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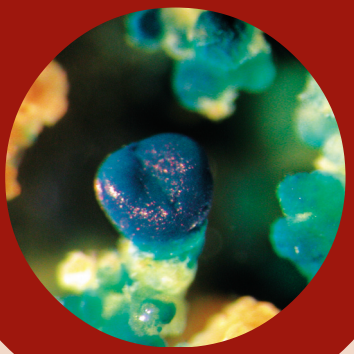
For quality of life

Clean vector technology for marker-free genetically modified plants

Wageningen Tissue Culture Centre, WTCC



Our clean vector technology enables the removal of undesired DNA sequences, like antibiotic resistance genes, from transgenic plants. Removal of selectable marker genes may be desirable for the production of genetically modified crops with increased consumer acceptance. In addition, marker removal enables stacking of transgenes by recurrent transformations.

Principle

pMF is a new plant transformation vector produced by Plant Research International, Wageningen for efficient production of genetically modified plants which are free from undesired DNA-sequences. The pMF vector provides an inducible site-specific recombination system for removal of undesired DNA sequences. A negative selection step using the *cod* A (cytosine deaminase) gene, ensures the ultimate production of completely marker-free plants (ref. Schaart *et al.* (2004) Plant Biotechnology J. 2: 223-240).

Transformation using **positive selection** (npt II)

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Induction of **Recombination R** activity for removal of marker gene

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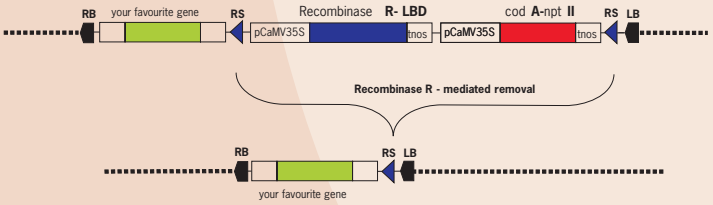
Selection for marker-free plants using **negative selection** (cod A)

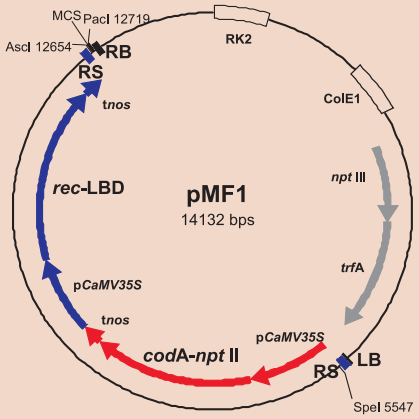
Chemically induced marker removal

The marker removal system fits easily in existing *Agrobacterium*-mediated transformation protocols. At any stage selection of transgenic tissue or plants, chemical induction of Recombinase R activity will result in elimination of DNA-sequences that are flanked by the recombination sites (RS). Subsequent (negative) selection prevents development of plant tissue still containing the *cod* A-gene.



Vector map

The pMF1 vector is equipped with the *npt* II gene for kanamycin selection of transgenic shoots. Two additional pMF-versions, pMF2 and pMF3 are available in which the selection gene *hpt* (hygromycin resistance) and *pat* (phosphinothricin resistance), respectively, have been introduced into a unique *Spe* I-restriction site of pMF1. All selection genes are removable.



RB, LB, RS,	Right and Left Border of T-DNA, respectively Recombination Site
***rec*-LBD,**	Fusion of Recombinase R-gene and Ligand Binding Domain
***cod* A-*npt* II,**	Fusion of *cod* A gene for negative selection on fluoro cytosine and *npt* II gene for positive selection on kanamycin
SpeI,	Restriction site in which *hpt* or *pat* genes have been inserted in pMF2 and pMF3, respectively
MCS,	Multiple Cloning Site

Ordering information

For further details or ordering information, please contact Dr J.G. Schaart (jan.schaart@wur.nl) or Dr. F.A. Krens (frans.krens@wur.nl) at Plant Research International, Wageningen UR or see the web-page http://www.pri.wur.nl/uk/products.

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