



**Service of Biosafety and Biotechnology**

**Dr. W. Moens**

Q./ref. : IPH/1520/SBB/03-0407

Y./ref. :

**Report on the molecular characterisation of the genetic map of event T25**

**16 June 2003**

## Table of Contents

<b>1. Introduction: T25 maize dossier .....</b>	<b>2</b>
<b>2. Overview on molecular data of event T25 .....</b>	<b>2</b>
<b>2.1. Plasmid used for transformation .....</b>	<b>2</b>
<b>2.2. Characterisation of the insert and flanking regions.....</b>	<b>2</b>
<b>2.3. Comparison of molecular data of T25 provided in dossier C/F/95/12-07         with data from CLO .....</b>	<b>3</b>
<b>3. Conclusions .....</b>	<b>3</b>
<b>4. Confidential information .....</b>	<b>4</b>

## **1. Introduction: T25 maize dossier**

Liberty-link® maize event T25 notification C/F/95/12-07 of AgrEvo (Bayer CropScience) is approved under Directive 90/220/EEC for marketing since 22 April 1998 (Commission Decision 98/293/EC). Products derived from T25 have been notified under Article 5 of the Regulation (EC) 258/97 on 21 October 1999.

A hybrid of T25 is still pending for approval for marketing. T25 x Mon810 notification C/NL/98/08 was submitted under Directive 90/220/EEC on 29 April 1999. The Scientific Committee gave a favourable opinion on this dossier on 6 June, 2000.

The molecular data of the notification C/F/95/12-07 have been discussed during the meeting of the Belgium Biosafety Advisory Council on 6 December 2002. An overview of the molecular data of the event T25 presented during this meeting and provided by the applicant and CLO (Centrum Landbouwkundig Onderzoek, Melle, Belgium) is given below.

## **2. Overview on molecular data of event T25**

### **2.1. Plasmid used for transformation**

The line T25 was obtained by protoplast transformation of the parental line He/89 using the plasmid pUC/Ac containing the *pat* gene derived from *Streptomyces viridochromogenes* Tü 494 and controlled by the cauliflower mosaic virus (CaMV) 35S transcription promoter and terminator (see fig 1). The plasmid includes the *bla* gene, conferring resistance to ampicillin.

### **2.2. Characterisation of the insert and flanking regions**

According to the data from the applicant and the CLO, the molecular analysis of the T25 maize insert shows that a single genetic insert is present (see annex 1, 2). PCR and sequence analysis indicate that the *pat* gene is surrounded by sequences from the plasmid vector pUC18. According to the dossier, at the 5' end, upstream from the 35S transcription promoter, a 2187 bp pUC fragment is present (see annex 1). This fragment ends up in the *bla* gene followed by a 353 bp fragment of the CaMV 35S transcription promoter, probably resulting from a duplication/recombination event. Results obtained by the CLO confirm these data. According to the applicant's data and the CLO, at the 3' end downstream of the 35S transcription terminator, a fragment derived from the pUC plasmid was found (see fig. 2). However, differences in the length of pUC plasmid transferred, were reported.

Aventis submitted data that describe the host flanking sequences of the T25 line (see annex 1). A 151 bp (5') and a 121 bp (3') fragment show homology (94 % identity) with the maize alcohol dehydrogenase *adh1* gene (GENBANK accession n° AF123535).

In summary, the *pat* gene, surrounded by sequences derived from the plasmid used for transformation, is integrated as a single genetic insert into the plant's *adh1* gene.

### 2.3. Comparison of molecular data of T25 provided in dossier C/F/95/12-07 with data from CLO

Table 1: Comparison of molecular data of event T25 according to dossier with data provided by CLO

Dossier <sup>#</sup>	CLO*	Remarks
1 transgene insert (p35S- <i>pat</i> -t35S) flanked by plasmid DNA at 5' (2187 bp) and at 3' (616 bp)	1 transgene insert (p35S- <i>pat</i> -t35S) flanked by plasmid DNA at 5' (2187 bp) and at 3' (798 bp)	Differences between the two reports concerning length of fragments mentioned
5': 353 bp 35S promoter followed by 151 bp flanking plant DNA ( <i>adh1</i> )	5': 298 bp 35S promoter fragment, no flanking plant DNA analysed	Sequence of 35S determined by CLO is shorter
3': 121 bp flanking plant DNA ( <i>adh1</i> )	3': no flanking plant DNA analysed	

<sup>#</sup> see annex 1

\* see annex 2

### 3. Conclusions

The molecular data presented in C/F/95/12-07 fulfil the Belgian requirements concerning molecular data. However, the sequence of the flanking regions (120 bp at 3', 150 bp at 5') is not provided by the notifier and might not be enough to exclude the presence of rearrangements, especially if not compared to the pre-insertion site. Also the exact length of cotransferred pUC18 derived plasmid DNA should be determined. In addition, bio-informatic analysis should be done to determine the presence of chimaeric open reading frames in the border integration sequences.

**Acknowledgements.** We would like to thank the experts of the working group 'Molecular Characterisation' of the SBB to contribute to this report.

#### **4. Confidential information**

List of figures:

Figure 1: Physical map of plasmid pUC/Ac

Figure 2: Physical map of the characterised insert of T25 and pUC/Ac

List of annexes:

Annex 1: Molecular data from dossier C/F/95/12/07

Annex 2: CLO Report p. 7-8