I
Sap I
Msc I
Xmn I
Swa I
Sca I
Acc65 I
Kpn I
Ssp I
Ava I
Xho I
Pac I
Avr II
Bsu36 I
Age I
Drd I
Tth111 I
BsaA I
Nae I
Bgl I
Pvu I
BspH I
Nhe I
SgrA I
Xba I
Hpa I
BsmB I
Nar I
Kas I
Apa I
Mlu I
4.5 Kb
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
4.0 Kb
T7 prom
mcs
T7 prom
His6
His6
GST
TEV
LIC
T7 term
lacI

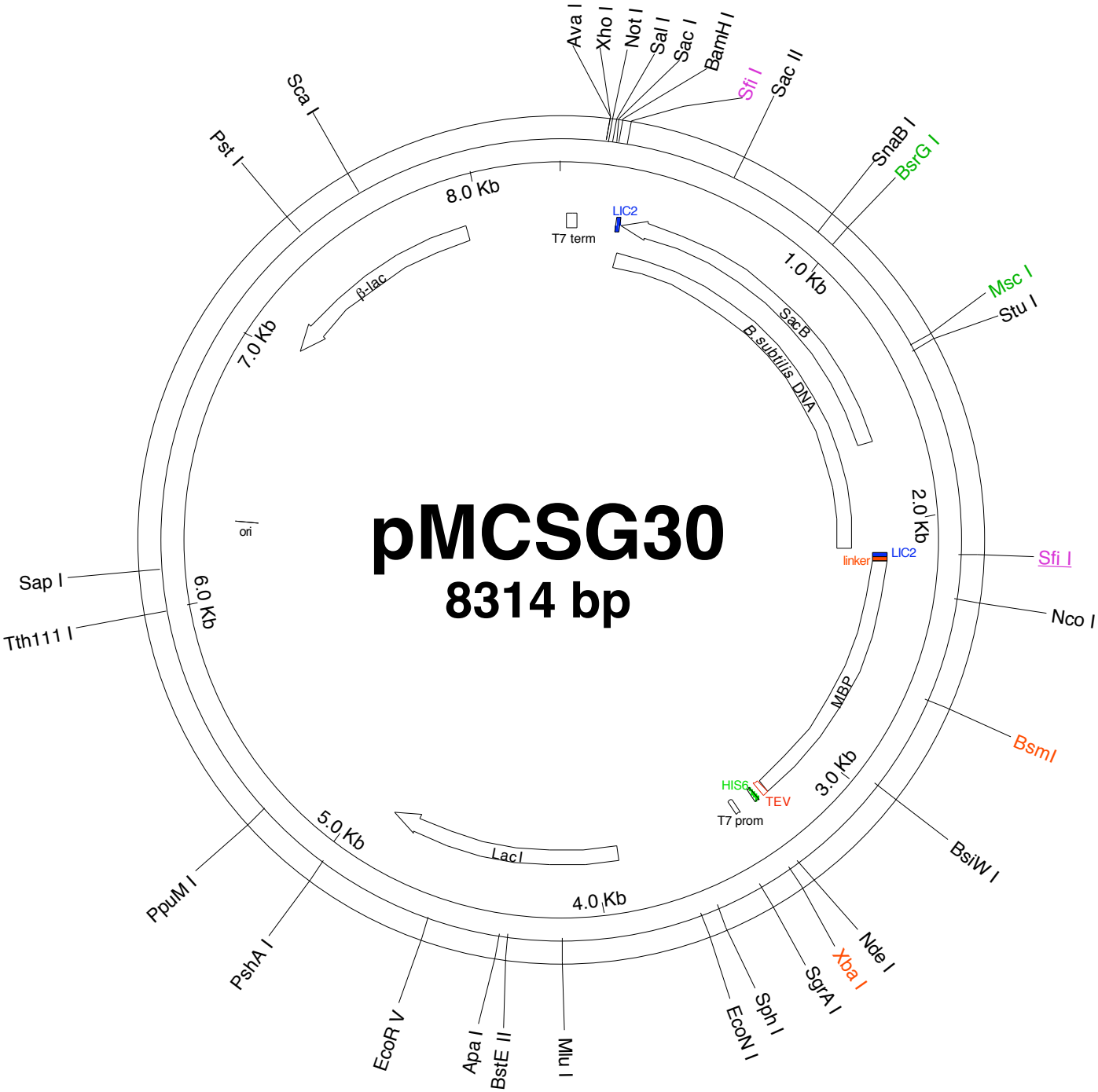

pMCSG25
5664 bp
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
Bgl II
Spe I
Xba I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Apa I
BssH II
Hpa I
PshA I
PpuM I
3.0 Kb
Sap I
BsaA I
Tth111 I
AlwN I
4.0 Kb
Bsa I
Pst I
Pvu I
Sca I
5.0 Kb
β-lac
1.0 Kb
Lac I
2.0 Kb
T7 term
LIC
His6
Protease
cds
TEV
T7 prom

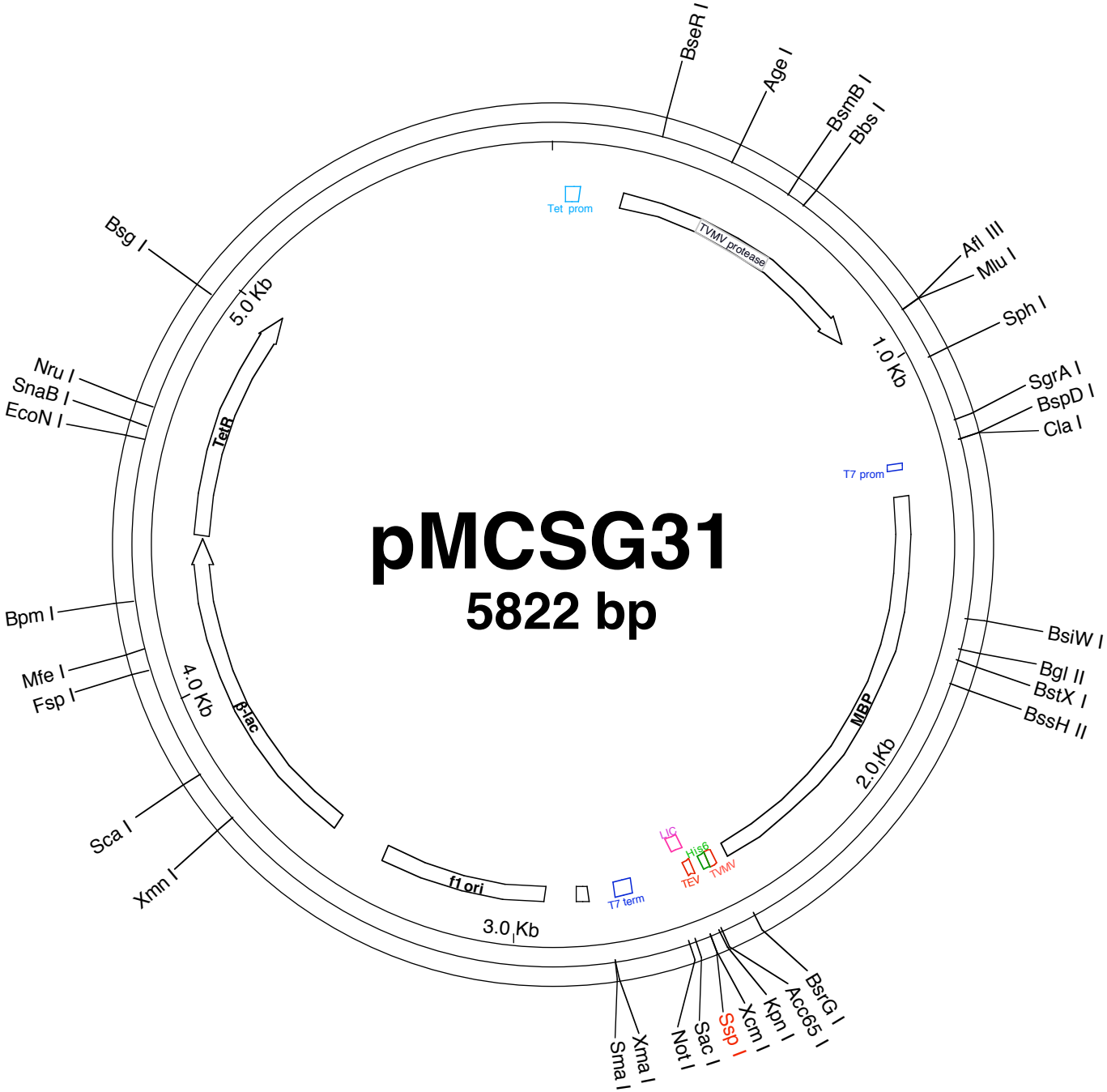

pMCSG26
5226 bp
Sty I
Blp I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
AlwN I
Bsa I
Pst I
Pvu I
Sca I
5.0 Kb
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
T7 Term
His6
T7 Prom
LIC
β-lac
Lac I

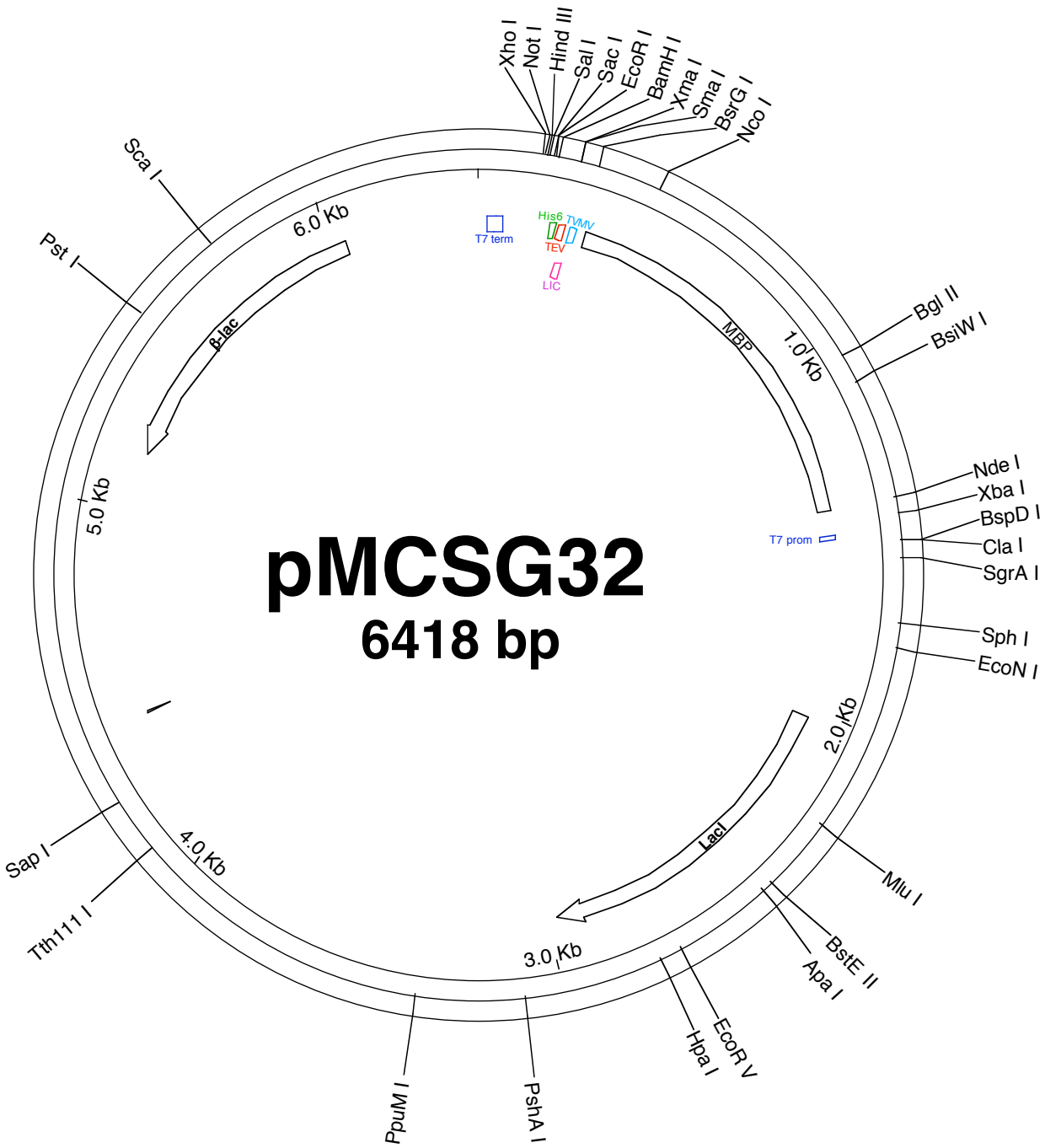

pMCSG27
6362 bp
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Nco I
Bgl II
BsiW I
Xma I
Sma I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
Mlu I
BstE II
Apa I
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
Pst I
Sca I
T7 term
MBP
1.0 Kb
His10
TVMV
LIC
T7 prom
Lac I
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb
β-lac

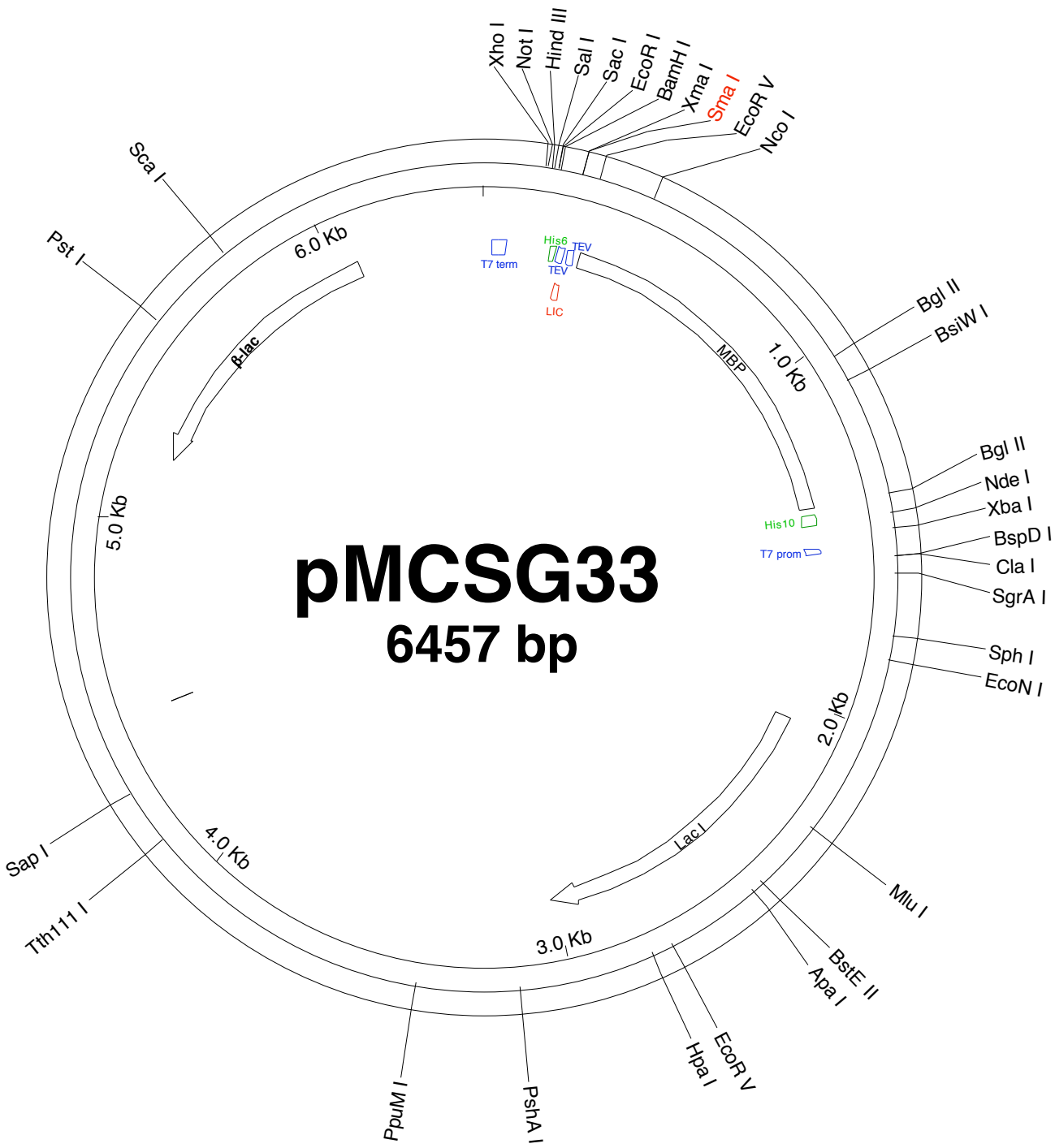

pMCSG28
5253 bp
Sty I
Blp I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
AlwN I
Bsa I
Pst I
Pvu I
Sca I
5.0 Kb
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
T7 term
His6
TEV
T7 prom
LIC
β-lac
LacI

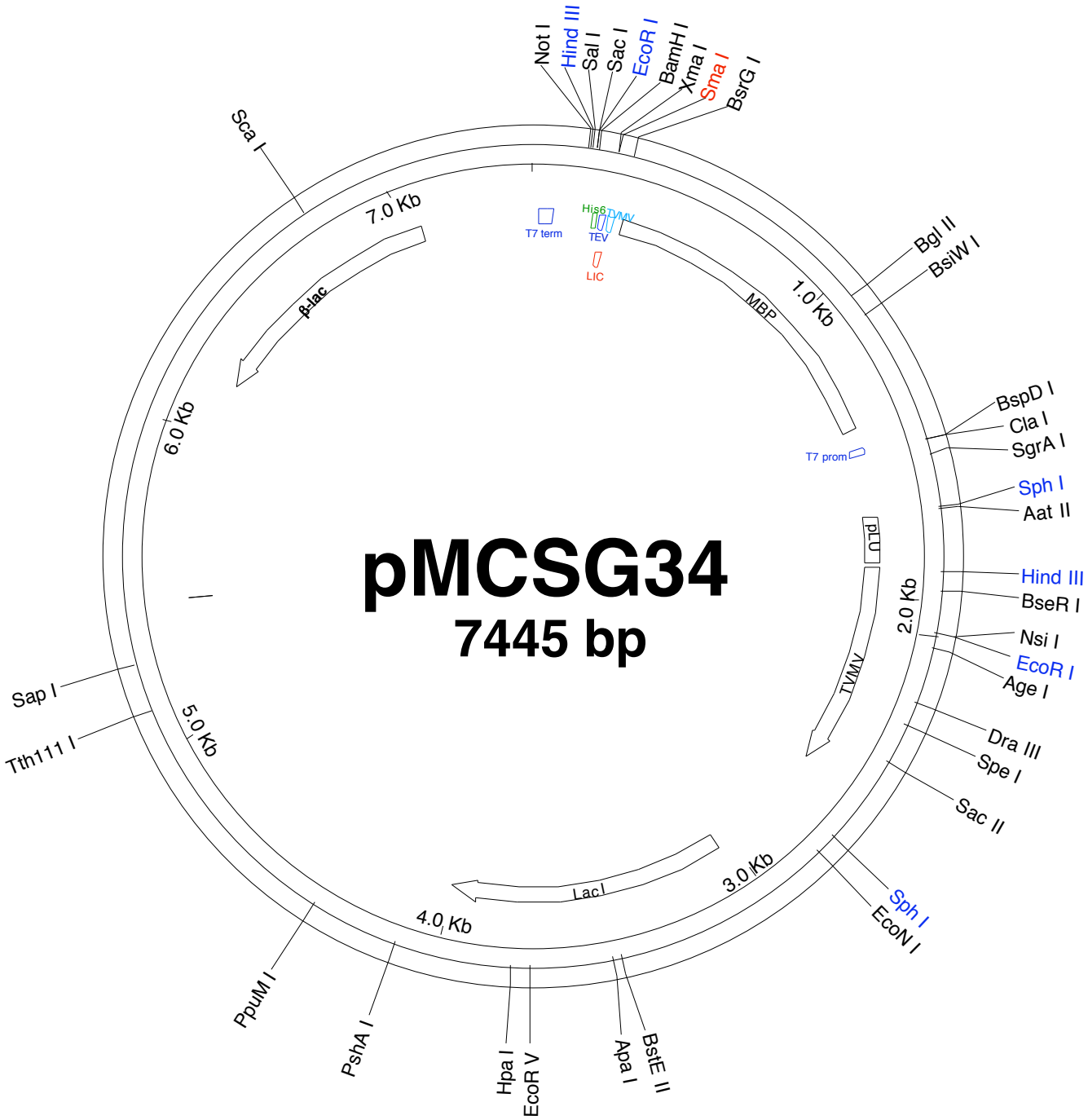

pMCSG29
6389 bp
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Nco I
Bgl II
BsiW I
Xma I
Sma I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
Mlu I
BstE II
Apa I
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
Pst I
Sca I
T7 term
MBP
1.0 Kb
TVMV
TEV
His10
LIC
T7 prom
LacI
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb
βlac

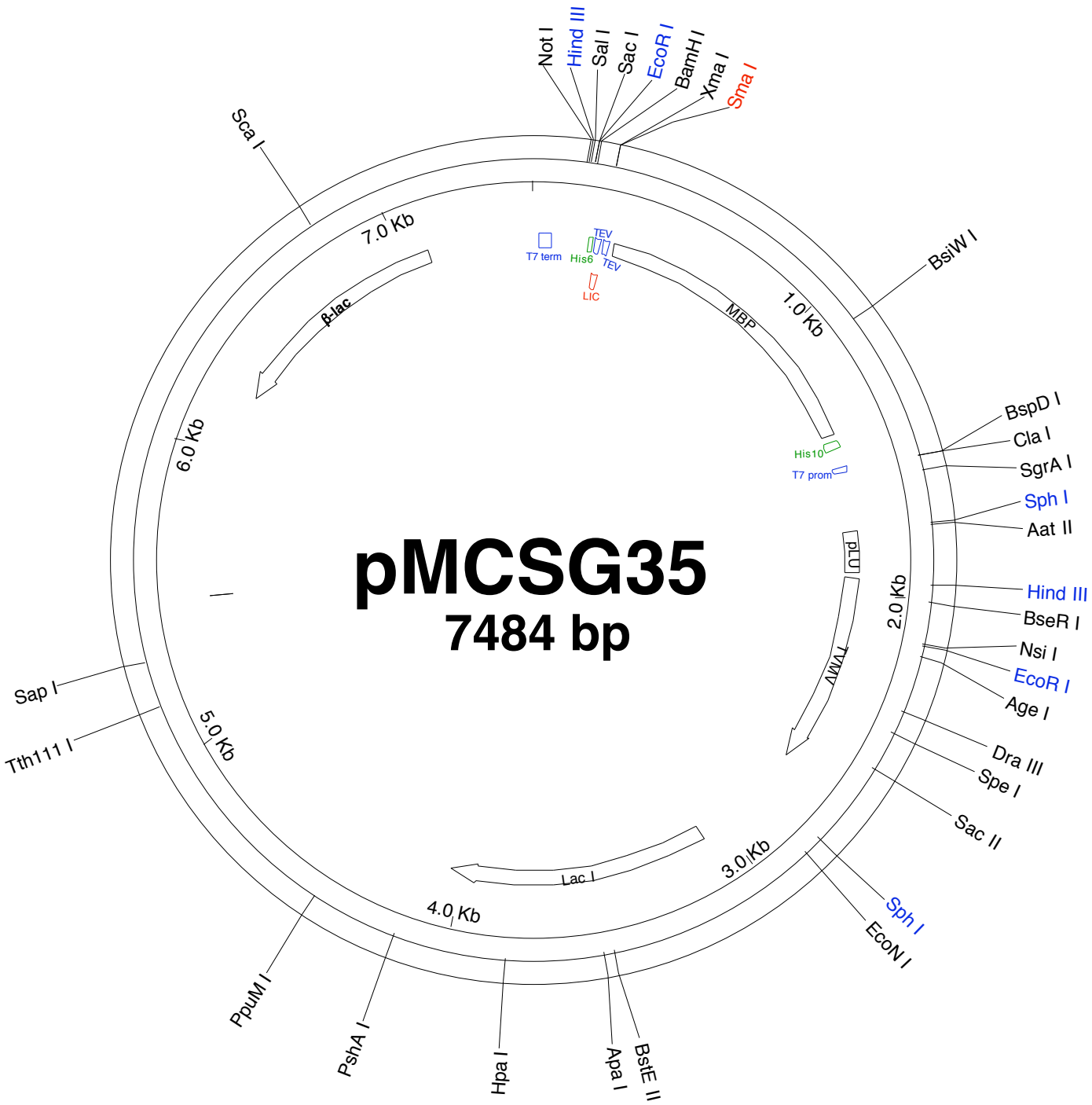

pMCSG30
8314 bp
Ava I
Xho I
Not I
Sal I
Sac I
BamH I
Sfi I
Sac II
SnaB I
BsrG I
Msc I
Stu I
Sfi I
Nco I
BsmI
BsiW I
Nde I
Xba I
SgrA I
Sph I
EcoN I
Mlu I
BstE II
Apa I
EcoR V
PshA I
PpuM I
Sap I
Tth111 I
Pst I
Sca I
8.0 Kb
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb
7.0 Kb
T7 term
LIC2
SacB
B.subtilis DNA
linker
LIC2
MBP
HIS6
TEV
T7 prom
LacI
β-lac
ori

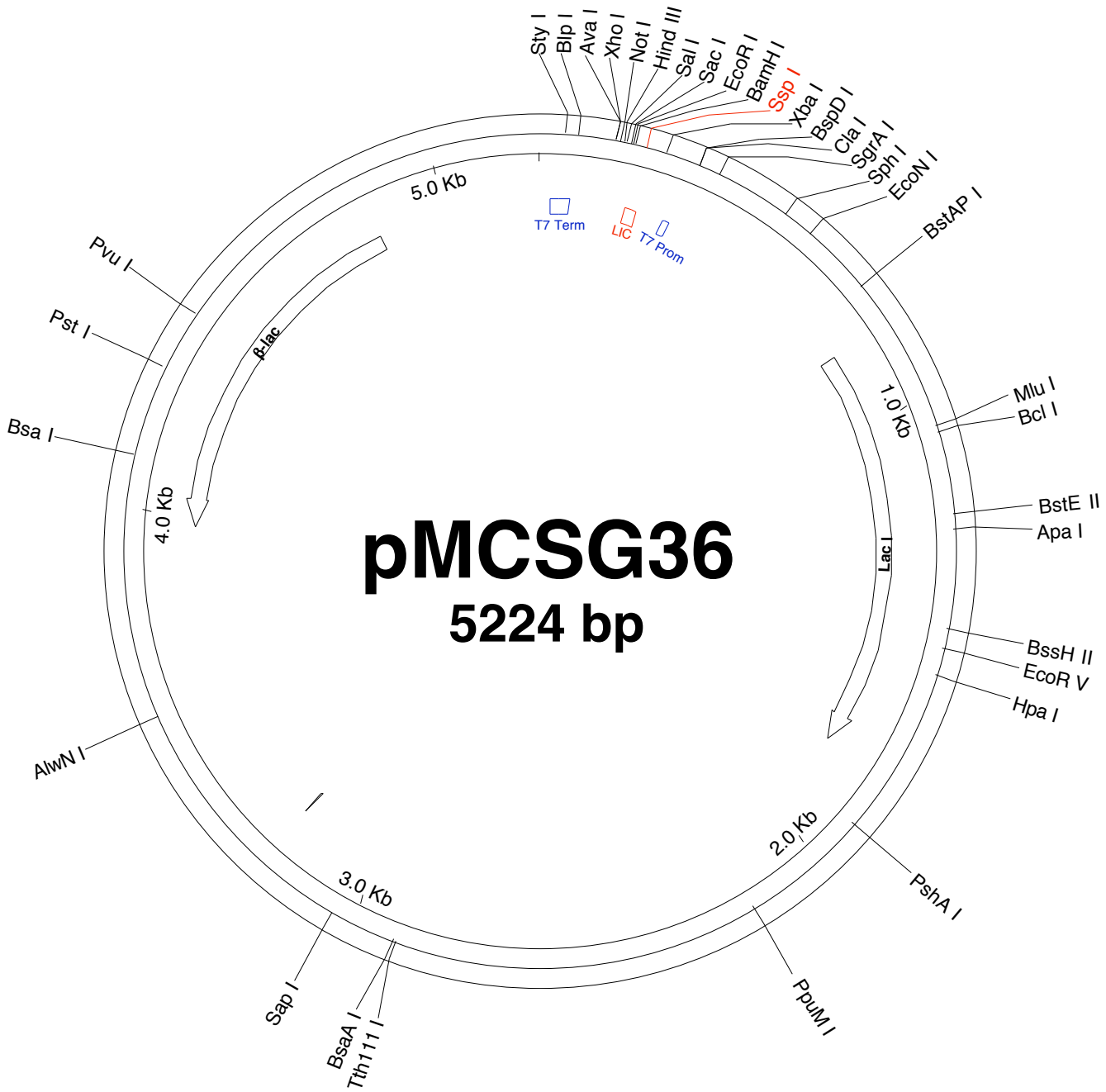

pMCSG31
5822 bp
BseR I
Age I
BsmB I
Bbs I
Afl III
Mlu I
Sph I
SgrA I
BspD I
Cla I
BsiW I
Bgl II
BstX I
BssH II
BsrG I
Acc65 I
Kpn I
Xcm I
Ssp I
Sac I
Not I
Xma I
Sma I
Xmn I
Sca I
Fsp I
Mfe I
Bpm I
EcoN I
SnaB I
Nru I
Bsg I
Tet prom
TVMV protease
1.0 Kb
T7 prom
MBP
2.0 Kb
LIC
His6
TEV
TVMV
T7 term
f1 ori
3.0 Kb
β-lac
4.0 Kb
TetR
5.0 Kb

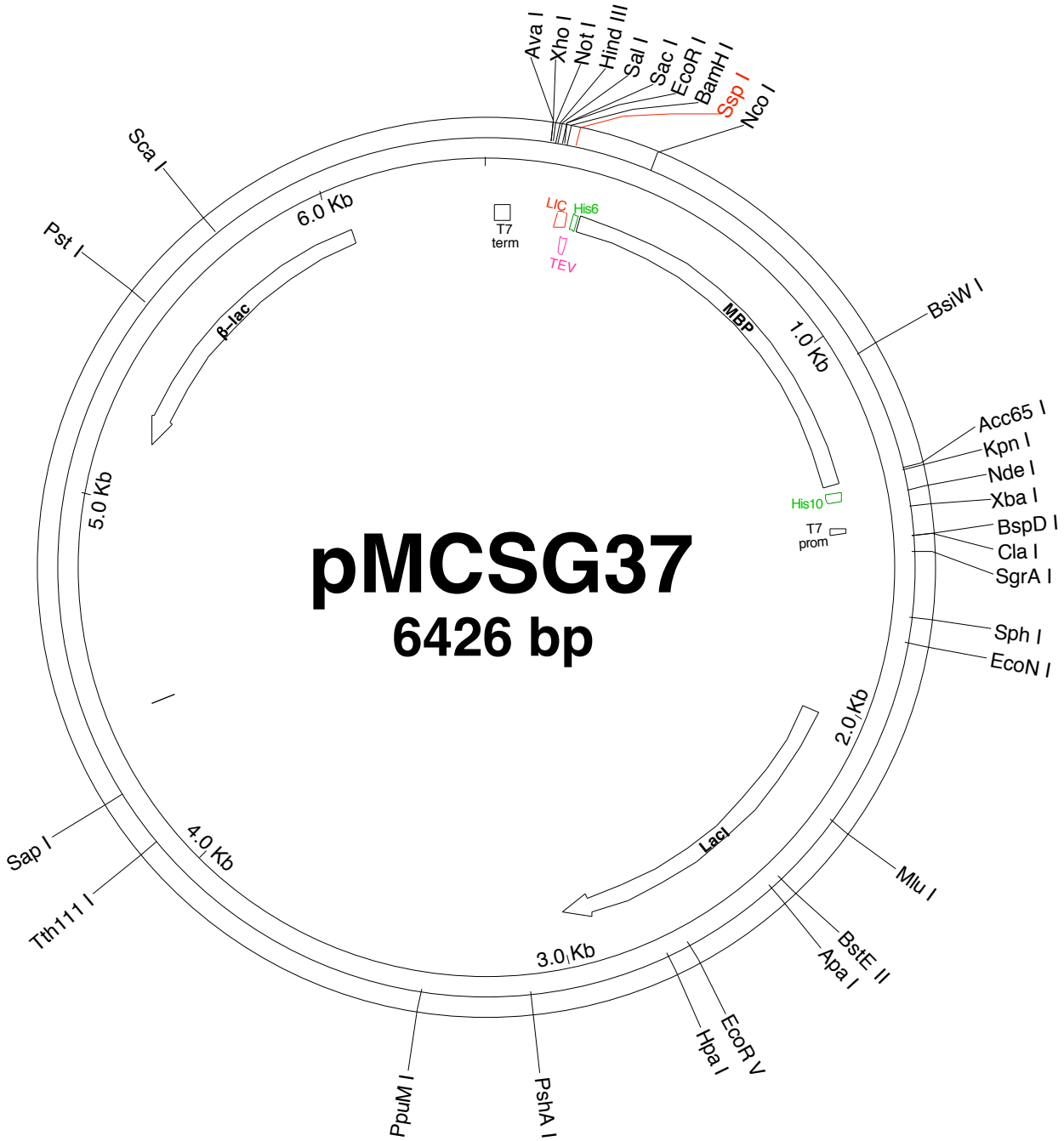

pMCSG32
6418 bp
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
BsrG I
Nco I
Bgl II
BsiW I
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
Mlu I
BstE II
Apa I
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
Pst I
Sca I
T7 term
His6
TVMV
TEV
LIC
MBP
1.0 Kb
T7 prom
2.0 Kb
LacI
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb
β-lac

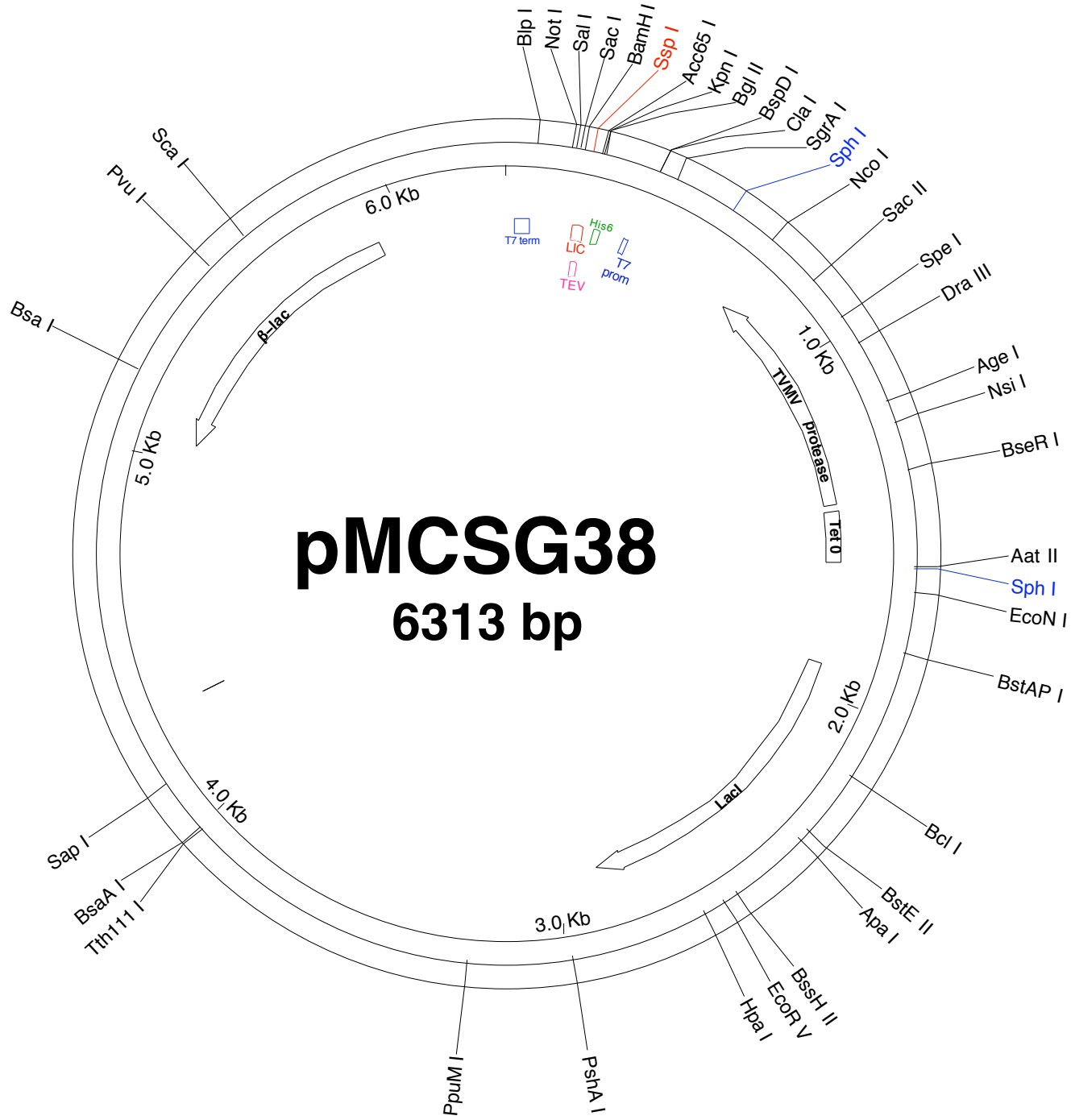

pMCSG33
6457 bp
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
EcoR V
Nco I
Bgl II
BsiW I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
Mlu I
BstE II
Apa I
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
Sca I
Pst I
6.0 Kb
5.0 Kb
4.0 Kb
3.0 Kb
2.0 Kb
1.0 Kb
β-lac
MBP
Lac I
T7 term
His6
TEV
TEV
LIC
His10
T7 prom

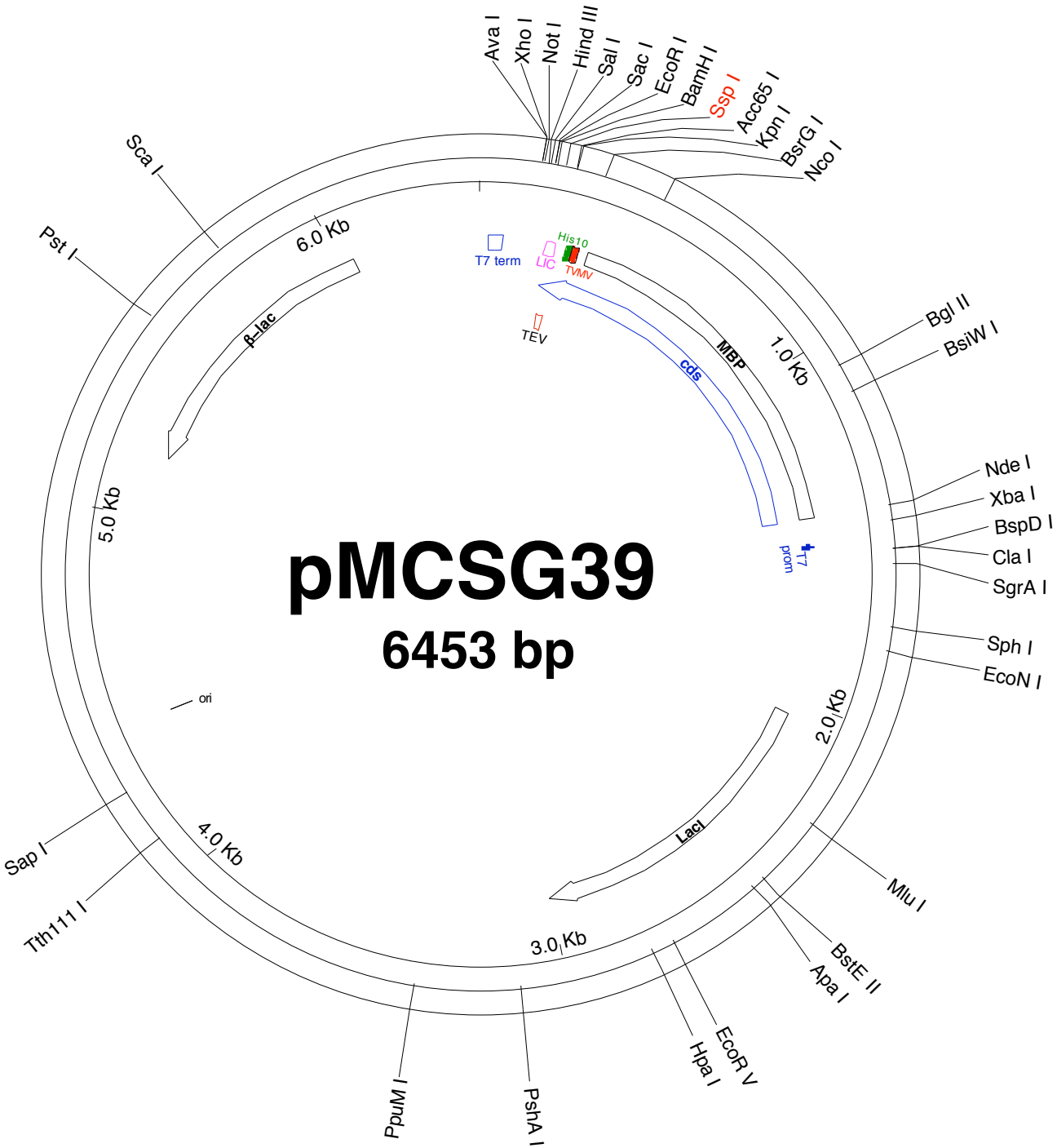

pMCSG34
7445 bp
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
BsrG I
Sca I
7.0 Kb
T7 term
His6
TEV
TVMV
LIC
MBP
1.0 Kb
Bgl II
BsiW I
β-lac
6.0 Kb
BspD I
Cla I
SgrA I
T7 prom
Sph I
Aat II
pLU
Hind III
BseR I
2.0 Kb
Nsi I
EcoR I
Age I
TVMV
Sap I
Tth111 I
5.0 Kb
Dra III
Spe I
Sac II
3.0 Kb
LacI
Sph I
EcoN I
4.0 Kb
PpuM I
PshA I
Hpa I
EcoR V
Apa I
BstE II

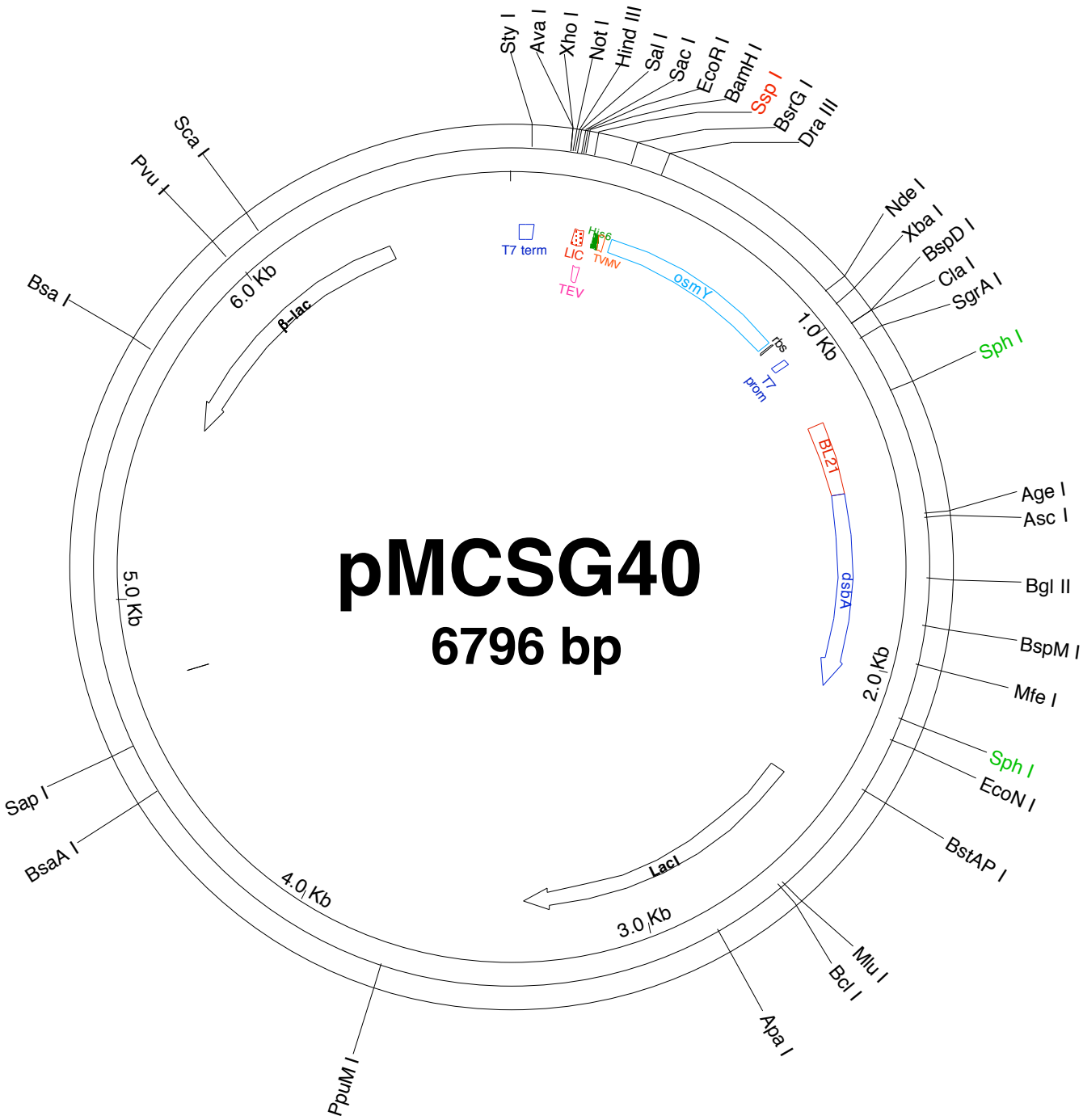

pMCSG35
7484 bp
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
BsiW I
BspD I
Cla I
SgrA I
Sph I
Aat II
Hind III
BseR I
Nsi I
EcoR I
Age I
Dra III
Spe I
Sac II
Sph I
EcoN I
BstE II
Apa I
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
Sca I
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb
7.0 Kb
MBP
TVMV
Lac I
β-lac
T7 term
His6
TEV
TEV
LIC
His10
T7 prom
pUC

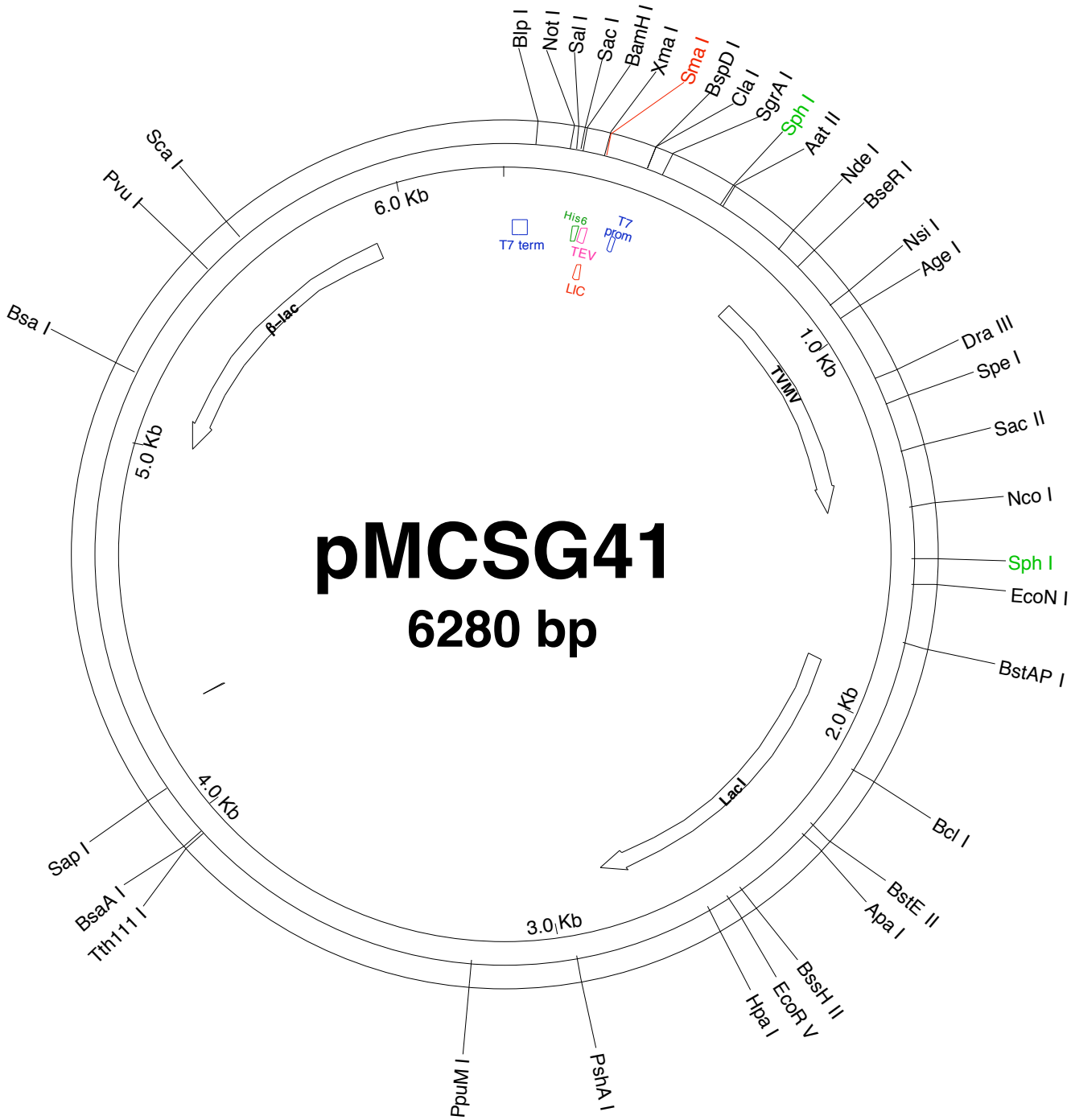

pMCSG36
5224 bp
Sty I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
2.0 Kb
3.0 Kb
Sap I
BsaA I
Tth111 I
AlwN I
4.0 Kb
Bsa I
Pst I
Pvu I
5.0 Kb
T7 Term
LIC
T7 Prom
β-lac
1.0 Kb
Lac I

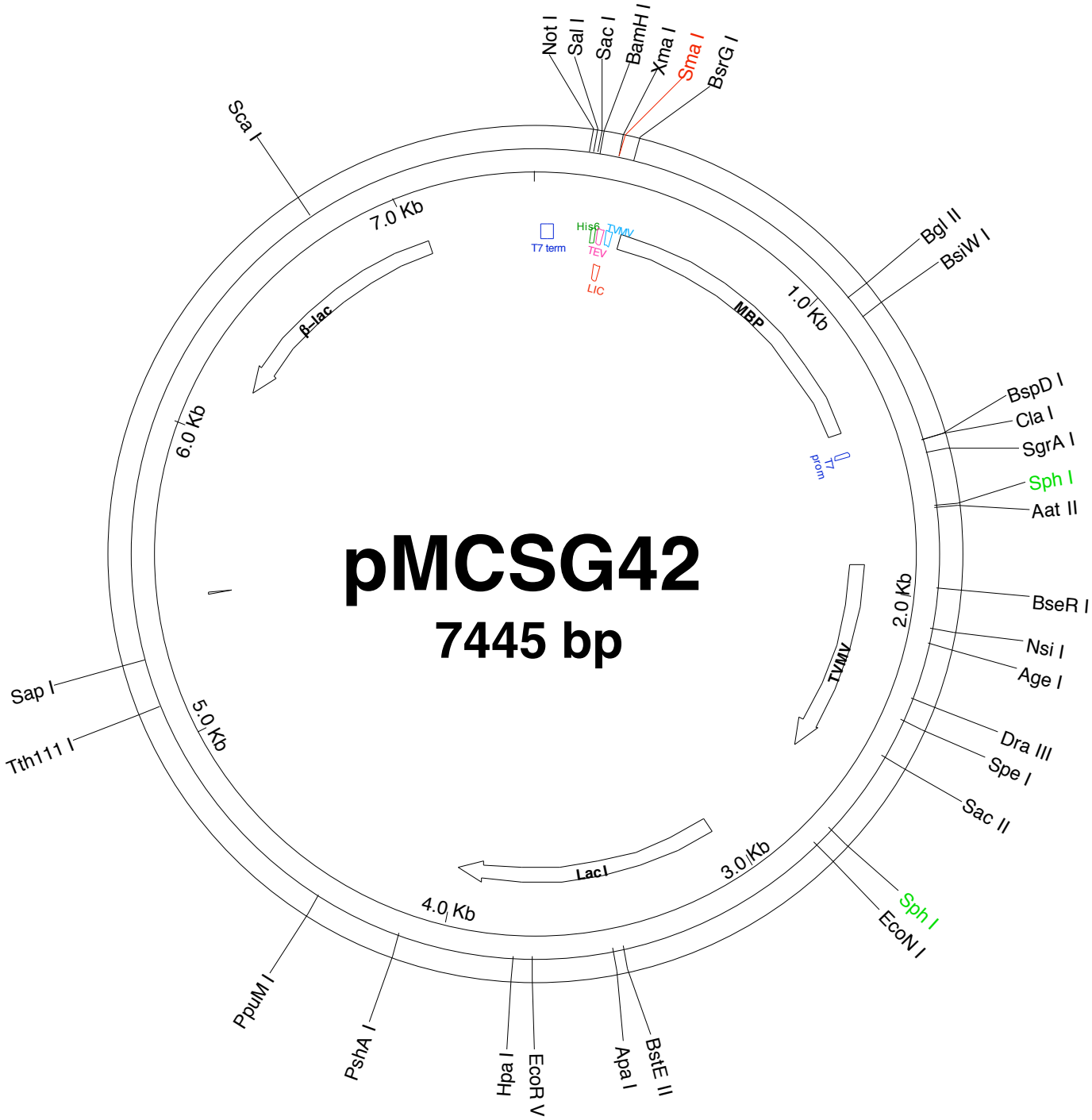

pMCSG37
6426 bp
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Nco I
BsiW I
Acc65 I
Kpn I
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
Mlu I
BstE II
Apa I
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
Pst I
Sca I
6.0 Kb
5.0 Kb
4.0 Kb
3.0 Kb
2.0 Kb
1.0 Kb
β-lac
MBP
LacI
T7
term
LIC
His6
TEV
His10
T7
prom

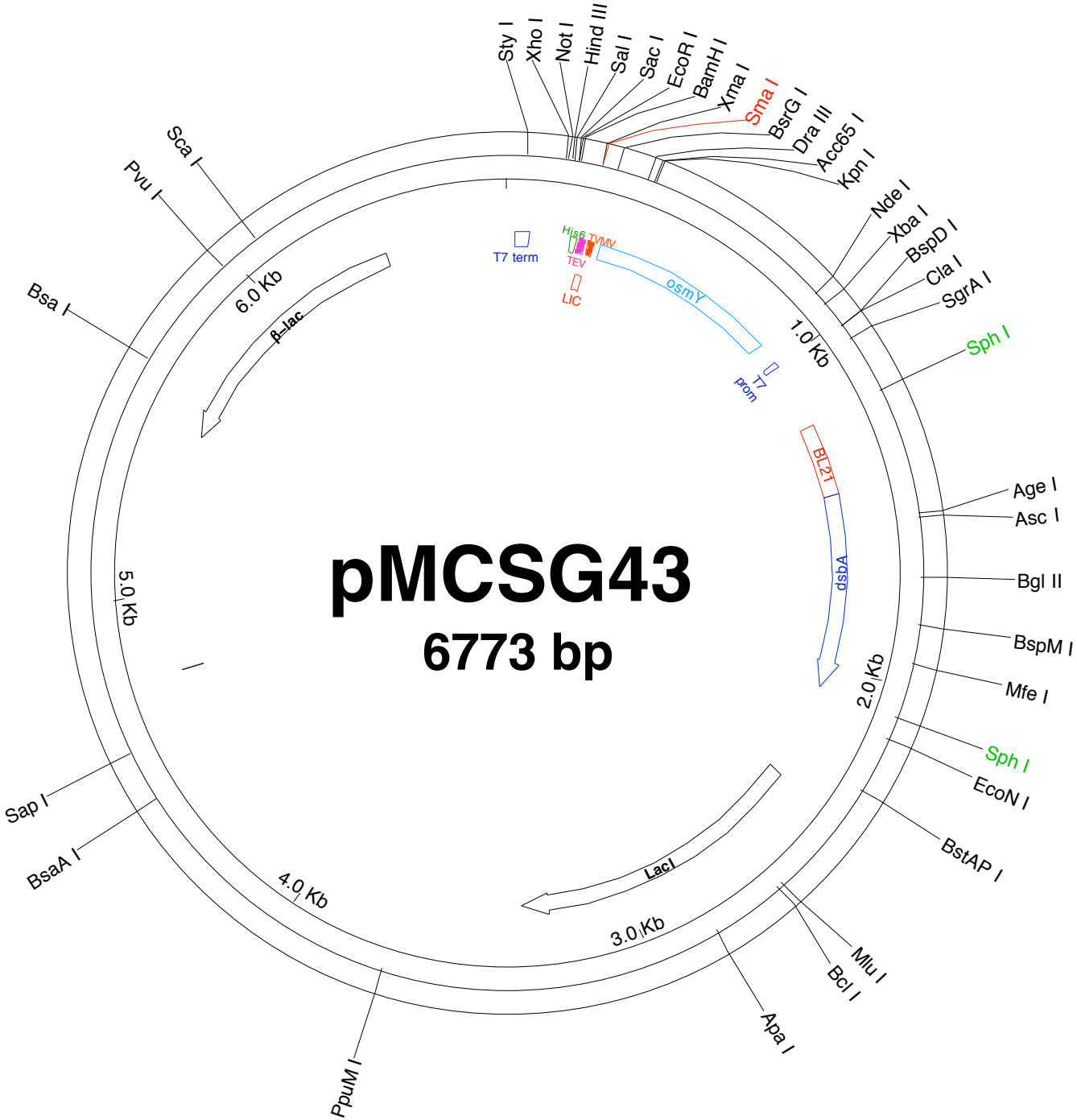

pMCSG38
6313 bp
Blp I
Not I
Sal I
Sac I
BamH I
Ssp I
Acc65 I
Kpn I
Bgl II
BspD I
Cla I
SgrA I
Sph I
Nco I
Sac II
Spe I
Dra III
Age I
Nsi I
BseR I
Aat II
Sph I
EcoN I
BstAP I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
Bsa I
Pvu I
Sca I
6.0 Kb
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
T7 term
His6
LIC
T7 prom
TEV
β-lac
TVMV protease
Tet 0
LacI

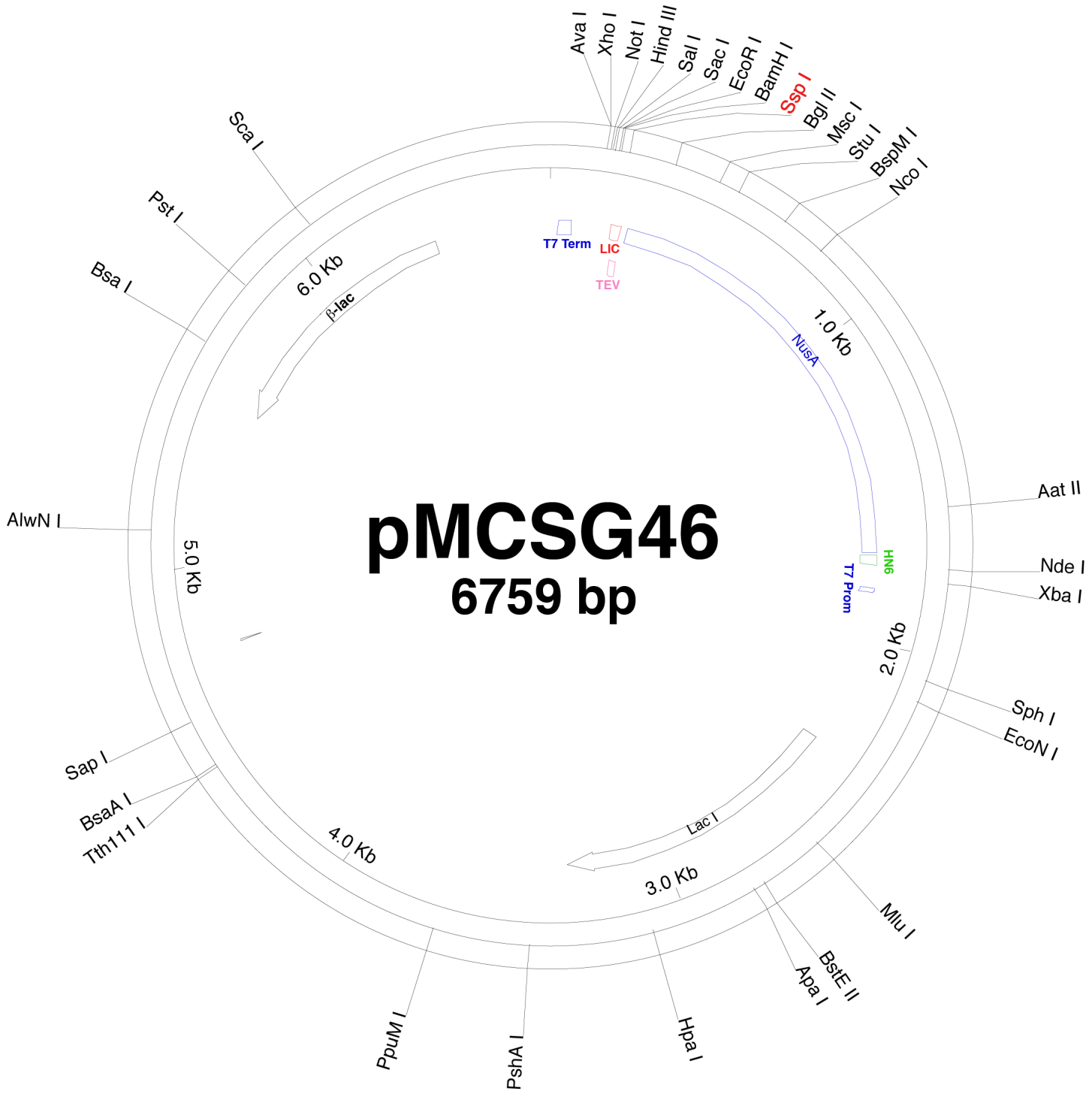

pMCSG39
6453 bp
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
BsrG I
Nco I
Bgl II
BsiW I
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
Mlu I
BstE II
Apa I
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
Pst I
Sca I
T7 term
LIC
His10
TVMV
TEV
cds
MBP
T7 prom
β-lac
LacI
ori
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb

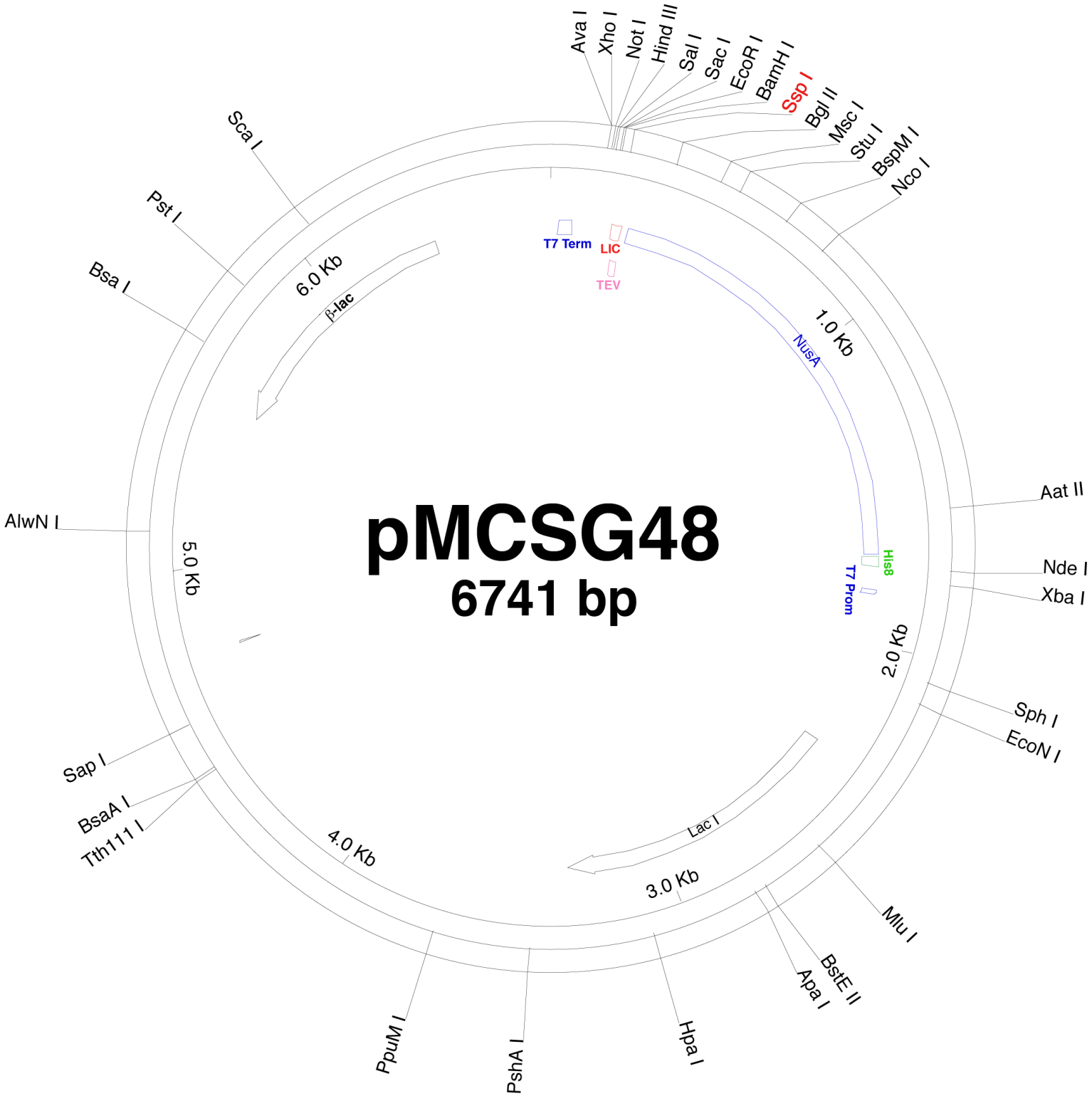

pMCSG40
6796 bp
Sty I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
BsrG I
Dra III
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
Age I
Asc I
Bgl II
BspM I
Mfe I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
Apa I
PpuM I
BsaA I
Sap I
Bsa I
Pvu I
Sca I
T7 term
LIC
His6
TVMV
TEV
osmY
rbs
T7 prom
BL21
dsbA
LacI
β-lac
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb

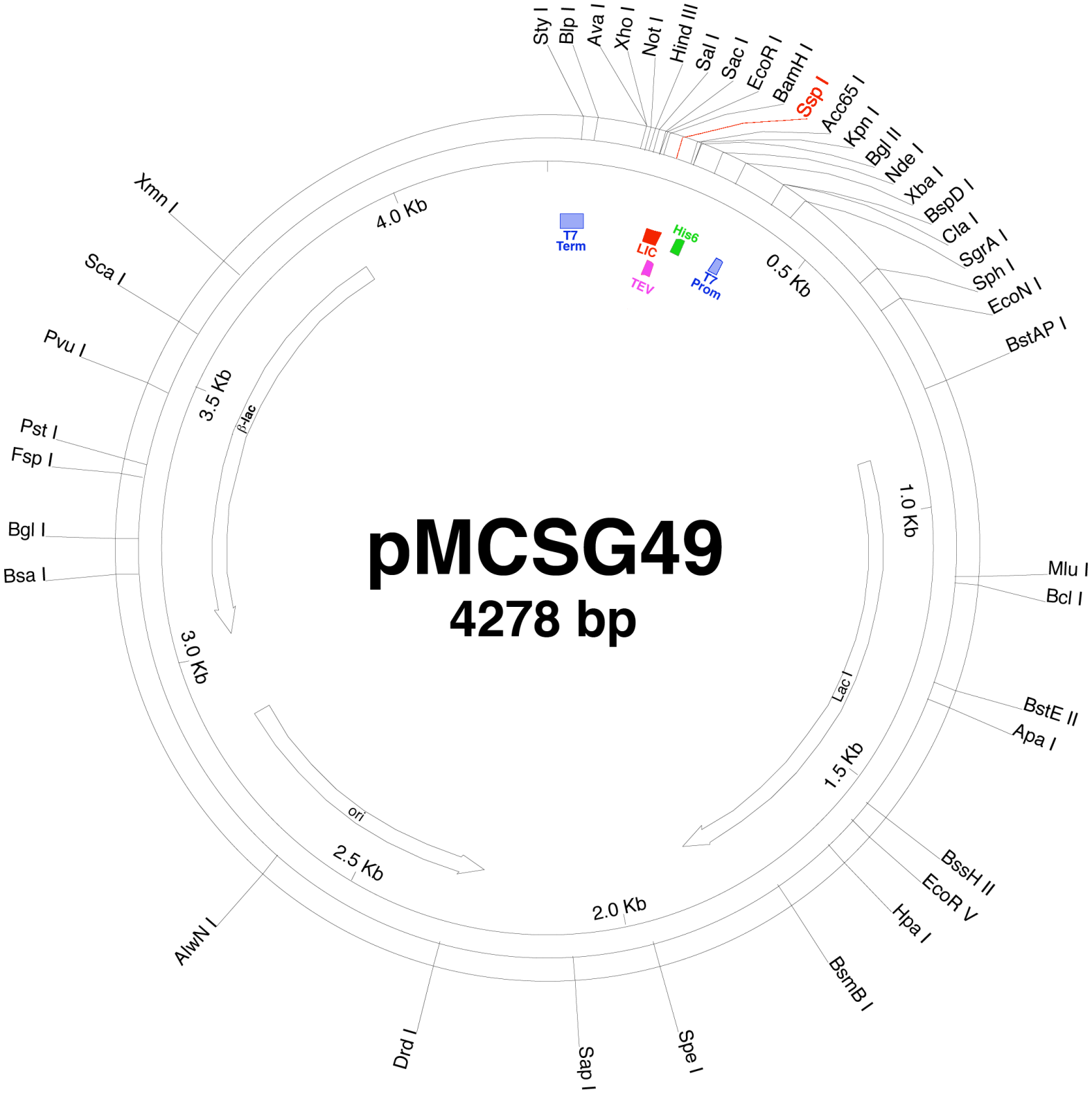

pMCSG41
6280 bp
Blp I
Not I
Sal I
Sac I
BamH I
Xma I
Sma I
BspD I
Cla I
SgrA I
Sph I
Aat II
Nde I
BseR I
Nsi I
Age I
Dra III
Spe I
Sac II
Nco I
Sph I
EcoN I
BstAP I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
Bsa I
Pvu I
Sca I
6.0 Kb
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
T7 term
His6
TEV
LIC
T7 prom
β-lac
TVMV
LacI

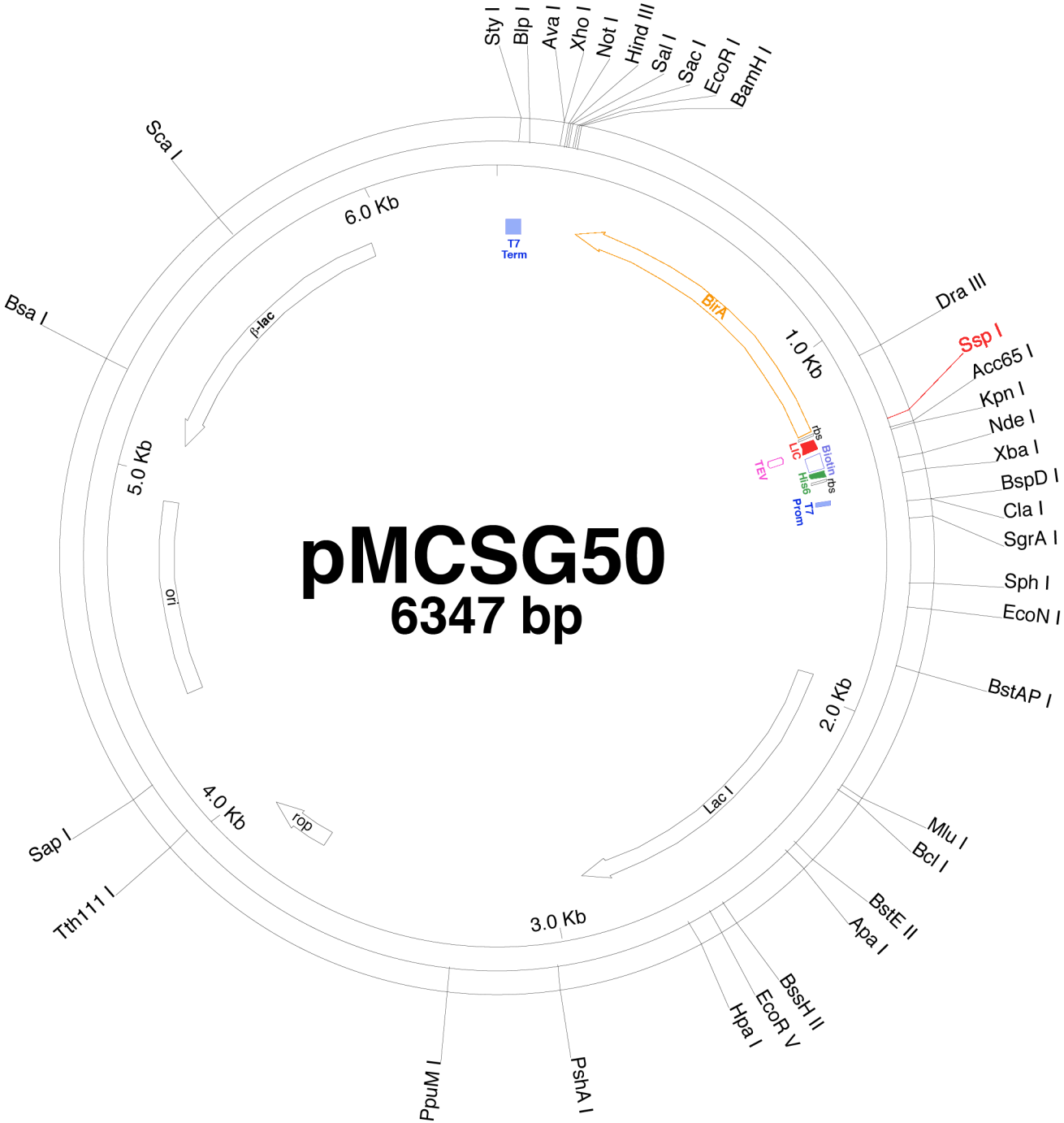

pMCSG42
7445 bp
Not I
Sal I
Sac I
BamH I
Xma I
Sma I
BsrG I
Sca I
Bgl II
BsiW I
BspD I
Cla I
SgrA I
Sph I
Aat II
BseR I
Nsi I
Age I
Dra III
Spe I
Sac II
Sph I
EcoN I
BstE II
Apa I
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
7.0 Kb
6.0 Kb
5.0 Kb
4.0 Kb
3.0 Kb
2.0 Kb
1.0 Kb
β-lac
MBP
TVMV
LacI
T7 term
His6
TVMV
TEV
LIC
T7 prom

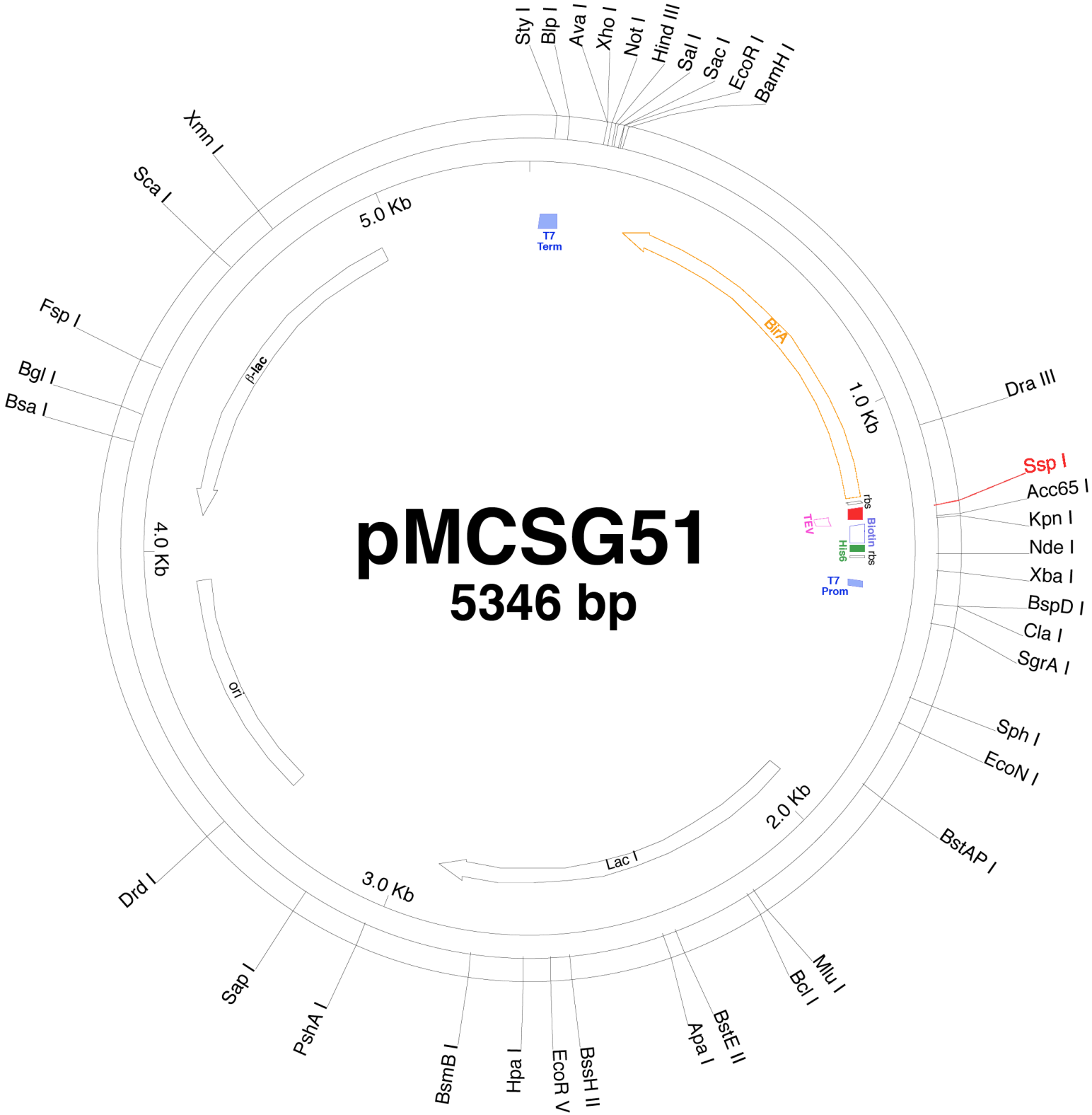

pMCSG43
6773 bp
Sty I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
BsrG I
Dra III
Acc65 I
Kpn I
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
Age I
Asc I
Bgl II
BspM I
Mfe I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
Apa I
PpuM I
BsaA I
Sap I
Bsa I
Pvu I
Sca I
T7 term
His6
TVMV
TEV
LIC
osmY
T7 prom
BL21
dsbA
β-lac
LacI
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb

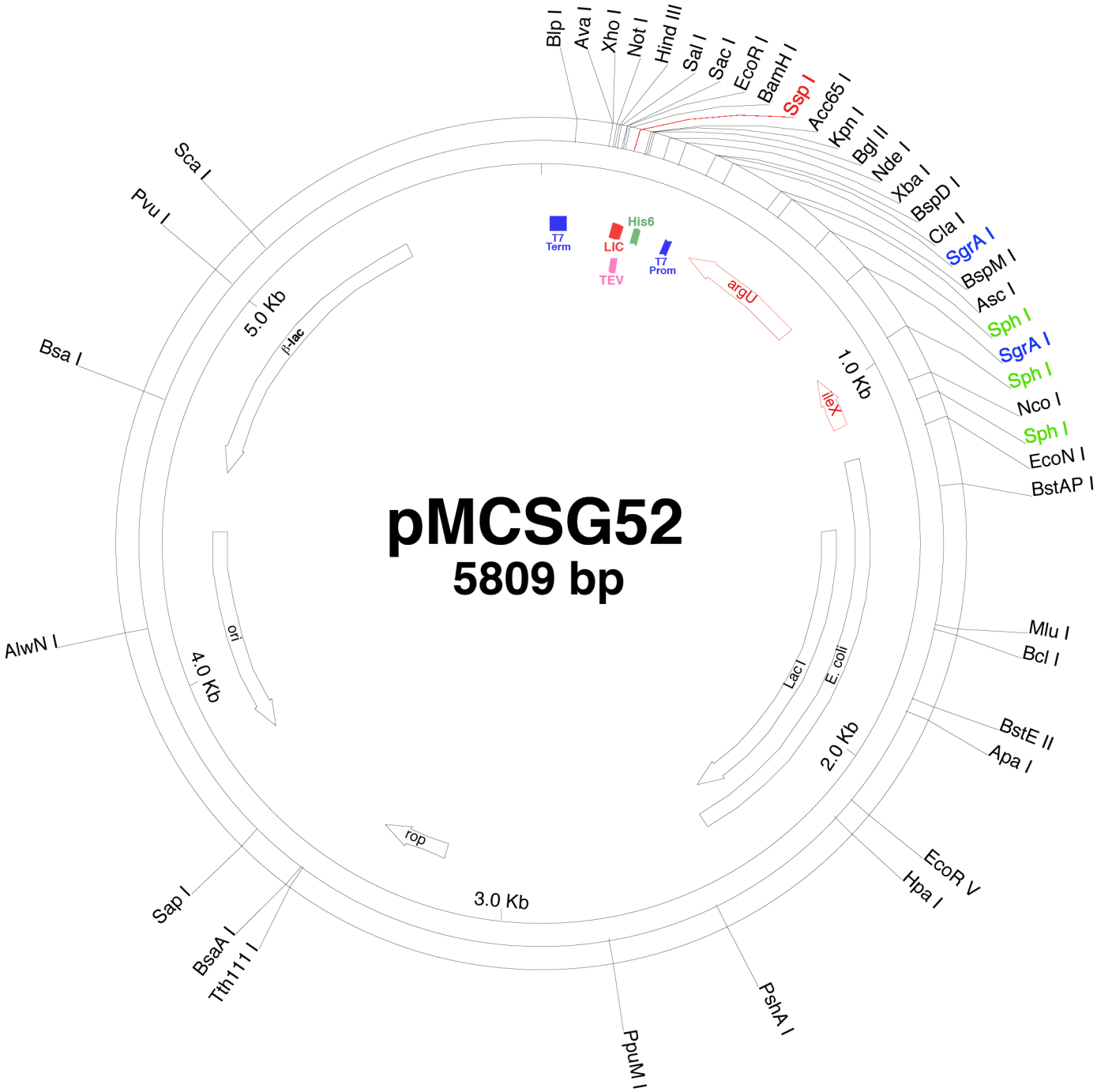

pMCSG46
6759 bp
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Bgl II
Msc I
Stu I
BspM I
Nco I
Aat II
Nde I
Xba I
Sph I
EcoN I
Mlu I
BstE II
Apa I
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
AlwN I
Bsa I
Pst I
Sca I
T7 Term
LIC
TEV
NusA
HN6
T7 Prom
Lac I
β-lac
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb

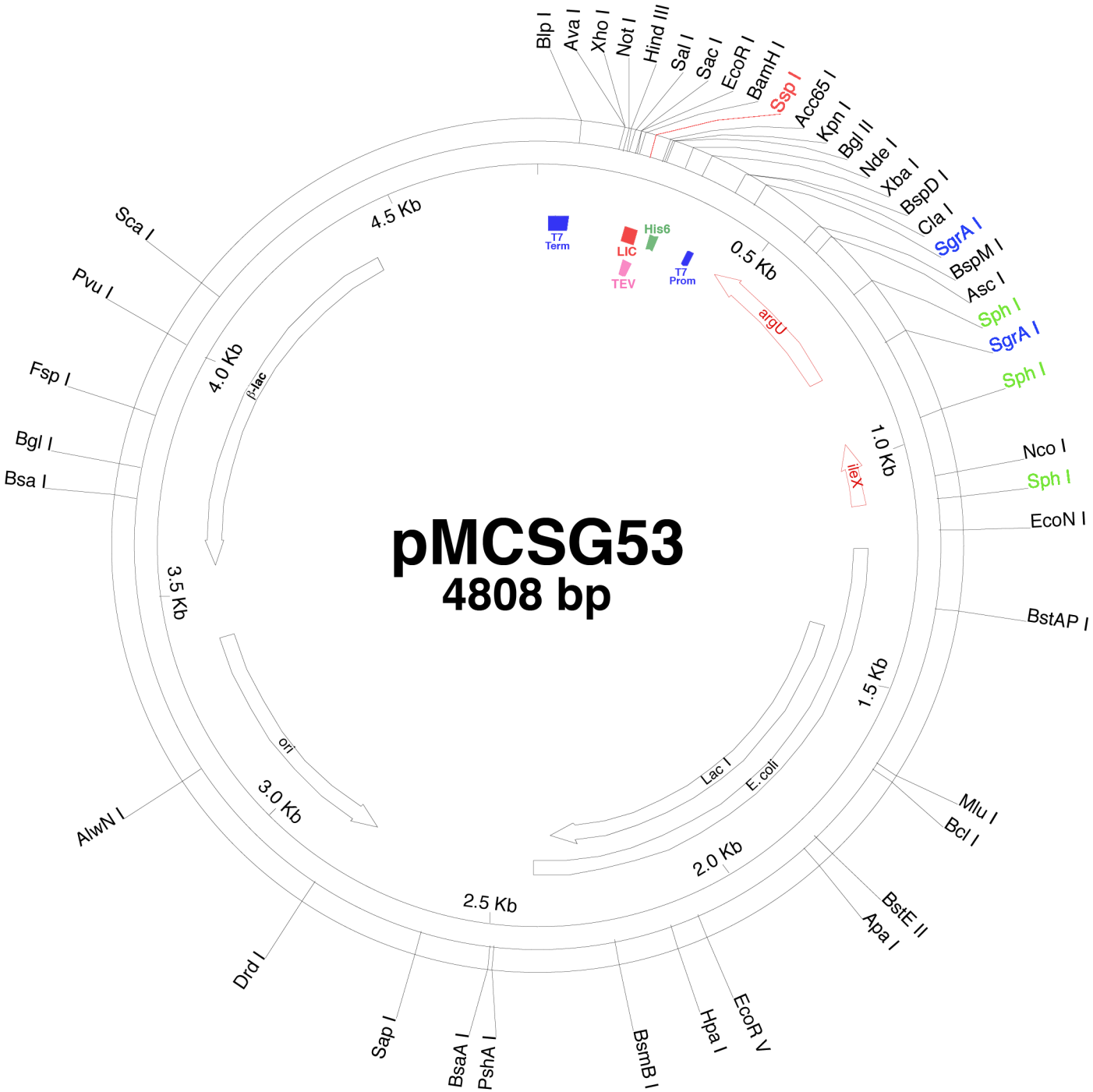

pMCSG48
6741 bp
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Bgl II
Msc I
Stu I
BspM I
Nco I
Aat II
Nde I
Xba I
Sph I
EcoN I
Mlu I
BstE II
Apa I
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
AlwN I
Bsa I
Pst I
Sca I
T7 Term
LIC
TEV
NusA
His8
T7 Prom
β-lac
Lac I
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb

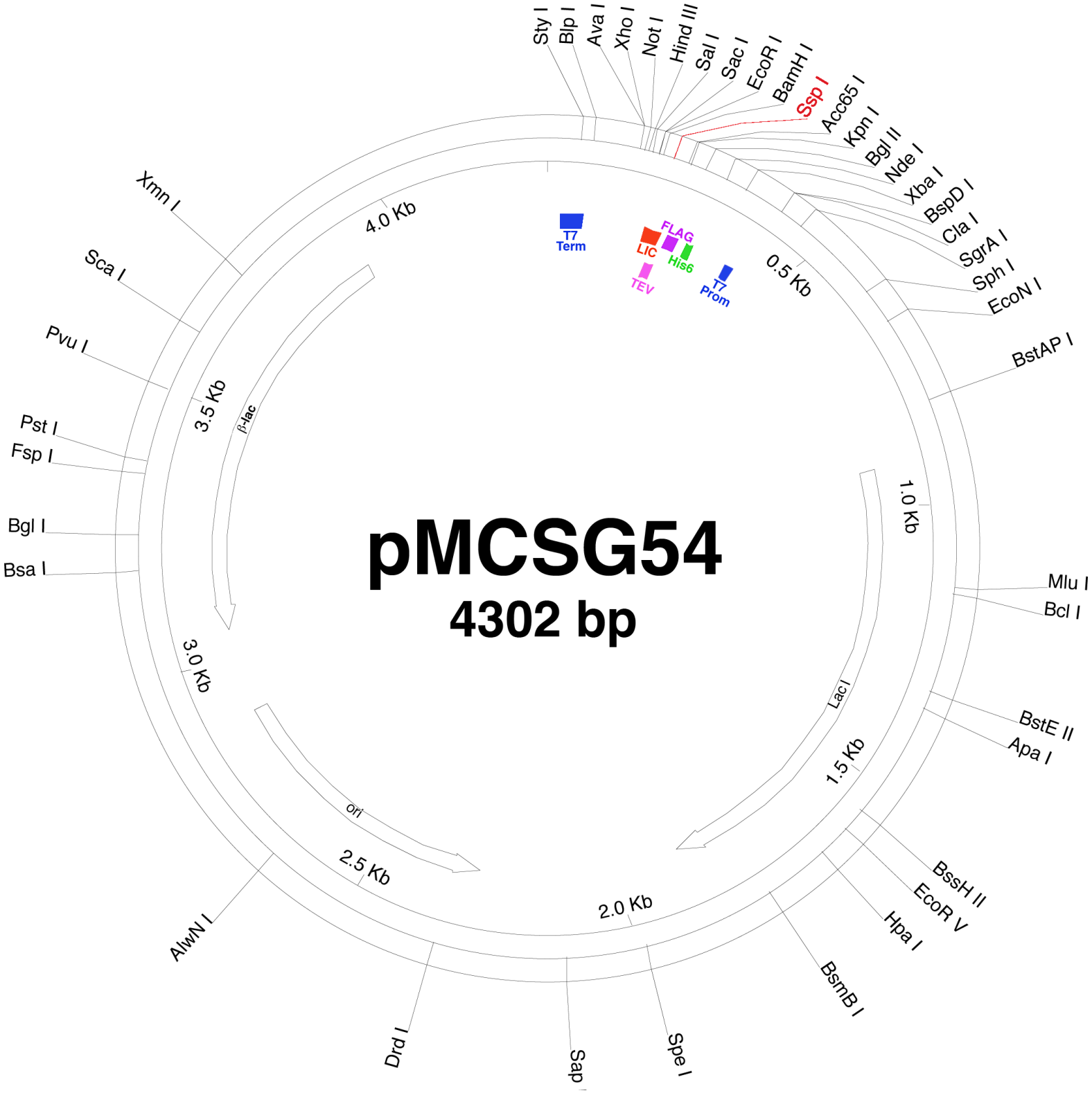

pMCSG49
4278 bp
Sty I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
BsmB I
Spe I
Sap I
Drd I
AlwN I
Bsa I
Bgl I
Fsp I
Pst I
Pvu I
Sca I
Xmn I
T7 Term
LIC
His6
TEV
T7 Prom
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
4.0 Kb
β-lac
Lac I
ori

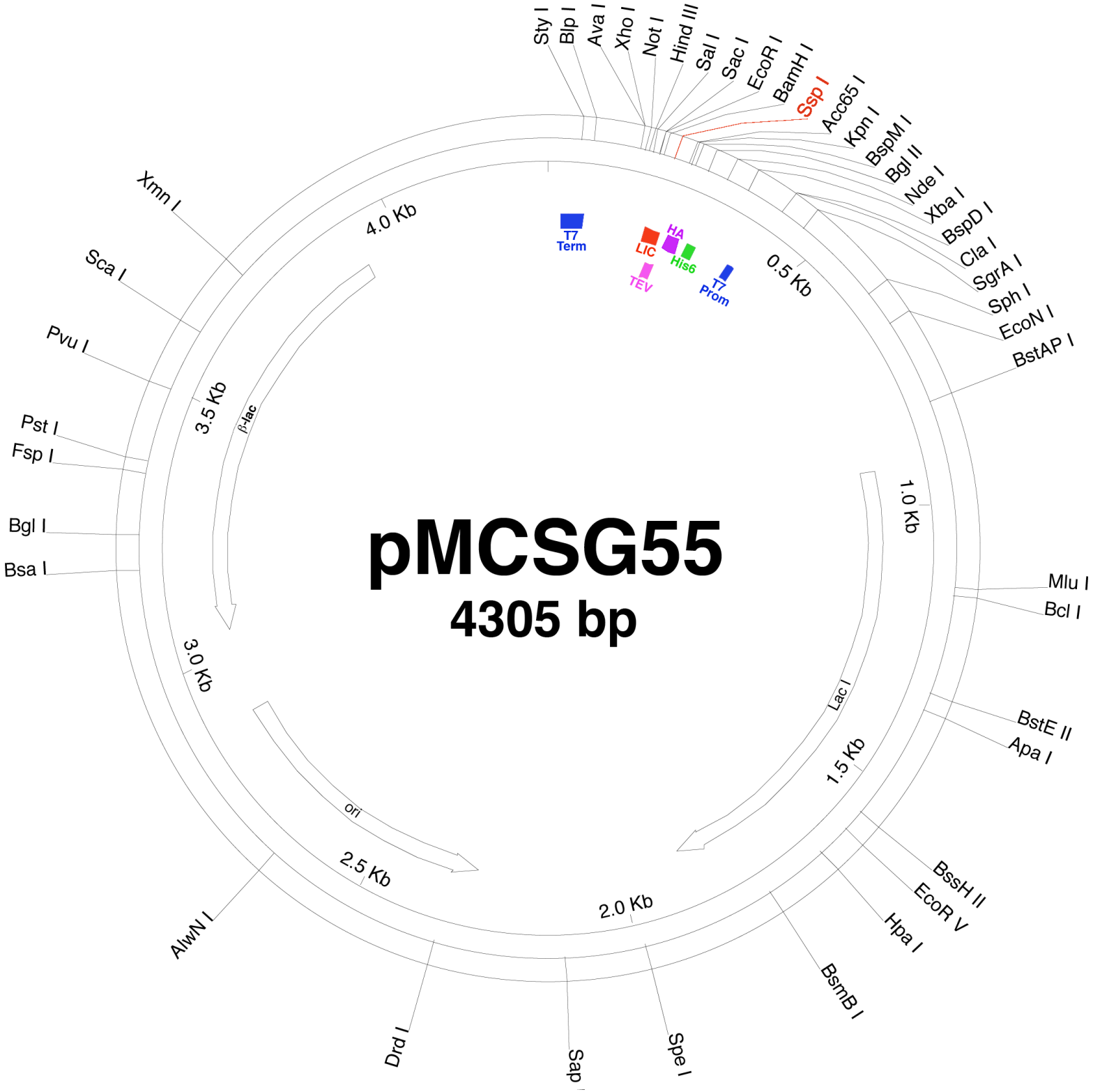

pMCSG50
6347 bp
Sty I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Sca I
Bsa I
Dra III
Ssp I
Acc65 I
Kpn I
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
6.0 Kb
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
T7
Term
BirA
β-lac
ori
rop
Lac I
rbs
LIC
TEV
Biotin
rbs
His6
T7
Prom

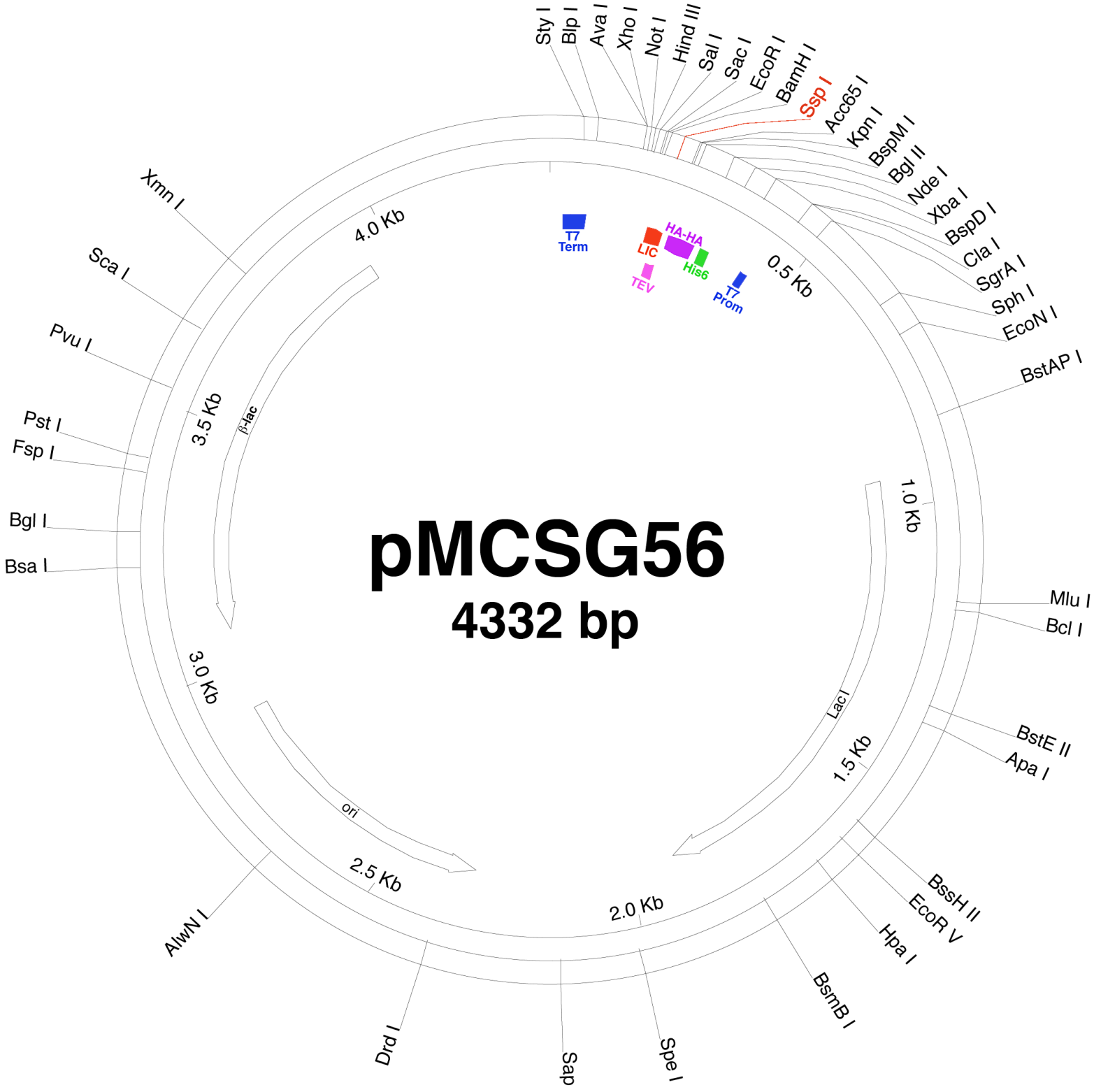

pMCSG51
5346 bp
Sty I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xmn I
Sca I
Fsp I
Bgl I
Bsa I
Dra III
Ssp I
Acc65 I
Kpn I
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
BsmB I
PshA I
Sap I
Drd I
5.0 Kb
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
T7
Term
BirA
rbs
Biotin
rbs
TEV
His6
T7
Prom
β-lac
ori
Lac I

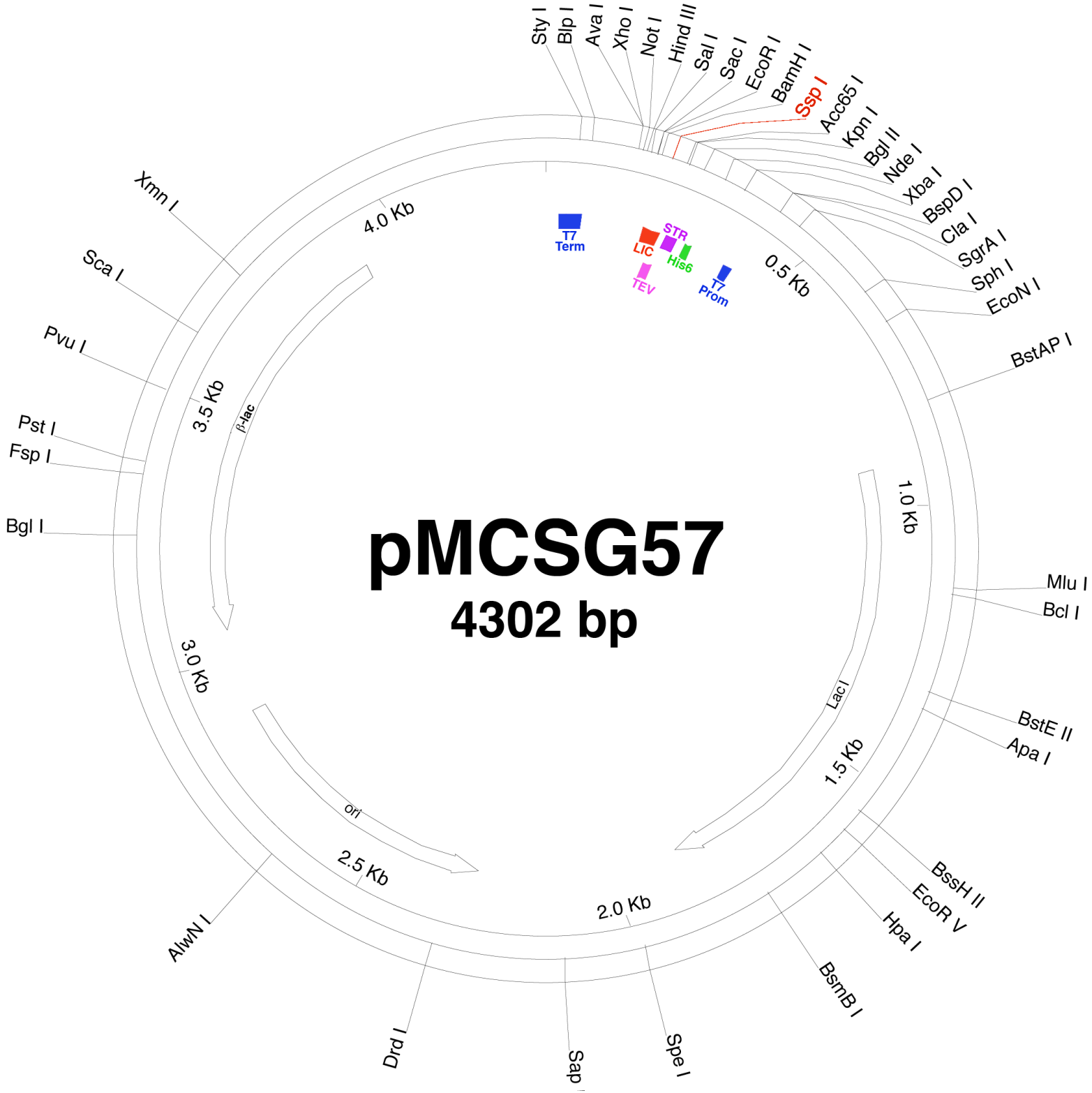

pMCSG52
5809 bp
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
BspM I
Asc I
Sph I
SgrA I
Sph I
Nco I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
AlwN I
Bsa I
Pvu I
Sca I
T7 Term
LIC
His6
TEV
T7 Prom
argU
ileX
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
β-lac
ori
rop
Lac I
E. coli

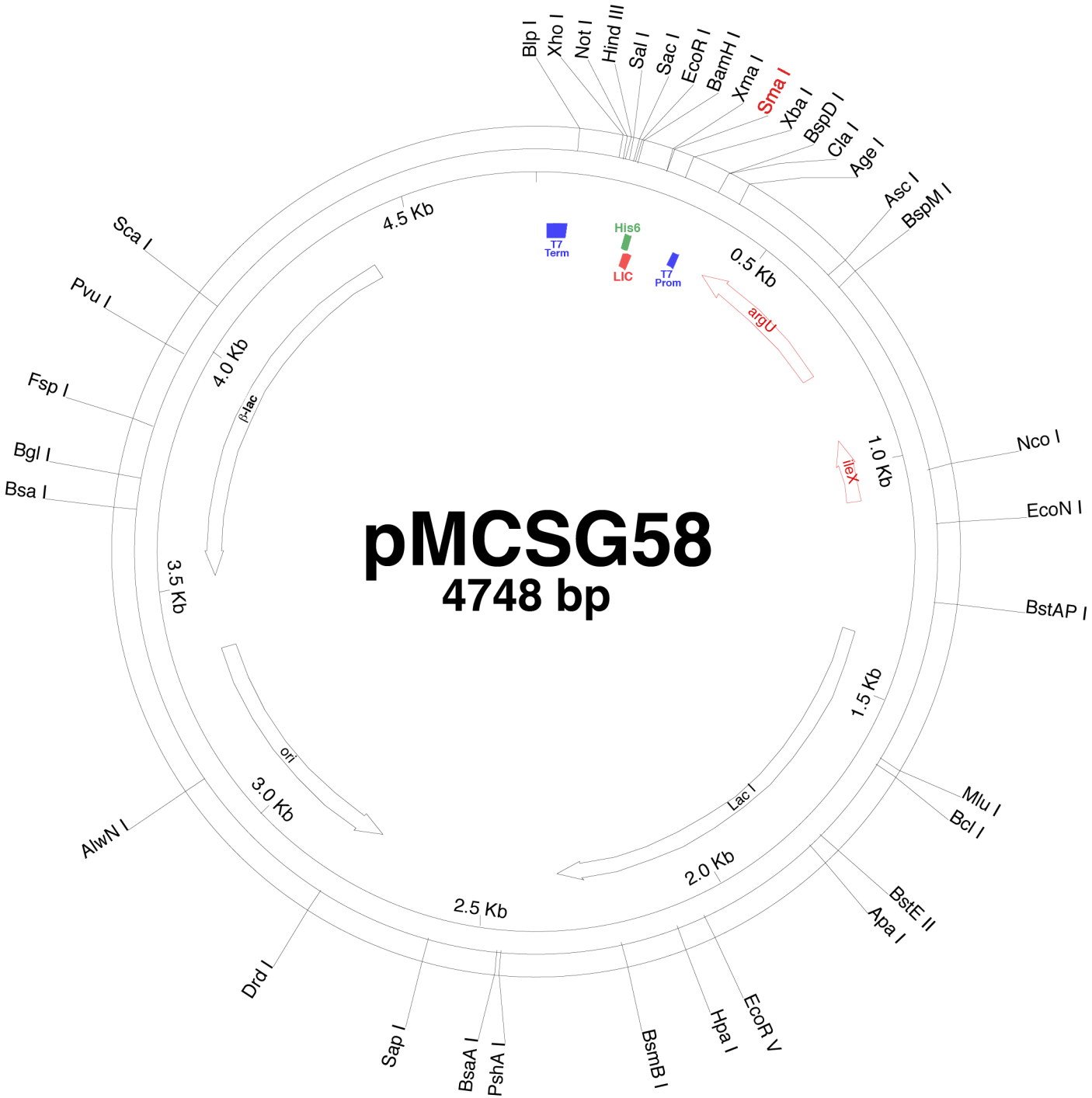

pMCSG53
4808 bp
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
BspM I
Asc I
Sph I
SgrA I
Sph I
Nco I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
EcoR V
Hpa I
BsmB I
BsaA I
PshA I
Sap I
Drd I
AlwN I
ori
Lac I
E. coli
β-lac
argU
ileX
T7 Term
LIC
His6
TEV
T7 Prom
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
4.0 Kb
4.5 Kb
Sca I
Pvu I
Fsp I
Bgl I
Bsa I

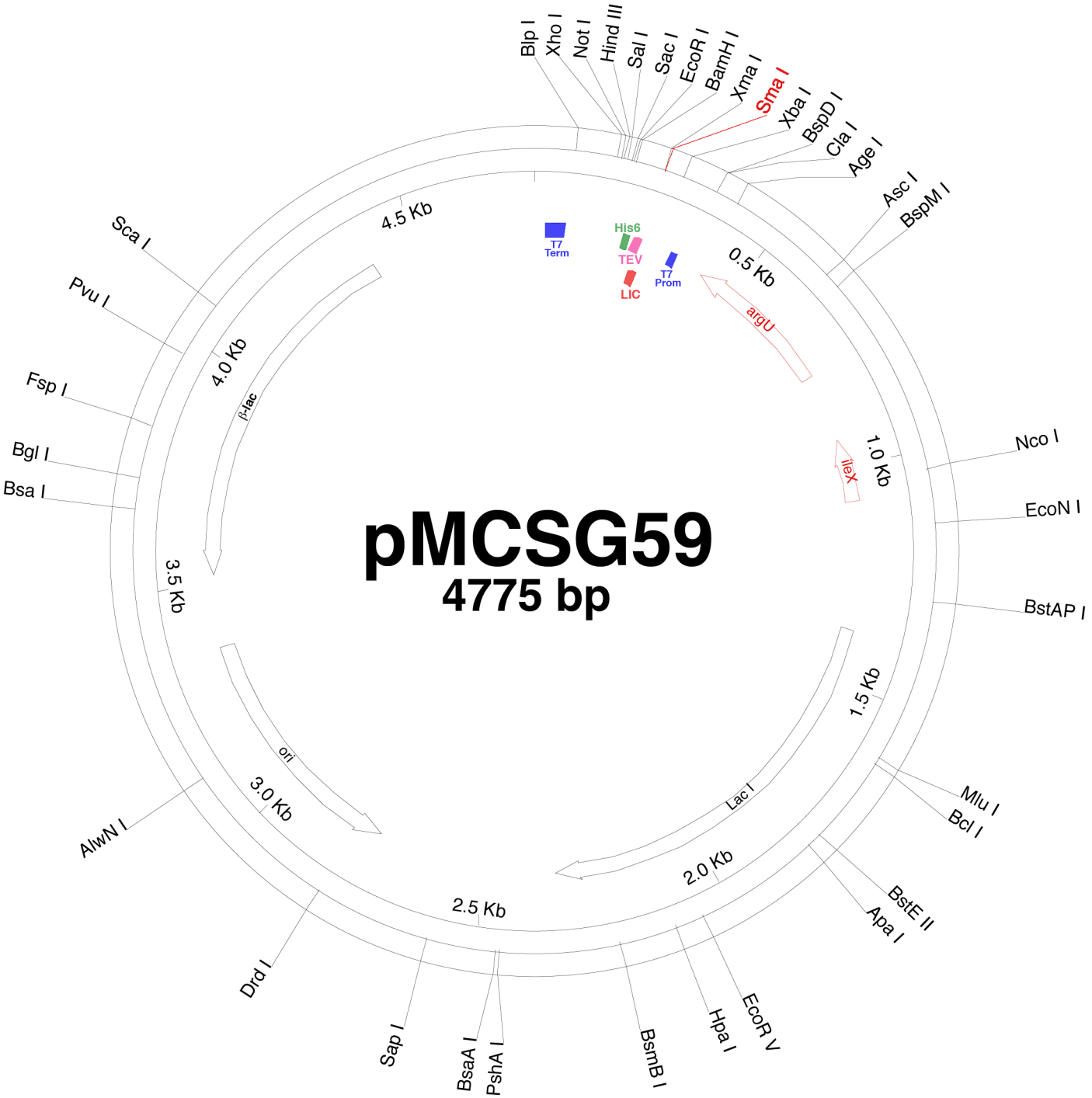

pMCSG54
4302 bp
Sty I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
BsmB I
Spe I
Sap
Drd I
AlwN I
Bsa I
Bgl I
Fsp I
Pst I
Pvu I
Sca I
Xmn I
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
4.0 Kb
T7 Term
LIC
FLAG
His6
TEV
T7 Prom
β-lac
LacI
ori

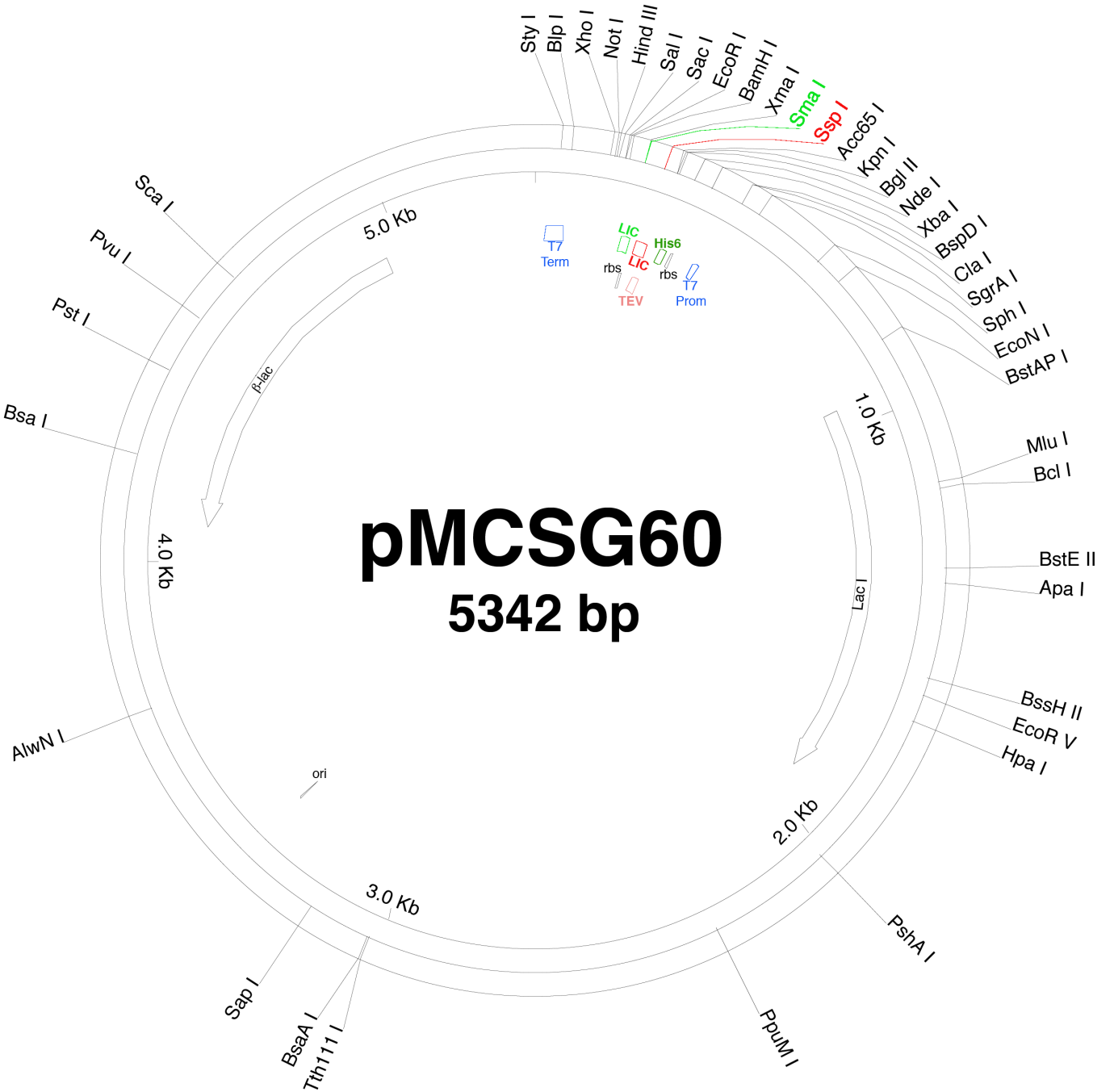

pMCSG55
4305 bp
Sty I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
BspM I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
BsmB I
Spe I
Sap
Drd I
AlwN I
Bsa I
Bgl I
Fsp I
Pst I
Pvu I
Sca I
Xmn I
T7 Term
LIC
HA
His6
TEV
T7 Prom
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
4.0 Kb
β-lac
Lac I
ori

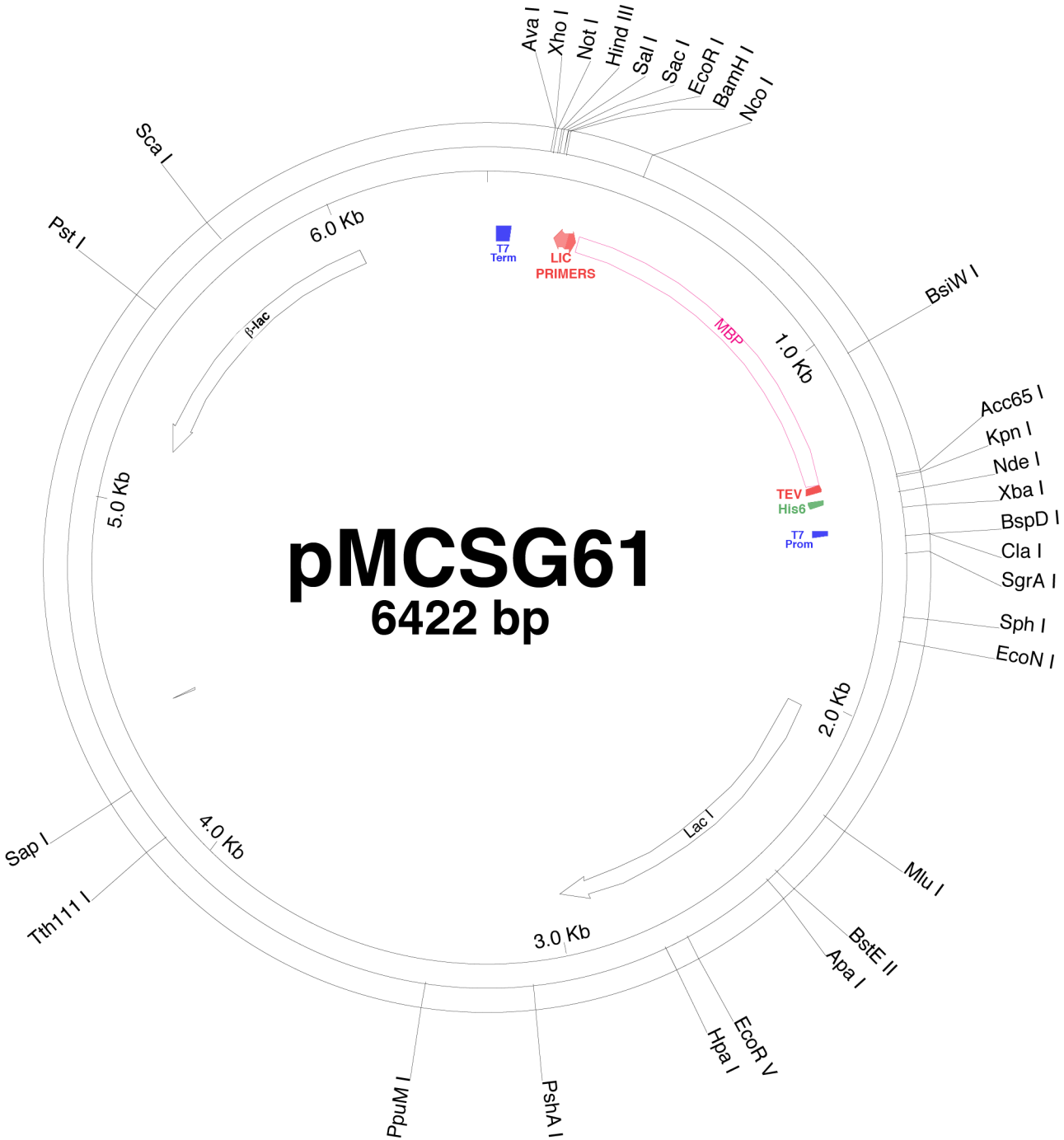

pMCSG56
4332 bp
Sty I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
BspM I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
BsmB I
Spe I
Sap
Drd I
AlwN I
Bsa I
Bgl I
Fsp I
Pst I
Pvu I
Sca I
Xmn I
T7 Term
LIC
HA-HA
His6
TEV
T7 Prom
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
4.0 Kb
β-lac
Lac I
ori

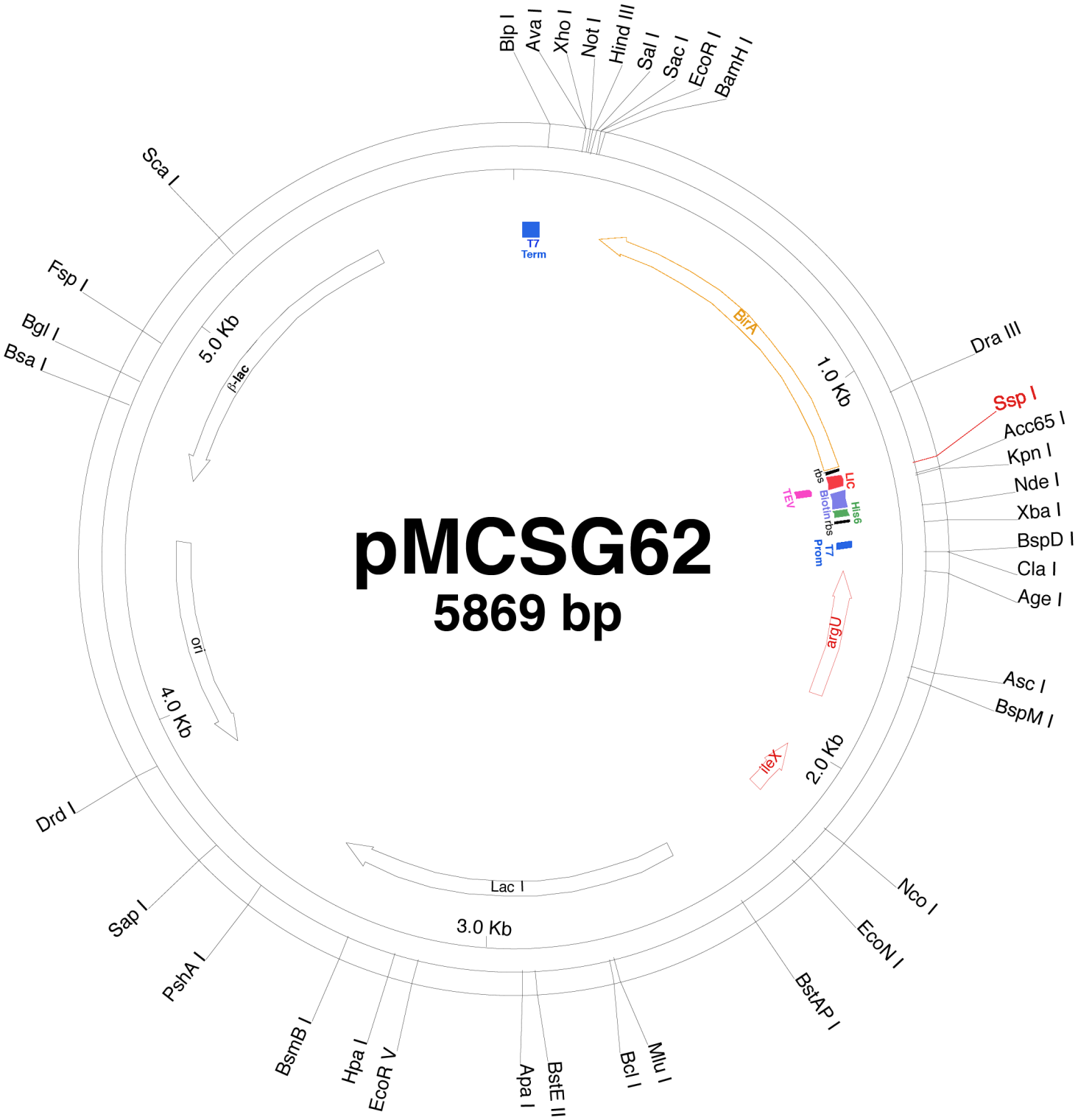

pMCSG57
4302 bp
Sty I
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
BsmB I
Spe I
Sap
Drd I
AlwN I
Bgl I
Fsp I
Pst I
Pvu I
Sca I
Xmn I
T7 Term
LIC
STR
His6
TEV
T7 Prom
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
4.0 Kb
β-lac
Lac I
ori

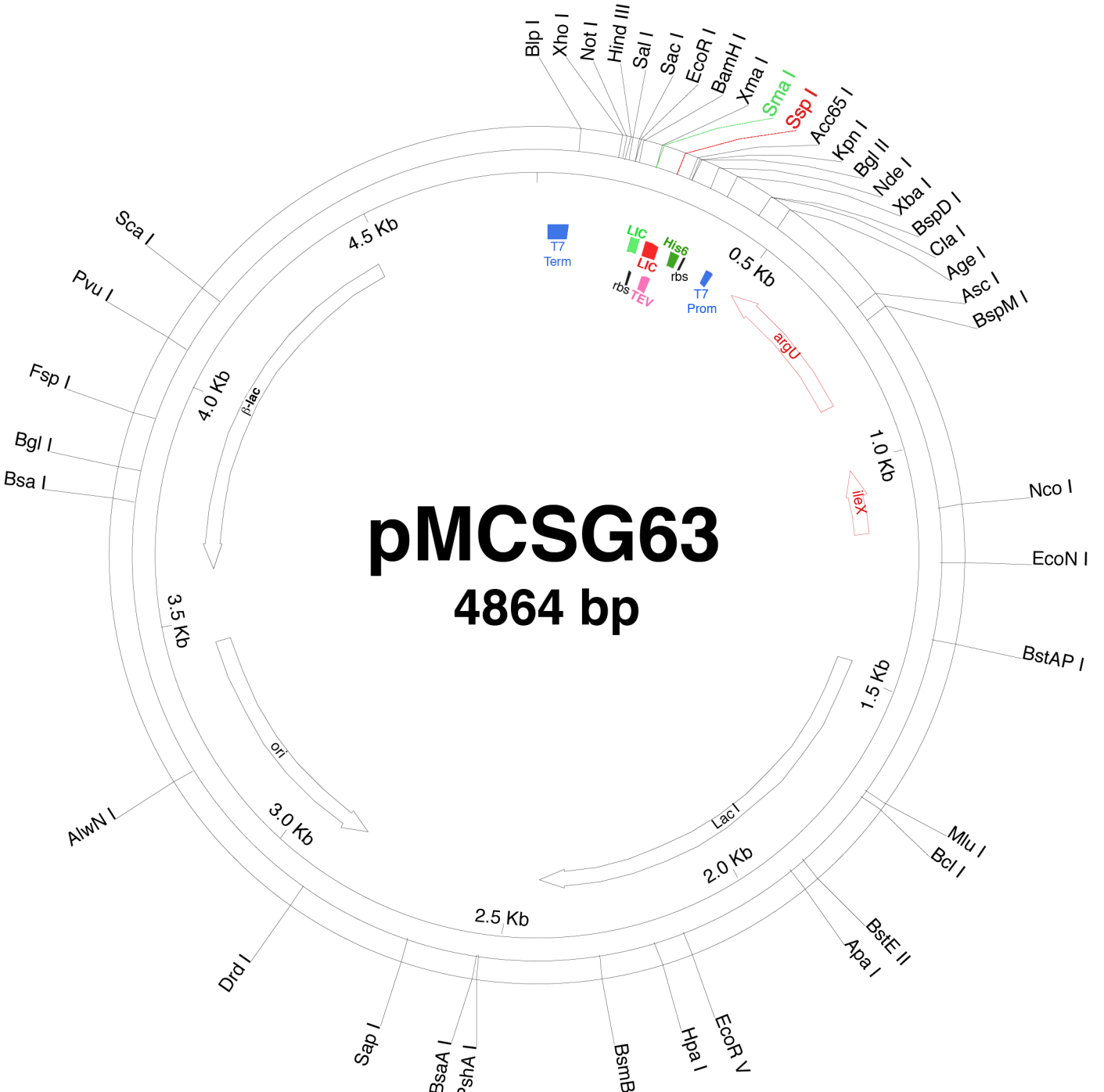

pMCSG58
4748 bp
Blp I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
Xba I
BspD I
Cla I
Age I
Asc I
BspM I
Nco I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
EcoR V
Hpa I
BsmB I
PshA I
BsaA I
Sap I
Drd I
AlwN I
ori
Lac I
ilex
argU
His6
LIC
T7 Term
T7 Prom
β-lac
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
4.0 Kb
4.5 Kb
Sca I
Pvu I
Fsp I
Bgl I
Bsa I

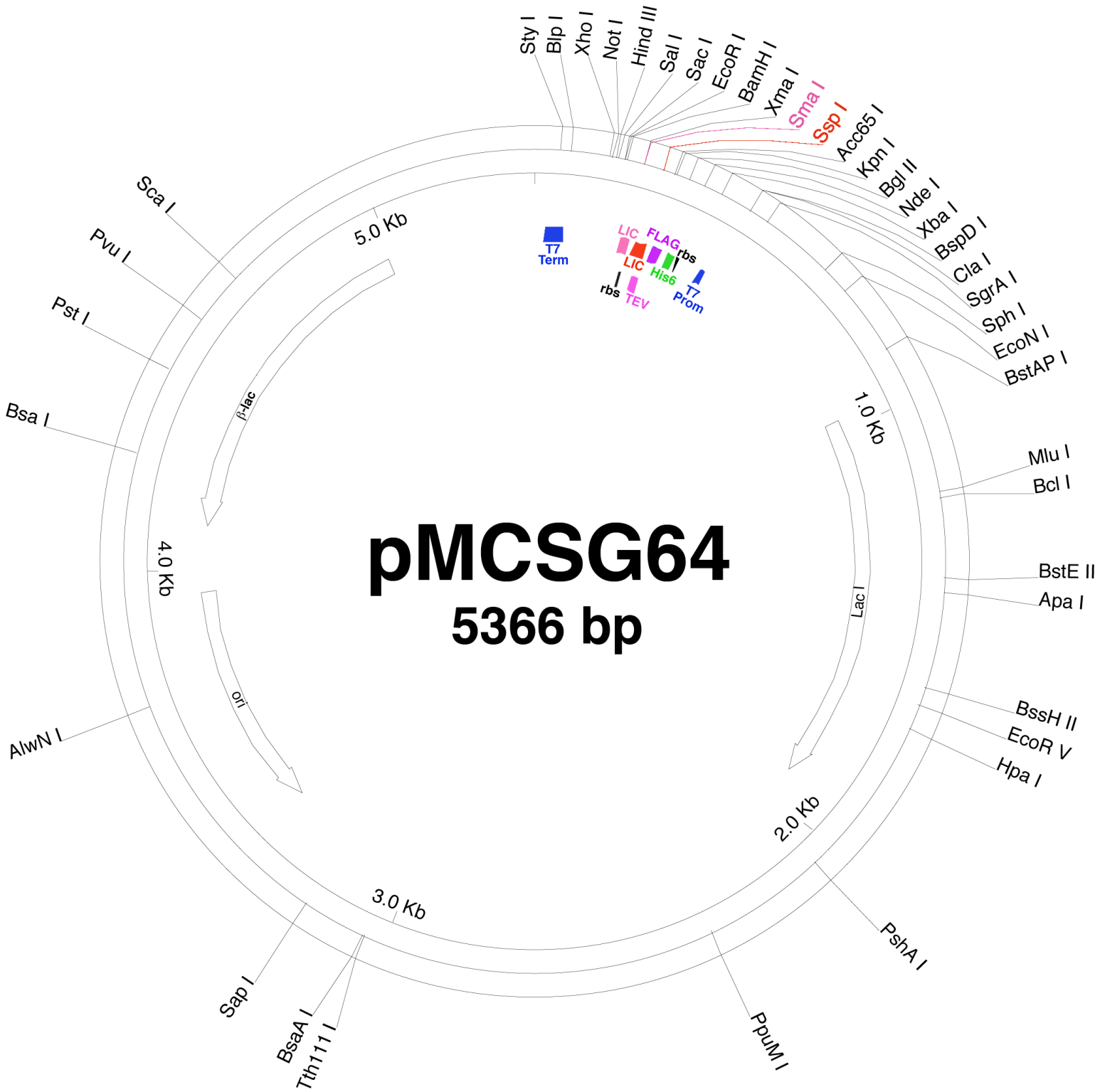

pMCSG59
4775 bp
Blp I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
Xba I
BspD I
Cla I
Age I
Asc I
BspM I
Nco I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
EcoR V
Hpa I
BsmB I
PshA I
BsaA I
Sap I
Drd I
AlwN I
Bsa I
Bgl I
Fsp I
Pvu I
Sca I
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
4.0 Kb
4.5 Kb
His6
TEV
LIC
T7 Term
T7 Prom
argU
ileX
Lac I
ori
β-lac

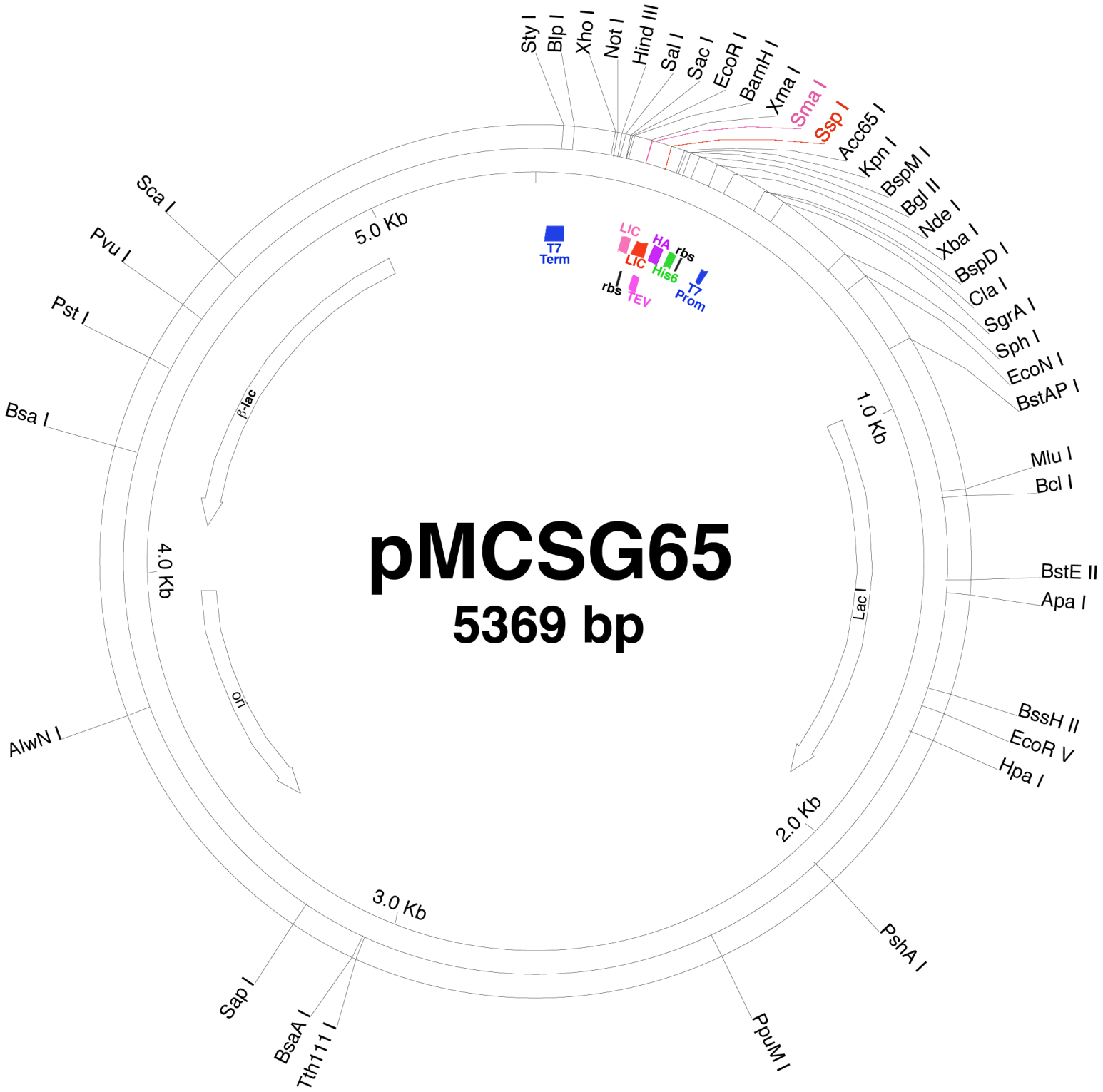

pMCSG60
5342 bp
Sty I
Blp I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
Ssp I
Acc65 I
Kpn I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
AlwN I
Bsa I
Pst I
Pvu I
Sca I
T7
Term
LIC
His6
LIC
rbs
rbs
T7
TEV
Prom
β-lac
Lac I
ori
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb

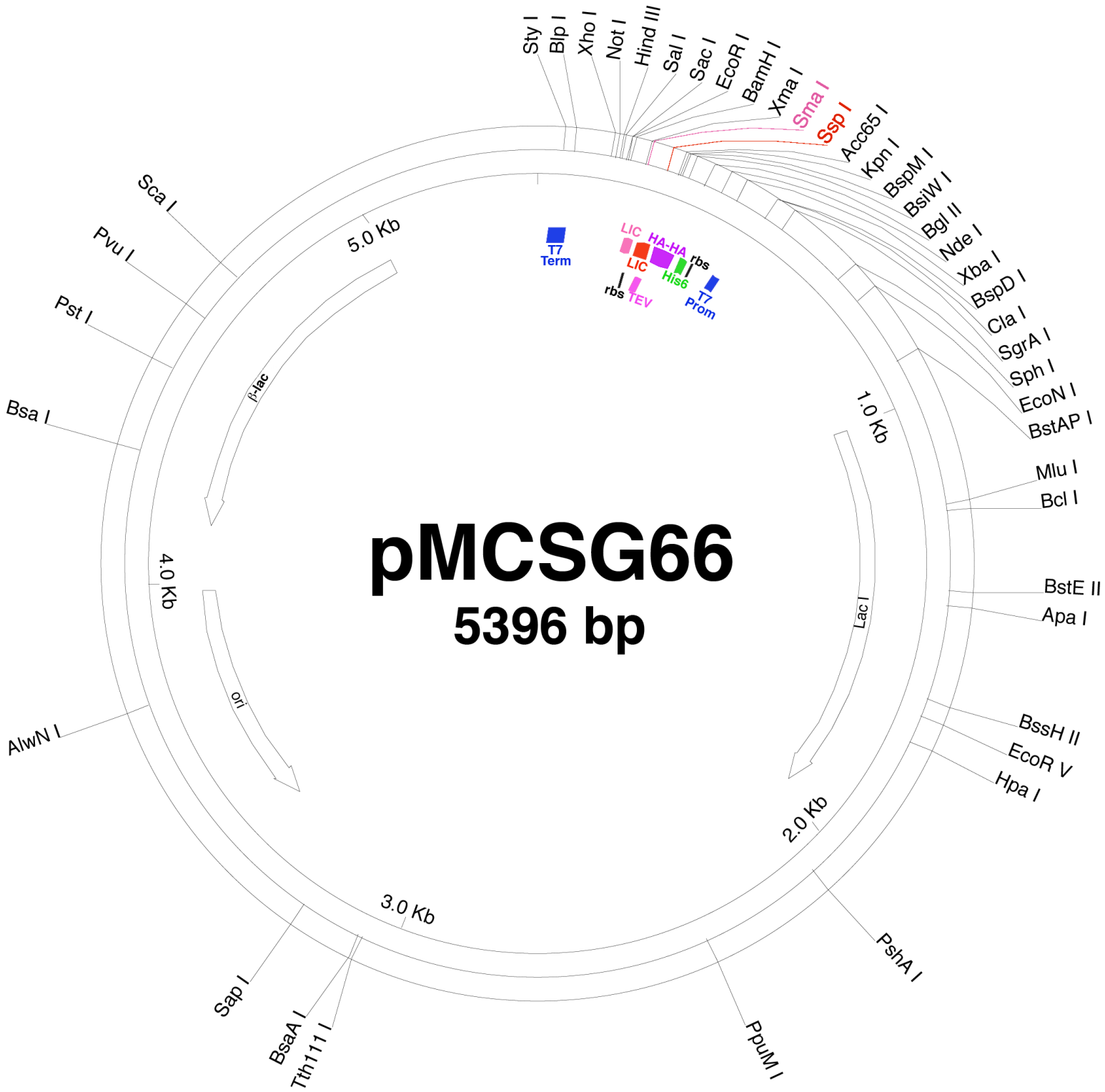

pMCSG61
6422 bp
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Nco I
BsiW I
Acc65 I
Kpn I
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
Mlu I
BstE II
Apa I
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
Sap I
Pst I
Sca I
T7
Term
LIC
PRIMERS
MBP
TEV
His6
T7
Prom
β-lac
Lac I
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb
6.0 Kb

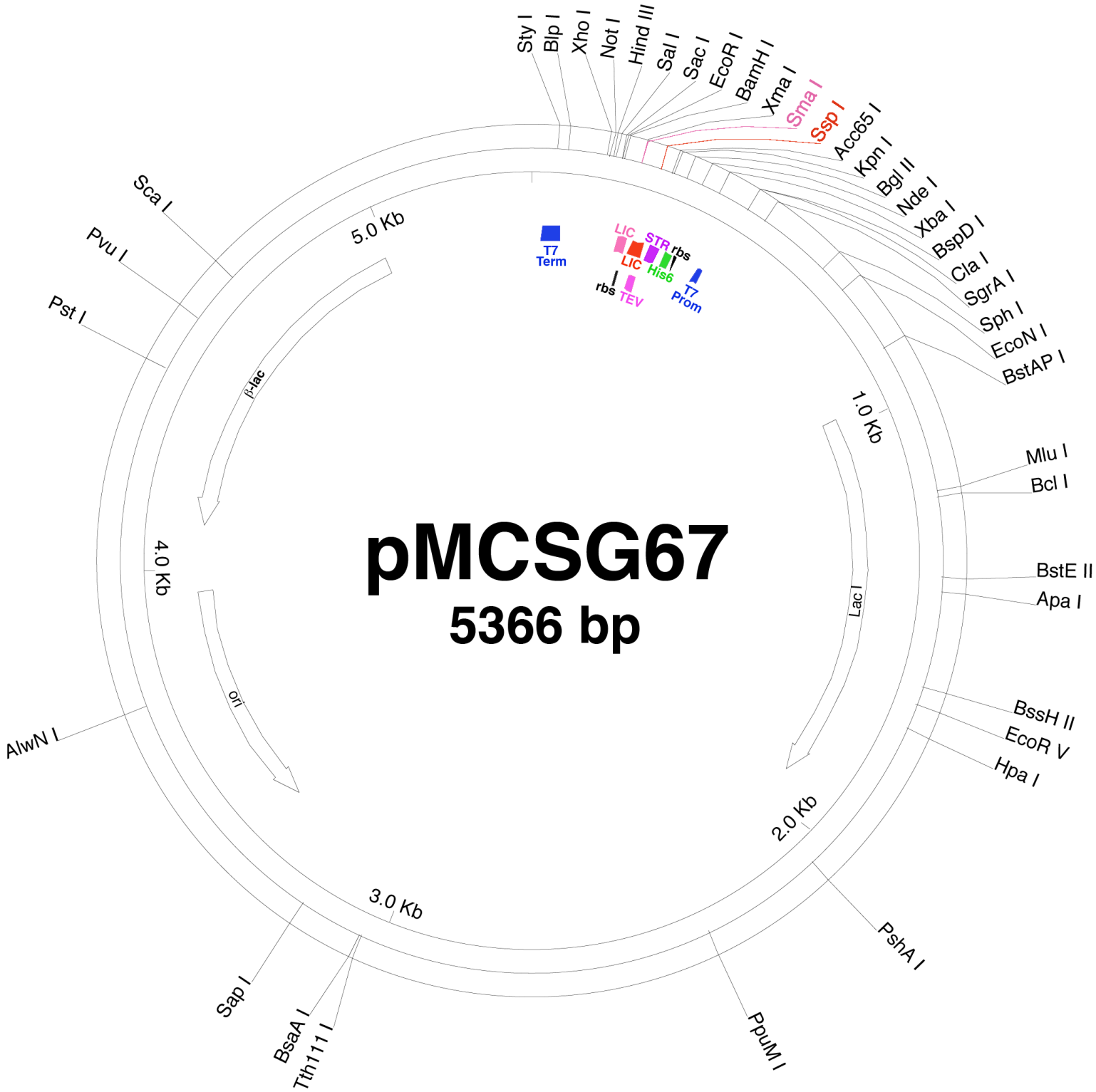

pMCSG62
5869 bp
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Dra III
Ssp I
Acc65 I
Kpn I
Nde I
Xba I
BspD I
Cla I
Age I
Asc I
BspM I
Nco I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
EcoR V
Hpa I
BsmB I
PshA I
Sap I
Drd I
4.0 Kb
ori
Bsa I
Bgl I
Fsp I
Sca I
5.0 Kb
β-lac
T7
Term
BirA
1.0 Kb
rbs
LIC
TEV
Biotin
His6
rbs
T7
Prom
argU
ileX
2.0 Kb
Lac I
3.0 Kb

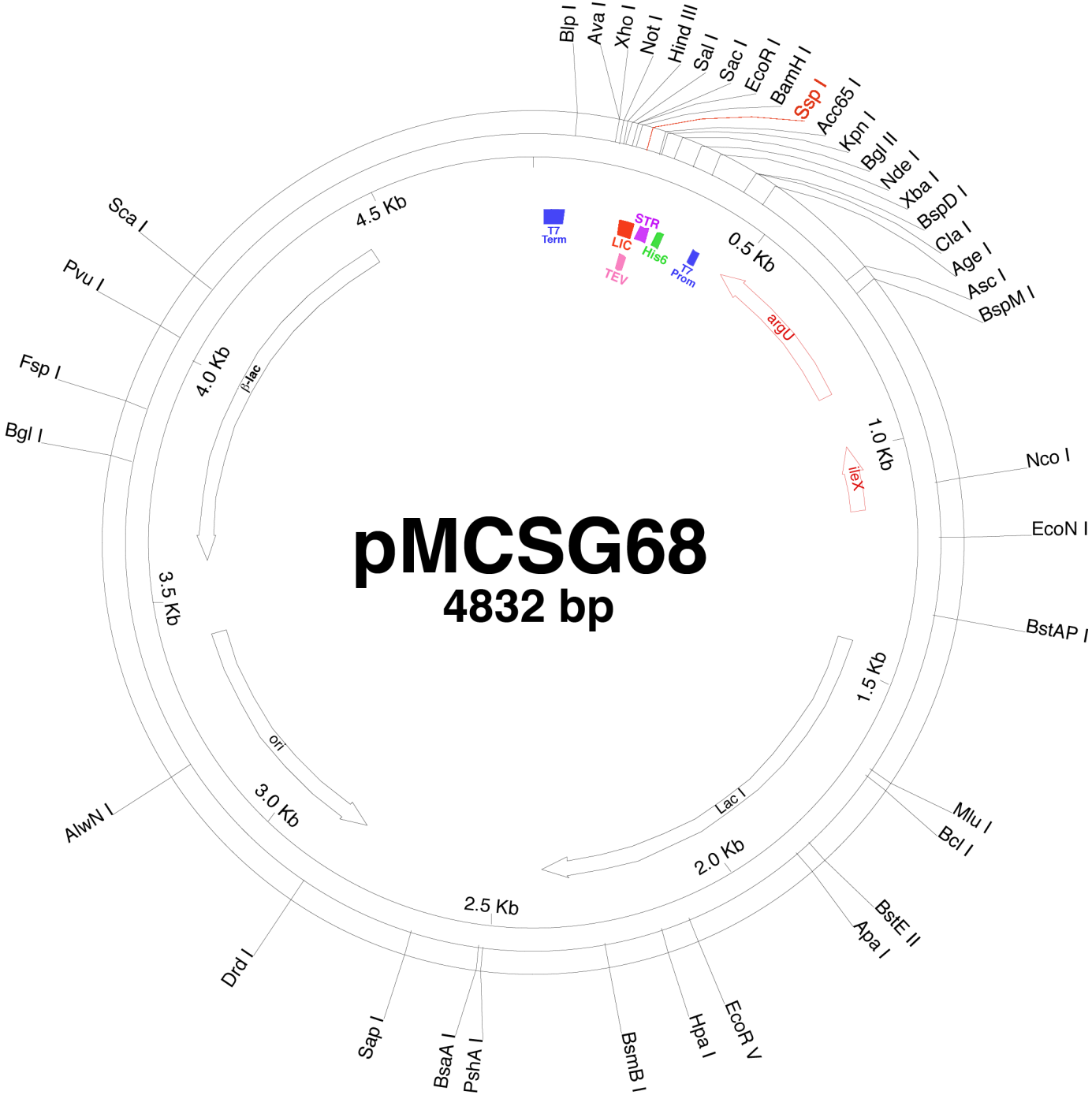

pMCSG63
4864 bp
Blp I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
Ssp I
Acc65 I
Kpn I
Bgl II
Nde I
Xba I
BspD I
Cla I
Age I
Asc I
BspM I
Nco I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
EcoR V
Hpa I
BsmB I
PshA I
BsaA I
Sap I
Drd I
AlwN I
Bsa I
Bgl I
Fsp I
Pvu I
Sca I
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
4.0 Kb
4.5 Kb
T7
Term
LIC
His6
LIC
rbs
rbs
TEV
T7
Prom
argU
ileX
Lac I
ori
β-lac

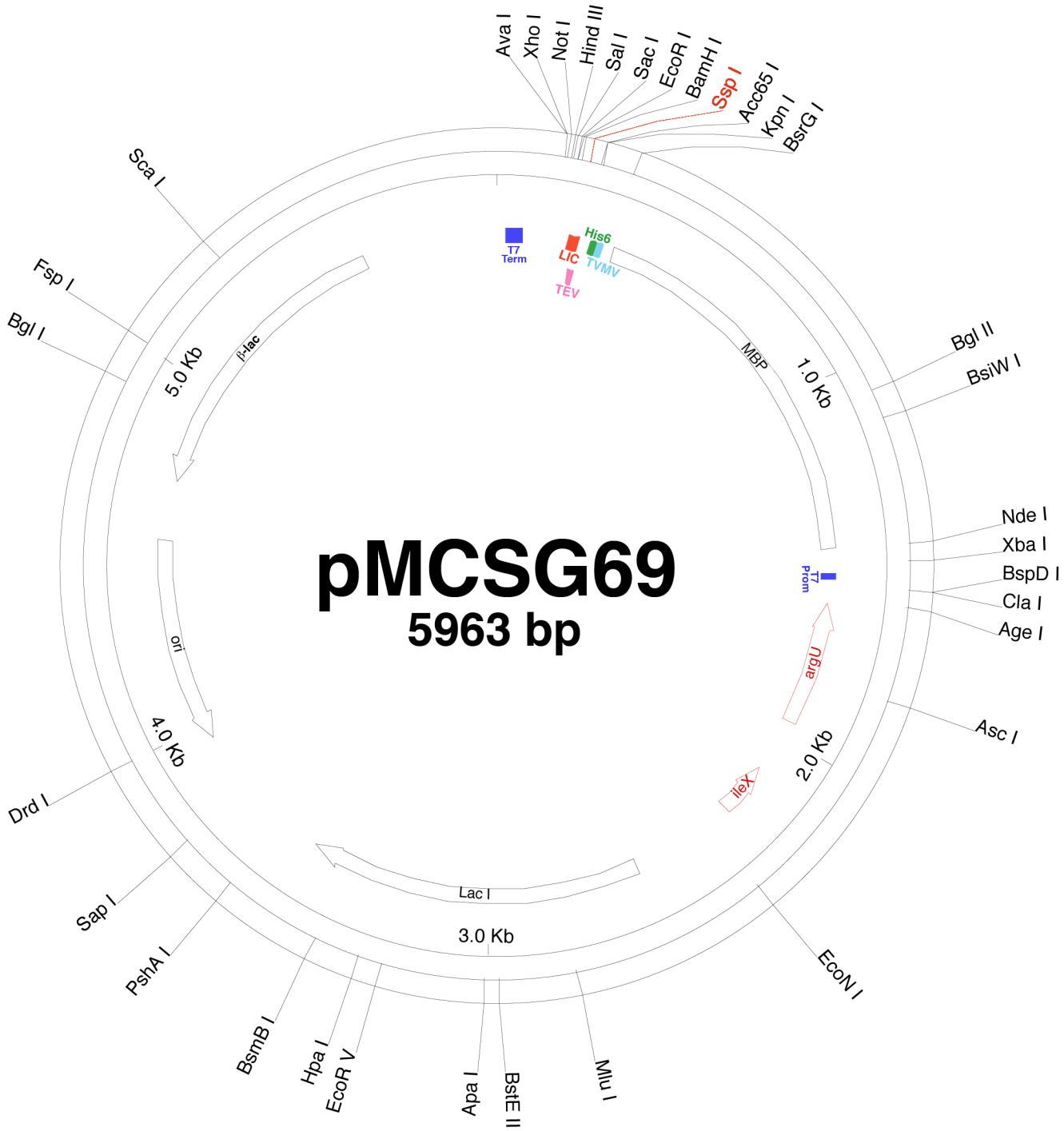

pMCSG64
5366 bp
Sty I
Blp I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
Ssp I
Acc65 I
Kpn I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
AlwN I
Bsa I
Pst I
Pvu I
Sca I
T7 Term
LIC
FLAG
rbs
LIC
His6
T7 Prom
rbs
TEV
β-lac
ori
Lac I
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb

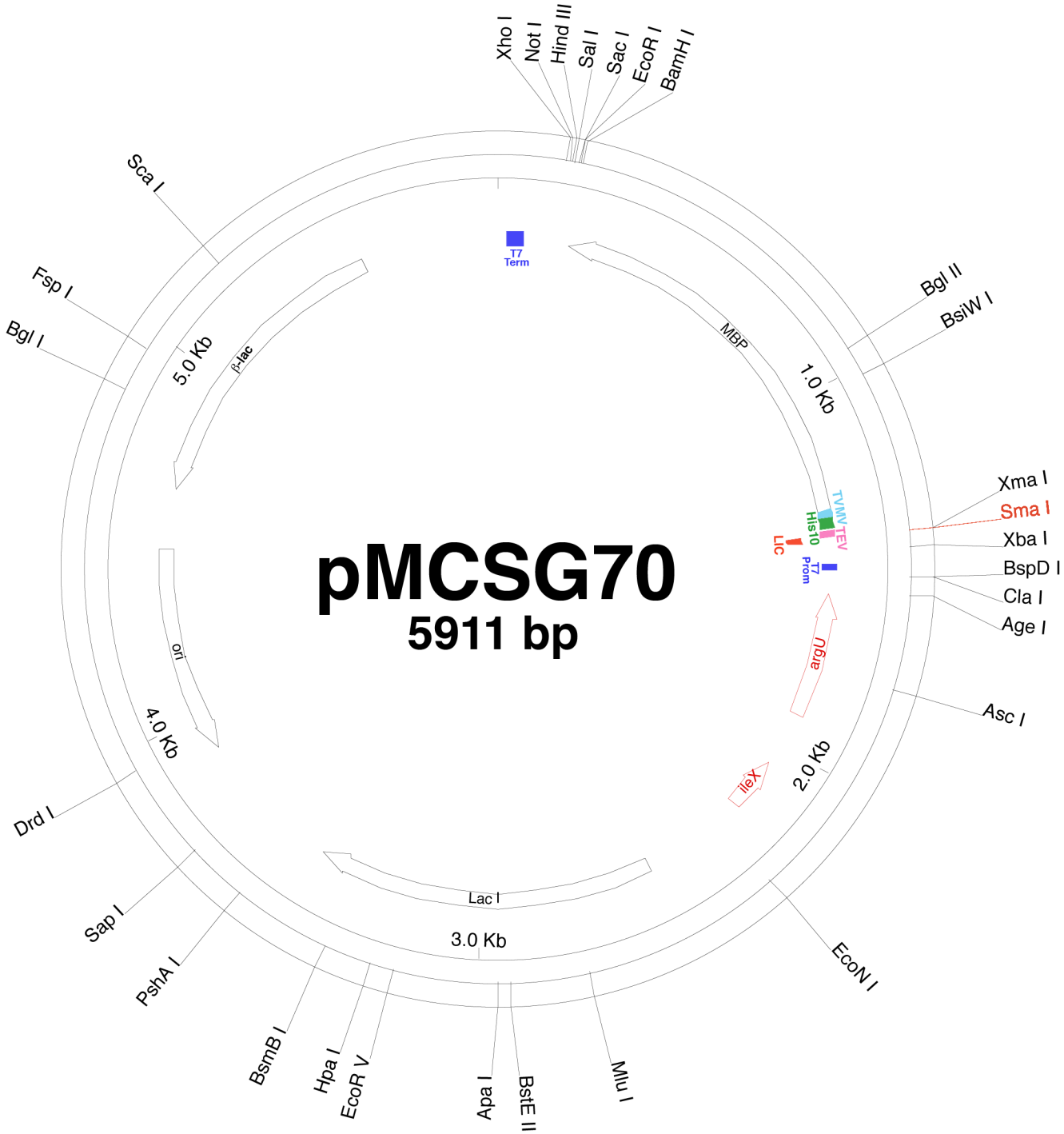

pMCSG65
5369 bp
Sty I
Blp I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
Ssp I
Acc65 I
Kpn I
BspM I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
AlwN I
Bsa I
Pst I
Pvu I
Sca I
T7 Term
LIC
HA
rbs
LIC
His6
T7 Prom
rbs
TEV
β-lac
ori
Lac I
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb

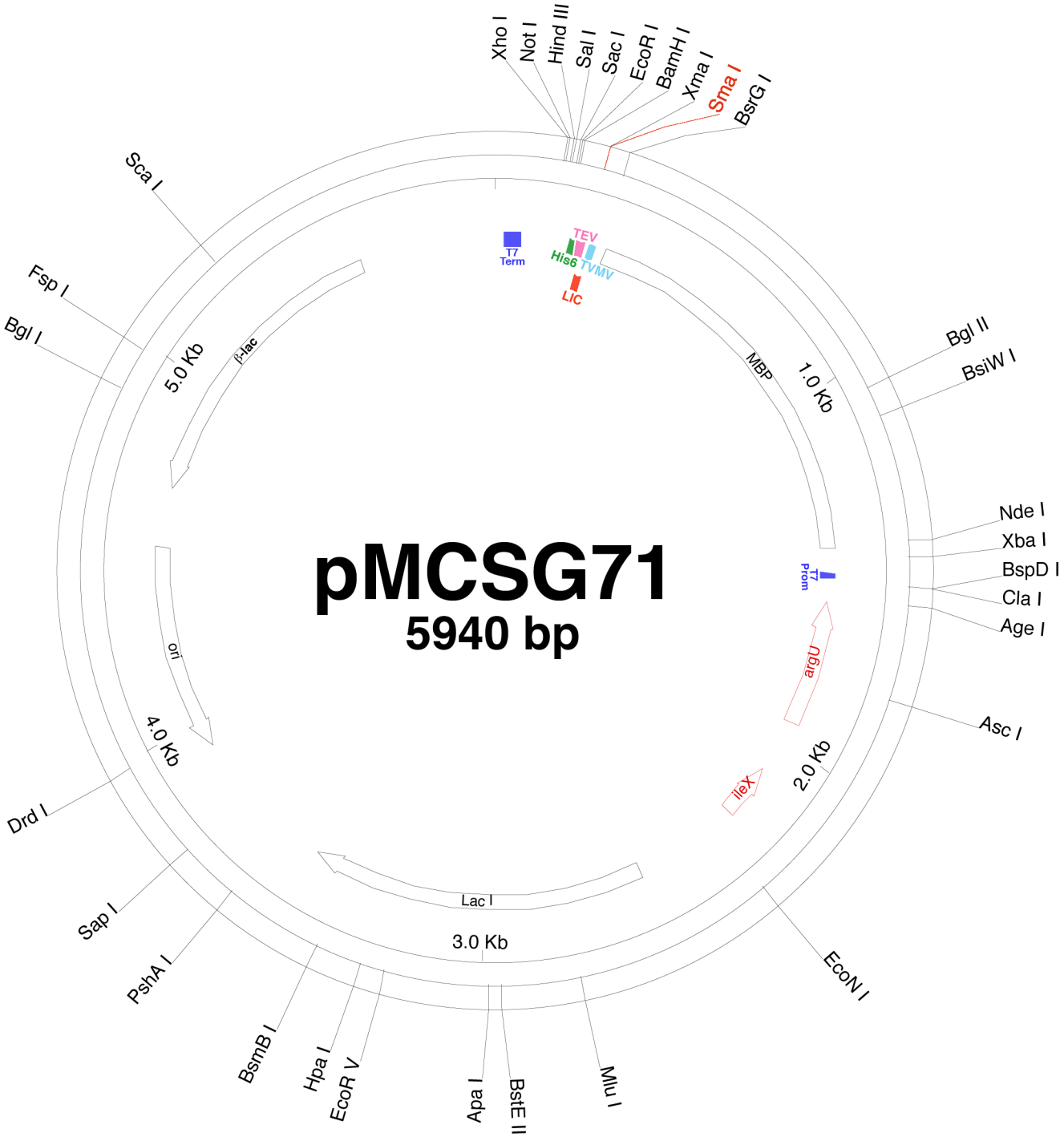

pMCSG66
5396 bp
Sty I
Blp I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
Ssp I
Acc65 I
Kpn I
BspM I
BsiW I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
AlwN I
Bsa I
Pst I
Pvu I
Sca I
T7 Term
LIC
HA-HA
rbs
LIC
His6
rbs
TEV
T7 Prom
β-lac
ori
Lac I
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb

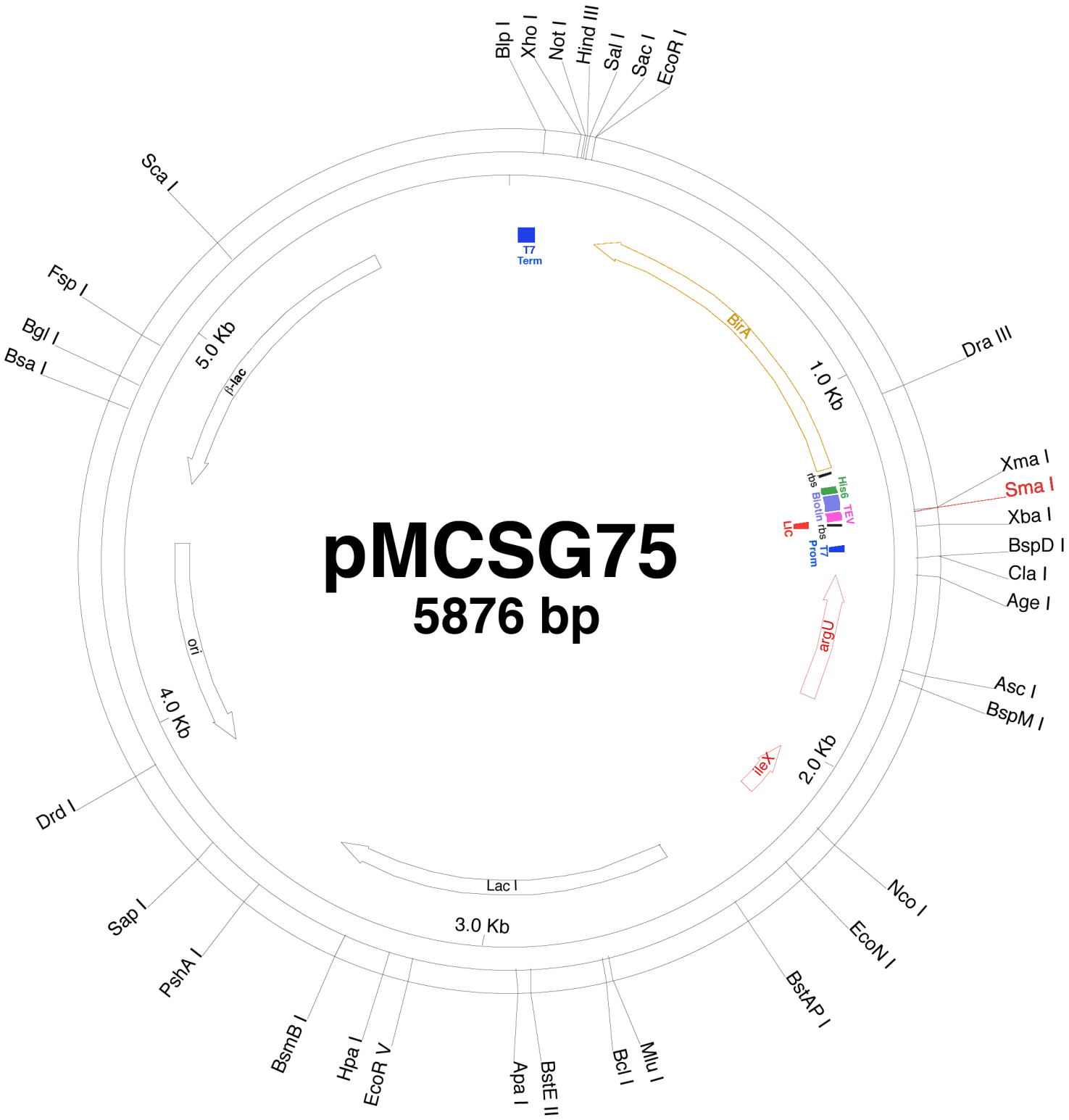

pMCSG67
5366 bp
Sty I
Blp I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
Ssp I
Acc65 I
Kpn I
Bgl II
Nde I
Xba I
BspD I
Cla I
SgrA I
Sph I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
BssH II
EcoR V
Hpa I
PshA I
PpuM I
Tth111 I
BsaA I
Sap I
AlwN I
Pst I
Pvu I
Sca I
T7 Term
LIC
STR
rbs
LIC
His6
rbs
TEV
T7 Prom
β-lac
ori
Lac I
1.0 Kb
2.0 Kb
3.0 Kb
4.0 Kb
5.0 Kb


pMCSG68
4832 bp
Blp I
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
Bgl II
Nde I
Xba I
BspD I
Cla I
Age I
Asc I
BspM I
Nco I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
EcoR V
Hpa I
BsmB I
PshA I
BsaA I
Sap I
Drd I
AlwN I
Bgl I
Fsp I
Pvu I
Sca I
T7 Term
LIC
TEV
STR
His6
T7 Prom
argU
ileX
β-lac
ori
Lac I
0.5 Kb
1.0 Kb
1.5 Kb
2.0 Kb
2.5 Kb
3.0 Kb
3.5 Kb
4.0 Kb
4.5 Kb


pMCSG69
5963 bp
Ava I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Ssp I
Acc65 I
Kpn I
BsrG I
Bgl II
BsiW I
Nde I
Xba I
BspD I
Cla I
Age I
Asc I
EcoN I
Mlu I
BstE II
Apa I
EcoR V
Hpa I
BsmB I
PshA I
Sap I
Drd I
Bgl I
Fsp I
Sca I
T7 Term
LIC
His6
TVMV
TEV
MBP
1.0 Kb
T7 Prom
argU
2.0 Kb
ileX
Lac I
3.0 Kb
ori
4.0 Kb
β-lac
5.0 Kb


pMCSG70
5911 bp
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Sca I
Fsp I
Bgl I
Bgl II
BsiW I
Xma I
Sma I
Xba I
BspD I
Cla I
Age I
Asc I
EcoN I
Mlu I
BstE II
Apa I
EcoR V
Hpa I
BsmB I
PshA I
Sap I
Drd I
T7
Term
MBP
1.0 Kb
TVMV
TEV
His10
LIC
T7
Prom
argU
2.0 Kb
IleX
Lac I
3.0 Kb
ori
4.0 Kb
β-lac
5.0 Kb


pMCSG71
5940 bp
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
BamH I
Xma I
Sma I
BsrG I
Sca I
Fsp I
Bgl I
Bgl II
BsiW I
Nde I
Xba I
BspD I
Cla I
Age I
Asc I
EcoN I
Mlu I
BstE II
Apa I
EcoR V
Hpa I
BsmB I
PshA I
Sap I
Drd I
T7 Term
His6
TEV
TVMV
LIC
MBP
1.0 Kb
T7 Prom
argU
2.0 Kb
ileX
Lac I
3.0 Kb
ori
4.0 Kb
β-lac
5.0 Kb


pMCSG75
5876 bp
Blp I
Xho I
Not I
Hind III
Sal I
Sac I
EcoR I
Sca I
Fsp I
Bgl I
Bsa I
Dra III
Xma I
Sma I
Xba I
BspD I
Cla I
Age I
Asc I
BspM I
Nco I
EcoN I
BstAP I
Mlu I
Bcl I
BstE II
Apa I
EcoR V
Hpa I
BsmB I
PshA I
Sap I
Drd I
T7
Term
BirA
1.0 Kb
rbs
HisG
Biotin
TEV
rbs
T7
Prom
LIC
argU
2.0 Kb
ileX
Lac I
3.0 Kb
ori
4.0 Kb
β-lac
5.0 Kb