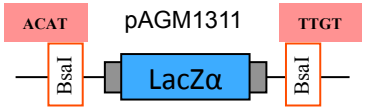
1 vector

Universal level -1 cloning vector

pUC19-derived backbone, Km^R



(BpiI)

BsaI

```
..gaag acat a gagacc...lacZ... ggtctc a ttgt cttc...
..cttc tgta t ctctgg...lacZ... ccagag t aaca gaag...
```

BsaI

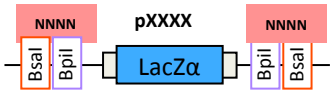
(BpiI)

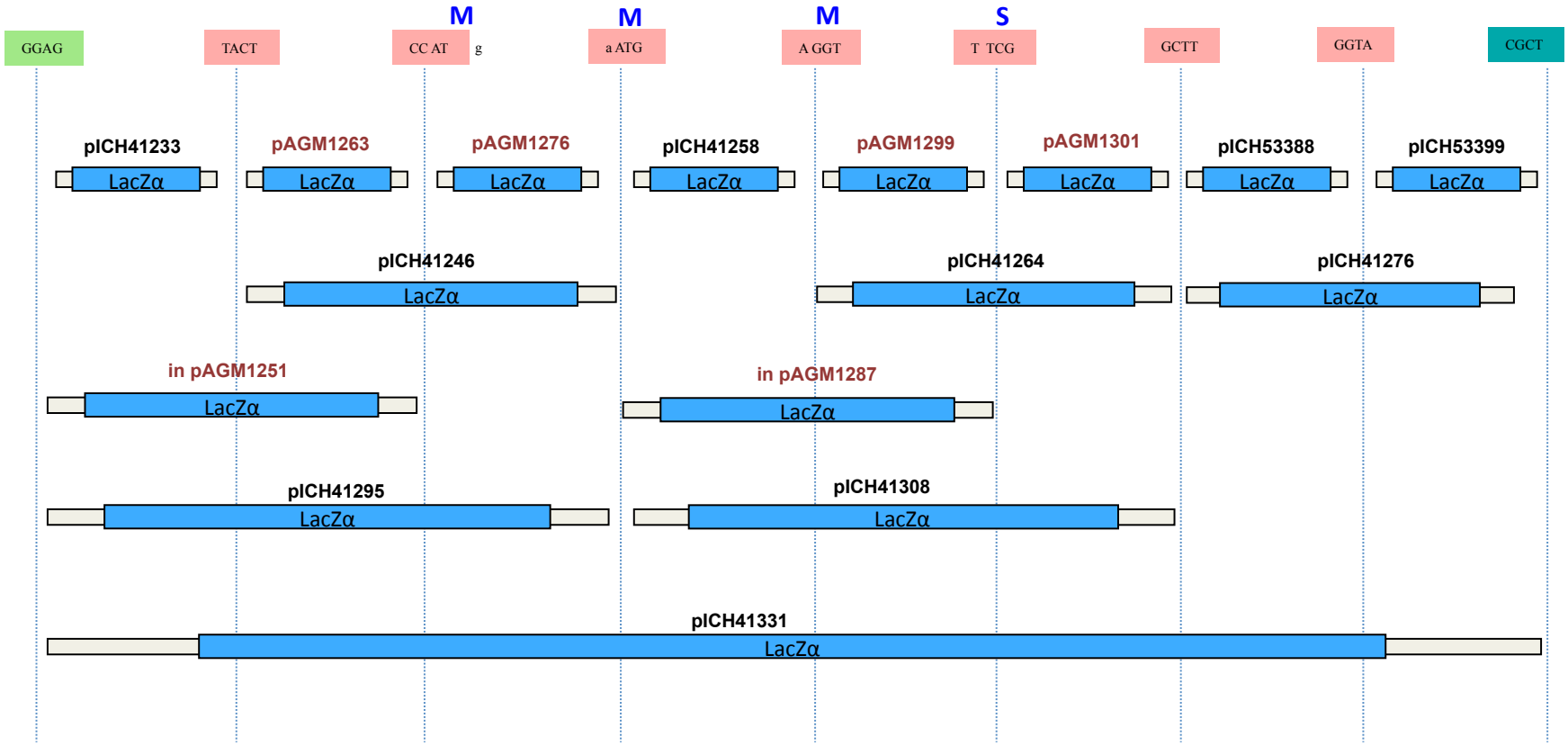
The clevage sites of the BpiI sites will be changed by the cloned PCR product !

16 vectors

Level 0 cloning vectors

pUC19-derived backbone, Spec^{R}

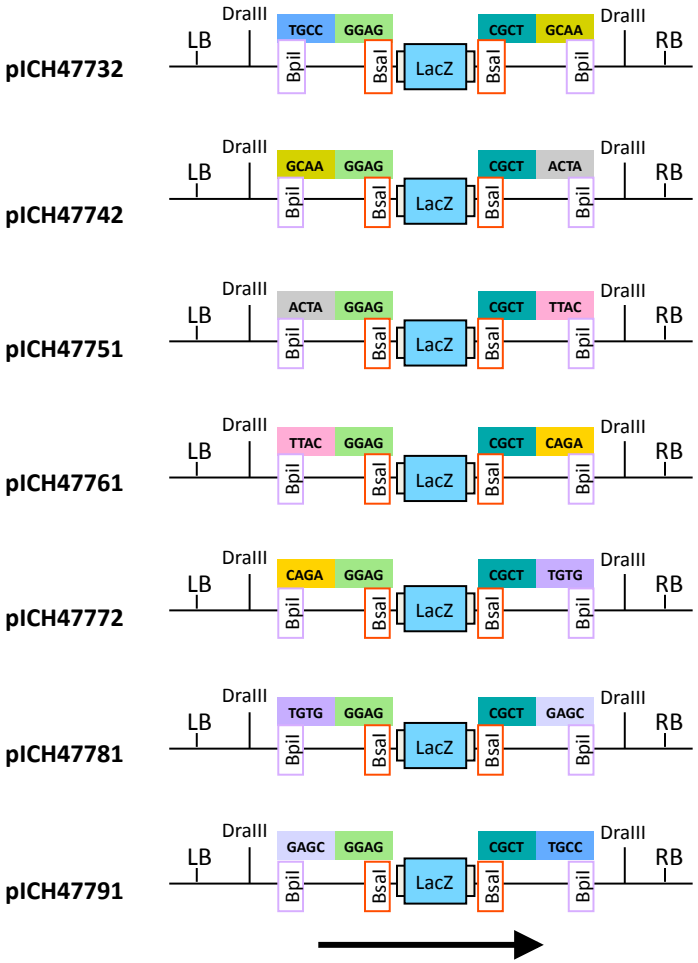




14 vectors

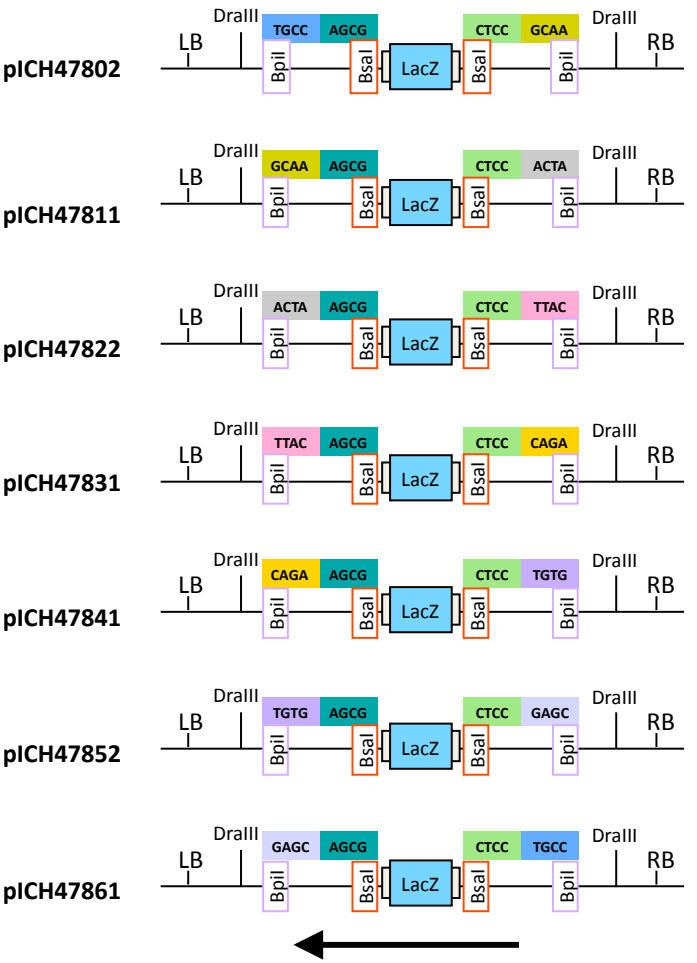
Level 1 cloning vectors, Amp^{R}

backbone derived from pBIN19 and pUC19, ColE1 and RK2 oris, replicate in *E.coli* and *Agrobacterium*



to clone transcription units in forward orientation

the DraIII fragment
can be subcloned in
new vector backbones



to clone transcription units in reverse orientation

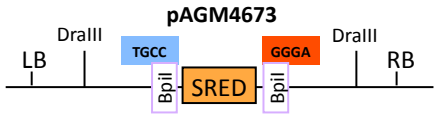
the DraIII fragment
can be subcloned in
new vector backbones

23 constructs

Level 2 vectors, Kan^R

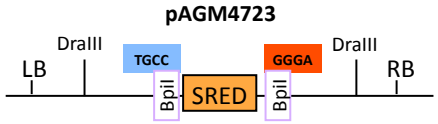
Replicate in E.coli and Agrobacterium

backbone derived from pBIN19 and pUC19, ColE1 and RK2 oris



the DraIII fragment can be subcloned in new vector backbones

backbone derived from pBIN19 and pPZP200, ColE1 and pVS1 oris



the DraIII fragment can be subcloned in new vector backbones

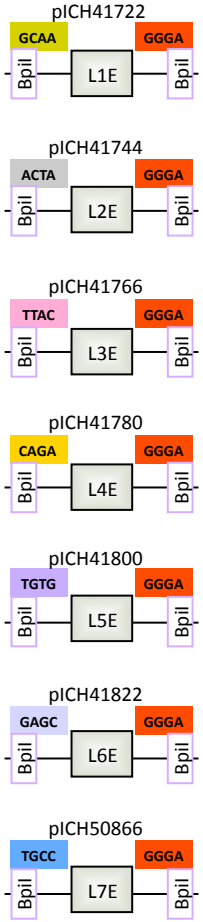
Level 2 vectors and end-linkers

Level 2 end-linkers

Spec^R

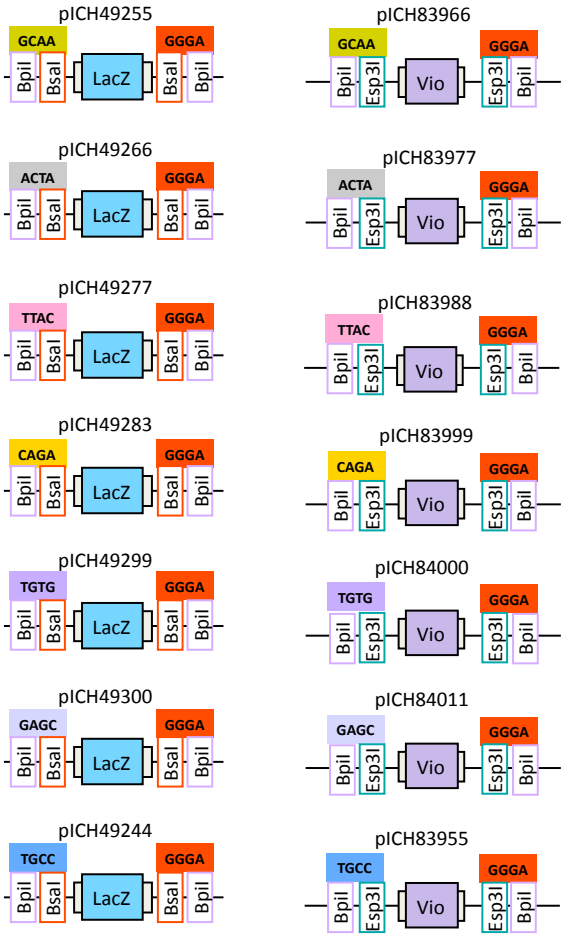
ColE1 ori

pUC19-derived backbone



Amp^R **ColE1 and RK2 oris**

backbone derived from pBIN19 (RK2 ori) and pUC19 (ColE1 ori). The RK2 ori taken from pBIN19 vector is not necessary in this backbone, but just happens to be there)

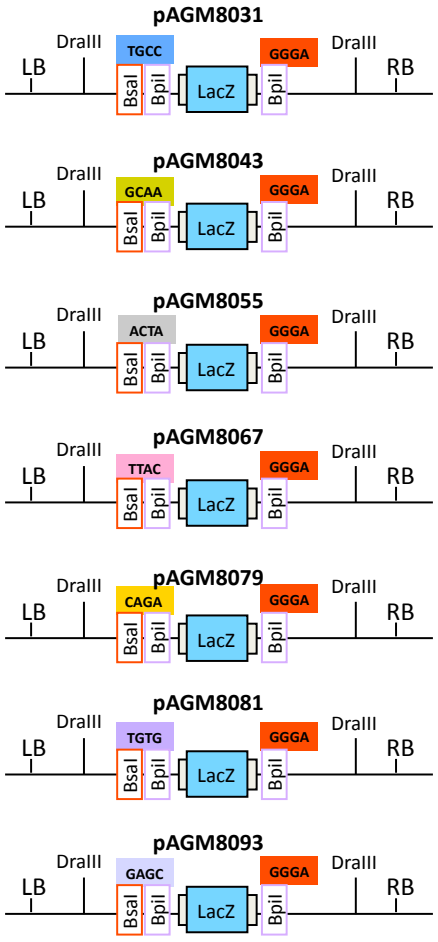


14 constructs

Level M vectors and end-linkers

Cloning vectors, Spec^R

oris: ColE1, Rk2

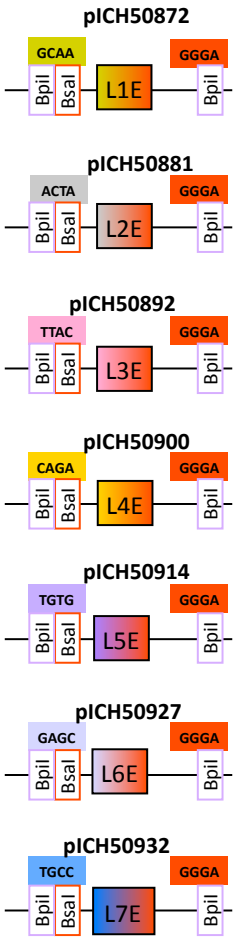


the DraIII fragment can be subcloned in new vector backbones

End linkers, Amp^R

ColE1 ori

pUC19-derived backbone



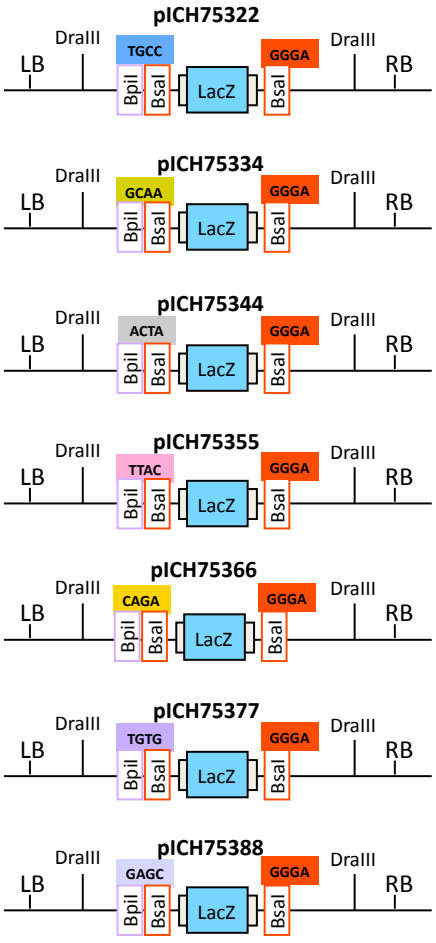
Level P vectors and end-linkers

14 constructs

Level P cloning vectors, Kan^R

ColE1 and pVS1 oris

backbone derived from pPZP200 (pVS1 ori) and pUC19 (ColE1 ori) replicate in E.coli and agrobacterium

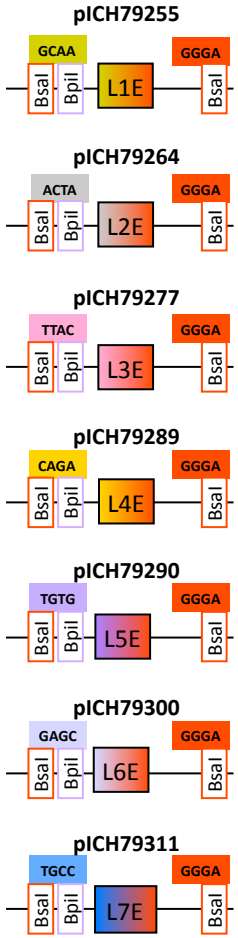


the DraIII fragment can be subcloned in new vector backbones

End linkers, Amp^R

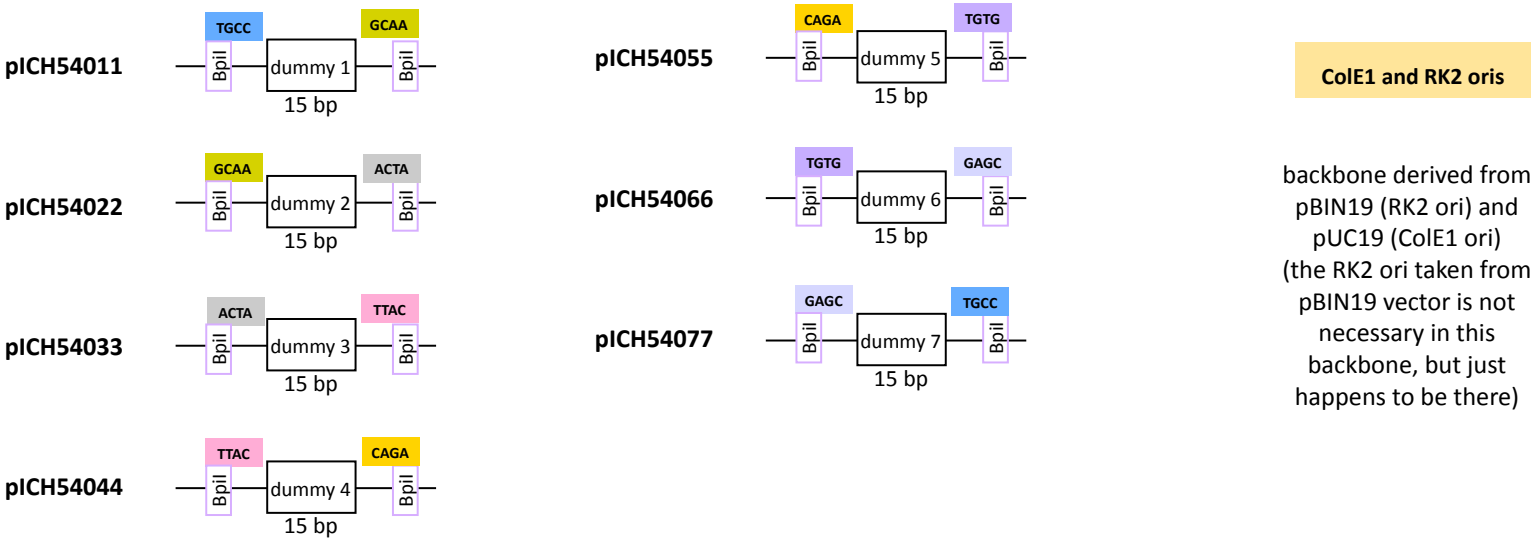
ColE1 ori

pUC19-derived backbone



Dummies, Amp^R

7 constructs

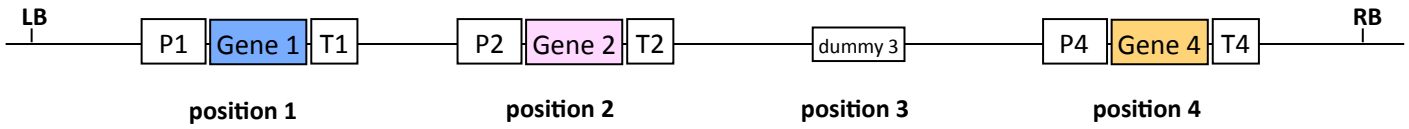


ColE1 and RK2 oris

backbone derived from pBIN19 (RK2 ori) and pUC19 (ColE1 ori) (the RK2 ori taken from pBIN19 vector is not necessary in this backbone, but just happens to be there)

Use of dummies:

Dummies can be used to assemble a multigene construct lacking a transcription unit at an internal position. For example, three transcription units previously cloned in level 1 vectors for positions 1,2 and 4, can be assembled in a multigene construct by using dummy 3 in the level 2 cloning reaction. The resulting construct is shown below. The multigene construct obtained will contain 15 bp of sequence at position 3. Dummies can also be used to assemble multigene constructs for levels M or P.

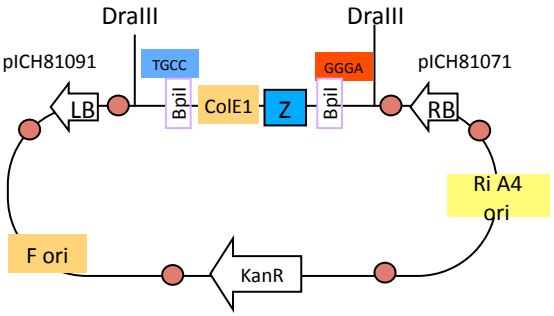


Other vectors

Medium-low copy vectors
(we are checking these vectors)

Level 2 vector, Kan^R

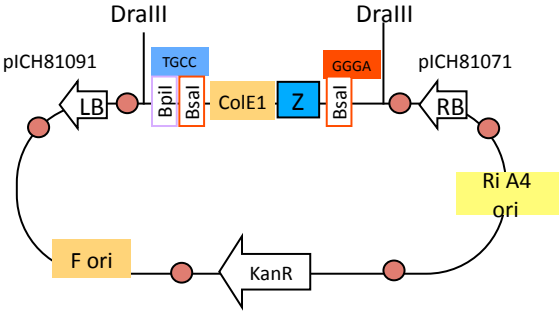
pICH89921



High copy before cloning an insert
low copy in *E.coli* (and *Agrobacterium*) after cloning

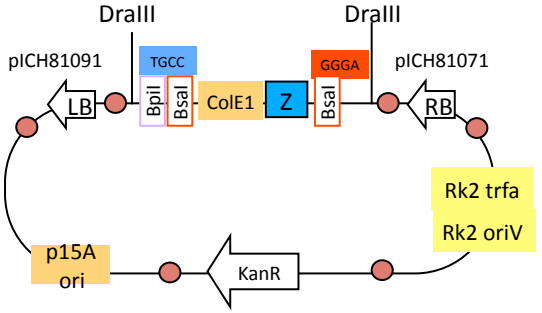
Level P vectors, Kan^R

pICH82113



High copy before cloning an insert
low copy in *E.coli* (and *Agrobacterium*) after cloning

pICH82094



High copy before cloning an insert
medium copy in *E.coli* (and *Agrobacterium*) after cloning

Level 2 vector, Kan^{R}

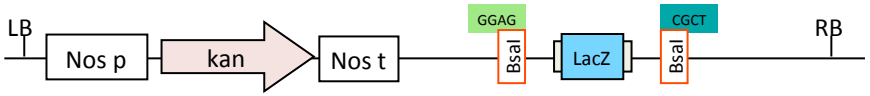
with selection marker for plant transformation

For direct cloning from level 0 to level 2
without cloning in level 1 vectors, allows cloning of only one transcription unit

ColE1 and pVS1 oris

backbone derived from pPZP200
(pVS1 ori) and pUC19 (ColE1 ori)
replicate in E.coli and agrobacterium

pICH86966



pICH86988