

**Method of Detection of Dried Killed Bacterial Biomass PL73
Brevibacterium Proposed by the Applicant to the Community Reference
Laboratory for GM Food and Feed**

Event	PLASMID PCABL			
Unique Identifier	Not allocated			
Applicant	Ajinomoto Eurolysine S.A.S.			
Procedure	Real-time TaqMan® PCR procedure for the event-specific detection of plasmid vector Pcabl in the bacterial biomass			
List of matrices relevant to the Method	Dried killed bacterial biomass used as feed material; by-product of L-lysine production by fermentation using a GM strain of <i>Brevibacterium lactofermentum</i>			
Method	Simplex format			
Optimisation of PCR assay	ABI Prism® 7700			
Amplification condition	UNG	50°C	120''	45 cycles
	Denaturation	95°C	600''	
	Amplification	95°C	15''	
		60°C	60''	
GM event amplicon	137-bp fragment of adjacent sequences (border sequences) between two genes of plasmid pCABL.			
Event-specific forward primer	5'- GCG CAA GGT GCT CAA GGA -3'			
Event-specific reverse primer	5'- GGA AGA AAA ACT TGG CGA TGA A -3'			
Event-specific Probe	6-FAM- CAA CCC TTC TCA CCT CGG CCG A -TAMRA			
Reference amplicon	Not applicable as no conventional counterpart to the dried killed bacterial biomass "PL73 <i>Brevibacterium</i> " exists on the market. The production of L-lysine using a conventional strain of <i>B. lactofermentum</i> by the applicant has been stopped nearly one decade ago.			
Reference-specific forward primer				
Reference-specific reverse primer				
Reference Probe				
LOD	At least 32 copies of plasmid pCABL			
LOQ	Not applicable			
Dynamic range	From 262144 to 32 copies of plasmid pCABL			

REACTION SETUP for the GM event specific system	REAGENTS	Final concentration	
	TaqMan Universal Master Mix (2x)	1x	
	Primers EvFw EvRev EvPr	439 nmol/L 439 nmol/L 146 nmol/L	
	water	#	
	DNA	1 ng	The use of 1 ng of template DNA per reaction extracted from the biomass is recommended
	Final Volume	25 µl	

The above method was submitted to the Community Reference Laboratory for GM Food and Feed of the Joint Research Centre in the context of application for authorisation according to Regulation (EC) No 1829/2003 on Genetically Modified Food and Feed.

The information given in this document has been extracted with no modifications from the correspondent application dossier. The Joint Research Centre cannot be held responsible for the truthfulness of the information provided in this document.

The entire risk as to the use, quality, analytical results and performance of the method proposed is with the final user of the method.

