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

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

REACTION SETUP for the GM event specific system	Reagents	Final concentration	
	Water	#	
	$MgCl_2$	3.0 mol/L	
	Taq buffer with $MgCl_2$	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	

REACTION SETUP for the target taxon reference system	Reagents	Final concentration	
	Water	#	
	$MgCl_2$	3.0 mol/L	
	Taq buffer with $MgCl_2$	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	