Universal level -1 cloning vector
pUC19-derived backbone, KmR

1 vector

The cleavage sites of the Bpil sites will be changed by the cloned PCR product!
16 vectors

Level 0 cloning vectors
pUC19-derived backbone, Spec<sup>R</sup>
14 vectors

Level 1 cloning vectors, **Amp<sup>R</sup>**
backbone derived from pBIN19 and pUC19, ColE1 and RK2 ori, replicate in *E.coli* and *Agrobacterium*.

- **pICH47732**
  - DralI
  - LB
  - GAGC
  - CGCT
  - GCAA
  - RB

- **pICH47742**
  - DralI
  - LB
  - GGAG
  - CGCT
  - TGCC
  - RB

- **pICH47751**
  - DralI
  - LB
  - ACTA
  - CGCT
  - TTAC
  - RB

- **pICH47761**
  - DralI
  - LB
  - TTAC
  - CGCT
  - CAGA
  - RB

- **pICH47772**
  - DralI
  - LB
  - CAGA
  - CGCT
  - TGCC
  - RB

- **pICH47781**
  - DralI
  - LB
  - TGCC
  - CGCT
  - GACC
  - RB

- **pICH47791**
  - DralI
  - LB
  - GACC
  - CGCT
  - TGGC
  - RB

The DraIII fragment can be subcloned in new vector backbones.

---

to clone transcription units in forward orientation

to clone transcription units in reverse orientation
23 constructs

Level 2 vectors, Kan^R
Replicate in E.coli and Agrobacterium

backbone derived from pBIN19 and pUC19, ColE1 and RK2 oris

pAGM4673

the Dralll fragment can be subcloned in new vector backbones

backbone derived from pBIN19 and pPZP200, ColE1 and pVS1 oris

pAGM4723

the Dralll 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

Cloning vectors, Spec\textsuperscript{R}

<table>
<thead>
<tr>
<th>Vector</th>
<th>DraIII</th>
<th>BpiI</th>
<th>BsaI</th>
<th>BpiI</th>
<th>DraIII</th>
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<tbody>
<tr>
<td>pAGM8031</td>
<td>TGGC</td>
<td>GCAA</td>
<td>ACTA</td>
<td>CAGA</td>
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<td>CAGA</td>
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<td>GAGC</td>
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<td>TGTG</td>
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<td>GAGC</td>
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</tbody>
</table>

the DraIII fragment can be subcloned in new vector backbones

Level M vectors and end-linkers

End linkers, Amp\textsuperscript{R}

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<thead>
<tr>
<th>Linker</th>
<th>pAGM8031</th>
<th>pAGM8043</th>
<th>pAGM8055</th>
<th>pAGM8067</th>
<th>pAGM8079</th>
<th>pAGM8081</th>
<th>pAGM8093</th>
</tr>
</thead>
<tbody>
<tr>
<td>ColE1 ori</td>
<td>pUC19-derived backbone</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>GCAA</td>
<td>L1E</td>
<td>GCAA</td>
<td>L2E</td>
<td>GCAA</td>
<td>L3E</td>
<td>GCAA</td>
<td>L4E</td>
</tr>
<tr>
<td>TTAC</td>
<td>L2E</td>
<td>GAA</td>
<td>L3E</td>
<td>GAA</td>
<td>L4E</td>
<td>GAA</td>
<td>L5E</td>
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<tr>
<td>ACTA</td>
<td>L2E</td>
<td>GAA</td>
<td>L3E</td>
<td>GAA</td>
<td>L4E</td>
<td>GAA</td>
<td>L5E</td>
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<tr>
<td>GAGC</td>
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<td>GAA</td>
<td>L4E</td>
<td>GAA</td>
<td>L5E</td>
</tr>
<tr>
<td>TGGC</td>
<td>L2E</td>
<td>GAA</td>
<td>L3E</td>
<td>GAA</td>
<td>L4E</td>
<td>GAA</td>
<td>L5E</td>
</tr>
<tr>
<td>GAGC</td>
<td>L2E</td>
<td>GAA</td>
<td>L3E</td>
<td>GAA</td>
<td>L4E</td>
<td>GAA</td>
<td>L5E</td>
</tr>
</tbody>
</table>

oris: ColE1, Rk2
Level P vectors and end-linkers

14 constructs

Level P cloning vectors, KanR

- ColE1 and pVS1 oris
- plICH75322
- plICH75334
- plICH75344
- plICH75355
- plICH75366
- plICH75377
- plICH75388

End linkers, AmpR

- ColE1 ori
- pUC19-derived backbone
- plICH79255
- plICH79264
- plICH79277
- plICH79289
- plICH79290
- plICH79300
- plICH79311

ColE1 and pVS1 oris

 optional

the DraIII fragment
can be subcloned in
new vector backbones

ColE1 and pVS1 ori and pUC19 (ColE1 ori) replicate in E.coli and agrobacterium.
7 constructs

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.

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

Level 2 vector, $\text{Kan}^R$

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

Level P vectors, $\text{Kan}^R$

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

High copy before cloning an insert medium copy in *E. coli* (and *Agrobacterium*) after cloning
Level 2 vector, $\text{Kan}^R$

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

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

ColE1 and pVS1 ori

LB

pICH86966

Nos p  kan  Nos t

GGAG  BsaI  GGAG  BsaI

LB

pICH86988

Nos p  kan  Nos t

35S-Ω  BsaI  35S-Ω  BsaI

LB

Ocs

RB