

**Method of Detection of pMT742 Proposed by the Applicant to the  
Community Reference Laboratory for  
GM Food and Feed**

<b>Event</b>	<b>pMT742</b>			
<b>Applicant</b>	Novo Nordisk A/S			
<b>Procedure</b>	Qualitative PCR procedure for the detection of the GM and reference sequences			
<b>List of matrices relevant to the method</b>	Yeast cream			
<b>Method</b>	Simplex format, end-point qualitative PCR procedure			
<b>Optimisation of PCR assay</b>	PTC-200 thermocycler (MJ Research)			
<b>Amplification condition</b>	Initial denaturation	95°C	240''	1 cycle
	Denaturation	95°C	30''	25 cycles
	Annealing	55°C	30''	
	Extension	72°C	15''	
	Final extension	72°C	300''	1 cycle
		4°C		
<b>GM event amplicon</b>	290-bp fragment of the region encoded by <i>Schizosaccharomyces pombe</i> <i>tp1</i> +POT gene			
<b>Event-specific forward primer</b>	5'-GGC ACG TAA ATT CTT TGT CG -3'			
<b>Event-specific reverse primer</b>	5'- GCT CGG AGT GAC CAG TCA AA -3'			
<b>Reference amplicon</b>	185-bp fragment of ACT1 (actin) gene from <i>Saccharomyces cerevisiae</i>			
<b>Reference-specific forward primer</b>	5'- TGG TAA CGA AAG ATT CAG AGC C -3'			
<b>Reference-specific reverse primer</b>	5'- TTT CGG CAA TAC CTG GGA A -3'			

<b>REACTION SETUP for the GM event specific system</b>	Reagents	Final concentration	
	Water	#	
	MgCl <sub>2</sub>	3.0 mol/L	
	Taq buffer with MgCl <sub>2</sub>	1x	
	dNTPs (ATP, CTP, GTP, TTP)	0.2 mmol/L each	
	Primers EvFw EvRev	300 nmol/L 300 nmol/L	
	Taq polymerase	0.075 U	
	DNA	1 µl	
	Final Volume	50 µl	

<b>REACTION SETUP for the target taxon reference system</b>	Reagents	Final concentration	
	Water	#	
	MgCl <sub>2</sub>	3.0 mol/L	
	Taq buffer with MgCl <sub>2</sub>	1x	
	dNTPs (ATP, CTP, GTP, TTP)	0.2 mmol/L each	
	Primers RefFw RefRev	300 nmol/L 300 nmol/L	
	Taq polymerase	0.075 U	
	DNA	1 µl	
	Final Volume	50 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.