

PureMlb™

■ Technology

- Clean gene technology for *Agrobacterium*-mediated transformation
- Vector carrying the “Multi-Left-Border”

■ Advantages

- Dramatically decreases read-through integration by *Agrobacterium*.
- Easy to use
- Addresses regulatory concerns

The letters “Mlb” in the PureMlb mark stand for “Multi-Left-Border”.



PureMlb™

- **Issue of *Agrobacterium* transformation**
 - Unnecessary integration of vector sequence
 - *Agrobacterium* sometimes fails to recognize the left border sequence.



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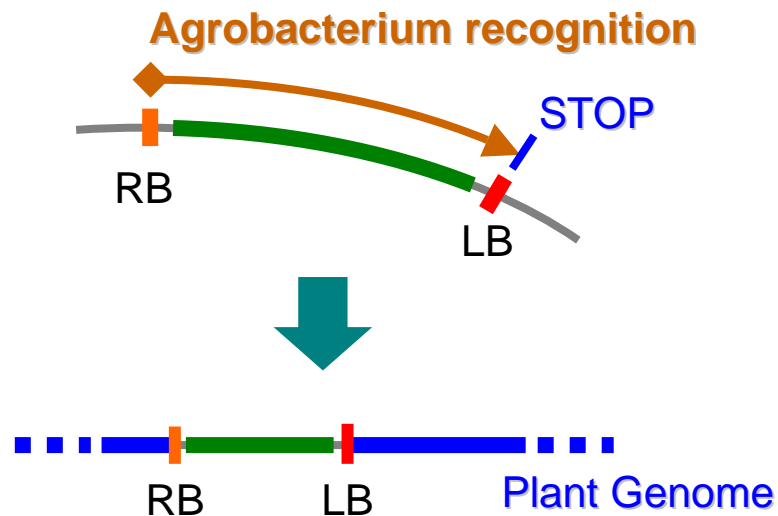
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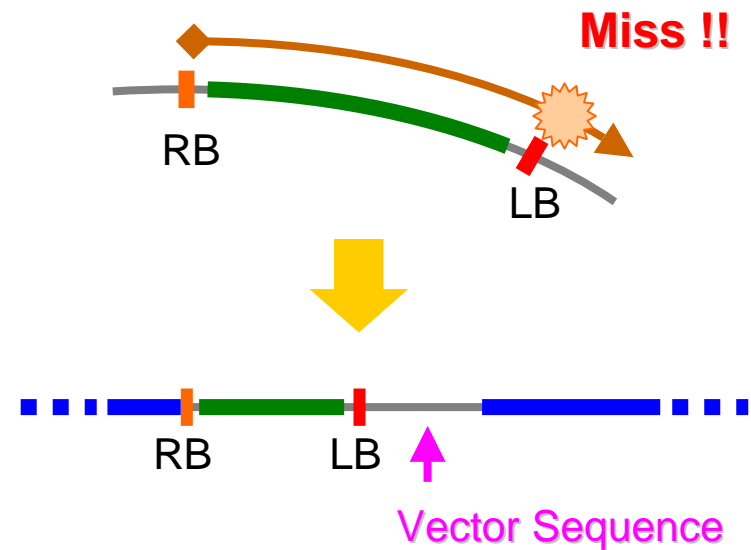
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■ Vector sequence integration

Normal T-DNA Integration



Read-through event



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- **Several publications reported the widespread occurrence of integration of unnecessary vector sequence.**
 - Martineau et al. (1994) *Plant Cell* **6**: 1032-1033
 - Non T-DNA plasmid sequences were found to have integrated in 20-30% of the transformants analyzed.
 - Ramanathan and Veluthambi (1995)
 - Cluster et al. (1996)
 - Kononov et al. (1997) *Plant J.* **11**: 945-957
 - As many as 75% of transformants were found to contain sequences from beyond the borders.
 - Wenck et al. (1997)

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- **Significant regulatory concerns raised on unidentified DNAs of GMOs in/near the market.**
 - e.g. “International appeal to scientists for help to identify the origin of unknown DNA” by Greenpeace (Aug. 2001)
 - P. Windels et al. (2001) Characterisation of the Roundup Ready soybean insert. Eur Food Res Technol 213:107-112

Large benefits in *PureMlb*™ to give better control of DNA insertion

Potentially faster and cheaper pre-commercial process

(registration, in-house monitoring, etc.)

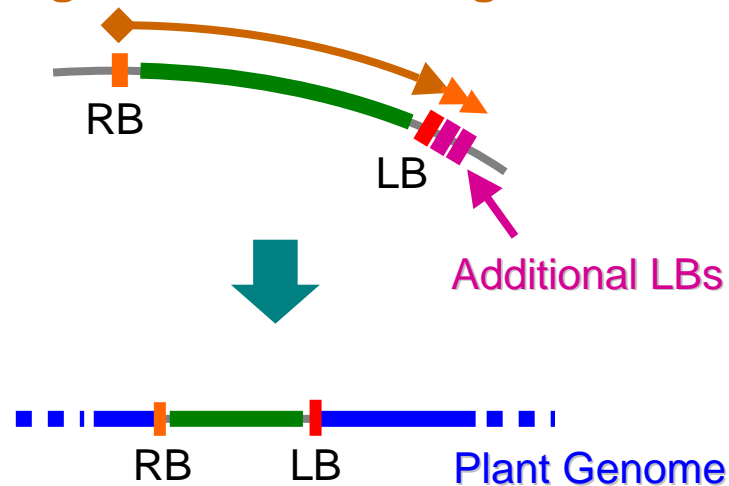
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- Multiple LBs dramatically decrease the “beyond the border” events.

Agrobacterium recognition



Effect of *PureMlb*

	Ratio of “Beyond the left border”
Single LB	20 – 75 %
Multiple LBs	Significant reduction

No additional work for construction once you obtain PureMlb vectors.

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■ Publications

■ Articles

- Kuraya Y, et al
 - Suppression of transfer of non-T-DNA 'vector backbone' sequences by multiple left border repeats in vectors for transformation of higher plants mediated by *Agrobacterium tumefaciens*. *Mol. Breeding* **14**: 309-320 (2004)

■ Patents

- Method of transforming plant and vector
WO 01/25459

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