

Original Research Paper

Molecular Characterization and Differentiation of Proteases Isolated from Different *Aspergillus* Fungal Species

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Abstract: A comparative molecular study of *Aspergillus* protease using sequence of protease gene representative 25 different *Aspergillus* species (*Aspergillus fumigatus*, *Aspergillus oryzae*, *Aspergillus flavus*, *Aspergillus novofumigatus*, *Aspergillus viridinutans*, *Aspergillus pseudotamarii*, *Aspergillus clavatus*, *Aspergillus sojae*, *Aspergillus fischeri*, *Aspergillus caelatus*, *Aspergillus novofumigatus*, *Aspergillus campestris*, *Aspergillus costaricaensis*, *Aspergillus candidus*, *Aspergillus neoniger*, *Aspergillus mulundensis*, *Aspergillus ibericus*, *Aspergillus niger*, *Aspergillus piperis*, *Aspergillus eucalypticola*, *Aspergillus vadensis*, *Aspergillus steynii*, and *Aspergillus lentulus*) have been described for several properties. To summarise the *Aspergillus* protease correlation, alignment of multiple sequence and phylogenetic tree assembly were carried out. 59 Protease gene amino acid sequence used for the constructed phylogenetic tree that showed the specific protease clusters of several species of *Aspergillus* (*Aspergillus oryzae*, *Aspergillus fumigatus*, *Aspergillus clavatus*, *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus viridinutans*, *Aspergillus flavus* and *Aspergillus fischeri*). Protease gene amino acid sequences acquired from NCBI. The homology sequence level was done using amino acid sequence. Among these proteases, the distributions of the commonly observed motifs were analysed. Protease protein sequences indicate that the structure and enzymatic function are involved.

Keywords: Protease, *Aspergillus*, Sequence, Amino Acid, Phylogenetic

Introduction

Proteases organise a wide and matrix cluster of digestive enzymes in the food, detergent and leather, medical, pharmaceutical and biotechnology industries with essential applications (Ramakrishna *et al.*, 2010). Protease enzymes are multipurpose and represent an essential variety of enzymes for the variability of their biotechnological and physiological functions (Silva *et al.*, 2011). Animals, plants and microorganisms, which constitute about 60% of the global market for enzymes, can produce those (Nirmal *et al.*, 2011). Filamentous fungi were being used for the development of different structural proteins. In particular, a few species of *Aspergillus* have been misused as essential sources of extracellular protease counting chemicals (De Castro and Sato, 2014a; Li *et al.*, 2014). *Aspergillus* produces aimed at classes for instance *Aspergillus Sojae*, *Aspergillus niger* and *Aspergillus oryzae* have approved their usage inside the food manufacturing assimilated a mostly documented as nontoxic grade as of the US medicine

management and food (Heerd *et al.*, 2012). It is possible to classify proteases as either acidic protease, impartial protease or soluble protease, corresponding to their ideal pH reactions. Proteases, especially impartial proteases, make up the world's largest amount of mechanical chemicals (Kasana *et al.*, 2011). Since their specific benefits, neutral proteases are broadly linked within the nourishment (Tavano, 2013; Yuzuki *et al.*, 2015), bolster (Zhang *et al.*, 2014), pharmaceutical (Umeadi *et al.*, 2008) and calfskin (Asker *et al.*, 2013), counting a gentle catalysis preparation and low level of contamination and high abdication. Impartial protease is often used in the food industry besides debiting soy sauce (Machida *et al.*, 2005) and brewing (Wang *et al.*, 2013). The unbiased protease, however, is largely destitute in warm solidity and is easily deactivated, limiting its application in a few companies requiring high temperatures (Xiao-Lin *et al.*, 2018). Much consideration has been given to the application of proteases to the hydrolysis of creature and plant proteins to extend their natural and useful properties. In a number of studies, antioxidant exercises

of protein hydrolysates are broadly detailed (Zhou *et al.*, 2012). Proteases hypothesized that the antioxidant characteristics of peptides come from their capacities to inactivate Responsive Oxygen Species (ROS), rummage free radicals, chelate prooxidative metals and diminish hydroperoxides (De Castro and Sato, 2014b). Alkaline soluble proteases are particularly intrigued by their potential applications as a cleaning substance added within the cleanser industry (Anwar and Saleemuddin, 1998; Gupta *et al.*, 2002). Proteases are clustered into four robotic classes: The cysteine, serine proteases, metallo and amino corrosive proteases. Possibly the best individuals were Chymotrypsin, Trypsin, Elastase and Subtilisin. Alkaline proteases were being clustered into 20 families, categorized into 6 clans with direct ancestors, depending on their own strong interaction (Barrett, 1994). The six particular catalytic proteases sorts that can be situated documented are the threonine, serine, aspartic, cysteine, metallo-proteases and glutamic. Similarities of amino acid sequences, three-dimensional constructions and proteolytic enzymes modes of action helped decipher their evolutionary course (Nishihira and Tachikawa, 1999; Morya *et al.*, 2012). Protein sequences of developed enzymes have been described to be characterised *in silico* (Yadav *et al.*, 2009; Dubey *et al.*, 2010; Yadav *et al.*, 2010). The characterization of *Silico* proteases produced by the *Aspergillus* species is reported in this study. Using bioinformatics tools, multiple sequence alignment, homology search, phylogenetic tree construction and superfamily distribution were analysed. Studies of the application of new sources of proteolysis enzymes are therefore critical for advancing knowledge about bioactive proteins.

Materials and Methods

Isolation

Sequences of protease genes were selected from NCBI as raw data to differentiate among the different species of *Aspergillus* (*Aspergillus oryzae*, *Aspergillus fumigatus*, *Aspergillus clavatus*, *Aspergillus pseudotamarii*, *Aspergillus novofumigatus*, *Aspergillus viridinutans*, *Aspergillus sojae*, *Aspergillus flavus*, *Aspergillus campestris*, *Aspergillus fischeri*, *Aspergillus novofumigatus*, *Aspergillus candidus*, *Aspergillus costaricaensis*, *Aspergillus mulundensis*, *Aspergillus neoniger*, *Aspergillus niger*, *Aspergillus ibericus*, *Aspergillus eucalypticola*, *Aspergillus piperis*, *Aspergillus vadensis*, *Aspergillus steynii*, *Aspergillus lentulus*, *Aspergillus fischeri* and *Aspergillus lentulus*). On the Proteomic database financial links {Clustal W, ProtParam, Protein calculator}, list of FASTA format was used for subsequent research. To determine the molecular weights of the different proteases diverse programs were used for calculation.

Detection of Restriction Site

Different program were used to detect the site of restriction enzymes (clone manger, restriction mapper)

Differentiation between Protease Genes

Statistical analyses were used to determine the differentiation between the different sp. of fungal isolates.

Results

Molecular Characterization of *Aspergillus* Protease using Nucleotide Sequences

25 different species of *Aspergillus* {*Aspergillus oryzae* (D00350.1, S79617.1, S75278.1, D10062.1, X54726.1 A. and X17561.1); *Aspergillus fumigatus* (AB807664.1, X66935.1, Z11580.1 and XM_746558.1); *Aspergillus novofumigatus* (XM_024823318.1); *Aspergillus flavus* (XM_002374250.1 and AF324246.1); *Aspergillus clavatus* (GQ925367.1 and XM_001272037.1); *Aspergillus pseudotamarii* (XM_032052352.1 and XM_032052352.1); *Aspergillus viridinutans* (AY590135.1 and AY590136.1); *Aspergillus sojae* (MG867728.1); *Aspergillus fischeri* (XM_001266851.1); *Aspergillus caelatus* (XM_032073130.1); *Aspergillus novofumigatus* (XM_024825725.1); *Aspergillus candidus* (XM_024813742.1); *Aspergillus campestris* (XM_024833434.1); *Aspergillus piperis* (XM_025662057.1); *Aspergillus mulundensis* (XM_026751684.1); *Aspergillus costaricaensis* (XM_025678012.1); *Aspergillus niger* (L19059.1); *Aspergillus ibericus* (XM_025719167.1); *Aspergillus neoniger* (XM_025625888.1); *Aspergillus vadensis* (XM_025711220.1); *Aspergillus eucalypticola* (XM_025536473.1); *Aspergillus steynii* (XM_024843524.1); *Aspergillus fischeri* (XM_001258144.1); *Aspergillus lentulus* (XM_033554602.1); *Aspergillus lentulus* (XM_033555156.1)} were used. Sequences of proteases acquired from GenBank symbolise diverse species of *Aspergillus* were designated as multiple sequence alignment, homology quest, phylogenetic tree creation and superfamily quest with different bioinformatics tools. Data on gene bank reflect that *Aspergillus oryzae* was the most organism used for protease production.

PCR-Restriction Fragment Length Polymorphism (PCR-RFLP)

Protease gene analysis with the use of various restriction enzymes (StuI, AflIII, Bsp1407I, EcoP15I, PstI, SpeI, Tsp45I, BfiI, KpnI, MmeI, HindII, AccI 33, ApsI, Hpy99I, TauI, BclI, CfrI, SapI, BsaBI, BtrI, MslI, PsiI, PvuII, StuI, ApoI, BclI, BglII, Bsp1407I, BspHI, EcoRI, Esp3I, HindIII, VspI, XbaI, BtsI, Eco57I,

PfIMI, SphI, AatII, AhoI, ArsI, BsgI, HaeIV, PstI and SacII) were done. The digested products were fractionated in the different *Aspergillus* species (presented in Table 1). Analysis result of these genes from NCBI showed variation in restriction sites between different *Aspergillus* species. To preserve the functional activity of the protein that reflects the variation between the digested protease genes related to genes listed as presented in the supplementary file (Tables 1-36 supplementary) protease genes, maintaining the core structural component of the active site is crucial.

Molecular Characterization of *Aspergillus* Protease using Amino Acid Sequences

For comparative motif analysis, 59 of amino acid sequences of different types of *Aspergillus* species were used to detect the variation between the different sources and by it can detect the structure of protease isolated from different *Aspergillus* species. The amino acid sequences

which were collected from NCBI were classified as follows: 20 Protease gene amino acid sequences isolated from *Aspergillus fumigatus* (XP_751651.1, CAA77666.1, A CAA75805.1, EDP50543.1, XP_750914.1, 1905286A1, B0Y708.1, EAL89613.1, XP_751651.1, CAA75806.1, Q4WH48, CAA75804.1, XP_749017.1, B0Y1V8.1, P28296.2, P41748.2, EAL86979.1, B0XRVO.1, O42630.1 POC959.1), 14 Protease gene amino acid sequences isolated from *Aspergillus oryzae* (Q2U319, Q2UDE1, Q8NKB2, Q2UUW3, AAC60533.1, Q8NKB2, Q8NKB6.1, AAB20819.1, Q06902.2, BAA00951.1, Q9Y8E3.2, P46076.2, Q2TWA0, Q8NKB5), 9 Protease gene amino acid sequences isolated from *Aspergillus niger* (A2R3L3.1, XP_001391470.1, P55325.1, P24665.1, A2Q7V4.1, CAK44878.1, Q00070, P55325.1, A2R2G1.1), 7 Protease gene amino acid sequences isolated from *Aspergillus clavatus* (A1CIA7.1, A1CBR4.1, ACX47962.1, A1CBR4.1, EAW10612.1, EAW10612).

Table 1: Characterization of protease genes extracted from Different *Aspergillus* spp. based on (no. of nucleotides, no. of amino acids obtained after translation

Organisms	Accession no	No. of nucleotide	No of amino acid	Expected molecular weight of protein (kDa)
<i>Aspergillus lentulus</i>	XM_033555156.1	1182	394	41.72
<i>Aspergillus fischeri</i>	XM_001258144.1	1935	645	68.29
<i>Aspergillus lentulus</i>	XM_033554602.1	1212	404	42.78
<i>Aspergillus steynii</i>	XM_024843524.1	1212	404	42.78
<i>Aspergillus vadensis</i>	XM_025711220.1	1254	418	44.26
<i>Aspergillus piperis</i>	XM_025662057.1	1682	561	59.37
<i>Aspergillus eucalypticola</i>	XM_025536473.1	1252	417	44.19
<i>Aspergillus ibericus</i>	XM_025719167.1	1447	482	51.07
<i>Aspergillus niger</i>	L19059.1	2993	998	105.64
<i>Aspergillus neoniger</i>	XM_025625888.1	1263	421	44.58
<i>Aspergillus mulundensis</i>	XM_026751684.1	1209	403	42.67
<i>Aspergillus costaricaensis</i>	XM_025678012.1	1251	417	44.15
<i>Aspergillus candidus</i>	XM_024813742.1	1560	520	55.06
<i>Aspergillus novofumigatus</i>	XM_024825725.1	1212	404	42.78
<i>Aspergillus campestris</i>	XM_024833434.1	1212	404	42.78
<i>Aspergillus fischeri</i>	XM_001266851.1	1212	404	42.78
<i>Aspergillus caelatus</i>	XM_032073130.1	1212	404	42.78
<i>Aspergillus sojae</i>	MG867728.1	1212	404	42.78
<i>Aspergillus viridinutans</i>	AY590136.1	1192	397	42.07
<i>Aspergillus viridinutans</i>	AY590135.1	1192	397	42.07
<i>Aspergillus pseudotamarii</i>	XM_032052352.1	2375	792	83.82
<i>Aspergillus clavatus</i>	GQ925367.1	2375	792	83.82
<i>Aspergillus clavatus</i>	XM_001272037.1	1212	404	42.78
<i>Aspergillus flavus</i>	XM_002374250.1	1212	404	42.78
<i>Aspergillus flavus</i>	AF324246.1	1886	629	66.57
<i>Aspergillus novofumigatus</i>	XM_024823318.1	2315	772	81.71
<i>Aspergillus fumigatus</i>	XM_746558.1	1212	404	42.78
<i>Aspergillus.fumigatus</i>	Z11580.1	2163	721	76.34
<i>Aspergillus.fumigatus</i>	X66935.1	758	253	26.75
<i>Aspergillus fumigatus</i>	AB807664.1	714	238	25.20
<i>Aspergillus oryzae</i>	X17561.1	1468	489	51.81
<i>Aspergillus oryzae</i>	X54726.1	1820	607	64.24
<i>Aspergillus oryzae</i>	D10062.1	2601	867	91.80
<i>Aspergillus oryzae</i>	S75278.1	1820	607	64.24
<i>Aspergillus oryzae</i>	S79617.1	2600	867	91.77
<i>Aspergillus oryzae</i>	D00350.1	1182	394	41.72

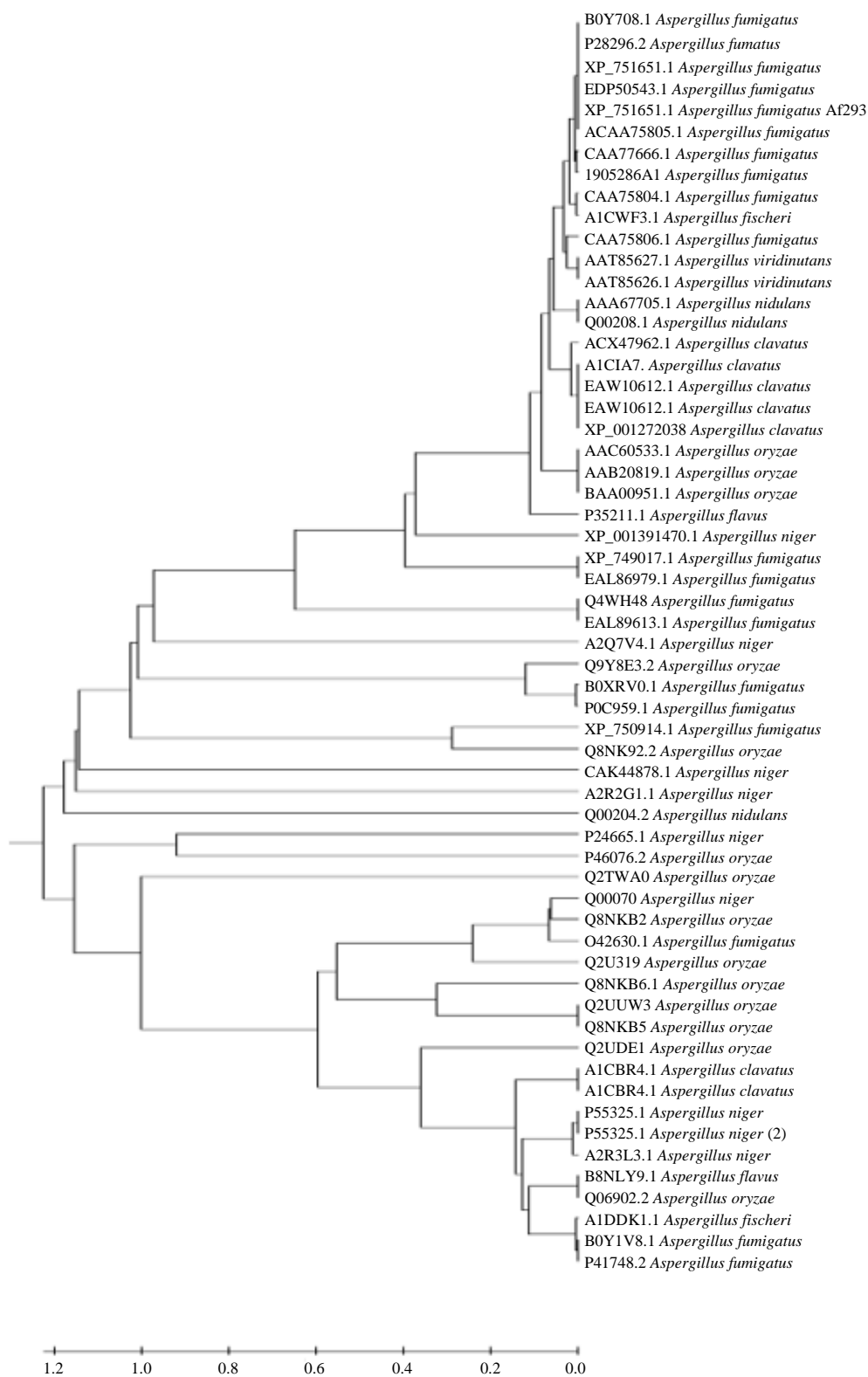


Fig. 1: Phylogenetic tree of amino acid sequence of protease gene isolated from *Aspergillus* spp. display the location amid the selected gene from NCBI based on amino acid sequence assessments

1, XP_001272038), 3 Protease gene amino acid sequences isolated from *Aspergillus nidulans* (Q00208.1, AAA67705.1, Q00204.2), 2 Protease gene amino acid sequences isolated from *Aspergillus viridinutans* (AAT85626.1, AAT85627.1), 2 Protease gene amino acid sequences isolated from *Aspergillus flavus* (B8NLY9.1, P35211.1) and 2 Protease gene amino acid sequences isolated from *Aspergillus fischeri* (A1DDK1.1, A1CWF3.1). Numbers of amino acid bases were varied (400-1030 amino acid bases) from one *Aspergillus* species to the other.

For molecular phylogenetic construction employing ClustalW, version 1.83 with ncbi database, sequence alignment orientations were performed. A phylogenetic tree was calculated for each protein based on the result of multiple sequence alignments by applying the maximum likelihood method implemented in the Mega 5 software Fig. 1. Moreover, graphic view was displayed based on comparison of some selected amino acid sequences (one of each cluster) to the target sequence protease genes from different *Aspergillus* sp. The bioinformatics examination appeared that each species of *Aspergillus* protein normal peptidase superfamily and contains particular space. It has been detailed that cuticle-degrading proteases delivered by a few nematocidal organisms have a place to the peptidase S8 superfamily26–28. To way better get it the work and advancement of this protein, we compared the all grouping with others. Proteases from *Aspergillus* sp. are isolated into distinctive branches exceptionally clearly, which reflects their diverse sources. The protein's aliphatic index could be a calculation of the relative volume of the subsequent amino acids working by the aliphatic side sequence: Isoleucine, valine, alanine and leucine.

Discussion

The sequence and structural homology methods primarily evaluate the global similarities between proteins compared to each other (Whisstock and Lesk, 2003). However, the molecular role of a protease in general is limited to its known active site, which may involve an interaction with the binding of protein peptides. Propagations exclusive the thermal permanence of globular proteins are recognized to cover the aliphatic index (Ikai, 1980; Rawlings *et al.*, 2006). The alkaline proteases of the examined *Aspergillus* species have maintained high aliphatic guide values to be thermostable. The variability guide deals with clarity when planning a protein's in vivo half-life (Guruprasad *et al.*, 1990). Proteins with an in vivo half-life of fewer than 5 h have been described to demonstration an guide of unpredictability of more

than 40, while those with an in vivo half-life of additional than 16 h (Rogers *et al.*, 1986) have an guide of unpredictability of less than 40. As a consequence, protein comparisons based on global sequence and structural similarity may miss proteins with active sites retained, but divergent sequences and structures may be missing (Powers *et al.*, 2006).

Conclusion

Characterization of *Aspergillus* protease protein sequences using bioinformatics programs (in silico) demonstrating the degree of similarity was remarkably found in *Aspergillus* species for sequence which could be applied for promising cloning of genes putative by designed primers from the conserved regions. Conserved motif amino acid sequence detect the secondary structures.

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Author Contributions

All authors equally contributed in this study.

Ethics

This article is unpublished and includes materials that is unpublished. The corresponding author states that the manuscript has been read and accepted by all the other writers and there are no ethical problems involved.

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Table 1: Restriction digestion of protease gene from D00350.1 *Aspergillus oryzae*

Name	Sequence	Site Length	Overhang	Frequency	Cut positions
BsaAI	YACGTR	6	blunt	1	1249
BsaBI	GATNNNNATC	6	blunt	1	565
BsrBI	CCGCTC	6	blunt	1	1061
BtrI	CACGTC	6	blunt	1	39
Eco47III	AGCGCT	6	blunt	1	1581
MslI	CAYNNNNRGTG	6	blunt	1	2463
PsiI	TTATAA	6	blunt	1	617
PvuII	CAGCTG	6	blunt	1	2594
StuI	AGGCCT	6	blunt	1	1307
XmnI	GAANNNTTC	6	blunt	1	1642
AflIII	CTTAAG	6	five_prime	1	2095
ApoI	RAATTY	6	five_prime	1	1718
AvaI	CYCGRG	6	five_prime	1	582
BclI	TGATCA	6	five_prime	1	2365
BglIII	AGATCT	6	five_prime	1	1119
Bsp1407I	TGTACA	6	five_prime	1	2257
BspHI	TCATGA	6	five_prime	1	2422
BspMI	ACCTGC	6	five_prime	1	2080
DraII	RGGNCCY	6	five_prime	1	153
EcoP15I	CAGCAG	6	five_prime	1	1223
EcoRI	GAATTC	6	five_prime	1	1718
Esp3I	CGTCTC	6	five_prime	1	1699
HindIII	AAGCTT	6	five_prime	1	816
NarI	GGCGCC	6	five_prime	1	928
PasI	CCCWGGG	7	five_prime	1	1161
PfoI	TCCNGGA	6	five_prime	1	821
SpeI	ACTAGT	6	five_prime	1	1220
VspI	ATTAAT	6	five_prime	1	211
XbaI	TCTAGA	6	five_prime	1	2438
XhoI	CTCGAG	6	five_prime	1	582
XhoII	RGATCY	6	five_prime	1	1119
BfiI	ACTGGG	6	three_prime	1	1486
BsmI	GAATGC	6	three_prime	1	1642
BtsI	GCACTG	6	three_prime	1	316
Eco57I	CTGAAG	6	three_prime	1	629
GsuI	CTGGAG	6	three_prime	1	936
PfiMI	CCANNNNNTGG	6	three_prime	1	2536
SphI	GCATGC	6	three_prime	1	550
Ball	TGGCCA	6	blunt	2	1347, 2031
NaeI	GCCGGC	6	blunt	2	1228, 1754
AvaII	GGWCC	5	five_prime	2	773, 1114
BsmAI	GTCTC	5	five_prime	2	310, 1699
BtgZI	GCGATG	6	five_prime	2	121, 1689
NeoI	CCATGG	6	five_prime	2	1348, 1933
AatII	GACGTC	6	three_prime	2	1858, 2044
AloI	GAACNNNNNTCC	7	three_prime	2	359, 391
ArsI	GACNNNNNTTYG	7	three_prime	2	2393, 2425
BcgI	CGANNNNNTGC	6	three_prime	2	1205, 1239
BglI	GCCNNNNNGGC	6	three_prime	2	1304, 1403
BsgI	GTGCAG	6	three_prime	2	810, 2373
Eco57MI	CTGRAG	6	three_prime	2	629, 936
HaeII	RGCGCY	6	three_prime	2	931, 1583
HaeIV	GAYNNNNNRTC	6	three_prime	2	476, 510
KpnI	GGTACC	6	three_prime	2	251, 1930
PstI	CTGCAG	6	three_prime	2	323, 2599
SacII	CCGCGG	6	three_prime	2	145, 772
TaqII	GACCGA	6	three_prime	2	1408, 1434
TsoI	TARCCA	6	three_prime	2	1595, 1926
TstI	CACNNNNNTCC	6	three_prime	2	44, 76
EcoRV	GATATC	6	blunt	3	55, 171, 1877
AcyI	GRCGYC	6	five_prime	3	928, 1855, 2041
CfrI	YGGCCR	6	five_prime	3	1345, 1754, 2029
SalI	GTCGAC	6	five_prime	3	326, 1271, 2399
SapI	GCTCTTC	7	five_prime	3	1803, 1904, 2157
StyI	CCWGGG	6	five_prime	3	1348, 1933, 2035

Table 1: Countinue

Tth111I	GACNNNGTC	6	five_prime	3	2043, 2452, 2465
BseMII	CTCAG	5	three_prime	3	210, 473, 802
BseSI	GKGCMC	6	three_prime	3	785, 1159, 1238
BsrDI	GCAATG	6	three_prime	3	496, 541, 1371
MmeI	TCCRAC	6	three_prime	3	705, 1858, 2554
NspI	RCATGY	6	three_prime	3	550, 630, 1334
TspDTI	ATGAA	5	three_prime	3	417, 2526, 2580
TspRI	CASTG	5	three_prime	3	323, 938, 1796
AccI	GTMKAC	6	five_prime	4	327, 1272, 2266, 2400
Cfr10I	RCCGGY	6	five_prime	4	1226, 1319, 1675, 1752
HgaI	GACGC	5	five_prime	4	313, 385, 909, 2423
TatI	WGTACW	6	five_prime	4	524, 829, 2257, 2557
Tsp45I	GTSAC	5	five_prime	4	86, 493, 836, 2006
BdaI	TGANNNNNTCA	6	three_prime	4	373, 407, 2402, 2436
BsrI	ACTGG	5	three_prime	4	938, 1481, 1825, 2140
FalI	AAGNNNNCTT	6	three_prime	4	726, 758, 1864, 1896
Hin4I	GAYNNNNVTC	6	three_prime	4	109, 141, 477, 509
TspGWI	ACGGA	5	three_prime	4	389, 559, 1444, 2196
HindII	GTyrAC	6	blunt	5	328, 410, 1273, 1853, 2401
FauI	CCCGC	5	five_prime	5	149, 444, 776, 807, 2006
SduI	GDGCHC	6	three_prime	5	469, 785, 1159, 1202, 1238
TauI	GCSGC	5	three_prime	5	147, 810, 990, 1296, 1800
SmlI	CTYRAG	6	five_prime	6	263, 582, 685, 1980, 2095, 2228
BseRI	GAGGAG	6	three_prime	6	171, 738, 741, 1038, 1124, 1295
HphI	GGTGA	5	three_prime	6	487, 894, 951, 1448, 1553, 2360
AgsI	TTSAA	5	three_prime	7	244, 510, 845, 1073, 1474, 1590, 1623
Hpy99I	CGWCG	5	three_prime	7	329, 925, 1011, 1109, 2046, 2116, 2332
BecI	CCATC	5	five_prime	8	649, 661, 738, 1127, 1711, 1794, 1926, 1986
EcoRII	CCWGG	5	five_prime	8	821, 885, 1092, 1160, 1431, 1887, 2102, 2535
FokI	GGATG	5	five_prime	9	78, 127, 279, 519, 617, 628, 1042, 1397, 2064
TfiI	GAWTC	5	five_prime	9	23, 869, 1028, 1439, 2120, 2174, 2250, 2419, 2500
BbvI	GCAGC	5	five_prime	11	333, 1185, 1303, 1458, 1468, 1507, 1659, 1961, 1988, 2074, 2581
TseI	GCWGC	5	five_prime	11	321, 1173, 1316, 1446, 1481, 1520, 1672, 1974, 2001, 2062, 2594
MboII	GAAGA	5	three_prime	11	112, 344, 705, 936, 1053, 1162, 1549, 1820, 1850, 1891, 2174
SfaNI	GCATC	5	five_prime	13	56, 144, 301, 476, 541, 639, 710, 1001, 1419, 1617, 1739, 1772, 2026

Table 2: Restriction digestion of protease gene from S75278.1 *Aspergillus oryzae*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsaAI	YACGTR	6	blunt	1	665
EcoRV	GATATC	6	blunt	1	1293
PsiI	TTATAA	6	blunt	1	36
StuI	AGGCCT	6	blunt	1	723
XmnI	GAANNNTTC	6	blunt	1	1058
AflIII	CTTAAG	6	five_prime	1	1511
ApoI	RAATTY	6	five_prime	1	1134
AvaI	CYCGRG	6	five_prime	1	1
BclI	TGATCA	6	five_prime	1	1781
BglII	AGATCT	6	five_prime	1	535
BsmAI	GTCTC	5	five_prime	1	1115
Bsp1407I	TGTACA	6	five_prime	1	1673
BspMI	ACCTGC	6	five_prime	1	1496
EcoP15I	CAGCAG	6	five_prime	1	639
EcoRI	GAATTC	6	five_prime	1	1134
Esp3I	CGTCTC	6	five_prime	1	1115
HgaI	GACGC	5	five_prime	1	325
HindIII	AAGCTT	6	five_prime	1	232
NarI	GGCGCC	6	five_prime	1	344
PasI	CCCWGGG	7	five_prime	1	577
PfoI	TCCNGGA	6	five_prime	1	237
PleI	GAGTC	5	five_prime	1	1001
SpeI	ACTAGT	6	five_prime	1	636
Tth111I	GACNNNGTC	6	five_prime	1	1459
XhoI	CTCGAG	6	five_prime	1	1
XhoII	RGATCY	6	five_prime	1	535
BfiI	ACTGGG	6	three_prime	1	902
BseMII	CTCAG	5	three_prime	1	218
BsmI	GAATGC	6	three_prime	1	1058
BsrDI	GCAATG	6	three_prime	1	787
Eco57I	CTGAAG	6	three_prime	1	48
GsuI	CTGGAG	6	three_prime	1	352
HaeII	RGCRCY	6	three_prime	1	347
KpnI	GGTACC	6	three_prime	1	1346

Table 2: Countiue

SacII	CCGCGG	6	three_prime	1	188
BalI	TGGCCA	6	blunt	2	763, 1447
NaeI	GCCGGC	6	blunt	2	644, 1170
AvaII	GGWCC	5	five_prime	2	189, 530
BtgZI	GCGATG	6	five_prime	2	457, 1105
NcoI	CCATGG	6	five_prime	2	764, 1349
SalI	GTCGAC	6	five_prime	2	687, 1815
TatI	WGTACW	6	five_prime	2	245, 1673
Tsp45I	GTSAC	5	five_prime	2	252, 1422
AatII	GACGTC	6	three_prime	2	1274, 1460
BcgI	CGANNNNNTGC	6	three_prime	2	621, 655
BglI	GCCNNNNNGGC	6	three_prime	2	720, 819
BsgI	GTGCAG	6	three_prime	2	226, 1789
Eco57MI	CTGRAG	6	three_prime	2	48, 352
MmeI	TCCRAC	6	three_prime	2	121, 1274
NspI	RCATGY	6	three_prime	2	49, 750
TaqII	GACCGA	6	three_prime	2	824, 850
TsoI	TARCCA	6	three_prime	2	1011, 1342
TspGWI	ACGGA	5	three_prime	2	860, 1612
TspRI	CASTG	5	three_prime	2	354, 1212
HindIII	GTYRAC	6	blunt	3	689, 1269, 1817
AccI	GTMKAC	6	five_prime	3	688, 1682, 1816
Acyl	GRCGYC	6	five_prime	3	344, 1271, 1457
CfrI	YGGCCR	6	five_prime	3	761, 1170, 1445
FauI	CCCCG	5	five_prime	3	192, 223, 1422
SapI	GCTCTTC	7	five_prime	3	1219, 1320, 1573
StyI	CCWWGG	6	five_prime	3	764, 1349, 1451
BseSI	GKGCMC	6	three_prime	3	201, 575, 654
Cfr10I	RCCGGY	6	five_prime	4	642, 735, 1091, 1168
FokI	GGATG	5	five_prime	4	36, 47, 813, 1480
BsrI	ACTGG	5	three_prime	4	354, 897, 1241, 1556
FalI	AAGNNNNCTT	6	three_prime	4	142, 174, 1280, 1312
SduI	GDGCHC	6	three_prime	4	201, 575, 618, 654
TauI	GCSGC	5	three_prime	4	226, 406, 712, 1216
SmlI	CTYRAG	6	five_prime	5	1, 101, 1396, 1511, 1644
AgsI	TTSAA	5	three_prime	5	261, 489, 890, 1006, 1039
BseRI	GAGGAG	6	three_prime	5	154, 157, 454, 540, 711
HphI	GGTGA	5	three_prime	5	310, 367, 864, 969, 1776
Hpy99I	CGWCG	5	three_prime	6	341, 427, 525, 1462, 1532, 1748
EcoRII	CCWGG	5	five_prime	7	237, 301, 508, 576, 847, 1303, 1518
TfiI	GAWTC	5	five_prime	7	285, 444, 855, 986, 1536, 1590, 1666
BccI	CCATC	5	five_prime	8	68, 80, 154, 543, 1127, 1210, 1342, 1402
SfaNI	GCATC	5	five_prime	8	58, 126, 417, 835, 1033, 1155, 1188, 1442
BbvI	GCAGC	5	five_prime	9	601, 719, 874, 884, 923, 1075, 1377, 1404, 1490
TseI	GCWGC	5	five_prime	9	589, 732, 862, 897, 936, 1088, 1390, 1417, 1478
MboII	GAAGA	5	three_prime	9	121, 352, 469, 578, 965, 1236, 1266, 1307, 1590

Table 3: Restriction digestion of protease gene from D10062.1 *Aspergillus oryzae*

Name	Sequence	Site Length	Overhang	Frequency	Cut Positions
BsaAI	YACGTR	6	blunt	1	1250
BsaBI	GATNNNNATC	6	blunt	1	566
BsrBI	CCGCTC	6	blunt	1	1062
BtrI	CACGTC	6	blunt	1	39
Eco47III	AGCGCT	6	blunt	1	1582
MslI	CAYNNNNRTG	6	blunt	1	2464
PsiI	TTATAA	6	blunt	1	618
PvuII	CAGCTG	6	blunt	1	2595
StuI	AGGCCT	6	blunt	1	1308
XmnI	GAANNNTTC	6	blunt	1	1643
AflII	CTTAAG	6	five_prime	1	2096
ApoI	RAATY	6	five_prime	1	1719
AvaI	CYCGRG	6	five_prime	1	583
BclI	TGATCA	6	five_prime	1	2366
BglII	AGATCT	6	five_prime	1	1120
Bsp1407I	TGTACA	6	five_prime	1	2258
BspMI	ACCTGC	6	five_prime	1	2081
DraII	RGNCY	6	five_prime	1	153
EcoP15I	CAGCAG	6	five_prime	1	1224
EcoRI	GAATTC	6	five_prime	1	1719
Esp3I	CGTCTC	6	five_prime	1	1700
HindIII	AAGCTT	6	five_prime	1	817
NarI	GGCGCC	6	five_prime	1	929
PasI	CCCWGGG	7	five_prime	1	1162

Table 3: Countinue

PfoI	TCCNGGA	6	five_prime	1	822
SpeI	ACTAGT	6	five_prime	1	1221
VspI	ATTAAT	6	five_prime	1	211
XbaI	TCTAGA	6	five_prime	1	2439
XhoI	CTCGAG	6	five_prime	1	583
XhoII	RGATCY	6	five_prime	1	1120
BfiI	ACTGGG	6	three_prime	1	1487
BsmI	GAATGC	6	three_prime	1	1643
BtsI	GCAGTG	6	three_prime	1	316
Eco57I	CTGAAG	6	three_prime	1	630
GsuI	CTGGAG	6	three_prime	1	937
PflMI	CCANNNNNTGG	6	three_prime	1	2537
SphI	GCATGC	6	three_prime	1	551
Ball	TGGCCA	6	blunt	2	1348, 2032
NaeI	GCCGGC	6	blunt	2	1229, 1755
AvaII	GGWCC	5	five_prime	2	774, 1115
BsmAI	GTCTC	5	five_prime	2	310, 1700
BspHI	TCATGA	6	five_prime	2	378, 2423
BtgZI	GCGATG	6	five_prime	2	121, 1690
NcoI	CCATGG	6	five_prime	2	1349, 1934
AatII	GACGTC	6	three_prime	2	1859, 2045
AloI	GAACNNNNNTCC	7	three_prime	2	359, 391
ArsI	GACNNNNNTTYG	7	three_prime	2	2394, 2426
BcgI	CGANNNNNTGC	6	three_prime	2	1206, 1240
BglI	GCCNNNNNGGC	6	three_prime	2	1305, 1404
BsgI	GTGCAG	6	three_prime	2	811, 2374
Eco57MI	CTGRAG	6	three_prime	2	630, 937
HaeII	RGCGCY	6	three_prime	2	932, 1584
HaeIV	GAYNNNNNRTC	6	three_prime	2	477, 511
KpnI	GGTACC	6	three_prime	2	251, 1931
PstI	CTGCAG	6	three_prime	2	323, 2600
SacII	CCGCGG	6	three_prime	2	145, 773
TaqII	GACCGA	6	three_prime	2	1409, 1435
TsoI	TARCCA	6	three_prime	2	1596, 1927
TstI	CACNNNNNTCC	6	three_prime	2	44, 76
EcoRV	GATATC	6	blunt	3	55, 171, 1878
AcyI	GRCGYC	6	five_prime	3	929, 1856, 2042
CfrI	YGGCCR	6	five_prime	3	1346, 1755, 2030
SalI	GTCGAC	6	five_prime	3	326, 1272, 2400
SapI	GCTCTTC	7	five_prime	3	1804, 1905, 2158
StyI	CCWWGG	6	five_prime	3	1349, 1934, 2036
Thl11I	GACNNNGTC	6	five_prime	3	2044, 2453, 2466
BseMII	CTCAG	5	three_prime	3	210, 474, 803
BseSI	GKGCMC	6	three_prime	3	786, 1160, 1239
BsrDI	GCAATG	6	three_prime	3	497, 542, 1372
MmeI	TCCRAC	6	three_prime	3	706, 1859, 2555
NspI	RCATGY	6	three_prime	3	551, 631, 1335
TspRI	CASTG	5	three_prime	3	323, 939, 1797
AccI	GTMKAC	6	five_prime	4	327, 1273, 2267, 2401
Cfr10I	RCCGGY	6	five_prime	4	1227, 1320, 1676, 1753
HgaI	GACGC	5	five_prime	4	313, 386, 910, 2424
TatI	WGTACW	6	five_prime	4	525, 830, 2258, 2558
Tsp45I	GTSAC	5	five_prime	4	86, 494, 837, 2007
BdaI	TGANNNNNNTCA	6	three_prime	4	374, 408, 2403, 2437
BsrI	ACTGG	5	three_prime	4	939, 1482, 1826, 2141
FalI	AAGNNNNNCTT	6	three_prime	4	727, 759, 1865, 1897
Hin4I	GAYNNNNNVTC	6	three_prime	4	109, 141, 478, 510
TspDTI	ATGAA	5	three_prime	4	367, 418, 2527, 2581
TspGWI	ACGGA	5	three_prime	4	390, 560, 1445, 2197
HindII	GTYRAC	6	blunt	5	328, 411, 1274, 1854, 2402
FauI	CCCCG	5	five_prime	5	149, 445, 777, 808, 2007
SduI	GDGCHC	6	three_prime	5	470, 786, 1160, 1203, 1239
TauI	GCSGC	5	three_prime	5	147, 811, 991, 1297, 1801
SmlI	CTYRAG	6	five_prime	6	263, 583, 686, 1981, 2096, 2229
BseRI	GAGGAG	6	three_prime	6	171, 739, 742, 1039, 1125, 1296
HphI	GGTGA	5	three_prime	6	488, 895, 952, 1449, 1554, 2361
AgsI	TTSAA	5	three_prime	7	244, 511, 846, 1074, 1475, 1591, 1624
Hpy99I	CGWCG	5	three_prime	7	329, 926, 1012, 1110, 2047, 2117, 2333
EcoRII	CCWGG	5	five_prime	8	822, 886, 1093, 1161, 1432, 1888, 2103, 2536
BccI	CCATC	5	five_prime	9	650, 662, 739, 1128, 1712, 1795, 1927, 1987, 2322
FokI	GGATG	5	five_prime	9	78, 127, 279, 520, 618, 629, 1043, 1398, 2065
TfiI	GAWTC	5	five_prime	9	23, 870, 1029, 1440, 2121, 2175, 2251, 2420, 2501
BbvI	GCAGC	5	five_prime	11	333, 1186, 1304, 1459, 1469, 1508, 1660, 1962, 1989, 2075, 2582
TseI	GCWGC	5	five_prime	11	321, 1174, 1317, 1447, 1482, 1521, 1673, 1975, 2002, 2063, 2595
MboII	GAAGA	5	three_prime	11	112, 344, 706, 937, 1054, 1163, 1550, 1821, 1851, 1892, 2175
SfaNI	GCATC	5	five_prime	13	56, 144, 301, 477, 542, 640, 711, 1002, 1420, 1618, 1740, 1773, 2027

Table 4: Restriction digestion of protease gene from X54726.1 *A. oryzae*

Name	Sequence	Site Length	Overhang	Frequency	Cut Positions
BsaAI	YACGTR	6	blunt	1	665
BsrBI	CCGCTC	6	blunt	1	477
EcoRV	GATATC	6	blunt	1	1293
NruI	TCGCGA	6	blunt	1	991
PsiI	TTATAA	6	blunt	1	36
StuI	AGGCCT	6	blunt	1	723
XmnI	GAANNNTTC	6	blunt	1	1058
AflII	CTTAAG	6	five_prime	1	1511
ApoI	RAATTY	6	five_prime	1	1134
AvaI	CYCGRG	6	five_prime	1	1
BclI	TGATCA	6	five_prime	1	1781
BglII	AGATCT	6	five_prime	1	535
BsmAI	GTCTC	5	five_prime	1	1115
BspI407I	TGTACA	6	five_prime	1	1673
BspMI	ACCTGC	6	five_prime	1	1496
BtgZI	GCGATG	6	five_prime	1	1105
EcoP15I	CAGCAG	6	five_prime	1	639
EcoRI	GAATTC	6	five_prime	1	1134
Esp3I	CGTCTC	6	five_prime	1	1115
HgaI	GACGC	5	five_prime	1	325
HindIII	AAGCTT	6	five_prime	1	232
NarI	GGCGCC	6	five_prime	1	344
PasI	CCCWGGG	7	five_prime	1	577
PfoI	TCCNGGA	6	five_prime	1	237
PleI	GAGTC	5	five_prime	1	1001
SpeI	ACTAGT	6	five_prime	1	636
Tth111I	GACNNGTCT	6	five_prime	1	1459
XhoI	CTCGAG	6	five_prime	1	1
XhoII	RGATCY	6	five_prime	1	535
BfiI	ACTGGG	6	three_prime	1	902
BseMIII	CTCAG	5	three_prime	1	218
BsmI	GAATGC	6	three_prime	1	1058
BsrDI	GCAATG	6	three_prime	1	787
Eco57I	CTGAAG	6	three_prime	1	48
GsuI	CTGGAG	6	three_prime	1	352
HaeII	RGCGCY	6	three_prime	1	347
KpnI	GGTACC	6	three_prime	1	1346
SacI	CCGCGG	6	three_prime	1	188
TspGWI	ACGGA	5	three_prime	1	1612
BalI	TGGCCA	6	blunt	2	763, 1447
NaeI	GCCGCG	6	blunt	2	644, 1170
AvaII	GGWCC	5	five_prime	2	189, 530
NcoI	CCATGG	6	five_prime	2	764, 1349
SalI	GTCGAC	6	five_prime	2	687, 1815
TatI	WGTACW	6	five_prime	2	245, 1673
Tsp45I	GTSAC	5	five_prime	2	252, 1422
AatII	GACGTC	6	three_prime	2	1274, 1460
BcgI	CGANNNNNTGC	6	three_prime	2	621, 655
BglI	GCCNNNNNGGC	6	three_prime	2	720, 819
BsgI	GTGCAG	6	three_prime	2	226, 1789
Eco57MI	CTGRAG	6	three_prime	2	48, 352
MmeI	TCCRAC	6	three_prime	2	121, 1274
NspI	RCATGY	6	three_prime	2	49, 750
TaqII	GACCGA	6	three_prime	2	824, 850
TsoI	TARCCA	6	three_prime	2	1011, 1342
TspRI	CASTG	5	three_prime	2	354, 1212
HindII	GTYRAC	6	blunt	3	689, 1269, 1817
AccI	GTMKAC	6	five_prime	3	688, 1682, 1816
AcyI	GRCGYC	6	five_prime	3	344, 1271, 1457
CfrI	YGGCCR	6	five_prime	3	761, 1170, 1445
FauI	CCCGC	5	five_prime	3	192, 223, 1422
SapI	GCTCTTC	7	five_prime	3	1219, 1320, 1573
StyI	CCWGGG	6	five_prime	3	764, 1349, 1451
BseSI	GKGCMC	6	three_prime	3	201, 575, 654
Cfr10I	RCCGGY	6	five_prime	4	642, 735, 1091, 1168
BsrI	ACTGG	5	three_prime	4	354, 897, 1241, 1556
FalI	AAGNNNNCTT	6	three_prime	4	142, 174, 1280, 1312
SduI	GDGCHC	6	three_prime	4	201, 575, 618, 654
TauI	GCSGC	5	three_prime	4	226, 406, 712, 1216
FokI	GGATG	5	five_prime	5	36, 47, 458, 813, 1480
SmlI	CTYRAG	6	five_prime	5	1, 101, 1396, 1511, 1644
AgsI	TTSAA	5	three_prime	5	261, 489, 890, 1006, 1039
BseRI	GAGGAG	6	three_prime	5	154, 157, 454, 540, 711
HphI	GGTGA	5	three_prime	5	310, 367, 864, 969, 1776
Hpy99I	CGWCG	5	three_prime	6	341, 427, 525, 1462, 1532, 1748
EcoRII	CCWGG	5	five_prime	7	237, 301, 508, 576, 847, 1303, 1518
TfiI	GAWTC	5	five_prime	7	285, 444, 855, 986, 1536, 1590, 1666
BccI	CCATC	5	five_prime	8	68, 80, 154, 543, 1127, 1210, 1342, 1402
SfaNI	GCATC	5	five_prime	8	58, 126, 417, 835, 1033, 1155, 1188, 1442
BbvI	GCAGC	5	five_prime	9	601, 719, 874, 884, 923, 1075, 1377, 1404, 1490
TseI	GCWGC	5	five_prime	9	589, 732, 862, 897, 936, 1088, 1390, 1417, 1478
MboII	GAAGA	5	three_prime	9	121, 352, 469, 578, 965, 1236, 1266, 1307, 1590

Table 5: Restriction digestion of protease gene from X17561.1 *A. oryzae*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
	YACGTR	6	blunt	1	529
EcoRV	GATATC	6	blunt	1	1042
StuI	AGGCCT	6	blunt	1	587
XmnI	GAANNNNTTC	6	blunt	1	863
AflII	CTTAAG	6	five_prime	1	1260
BglII	AGATCT	6	five_prime	1	399
Bsp1407I	TGTACA	6	five_prime	1	1422
BspMI	ACCTGC	6	five_prime	1	1245
EcoP15I	CAGCAG	6	five_prime	1	503
HgaI	GACGC	5	five_prime	1	239
HindIII	AAGCTT	6	five_prime	1	146
NarI	GGCGCC	6	five_prime	1	258
PasI	CCCWGGG	7	five_prime	1	441
PfoI	TCCNGGA	6	five_prime	1	151
SalI	GTCGAC	6	five_prime	1	551
SpeI	ACTAGT	6	five_prime	1	500
Thl111I	GACNNNGTC	6	five_prime	1	1208
XhoII	RGATCY	6	five_prime	1	399
BfiI	ACTGGG	6	three_prime	1	766
BseMII	CTCAG	5	three_prime	1	132
BsgI	GTGCAG	6	three_prime	1	140
BsmI	GAATGC	6	three_prime	1	863
BsrDI	GCAATG	6	three_prime	1	651
Eco57MI	CTGRAG	6	three_prime	1	266
GsuI	CTGGAG	6	three_prime	1	266
HaeII	RGCGCY	6	three_prime	1	261
KpnI	GGTACC	6	three_prime	1	1095
NspI	RCATGY	6	three_prime	1	614
SacII	CCGCGG	6	three_prime	1	102
Ball	TGGCCA	6	blunt	2	627, 1196
HindII	GTYRAC	6	blunt	2	553, 1018
NaeI	GCCGGC	6	blunt	2	508, 919
AccI	GTMKAC	6	five_prime	2	552, 1431
AvaII	GGWCC	5	five_prime	2	103, 394
FokI	GGATG	5	five_prime	2	677, 1229
NcoI	CCATGG	6	five_prime	2	628, 1098
TatI	WGTACW	6	five_prime	2	159, 1422
Tsp45I	GTSAC	5	five_prime	2	166, 1171
AatII	GACGTC	6	three_prime	2	1023, 1209
BglI	GCCNNNNNGGC	6	three_prime	2	584, 683
MmeI	TCCRAC	6	three_prime	2	35, 1023
TaqII	GACCGA	6	three_prime	2	688, 714
TsoI	TARCCA	6	three_prime	2	816, 1091
TspGWI	ACGGA	5	three_prime	2	724, 1361
TspRI	CASTG	5	three_prime	2	268, 961
AcyI	GRCGYC	6	five_prime	3	258, 1020, 1206
CfrI	YGGCCR	6	five_prime	3	625, 919, 1194
FauI	CCCGC	5	five_prime	3	106, 137, 1171
SapI	GCTCTTC	7	five_prime	3	968, 1069, 1322
StyI	CCWWGG	6	five_prime	3	628, 1098, 1200
AgsI	TTSAA	5	three_prime	3	175, 754, 844
BseSI	GKGCMC	6	three_prime	3	115, 439, 518
HphI	GGTGA	5	three_prime	3	224, 281, 728
Cfr10I	RCCGGY	6	five_prime	4	506, 599, 896, 917
SmlI	CTYRAG	6	five_prime	4	15, 1145, 1260, 1393
BcgI	CGANNNNNNTGC	6	three_prime	4	359, 393, 485, 519
BsrI	ACTGG	5	three_prime	4	268, 761, 990, 1305
FalI	AAGNNNNCTT	6	three_prime	4	56, 88, 1029, 1061
SduI	GDGCHC	6	three_prime	4	115, 439, 482, 518
TauI	GCSGC	5	three_prime	4	140, 320, 576, 965
BccI	CCATC	5	five_prime	5	68, 407, 959, 1091, 1151
BseRI	GAGGAG	6	three_prime	5	68, 71, 368, 404, 575
Hpy99I	CGWCG	5	three_prime	5	255, 341, 389, 1211, 1281
EcoRII	CCWGG	5	five_prime	6	151, 215, 440, 711, 1052, 1267
TfiI	GAWTC	5	five_prime	6	199, 358, 719, 1285, 1339, 1415
SfaNI	GCATC	5	five_prime	7	40, 331, 699, 838, 904, 937, 1191
MboII	GAAGA	5	three_prime	8	35, 266, 383, 442, 985, 1015, 1056, 1339
BbvI	GCAGC	5	five_prime	9	465, 583, 738, 748, 787, 880, 1126, 1153, 1239
TseI	GCWGC	5	five_prime	9	453, 596, 726, 761, 800, 893, 1139, 1166, 1227

Table 6: Restriction digestion of protease gene from AB807664.1 *Aspergillus fumigatus*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BalI	TGGCCA	6	blunt	1	181
BsrBI	CCGCTC	6	blunt	1	583
BtrI	CACGTC	6	blunt	1	63
Eco47III	AGCGCT	6	blunt	1	9
EcoRV	GATATC	6	blunt	1	684
XmnI	GAANNNTTC	6	blunt	1	433
AflIII	ACRYGT	6	five_prime	1	539
BspMI	ACCTGC	6	five_prime	1	512
BtgZI	GCGATG	6	five_prime	1	68
EcoP15I	CAGCAG	6	five_prime	1	436
FauI	CCCGC	5	five_prime	1	634
FokI	GGATG	5	five_prime	1	229
PleI	GAGTC	5	five_prime	1	231
StyI	CCWWGG	6	five_prime	1	321
BciVI	GTATCC	6	three_prime	1	496
BfiI	ACTGGG	6	three_prime	1	270
BsrDI	GCAATG	6	three_prime	1	516
HaeII	RGCGCY	6	three_prime	1	11
KpnI	GGTACC	6	three_prime	1	155
NspI	RCATGY	6	three_prime	1	543
TsoI	TARCCA	6	three_prime	1	386
TspRI	CASTG	5	three_prime	1	48
AccI	GTMKAC	6	five_prime	2	38, 659
BccI	CCATC	5	five_prime	2	247, 371
CfrI	YGGCCR	6	five_prime	2	129, 179
EcoRII	CCWGG	5	five_prime	2	215, 694
SalI	GTCGAC	6	five_prime	2	37, 658
BaeI	ACNNNGTAYC	7	three_prime	2	468, 501
BsrI	ACTGG	5	three_prime	2	265, 358
FalI	AAGNNNNCTT	6	three_prime	2	434, 466
Hin4I	GAYNNNNVTC	6	three_prime	2	273, 305
Hpy99I	CGWCG	5	three_prime	2	67, 463
MmeI	TCCRAC	6	three_prime	2	511, 665
TspDTI	ATGAA	5	three_prime	2	455, 527
AgsI	TTSAA	5	three_prime	3	258, 351, 414
MboII	GAAGA	5	three_prime	3	198, 204, 657
HindIII	GTYRAC	6	blunt	4	39, 57, 120, 660
BbvI	GCAGC	5	five_prime	4	122, 291, 450, 591
SfaNI	GCATC	5	five_prime	4	56, 91, 134, 546
TseI	GCWGC	5	five_prime	4	110, 304, 463, 604
TauI	GCSGC	5	three_prime	4	80, 103, 164, 374
TspGWI	ACGGA	5	three_prime	4	149, 188, 192, 642

Table 7: Restriction digestion of protease gene from X66935.1 *Aspergillus fumigatus*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BalI	TGGCCA	6	blunt	1	199
BsrBI	CCGCTC	6	blunt	1	602
BtrI	CACGTC	6	blunt	1	81
Eco47III	AGCGCT	6	blunt	1	27
XmnI	GAANNNTTC	6	blunt	1	452
AflIII	ACRYGT	6	five_prime	1	558
BspMI	ACCTGC	6	five_prime	1	531
BtgZI	GCGATG	6	five_prime	1	86
EcoP15I	CAGCAG	6	five_prime	1	455
FauI	CCCGC	5	five_prime	1	653
FokI	GGATG	5	five_prime	1	247
PleI	GAGTC	5	five_prime	1	249
StyI	CCWWGG	6	five_prime	1	339
BciVI	GTATCC	6	three_prime	1	515
BfiI	ACTGGG	6	three_prime	1	288
BtsI	GCAGTG	6	three_prime	1	729
HaeII	RGCGCY	6	three_prime	1	29
KpnI	GGTACC	6	three_prime	1	173
NmeAIII	GCCGAG	6	three_prime	1	58
NspI	RCATGY	6	three_prime	1	562
TsoI	TARCCA	6	three_prime	1	405
TspDTI	ATGAA	5	three_prime	1	474
AccI	GTMKAC	6	five_prime	2	56, 678
EcoRII	CCWGG	5	five_prime	2	233, 713
SalI	GTCGAC	6	five_prime	2	55, 677
BaeI	ACNNNGTAYC	7	three_prime	2	487, 520
BsrI	ACTGG	5	three_prime	2	283, 376
FalI	AAGNNNNCTT	6	three_prime	2	453, 485
Hin4I	GAYNNNNVTC	6	three_prime	2	291, 323
Hpy99I	CGWCG	5	three_prime	2	85, 482
MmeI	TCCRAC	6	three_prime	2	530, 684

Table 7: Countinue

TspRI	CASTG	5	three_prime	2	66, 736
BccI	CCATC	5	five_prime	3	265, 390, 752
CfrI	YGGCCR	6	five_prime	3	30, 147, 197
AgsI	TTSAA	5	three_prime	3	276, 369, 433
MboII	GAAGA	5	three_prime	3	216, 222, 676
HindII	GTYRAC	6	blunt	4	57, 75, 138, 679
BbvI	GCAGC	5	five_prime	4	140, 309, 469, 610
SfaNI	GCATC	5	five_prime	4	74, 109, 152, 565
TseI	GCWGC	5	five_prime	4	128, 322, 482, 623
TauI	GCSGC	5	three_prime	4	98, 121, 182, 393
TspGWI	ACGGA	5	three_prime	4	167, 206, 210, 661

Table 8: Restriction digestion of protease gene from Z11580.1 *Aspergillus.fumigatus*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsaBI	GATNNNNATC	6	blunt	1	1940
BtrI	CACGTC	6	blunt	1	860
Eco47III	AGCGCT	6	blunt	1	806
EcoRV	GATATC	6	blunt	1	1478
MslI	CAYNNNNRTG	6	blunt	1	142
PsiI	TTATAA	6	blunt	1	195
ScaI	AGTACT	6	blunt	1	626
XmnI	GAANNNTTC	6	blunt	1	1227
AclI	AACGTT	6	five_prime	1	1654
AflII	CTTAAG	6	five_prime	1	1696
AflIII	ACRYGT	6	five_prime	1	1333
ApaLI	GTGCAC	6	five_prime	1	670
AscI	GGCGCGCC	8	five_prime	1	355
AvaII	GGWCC	5	five_prime	1	695
BglIII	AGATCT	6	five_prime	1	218
BsePI	GCGCGC	6	five_prime	1	355
DraII	RGGNCCY	6	five_prime	1	746
Esp3I	CGTCTC	6	five_prime	1	610
FauI	CCCGC	5	five_prime	1	1428
NarI	GGCGCC	6	five_prime	1	736
PfoI	TCCNGGA	6	five_prime	1	364
XhoII	RGATCY	6	five_prime	1	218
AatII	GACGTC	6	three_prime	1	622
AlwNI	CAGNNNCTG	6	three_prime	1	289
BciVI	GTATCC	6	three_prime	1	1290
BfiI	ACTGGG	6	three_prime	1	1067
BseRI	GAGGAG	6	three_prime	1	705
BsmI	GAATGC	6	three_prime	1	69
BsrI	ACTGG	5	three_prime	1	1062
DrdI	GACNNNNNGTC	6	three_prime	1	240
HaeIV	GAYNNNNNRTC	6	three_prime	1	286
HphI	GGTGA	5	three_prime	1	129
PstI	CTGCAG	6	three_prime	1	1607
TsoI	TARCCA	6	three_prime	1	1180
XcmI	CCANNNNNNNTGG	6	three_prime	1	1760
BsrBI	CCGCTC	6	blunt	2	1377, 1614
AceI	GTMKAC	6	five_prime	2	835, 1453
BseYI	CCCAGC	6	five_prime	2	254, 639
BspMI	ACCTGC	6	five_prime	2	210, 1306
EcoP15I	CAGCAG	6	five_prime	2	1230, 1572
HindIII	AAGCTT	6	five_prime	2	1674, 1699
NcoI	CCATGG	6	five_prime	2	741, 1534
SalI	GTCGAC	6	five_prime	2	834, 1452
SapI	GCTTTC	7	five_prime	2	531, 2091
AlfI	GCANNNNNTGTC	6	three_prime	2	302, 336
ArsI	GACNNNNNTTYG	7	three_prime	2	508, 540
BaeI	ACNNNNGTAYC	7	three_prime	2	1262, 1295
BplI	GAGNNNNNCTC	6	three_prime	2	1575, 1607
BseMIII	CTCAG	5	three_prime	2	123, 399
BseSI	GKGC MC	6	three_prime	2	429, 674
BstXI	CCANNNNNTGG	6	three_prime	2	1760, 2091
Eco57I	CTGAAG	6	three_prime	2	178, 615
FalI	AAGNNNNNCTT	6	three_prime	2	1228, 1260
GsuI	CTGGAG	6	three_prime	2	197, 510
HaeII	RGCGCY	6	three_prime	2	739, 808
KpnI	GGTACC	6	three_prime	2	710, 952
SphI	GCAATGC	6	three_prime	2	5, 2162
Ball	TGGCCA	6	blunt	3	978, 1539, 1632
BtgZI	GCGATG	6	five_prime	3	45, 865, 2072
SmlI	CTYRAG	6	five_prime	3	1581, 1696, 2034
TatI	WGTACW	6	five_prime	3	403, 624, 1567
TfiI	GAWTC	5	five_prime	3	128, 392, 1801
Tsp45I	GTSAC	5	five_prime	3	117, 410, 1607

Table 8: Countinue

BtsI	GCAGTG	6	three_prime	3	64, 1504, 1612
HindII	GTYRAC	6	blunt	4	836, 854, 917, 1454
AcyI	GRCGYC	6	five_prime	4	586, 619, 715, 736
CfrI	YGGCCR	6	five_prime	4	926, 976, 1537, 1630
PleI	GAGTC	5	five_prime	4	294, 455, 1028, 1753
BsaXI	ACNNNNCTCC	6	three_prime	4	558, 588, 1506, 1536
BsrDI	GCAATG	6	three_prime	4	50, 1310, 1697, 1838
Eco57MI	CTGRAG	6	three_prime	4	178, 197, 510, 615
NspI	RCATGY	6	three_prime	4	5, 1337, 1899, 2162
SduI	GDGCHC	6	three_prime	4	429, 674, 1783, 1813
EcoRII	CCWGG	5	five_prime	5	364, 395, 748, 1012, 1488
HgaI	GACGC	5	five_prime	5	483, 594, 723, 2011, 2054
StyI	CCWGGG	6	five_prime	5	326, 741, 1118, 1534, 2084
AgsI	TTSAA	5	three_prime	5	419, 1055, 1146, 1208, 1952
MmeI	TCCRAC	6	three_prime	5	44, 269, 1305, 1459, 1884
BsmAI	GTCTC	5	five_prime	6	100, 112, 176, 454, 610, 2037
FokI	GGATG	5	five_prime	6	86, 1026, 1772, 1948, 2002, 2094
BcgI	CGANNNNNNTGC	6	three_prime	6	389, 423, 1884, 1918, 2062, 2096
Hin4I	GA YNNNNNVTC	6	three_prime	6	253, 285, 1070, 1102, 1786, 1818
TauI	GCSGC	5	three_prime	6	514, 564, 877, 900, 961, 1168
TspDTI	ATGAA	5	three_prime	6	154, 181, 1249, 1321, 2075, 2096
SfaNI	GCATC	5	five_prime	7	22, 277, 853, 888, 931, 1340, 1627
Hpy99I	CGWCG	5	three_prime	7	25, 379, 499, 528, 864, 1257, 2048
TspRI	CASTG	5	three_prime	7	71, 473, 845, 1511, 1612, 1988, 2074
TspGWI	ACGGA	5	three_prime	8	946, 985, 989, 1436, 1553, 1798, 1948, 2100
BbvI	GCAGC	5	five_prime	9	307, 368, 766, 919, 1088, 1244, 1385, 1589, 1675
TseI	GCWGC	5	five_prime	9	320, 381, 754, 907, 1101, 1257, 1398, 1602, 1663
BceI	CCATC	5	five_prime	10	107, 600, 1044, 1165, 1527, 1568, 1750, 1754, 1921, 1930
MboII	GAAGA	5	three_prime	12	171, 191, 401, 465, 548, 621, 995, 1001, 1451, 1722, 2074, 2108

Table 9: Restriction digestion of protease gene from XM_746558.1 *Aspergillus fumigatus* Af293

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BtrI	CACGTC	6	blunt	1	507
Eco47III	AGCGCT	6	blunt	1	453
EcoRV	GATATC	6	blunt	1	990
XmnI	GAANNNNTTC	6	blunt	1	811
AclI	AACGTT	6	five_prime	1	1166
AscI	GGCGGCC	8	five_prime	1	59
AvaII	GGWCC	5	five_prime	1	342
BsePI	GCGCGC	6	five_prime	1	59
BsmAI	GTCTC	5	five_prime	1	158
BtgZI	GCGATG	6	five_prime	1	512
DraII	RGGNCCY	6	five_prime	1	393
FauI	CCCGC	5	five_prime	1	940
FokI	GGATG	5	five_prime	1	673
HindIII	AAGCTT	6	five_prime	1	1186
NarI	GGCGCC	6	five_prime	1	383
PfoI	TCCNGGA	6	five_prime	1	68
SapI	GCTCTTC	7	five_prime	1	235
SmlI	CTYRAG	6	five_prime	1	1093
TatI	WGTACW	6	five_prime	1	107
TfiI	GAWTC	5	five_prime	1	96
BfiI	ACTGGG	6	three_prime	1	714
BseMII	CTCAG	5	three_prime	1	103
BseRI	GAGGAG	6	three_prime	1	352
BseSI	GKCMC	6	three_prime	1	133
BsrDI	GCAATG	6	three_prime	1	1209
BsrI	ACTGG	5	three_prime	1	709
Eco57MI	CTGRAG	6	three_prime	1	214
GsuI	CTGGAG	6	three_prime	1	214
MmeI	TCCRAC	6	three_prime	1	971
PstI	CTGCAG	6	three_prime	1	1119
SduI	GDGCHC	6	three_prime	1	133
TsoI	TARCCA	6	three_prime	1	764
TspDTI	ATGAA	5	three_prime	1	833
BsrBI	CCGCTC	6	blunt	2	889, 1126
EcoP15I	CAGCAG	6	five_prime	2	814, 1084
NcoI	CCATGG	6	five_prime	2	388, 1046
PleI	GAGTC	5	five_prime	2	159, 675
SalI	GTCGAC	6	five_prime	2	481, 964
Tsp45I	GTSAC	5	five_prime	2	114, 1119
AlfI	GCANNNNNNTGC	6	three_prime	2	6, 40
ArsI	GACNNNNNTTYG	7	three_prime	2	212, 244
BcgI	CGANNNNNNTGC	6	three_prime	2	93, 127
BplI	GAGNNNNNTCTC	6	three_prime	2	1087, 1119
BtsI	GCAGTG	6	three_prime	2	1016, 1124
FalI	AAGNNNNNTCT	6	three_prime	2	812, 844

Table 9: Countiue

HaeII	RGCGCY	6	three_prime	2	386, 455
Hin4I	GAYNNNNVTC	6	three_prime	2	717, 749
KpnI	GGTACC	6	three_prime	2	357, 599
Ball	TGGCCA	6	blunt	3	625, 1051, 1144
AccI	GTMKAC	6	five_prime	3	482, 965, 1079
AcyI	GRCGYC	6	five_prime	3	290, 362, 383
HgaI	GACGC	5	five_prime	3	187, 298, 370
AgsI	TTSAA	5	three_prime	3	123, 702, 792
HindII	GTyrAC	6	blunt	4	483, 501, 564, 966
BccI	CCATC	5	five_prime	4	304, 691, 1039, 1080
CfrI	YGGCCR	6	five_prime	4	573, 623, 1049, 1142
StyI	CCWWGG	6	five_prime	4	30, 388, 765, 1046
BsaXI	ACNNNNCTCC	6	three_prime	4	262, 292, 1018, 1048
EcoRII	CCWGG	5	five_prime	5	68, 99, 395, 659, 1000
SfaNI	GCATC	5	five_prime	5	500, 535, 578, 852, 1139
Hpy99I	CGWCG	5	three_prime	5	83, 203, 232, 511, 841
TauI	GCSGC	5	three_prime	5	218, 268, 524, 547, 608
TspGWI	ACGGA	5	three_prime	5	593, 632, 636, 948, 1065
TspRI	CASTG	5	three_prime	5	177, 492, 864, 1023, 1124
MboII	GAAGA	5	three_prime	6	105, 169, 252, 642, 648, 963
BbvI	GCAGC	5	five_prime	9	11, 72, 413, 566, 735, 828, 897, 1101, 1187
TseI	GCWGC	5	five_prime	9	24, 85, 401, 554, 748, 841, 910, 1114, 1175

Table 10: Restriction digestion of protease gene from XM_024823318.1 *Aspergillus novofumigatus*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsaAI	YACGTR	6	blunt	1	15
Eco47III	AGCGCT	6	blunt	1	412
EcoRV	GATATC	6	blunt	1	1812
FspAI	RTGCGCAY	8	blunt	1	1790
StuI	AGGCCT	6	blunt	1	145
SwaI	ATTTAAAT	8	blunt	1	1968
AarI	CACCTGC	7	five_prime	1	2241
AclI	AACGTT	6	five_prime	1	2116
AflIII	ACRYGT	6	five_prime	1	12
AgeI	ACCGGT	6	five_prime	1	2029
BclI	TGATCA	6	five_prime	1	2102
BglII	AGATCT	6	five_prime	1	1058
BspMI	ACCTGC	6	five_prime	1	2241
EcoNI	CCTNNNNNAGG	6	five_prime	1	1318
PasI	CCCWGGG	7	five_prime	1	831
PfoI	TCCNGGA	6	five_prime	1	115
PspXI	VCTCGAGB	8	five_prime	1	322
SgrAI	CRCCGGYG	8	five_prime	1	1698
SgrDI	CGTCGACG	8	five_prime	1	859
StyI	CCWWGG	6	five_prime	1	734
Tth111I	GACNNNGTC	6	five_prime	1	1639
VspI	ATTAAT	6	five_prime	1	2016
XhoI	CTCGAG	6	five_prime	1	322
AlwNI	CAGNNNCTG	6	three_prime	1	907
BglI	GCCNNNNNGGC	6	three_prime	1	1238
BtsI	GCAGTG	6	three_prime	1	628
EciI	GGCGGA	6	three_prime	1	330
Eco57I	CTGAAG	6	three_prime	1	871
KpnI	GGTACC	6	three_prime	1	1539
SacI	GAGCTC	6	three_prime	1	570
SacII	CCGCGG	6	three_prime	1	1694
Sse8387I	CCTGCAGG	8	three_prime	1	1238
BsrBI	CCGCTC	6	blunt	2	230, 1737
HindII	GTyrAC	6	blunt	2	861, 1757
MslI	CAYNNNNRTG	6	blunt	2	663, 1968
PsiI	TTATAA	6	blunt	2	998, 2240
PvuII	CAGCTG	6	blunt	2	34, 46
SmaI	CCCGGG	6	blunt	2	1533, 1832
SspI	AATATT	6	blunt	2	711, 2262
ApoI	RAATTY	6	five_prime	2	824, 2155
AvaII	GGWCC	5	five_prime	2	1071, 1407
BamHI	GGATCC	6	five_prime	2	1042, 1872
BsePI	GCGCGC	6	five_prime	2	1479, 1521
BseYI	CCCAGC	6	five_prime	2	648, 1350
BspHI	TCATGA	6	five_prime	2	198, 1925
BstEII	GGTNACC	6	five_prime	2	1291, 1878
BtgZI	GCGATG	6	five_prime	2	1173, 1924
Cfr10I	RCCGGY	6	five_prime	2	1698, 2029
EcoP15I	CAGCAG	6	five_prime	2	618, 1593
NarI	GGCGCC	6	five_prime	2	1169, 1696
SalI	GTCGAC	6	five_prime	2	859, 1755
TfiI	GAWTC	5	five_prime	2	5, 904
AatII	GACGTC	6	three_prime	2	1334, 1622
BaeI	ACNNNGTAYC	7	three_prime	2	1594, 1627
BciVI	GTATCC	6	three_prime	2	228, 941
BfiI	ACTGGG	6	three_prime	2	489, 2261

Table 10: Countiue

BpII	GAGNNNNCTC	6	three_prime	2	305, 337
BsaXI	ACNNNNCTCC	6	three_prime	2	1045, 1075
DrdI	GACNNNNNGTC	6	three_prime	2	1273, 1642
NspI	RCATGY	6	three_prime	2	1665, 1987
SphI	GCATGC	6	three_prime	2	1665, 1987
AccI	GTMKAC	6	five_prime	3	860, 1130, 1756
CfrI	YGGCCR	6	five_prime	3	943, 1625, 1709
SmlI	CTYRAG	6	five_prime	3	322, 616, 869
BseMII	CTCAG	5	three_prime	3	320, 370, 398
BseRI	GAGGAG	6	three_prime	3	139, 1747, 1937
BseSI	GKGC MC	6	three_prime	3	838, 1684, 2058
GsuI	CTGGAG	6	three_prime	3	1102, 1972, 1979
MmeI	TCCRAC	6	three_prime	3	365, 914, 1895
PstI	CTGCAG	6	three_prime	3	33, 45, 1238
AvaI	CYCGRG	6	five_prime	4	322, 1315, 1531, 1830
Esp3I	CGTCTC	6	five_prime	4	121, 239, 1248, 1997
PleI	GAGTC	5	five_prime	4	333, 565, 1261, 1887
Tsp4SI	GTSAC	5	five_prime	4	1291, 1327, 1434, 1878
BcgI	CGANNNNNNTGC	6	three_prime	4	76, 110, 262, 296
BsrDI	GCAATG	6	three_prime	4	379, 467, 658, 747
Eco57MI	CTGRAG	6	three_prime	4	871, 1102, 1972, 1979
TspGWI	ACGGA	5	three_prime	4	136, 246, 1055, 1236
Eco3II	GGTCTC	6	five_prime	5	175, 1501, 2078, 2099, 2155
HgaI	GACGC	5	five_prime	5	1116, 1230, 1263, 1613, 1677
XhoII	RGATCY	6	five_prime	5	762, 789, 1042, 1058, 1872
BsrI	ACTGG	5	three_prime	5	495, 1011, 1119, 1206, 2256
HaeII	RGCGCY	6	three_prime	5	414, 869, 1172, 1203, 1699
HphI	GGTGA	5	three_prime	5	1303, 1339, 1362, 1890, 2097
MboII	GAAGA	5	three_prime	5	274, 795, 1326, 1435, 1641
TspRI	CASTG	5	three_prime	5	506, 635, 1011, 1126, 1213
AgsI	TTSAA	5	three_prime	6	580, 922, 1653, 2022, 2206, 2266
BdaI	TGANNNNNNTCA	6	three_prime	6	1707, 1741, 1993, 2027, 2158, 2192
HaeIV	GAYNNNNNRTC	6	three_prime	6	235, 269, 287, 321, 959, 993
NmeAIII	GCCGAG	6	three_prime	6	1197, 1208, 1239, 1379, 1599, 1834
SduI	GDGCHC	6	three_prime	6	56, 570, 838, 1151, 1684, 2058
AcyI	GRCGYC	6	five_prime	7	1127, 1169, 1241, 1274, 1331, 1619, 1696
EcoRII	CCWGG	5	five_prime	7	20, 115, 290, 830, 911, 1720, 2213
SfaNI	GCATC	5	five_prime	7	359, 368, 542, 761, 1020, 1267, 1800
BbvI	GCAGC	5	five_prime	8	15, 27, 43, 55, 590, 632, 1577, 1845
BceI	CCATC	5	five_prime	8	204, 853, 964, 1162, 1195, 1636, 1720, 1862
FauI	CCCGC	5	five_prime	8	235, 608, 693, 1082, 1098, 1375, 1420, 1450
TseI	GCWGC	5	five_prime	8	28, 31, 40, 43, 603, 645, 1565, 1858
TspDTI	ATGAA	5	three_prime	8	104, 578, 647, 1237, 1436, 1914, 2167, 2186
BsmAI	GTCTC	5	five_prime	10	121, 175, 239, 289, 1248, 1501, 1997, 2078, 2099, 2155
FokI	GGATG	5	five_prime	12	390, 784, 827, 832, 943, 1233, 1245, 1432, 1699, 1778, 1880, 1989
Hpy99I	CGWCG	5	three_prime	12	98, 262, 862, 865, 1099, 1252, 1255, 1258, 1279, 1624, 1645, 1671
TauI	GCSGC	5	three_prime	15	609, 624, 948, 1103, 1127, 1169, 1205, 1457, 1583, 1586, 1627, 1693, 1696, 1711, 1740
Hin4I	GAYNNNNNVTC	6	three_prime	16	130, 153, 162, 185, 214, 236, 246, 268, 288, 320, 322, 354, 960, 992, 1050, 1082

Table 11: Restriction digestion of protease gene from XM_002374250.1 *Aspergillus flavus*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsaAI	YACGTR	6	blunt	1	477
EcoRV	GATATC	6	blunt	1	990
StuI	AGGCCT	6	blunt	1	535
XmnI	GAANNNTTC	6	blunt	1	811
AccI	GTMKAC	6	five_prime	1	500
BgIII	AGATCT	6	five_prime	1	347
BspMI	ACCTGC	6	five_prime	1	1193
EcoP15I	CAGCAG	6	five_prime	1	451
HgaI	GACGC	5	five_prime	1	187
HindIII	AAGCTT	6	five_prime	1	94
NarI	GGCGCC	6	five_prime	1	206
PasI	CCCWGGG	7	five_prime	1	389
PfoI	TCCNGGA	6	five_prime	1	99
SalI	GTCGAC	6	five_prime	1	499
SmlI	CTYRAG	6	five_prime	1	1093
SpeI	ACTAGT	6	five_prime	1	448
TatI	WGTACW	6	five_prime	1	107
Tth111I	GACNNNGTC	6	five_prime	1	1156
XhoII	RGATCY	6	five_prime	1	347
BfiI	ACTGGG	6	three_prime	1	714
BseMIII	CTCAG	5	three_prime	1	80
BsgI	GTCGAG	6	three_prime	1	88
BsmI	GAATGC	6	three_prime	1	811
BsrDI	GCAATG	6	three_prime	1	599
Eco57MI	CTGRAG	6	three_prime	1	214
GsuI	CTGGAG	6	three_prime	1	214
HaeII	RGCGCY	6	three_prime	1	209
KpnI	GGTACC	6	three_prime	1	1043
MmeI	TCCRAC	6	three_prime	1	971

Table 11: Countiue

NspI	RCATGY	6	three_prime	1	562
SacII	CCGCGG	6	three_prime	1	50
TspGWI	ACGGA	5	three_prime	1	672
Ball	TGGCCA	6	blunt	2	575, 1144
HindII	GTyrAC	6	blunt	2	501, 966
NaeI	GCCGGC	6	blunt	2	456, 867
AvaII	GGWCC	5	five_prime	2	51, 342
FokI	GGATG	5	five_prime	2	625, 1177
NcoI	CCATGG	6	five_prime	2	576, 1046
SapI	GCTCTTC	7	five_prime	2	916, 1017
Tsp45I	GTSAC	5	five_prime	2	114, 1119
AatII	GACGTC	6	three_prime	2	971, 1157
BglI	GCCNNNNNGGC	6	three_prime	2	532, 631
FalI	AAGNNNNNCTT	6	three_prime	2	977, 1009
TaqII	GACCGA	6	three_prime	2	636, 662
TsoI	TARCCA	6	three_prime	2	764, 1039
TspRI	CASTG	5	three_prime	2	216, 909
AcyI	GRCGYC	6	five_prime	3	206, 968, 1154
CfrI	YGGCCR	6	five_prime	3	573, 867, 1142
FauI	CCCGC	5	five_prime	3	54, 85, 1119
StyI	CCWWGG	6	five_prime	3	576, 1046, 1148
TfiI	GAWTC	5	five_prime	3	147, 306, 667
AgsI	TTSAA	5	three_prime	3	123, 702, 792
BseSI	GKGCMC	6	three_prime	3	63, 387, 466
BsrI	ACTGG	5	three_prime	3	216, 709, 938
HphI	GGTGA	5	three_prime	3	172, 229, 676
Cfr10I	RCCGGY	6	five_prime	4	454, 547, 844, 865
BglI	CGANNNNNNTGC	6	three_prime	4	307, 341, 433, 467
Hpy99I	CGWCG	5	three_prime	4	203, 289, 337, 1159
SduI	GDGCHC	6	three_prime	4	63, 387, 430, 466
TauI	GCSGC	5	three_prime	4	88, 268, 524, 913
BecI	CCATC	5	five_prime	5	16, 355, 907, 1039, 1099
EcoRII	CCWGG	5	five_prime	5	99, 163, 388, 659, 1000
BseRI	GAGGAG	6	three_prime	5	16, 19, 316, 352, 523
SfaNI	GCATC	5	five_prime	6	279, 647, 786, 852, 885, 1139
MboII	GAAGA	5	three_prime	6	214, 331, 390, 933, 963, 1004
BbvI	GCAGC	5	five_prime	9	413, 531, 686, 696, 735, 828, 1074, 1101, 1187
TseI	GCWGC	5	five_prime	9	401, 544, 674, 709, 748, 841, 1087, 1114, 1175

Table 12: Restriction digestion of protease gene from AF324246.1 *Aspergillus flavus*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsaAI	YACGTR	6	blunt	1	668
BsrBI	CCGCTC	6	blunt	1	480
Eco47III	AGCGCT	6	blunt	1	1000
EcoRV	GATATC	6	blunt	1	1296
PsiI	TTATAA	6	blunt	1	36
StuI	AGGCCT	6	blunt	1	726
XmnI	GAANNNNTTC	6	blunt	1	1061
AflIII	CTTAAG	6	five_prime	1	1514
ApoI	RAATTY	6	five_prime	1	1137
AvaI	CYCGRG	6	five_prime	1	1
BclI	TGATCA	6	five_prime	1	1784
BglIII	AGATCT	6	five_prime	1	538
BsmAI	GTCTC	5	five_prime	1	1118
Bsp1407I	TGTACA	6	five_prime	1	1676
BspHI	TCATGA	6	five_prime	1	1841
BspMI	ACCTGC	6	five_prime	1	1499
BtgZI	GCGATG	6	five_prime	1	1108
EcoP15I	CAGCAG	6	five_prime	1	642
EcoRI	GAATTC	6	five_prime	1	1137
Esp3I	CGTCTC	6	five_prime	1	1118
HindIII	AAGCTT	6	five_prime	1	235
NarI	GGCGCC	6	five_prime	1	347
PasI	CCCWGGG	7	five_prime	1	580
PfoI	TCCNGGA	6	five_prime	1	240
SpeI	ACTAGT	6	five_prime	1	639
XbaI	TCTAGA	6	five_prime	1	1857
XhoI	CTCGAG	6	five_prime	1	1
XhoII	RGATCY	6	five_prime	1	538
BfiI	ACTGGG	6	three_prime	1	905
BsmI	GAATGC	6	three_prime	1	1061
BsrDI	GCAATG	6	three_prime	1	790
Eco57I	CTGAAG	6	three_prime	1	48
GsuI	CTGGAG	6	three_prime	1	355
KpnI	GGTACC	6	three_prime	1	1349
SacII	CCGCGG	6	three_prime	1	191
Ball	TGGCCA	6	blunt	2	766, 1450

Table 12: Countiue

NaeI	GCCGGC	6	blunt	2	647, 1173
AvaII	GGWCC	5	five_prime	2	192, 533
HgaI	GACGC	5	five_prime	2	328, 1842
NcoI	CCATGG	6	five_prime	2	767, 1352
Sall	GTCGAC	6	five_prime	2	690, 1818
TatI	WGTACW	6	five_prime	2	248, 1676
Tsp45I	GTSAC	5	five_prime	2	255, 1425
Tth111I	GACNNNGTC	6	five_prime	2	1462, 1871
AatII	GACGTC	6	three_prime	2	1277, 1463
ArsI	GACNNNNNTTYG	7	three_prime	2	1812, 1844
BcgI	CGANNNNTGTC	6	three_prime	2	624, 658
BdaI	TGANNNNTCA	6	three_prime	2	1821, 1855
BglI	GCCNNNNGGC	6	three_prime	2	723, 822
BseMII	CTCAG	5	three_prime	2	221, 1787
BsgI	GTGCAG	6	three_prime	2	229, 1792
Eco57MI	CTGRAG	6	three_prime	2	48, 355
HaeII	RGCGCY	6	three_prime	2	350, 1002
MmeI	TCCRAC	6	three_prime	2	124, 1277
NspI	RCATGY	6	three_prime	2	49, 753
TaqII	GACCGA	6	three_prime	2	827, 853
TsoI	TARCCA	6	three_prime	2	1014, 1345
TspGWI	ACGGA	5	three_prime	2	863, 1615
TspRI	CASTG	5	three_prime	2	357, 1215
HindII	GTYRAC	6	blunt	3	692, 1272, 1820
AccI	GTMKAC	6	five_prime	3	691, 1685, 1819
AcyI	GRCGYC	6	five_prime	3	347, 1274, 1460
CfrI	YGGCCR	6	five_prime	3	764, 1173, 1448
FauI	CCCGC	5	five_prime	3	195, 226, 1425
SapI	GCTCTTC	7	five_prime	3	1222, 1323, 1576
StyI	CCWWGG	6	five_prime	3	767, 1352, 1454
BseSI	GKGCMC	6	three_prime	3	204, 578, 657
Cfr10I	RCCGGY	6	five_prime	4	645, 738, 1094, 1171
BsrI	ACTGG	5	three_prime	4	357, 900, 1244, 1559
FalI	AAGNNNNCTT	6	three_prime	4	145, 177, 1283, 1315
SduI	GDGCHC	6	three_prime	4	204, 578, 621, 657
TauI	GCSGC	5	three_prime	4	229, 409, 715, 1219
FokI	GGATG	5	five_prime	5	36, 47, 461, 816, 1483
SmlI	CTYRAG	6	five_prime	5	1, 104, 1399, 1514, 1647
AgsI	TTSAA	5	three_prime	5	264, 492, 893, 1009, 1042
BseRI	GAGGAG	6	three_prime	5	157, 160, 457, 543, 714
HphI	GGTGA	5	three_prime	5	313, 370, 867, 972, 1779
Hpy99I	CGWCG	5	three_prime	6	344, 430, 528, 1465, 1535, 1751
EcoRII	CCWGG	5	five_prime	7	240, 304, 511, 579, 850, 1306, 1521
TfiI	GAWTC	5	five_prime	7	288, 447, 858, 1539, 1593, 1669, 1838
BclI	CCATC	5	five_prime	8	68, 80, 157, 546, 1130, 1213, 1345, 1405
SfaNI	GCATC	5	five_prime	8	58, 129, 420, 838, 1036, 1158, 1191, 1445
BbvI	GCAGC	5	five_prime	9	604, 722, 877, 887, 926, 1078, 1380, 1407, 1493
TseI	GCWGC	5	five_prime	9	592, 735, 865, 900, 939, 1091, 1393, 1420, 1481
MboII	GAAGA	5	three_prime	9	124, 355, 472, 581, 968, 1239, 1269, 1310, 1593

Table 13: Restriction digestion of protease gene from GQ925367.1 *Aspergillus clavatus*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsrBI	CCGCTC	6	blunt	1	889
HpaI	GTTAAC	6	blunt	1	501
NaeI	GCCGGC	6	blunt	1	47
PvuII	CAGCTG	6	blunt	1	1140
StuI	AGGCCT	6	blunt	1	535
XmnI	GAANNNNTTC	6	blunt	1	244
AccI	AACGTT	6	five_prime	1	497
AcyI	GRCGYC	6	five_prime	1	1154
AflIII	ACRYGT	6	five_prime	1	863
BglII	AGATCT	6	five_prime	1	347
BstEII	GGTNACC	6	five_prime	1	553
DraII	RGGNCCY	6	five_prime	1	278
Eco31I	GGTCTC	6	five_prime	1	400
FauI	CCCGC	5	five_prime	1	940
MluI	ACGCGT	6	five_prime	1	863
NcoI	CCATGG	6	five_prime	1	1046
NheI	GCTAGC	6	five_prime	1	424
PpuMI	RGGWCCY	7	five_prime	1	278
Sall	GTCGAC	6	five_prime	1	859
SgrAI	CRCCGGYG	8	five_prime	1	454
SmlI	CTYRAG	6	five_prime	1	1093
TatI	WGTACW	6	five_prime	1	107
XhoII	RGATCY	6	five_prime	1	347
BfiI	ACTGGG	6	three_prime	1	714
BseSI	GKGCMC	6	three_prime	1	387

Table 13: Countiue

BsrDI	GCAATG	6	three_prime	1	933
BstXI	CCANNNNNNTGG	6	three_prime	1	1066
BtsI	GCAGTG	6	three_prime	1	1016
DrdI	GACNNNNNGTC	6	three_prime	1	375
EciI	GGCGGA	6	three_prime	1	987
HaeII	RGCGCY	6	three_prime	1	455
NmeAIII	GCCGAG	6	three_prime	1	377
TspGWI	ACGGA	5	three_prime	1	593
BalI	TGGCCA	6	blunt	2	625, 983
AccI	GTMKAC	6	five_prime	2	860, 1079
AvaII	GGWCC	5	five_prime	2	278, 1108
BseYI	CCCAGC	6	five_prime	2	274, 1124
BsmAI	GTCTC	5	five_prime	2	400, 1107
CfrI	YGGCCR	6	five_prime	2	623, 981
EcoP15I	CAGCAG	6	five_prime	2	199, 1084
FokI	GGATG	5	five_prime	2	82, 156
BcgI	CGANNNNNNTGC	6	three_prime	2	208, 242
BglII	GCCNNNNNGGC	6	three_prime	2	212, 740
BpII	GAGNNNNNCTC	6	three_prime	2	1087, 1119
BsaXI	ACNNNNNCTCC	6	three_prime	2	1018, 1048
BsrI	ACTGG	5	three_prime	2	671, 709
HaeIV	GAYNNNNNRTC	6	three_prime	2	978, 1012
Hin4I	GAYNNNNNVTC	6	three_prime	2	979, 1011
HphI	GGTGA	5	three_prime	2	70, 565
PpiI	GAACNNNNNCTC	7	three_prime	2	229, 261
PsrI	GAACNNNNNTAC	7	three_prime	2	660, 692
SduI	GDGCHC	6	three_prime	2	39, 387
TaqII	GACCGA	6	three_prime	2	672, 698
TsoI	TARCCA	6	three_prime	2	517, 1039
TspRI	CASTG	5	three_prime	2	138, 1023
TstI	CACNNNNNTCC	6	three_prime	2	70, 102
HindIII	GTYRAC	6	blunt	3	483, 501, 861
PleI	GAGTC	5	five_prime	3	159, 478, 770
Tsp45I	GTSAC	5	five_prime	3	553, 574, 1119
Eco57MI	CTGRAG	6	three_prime	3	214, 996, 1120
GsuI	CTGGAG	6	three_prime	3	214, 996, 1120
KpnI	GGTACC	6	three_prime	3	357, 599, 1043
BccI	CCATC	5	five_prime	4	16, 304, 688, 1039
Cfr10I	RCCGGY	6	five_prime	4	45, 454, 592, 844
HgaI	GACGC	5	five_prime	4	187, 854, 871, 1143
AgsI	TTSAA	5	three_prime	4	123, 249, 702, 792
AlfI	GCANNNNNNTGC	6	three_prime	4	6, 40, 364, 398
Hpy99I	CGWCG	5	three_prime	4	203, 481, 865, 910
MmeI	TCCRAC	6	three_prime	4	113, 197, 313, 971
SfaNI	GCATC	5	five_prime	5	178, 242, 351, 413, 500
StyI	CCWWGG	6	five_prime	5	30, 388, 530, 570, 1046
MboII	GAAGA	5	three_prime	6	77, 252, 349, 352, 648, 963
EcoRII	CCWGG	5	five_prime	7	99, 163, 191, 515, 978, 983, 1000
TauI	GCSGC	5	three_prime	7	145, 206, 218, 268, 524, 608, 844
BbvI	GCAGC	5	five_prime	8	45, 61, 413, 696, 735, 887, 1101, 1191
TseI	GCWGC	5	five_prime	8	49, 58, 401, 709, 748, 875, 1114, 1204

Table 14: Restriction digestion of protease gene from XM_001272037.1 *Aspergillus clavatus*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsrBI	CCGCTC	6	blunt	1	889
BtrI	CACGTC	6	blunt	1	561
EcoRV	GATATC	6	blunt	1	990
NaeI	GCCGGC	6	blunt	1	47
PvuII	CAGCTG	6	blunt	1	1140
StuI	AGGCCT	6	blunt	1	535
AclI	AACGTT	6	five_prime	1	497
AcyI	GRCGYC	6	five_prime	1	1154
AgeI	ACCGGT	6	five_prime	1	670
AvaII	GGWCC	5	five_prime	1	1108
BglIII	AGATCT	6	five_prime	1	347
BstEII	GGTNACC	6	five_prime	1	553
Eco31I	GGTCTC	6	five_prime	1	400
NcoI	CCATGG	6	five_prime	1	1046
NheI	GCTAGC	6	five_prime	1	424
SalI	GTCGAC	6	five_prime	1	859
SgrAI	CRCCGGYG	8	five_prime	1	454
SmlI	CTYRAG	6	five_prime	1	1093
TatI	WGTACW	6	five_prime	1	107
Thh111I	GACNNNGTC	6	five_prime	1	559
XhoII	RGATCY	6	five_prime	1	347
BfiI	ACTGGG	6	three_prime	1	714
BseMII	CTCAG	5	three_prime	1	1138

Table 14: Counttue

BseRI	GAGGAG	6	three_prime	1	16
BseSI	GKGC MC	6	three_prime	1	387
BsrDI	GCAATG	6	three_prime	1	933
BsrI	ACTGG	5	three_prime	1	709
BstXI	CCANNNNNNTGG	6	three_prime	1	1066
BtsI	GCAGTG	6	three_prime	1	1016
DrdI	GACNNNNNGT C	6	three_prime	1	375
EciI	GGCGGA	6	three_prime	1	987
HaeII	RGCGCY	6	three_prime	1	455
NmeAIII	GCCGAG	6	three_prime	1	377
TspGWI	ACGGA	5	three_prime	1	593
TspRI	CASTG	5	three_prime	1	1023
Ball	TGGCCA	6	blunt	2	983, 1144
AccI	GTMKAC	6	five_prime	2	860, 1079
FauI	CCCCG	5	five_prime	2	208, 940
AlfI	GCANNNNNNTGC	6	three_prime	2	364, 398
BcgI	CGANNNNNNTGC	6	three_prime	2	307, 341
BglI	GCCNNNNNGGC	6	three_prime	2	212, 740
BplI	GAGNNNNNCTC	6	three_prime	2	1087, 1119
BsaXI	ACNNNNNTCC	6	three_prime	2	1018, 1048
Eco57MI	CTGRAG	6	three_prime	2	214, 1120
GsuI	CTGGAG	6	three_prime	2	214, 1120
HaeIV	GAYNNNNNRTC	6	three_prime	2	978, 1012
HphI	GGTGA	5	three_prime	2	70, 565
KpnI	GGTACC	6	three_prime	2	357, 599
Ppil	GAACNNNNNCTC	7	three_prime	2	229, 261
PsrI	GAACNNNNNTAC	7	three_prime	2	660, 692
SduI	GDGCHC	6	three_prime	2	39, 387
TaqII	GACCGA	6	three_prime	2	672, 698
TsoI	TARCCA	6	three_prime	2	613, 1039
TstI	CACNNNNNTCC	6	three_prime	2	70, 102
HindII	GTYRAC	6	blunt	3	483, 501, 861
BsmAI	GTCTC	5	five_prime	3	158, 400, 1107
CfrI	YGGCCR	6	five_prime	3	65, 981, 1142
EcoP15I	CAGCAG	6	five_prime	3	12, 199, 1084
FokI	GGATG	5	five_prime	3	82, 156, 706
HgaI	GACGC	5	five_prime	3	187, 871, 1143
Tsp45I	GTSAC	5	five_prime	3	553, 574, 1119
MmeI	TCCRAC	6	three_prime	3	113, 197, 971
BceI	CCATC	5	five_prime	4	16, 304, 688, 1039
PleI	GAGTC	5	five_prime	4	159, 478, 770, 1116
AgsI	TTSAA	5	three_prime	4	123, 249, 702, 792
Hin4I	GAYNNNNNVTC	6	three_prime	4	138, 170, 979, 1011
Cfr10I	RCCGGY	6	five_prime	5	45, 454, 592, 670, 844
SfaNI	GCATC	5	five_prime	5	178, 242, 351, 413, 500
StyI	CCWWGG	6	five_prime	5	30, 388, 530, 570, 1046
Hpy99I	CGWCG	5	three_prime	5	203, 481, 565, 865, 910
MboII	GAAGA	5	three_prime	5	77, 331, 352, 648, 963
TauI	GCSGC	5	three_prime	7	145, 206, 268, 524, 608, 737, 844
EcoRII	CCWGG	5	five_prime	8	67, 99, 163, 191, 515, 978, 983, 1000
BbvI	GCAGC	5	five_prime	9	29, 45, 61, 413, 696, 735, 887, 1101, 1191
TseI	GCWGC	5	five_prime	9	42, 49, 58, 401, 709, 748, 875, 1114, 1204

Table 15: Restriction digestion of protease gene from XM_032052352.1 *Aspergillus pseudotamarii*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsaAI	YACGTR	6	blunt	1	1085
BsrBI	CCGCTC	6	blunt	1	2094
HpaI	GTTAAC	6	blunt	1	1790
MsiI	CAYNNNNRGTG	6	blunt	1	1867
OliI	CACNNNNGTG	6	blunt	1	1867
PsiI	TTATAA	6	blunt	1	503
Smal	CCCGGG	6	blunt	1	2236
StuI	AGGCCT	6	blunt	1	1143
XmnI	GAANNNNTTC	6	blunt	1	1013
AflIII	CTTAAG	6	five_prime	1	1816
Bpu10I	CCTNAGC	6	five_prime	1	147
BspHI	TCATGA	6	five_prime	1	2142
BspMI	ACCTGC	6	five_prime	1	1801
BtgZI	GCGATG	6	five_prime	1	4
NarI	GGCGCC	6	five_prime	1	814
PasI	CCCWGGG	7	five_prime	1	997
PfoI	TCCNGGA	6	five_prime	1	707
SexAI	ACCWGGT	7	five_prime	1	1823
SgrAI	CRCCGGY G	8	five_prime	1	819
VspI	ATTAAT	6	five_prime	1	94
XhoII	RGATCY	6	five_prime	1	913
AatII	GACGTC	6	three_prime	1	1765

Table 15: Countiue

BfiI	ACTGGG	6	three_prime	1	1322
EciI	GGCGGA	6	three_prime	1	1595
Eco57I	CTGAAG	6	three_prime	1	288
GsuI	CTGGAG	6	three_prime	1	822
HaeII	RGCGCY	6	three_prime	1	817
NmeAIII	GCCGAG	6	three_prime	1	722
PvuI	CGATCG	6	three_prime	1	2054
SphI	GCATGC	6	three_prime	1	436
TsoI	TARCCA	6	three_prime	1	1372
BsaBI	GATNNNNATC	6	blunt	2	2084, 2203
NaeI	GCCGGC	6	blunt	2	1064, 1475
AccI	GTMKAC	6	five_prime	2	1108, 1573
AcyI	GRCGYC	6	five_prime	2	814, 1762
BsmAI	GTCTC	5	five_prime	2	193, 675
Eco31I	GGTCTC	6	five_prime	2	193, 675
EcoP15I	CAGCAG	6	five_prime	2	1125, 1692
HgaI	GACGC	5	five_prime	2	269, 795
NcoI	CCATGG	6	five_prime	2	1184, 1654
PpuMI	RGGWCCY	7	five_prime	2	36, 2188
SalI	GTCGAC	6	five_prime	2	1107, 1572
XhoI	CTCGAG	6	five_prime	2	209, 468
AlfI	GCANNNNNNTGC	6	three_prime	2	574, 608
AloI	GAACNNNNNTCC	7	three_prime	2	242, 274
BdaI	TGANNNNNNTCA	6	three_prime	2	257, 291
BglI	GCCNNNNNGGC	6	three_prime	2	1140, 1239
BsaXI	ACNNNNNTCC	6	three_prime	2	242, 272
BseSI	GKGC MC	6	three_prime	2	671, 1074
BsgI	GTGCAG	6	three_prime	2	696, 2093
BsmI	GAATGC	6	three_prime	2	995, 1421
Eco57MI	CTGRAG	6	three_prime	2	288, 822
KpnI	GGTACC	6	three_prime	2	134, 1651
NspI	RCATGY	6	three_prime	2	436, 516
PpiI	GAACNNNNNTC	7	three_prime	2	242, 274
SacII	CCGCGG	6	three_prime	2	28, 658
TaqII	GACCGA	6	three_prime	2	1244, 1270
BalI	TGGCCA	6	blunt	3	1659, 1752, 2123
EcoRV	GATATC	6	blunt	3	54, 1598, 2326
HindII	GTYRAC	6	blunt	3	1109, 1574, 1790
AvaI	CYCGRG	6	five_prime	3	209, 468, 2234
Bsp1407I	TGTACA	6	five_prime	3	438, 1829, 1978
DraII	RGGNCCY	6	five_prime	3	36, 1001, 2188
HindIII	AAGCTT	6	five_prime	3	597, 702, 2300
Tth111I	GACNNNGTC	6	five_prime	3	1764, 2173, 2186
BsrDI	GCAATG	6	three_prime	3	382, 427, 1207
MmeI	TCCRAC	6	three_prime	3	591, 1579, 1923
TspDTI	ATGAA	5	three_prime	3	2022, 2096, 2257
AvaII	GGWCC	5	five_prime	4	36, 659, 950, 2188
Cfr10I	RCCGGY	6	five_prime	4	819, 1062, 1452, 1473
StyI	CCWWGG	6	five_prime	4	1184, 1390, 1654, 1756
Tsp45I	GTSAC	5	five_prime	4	379, 722, 1727, 2175
BseMII	CTCAG	5	three_prime	4	93, 138, 357, 1724
BsrI	ACTGG	5	three_prime	4	1057, 1317, 1546, 2311
FalI	AAGNNNNNTT	6	three_prime	4	612, 644, 2177, 2209
Hin4I	GAYNNNNVTC	6	three_prime	4	203, 235, 565, 597
TspGWI	ACGGA	5	three_prime	4	273, 1430, 1917, 2076
TstI	CACNNNNNTCC	6	three_prime	4	471, 503, 2055, 2087
CfrI	YGGCCR	6	five_prime	5	1181, 1475, 1657, 1750, 2121
FokI	GGATG	5	five_prime	5	292, 405, 514, 1785, 2092
SmlI	CTYRAG	6	five_prime	5	209, 468, 1701, 1816, 1949
TatI	WGTACW	6	five_prime	5	410, 438, 715, 1829, 1978
TfiI	GAWTC	5	five_prime	5	755, 1275, 1895, 1971, 2139
TspRI	CASTG	5	three_prime	5	1064, 1517, 1718, 1855, 1871
AgsI	TTSAA	5	three_prime	6	127, 396, 731, 1310, 1400, 2133
BcgI	CGANNNNNNTGC	6	three_prime	6	156, 190, 915, 949, 1041, 1075
HphI	GGTGA	5	three_prime	6	373, 780, 837, 1284, 1881, 2080
TauI	GCSGC	5	three_prime	6	30, 696, 876, 1132, 1359, 1521
BccI	CCATC	5	five_prime	7	426, 535, 547, 624, 963, 1299, 1647
EcoRII	CCWGG	5	five_prime	7	707, 771, 996, 1003, 1267, 1608, 1823
FauI	CCCGC	5	five_prime	7	32, 265, 662, 693, 1205, 1548, 2099
Hpy99I	CGWCG	5	three_prime	7	747, 811, 897, 945, 1575, 1767, 1904
SduI	GDGCHC	6	three_prime	7	179, 255, 353, 492, 671, 1038, 1074
BbvI	GCAGC	5	five_prime	8	528, 1139, 1294, 1304, 1436, 1682, 1709, 1795
TseI	GCWGC	5	five_prime	8	516, 1152, 1282, 1317, 1449, 1695, 1722, 1783
BseRI	GAGGAG	6	three_prime	8	54, 624, 627, 924, 960, 1131, 1610, 1613
SfaNI	GCATC	5	five_prime	9	27, 748, 887, 1186, 1394, 1460, 1493, 1747, 2213
MboII	GAAGA	5	three_prime	9	764, 822, 939, 998, 1571, 1895, 2095, 2185, 2303

Table 16: Restriction digestion of protease gene from XM_032052352.1 *Aspergillus pseudotamarii*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsaAI	YACGTR	6	blunt	1	1085
BsrBI	CCGCTC	6	blunt	1	2094
HpaI	GTTAAC	6	blunt	1	1790
MsiI	CAYNNNNRTG	6	blunt	1	1867
OliI	CACNNNNGTG	6	blunt	1	1867
PsiI	TTATAA	6	blunt	1	503
SmaI	CCCGGG	6	blunt	1	2236
StuI	AGGCCT	6	blunt	1	1143
XmnI	GAANNNTTC	6	blunt	1	1013
AflIII	CTTAAG	6	five_prime	1	1816
Bpu10I	CCTNAGC	6	five_prime	1	147
BspHI	TCATGA	6	five_prime	1	2142
BspMI	ACCTGC	6	five_prime	1	1801
BtgZI	GCGATG	6	five_prime	1	4
NarI	GCGGCC	6	five_prime	1	814
PasI	CCCWGGG	7	five_prime	1	997
PfoI	TCCNGGA	6	five_prime	1	707
SexAI	ACCWGGT	7	five_prime	1	1823
SgrAI	CRCCGGYG	8	five_prime	1	819
VspI	ATTAAT	6	five_prime	1	94
XhoII	RGATCY	6	five_prime	1	913
AatII	GACGTC	6	three_prime	1	1765
BfiI	ACTGGG	6	three_prime	1	1322
EciI	GGCGGA	6	three_prime	1	1595
Eco57I	CTGAAG	6	three_prime	1	288
GsuI	CTGGAG	6	three_prime	1	822
HaeII	RGCGCY	6	three_prime	1	817
NmeAIII	GCCGAG	6	three_prime	1	722
PvuI	CGATCG	6	three_prime	1	2054
SphI	GCATGC	6	three_prime	1	436
TsoI	TARCCA	6	three_prime	1	1372
BsaBI	GATNNNNATC	6	blunt	2	2084, 2203
NaeI	GCCGGC	6	blunt	2	1064, 1475
AccI	GTMKAC	6	five_prime	2	1108, 1573
AcyI	GRCGYC	6	five_prime	2	814, 1762
BsmAI	GTCTC	5	five_prime	2	193, 675
Eco3II	GGTCTC	6	five_prime	2	193, 675
EcoP15I	CAGCAG	6	five_prime	2	1125, 1692
HgaI	GACGC	5	five_prime	2	269, 795
NcoI	CCATGG	6	five_prime	2	1184, 1654
PpuMI	RGGWCCY	7	five_prime	2	36, 2188
SalI	GTCGAC	6	five_prime	2	1107, 1572
XhoI	CTCGAG	6	five_prime	2	209, 468
AlfI	GCANNNNNNTGC	6	three_prime	2	574, 608
AloI	GAACNNNNNTCC	7	three_prime	2	242, 274
BdaI	TGANNNNNNTCA	6	three_prime	2	257, 291
BglI	GCCNNNNNGGC	6	three_prime	2	1140, 1239
BsaXI	ACNNNNNTCC	6	three_prime	2	242, 272
BseSI	GKGCMC	6	three_prime	2	671, 1074
BsgI	GTGCAG	6	three_prime	2	696, 2093
BsmI	GAATGC	6	three_prime	2	995, 1421
Eco57MI	CTGRAG	6	three_prime	2	288, 822
KpnI	GGTACC	6	three_prime	2	134, 1651
NspI	RCATGY	6	three_prime	2	436, 516
PpiI	GAACNNNNNTCTC	7	three_prime	2	242, 274
SacII	CCGCGG	6	three_prime	2	28, 658
TaqII	GACCGA	6	three_prime	2	1244, 1270
BalI	TGGCCA	6	blunt	3	1659, 1752, 2123
EcoRV	GATATC	6	blunt	3	54, 1598, 2326
HindII	GTyrAC	6	blunt	3	1109, 1574, 1790
AvaI	CYCGRG	6	five_prime	3	209, 468, 2234
Bsp1407I	TGTACA	6	five_prime	3	438, 1829, 1978
DraII	RGGNCCY	6	five_prime	3	36, 1001, 2188
HindIII	AAGCTT	6	five_prime	3	597, 702, 2300
Tth111I	GACNNNGTC	6	five_prime	3	1764, 2173, 2186
BsrDI	GCAATG	6	three_prime	3	382, 427, 1207
MmeI	TCCRAC	6	three_prime	3	591, 1579, 1923
TspDTI	ATGAA	5	three_prime	3	2022, 2096, 2257
AvaII	GGWCC	5	five_prime	4	36, 659, 950, 2188
Cfr10I	RCCGGY	6	five_prime	4	819, 1062, 1452, 1473
StyI	CCWWGG	6	five_prime	4	1184, 1390, 1654, 1756
Tsp45I	GTSAC	5	five_prime	4	379, 722, 1727, 2175
BseMII	CTCAG	5	three_prime	4	93, 138, 357, 1724
BsrI	ACTGG	5	three_prime	4	1057, 1317, 1546, 2311
FalI	AAGNNNNNTT	6	three_prime	4	612, 644, 2177, 2209
Hin4I	GAYNNNNNVTC	6	three_prime	4	203, 235, 565, 597

Table 16: Countiue

TspGWI	ACGGA	5	three_prime	4	273, 1430, 1917, 2076
TstI	CACNNNNNTCC	6	three_prime	4	471, 503, 2055, 2087
CfrI	YGGCCR	6	five_prime	5	1181, 1475, 1657, 1750, 2121
FokI	GGATG	5	five_prime	5	292, 405, 514, 1785, 2092
SmlI	CTYRAG	6	five_prime	5	209, 468, 1701, 1816, 1949
TatI	WGTACW	6	five_prime	5	410, 438, 715, 1829, 1978
TfiI	GAWTC	5	five_prime	5	755, 1275, 1895, 1971, 2139
TspRI	CASTG	5	three_prime	5	1064, 1517, 1718, 1855, 1871
AgsI	TTSAA	5	three_prime	6	127, 396, 731, 1310, 1400, 2133
BcgI	CGANNNNNNTGC	6	three_prime	6	156, 190, 915, 949, 1041, 1075
HphI	GGTGA	5	three_prime	6	373, 780, 837, 1284, 1881, 2080
TauI	GCSGC	5	three_prime	6	30, 696, 876, 1132, 1359, 1521
BccI	CCATC	5	five_prime	7	426, 535, 547, 624, 963, 1299, 1647
EcoRII	CCWGG	5	five_prime	7	707, 771, 996, 1003, 1267, 1608, 1823
FauI	CCCGC	5	five_prime	7	32, 265, 662, 693, 1205, 1548, 2099
Hpy99I	CGWCG	5	three_prime	7	747, 811, 897, 945, 1575, 1767, 1904
SduI	GDGCHC	6	three_prime	7	179, 255, 353, 492, 671, 1038, 1074
BbvI	GCAGC	5	five_prime	8	528, 1139, 1294, 1304, 1436, 1682, 1709, 1795
TseI	GCWGC	5	five_prime	8	516, 1152, 1282, 1317, 1449, 1695, 1722, 1783
BseRI	GAGGAG	6	three_prime	8	54, 624, 627, 924, 960, 1131, 1610, 1613
SfaNI	GCATC	5	five_prime	9	27, 748, 887, 1186, 1394, 1460, 1493, 1747, 2213
MboII	GAAGA	5	three_prime	9	764, 822, 939, 998, 1571, 1895, 2095, 2185, 2303

Table 17: Restriction digestion of protease gene from AY590135.1 *Aspergillus viridinutans* strain MK246

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BtrI	CACGTC	6	blunt	1	384
Eco47III	AGCGCT	6	blunt	1	330
NaeI	GCCGGC	6	blunt	1	96
ScaI	AGTACT	6	blunt	1	150
AcyI	GRCGYC	6	five_prime	1	260
AvaII	GGWCC	5	five_prime	1	219
Bpu10I	CCTNAGC	6	five_prime	1	1183
BsmAI	GTCTC	5	five_prime	1	1110
BspHI	TCATGA	6	five_prime	1	172
BstEII	GGTNACC	6	five_prime	1	430
BtgZI	GCGATG	6	five_prime	1	53
Cfr10I	RCCGGY	6	five_prime	1	94
Eco31I	GGTCTC	6	five_prime	1	1110
FauI	CCCGC	5	five_prime	1	954
FokI	GGATG	5	five_prime	1	390
NarI	GGCGCC	6	five_prime	1	260
NdeI	CATATG	6	five_prime	1	829
SmlI	CTYRAG	6	five_prime	1	1107
TatI	WGTACW	6	five_prime	1	148
TfiI	GAWTC	5	five_prime	1	1112
XhoI	CTCGAG	6	five_prime	1	1107
BciVI	GTATCC	6	three_prime	1	816
BfiI	ACTGGG	6	three_prime	1	591
BseMII	CTCAG	5	three_prime	1	169
BsrDI	GCAATG	6	three_prime	1	835
BstXI	CCANNNNNNTGG	6	three_prime	1	1080
BtsI	GCAGTG	6	three_prime	1	1030
EciI	GGCGGA	6	three_prime	1	1001
Eco57MI	CTGRAG	6	three_prime	1	31
GsuI	CTGGAG	6	three_prime	1	31
HphI	GGTGA	5	three_prime	1	1166
KpnI	GGTACC	6	three_prime	1	476
SduI	GDGCHC	6	three_prime	1	307
Ball	TGGCCA	6	blunt	2	502, 1065
BsrBI	CCGCTC	6	blunt	2	903, 1140
XmnI	GAANNNTTC	6	blunt	2	753, 766
AsuII	TTCGAA	6	five_prime	2	619, 817
AvaI	CYCGRG	6	five_prime	2	1107, 1118
DraII	RGGNCCY	6	five_prime	2	270, 1122
HgaI	GACGC	5	five_prime	2	4, 169
NcoI	CCATGG	6	five_prime	2	265, 1060
PleI	GAGTC	5	five_prime	2	552, 647
Thi111I	GACNNNGTC	6	five_prime	2	223, 445
AjuI	GAANNNNNNTGG	7	three_prime	2	751, 783
AlfI	GCANNNNNNTGC	6	three_prime	2	643, 677
BaeI	ACNNNGTAYC	7	three_prime	2	788, 821
BpII	GAGNNNNCTC	6	three_prime	2	1101, 1133
BseRI	GAGGAG	6	three_prime	2	143, 229
FalI	AAGNNNNCTT	6	three_prime	2	754, 786
HaeIV	GAYNNNNRNTC	6	three_prime	2	641, 675
MmeI	TCCRAC	6	three_prime	2	985, 1051
TspDTI	ATGAA	5	three_prime	2	161, 846

Table 17: Countiue

AccI	GTMKAC	6	five_prime	3	359, 440, 979
EcoRII	CCWGG	5	five_prime	3	407, 536, 1014
SalI	GTCGAC	6	five_prime	3	358, 439, 978
StyI	CCWWGG	6	five_prime	3	265, 273, 1060
HaeII	RGCGCY	6	three_prime	3	263, 332, 338
Hpy99I	CGWCG	5	three_prime	3	20, 52, 388
TsoI	TARCCA	6	three_prime	3	261, 448, 706
BccI	CCATC	5	five_prime	4	124, 691, 1053, 1094
CfrI	YGGCCR	6	five_prime	4	396, 450, 500, 1063
Tsp45I	GTSAC	5	five_prime	4	182, 914, 1133, 1172
AgsI	TTSAA	5	three_prime	4	579, 681, 734, 883
BsaXI	ACNNNNNCTCC	6	three_prime	4	82, 112, 223, 253
BsrI	ACTGG	5	three_prime	4	369, 586, 678, 869
Hin4I	GAYNNNNNVTC	6	three_prime	4	594, 626, 642, 674
TspRI	CASTG	5	three_prime	4	187, 369, 450, 1037
HindII	GTYRAC	6	blunt	5	360, 378, 441, 591, 980
EcoP15I	CAGCAG	6	five_prime	5	60, 664, 756, 1098, 1101
MboII	GAAGA	5	three_prime	5	72, 519, 525, 806, 977
TauI	GCSGC	5	three_prime	5	38, 88, 401, 424, 485
TspGWI	ACGGA	5	three_prime	5	470, 509, 513, 962, 1079
SfaNI	GCATC	5	five_prime	8	30, 96, 377, 412, 641, 866, 1153, 1169
BbvI	GCAGC	5	five_prime	9	44, 96, 290, 612, 678, 770, 911, 1115, 1163
TseI	GCWGC	5	five_prime	9	32, 109, 278, 625, 691, 783, 924, 1128, 1151

Table 18: Restriction digestion of protease gene from AY590136.1 *Aspergillus viridinutans* strain MK284

Name	Sequence	Site Length	Overhang	Frequency	Cut Positions
BtrI	CACGTC	6	blunt	1	384
Eco47III	AGCGCT	6	blunt	1	330
NaeI	GCCGGC	6	blunt	1	96
ScaI	AGTACT	6	blunt	1	150
XmnI	GAANNNTTC	6	blunt	1	753
AcyI	GRCGYC	6	five_prime	1	260
AsuII	TTCGAA	6	five_prime	1	817
Bpu10I	CCTNAGC	6	five_prime	1	1183
BseYI	CCCAGC	6	five_prime	1	893
BsmAI	GTCTC	5	five_prime	1	1110
BspHI	TCATGA	6	five_prime	1	172
BspMI	ACCTGC	6	five_prime	1	1133
BtgZI	GCGATG	6	five_prime	1	53
Cfr10I	RCCGGY	6	five_prime	1	94
DraII	RGGNCCY	6	five_prime	1	1122
Eco31I	GGTCTC	6	five_prime	1	1110
FauI	CCCGC	5	five_prime	1	954
NarI	GGCGCC	6	five_prime	1	260
PpuMI	RGGWCCY	7	five_prime	1	1122
SmlI	CTYRAG	6	five_prime	1	1107
TatI	WGTACW	6	five_prime	1	148
TfiI	GAWTC	5	five_prime	1	1112
XhoI	CTCGAG	6	five_prime	1	1107
BciVI	GTATCC	6	three_prime	1	816
BfiI	ACTGGG	6	three_prime	1	591
BseMII	CTCAG	5	three_prime	1	169
BsrDI	GCAATG	6	three_prime	1	835
BstXI	CCANNNNNTGG	6	three_prime	1	1080
BtsI	GCAGTG	6	three_prime	1	1030
EciI	GGCGGA	6	three_prime	1	1001
Eco57MI	CTGRAG	6	three_prime	1	31
GsuI	CTGGAG	6	three_prime	1	31
HphI	GGTGA	5	three_prime	1	1166
KpnI	GGTACC	6	three_prime	1	476
SduI	GDGCHC	6	three_prime	1	307
BalI	TGGCCA	6	blunt	2	502, 1065
BsrBI	CCGCTC	6	blunt	2	903, 1140
AvaI	CYCGRG	6	five_prime	2	1107, 1118
AvaII	GGWCC	5	five_prime	2	219, 1122
NcoI	CCATGG	6	five_prime	2	265, 1060
PleI	GAGTC	5	five_prime	2	552, 647
StyI	CCWWGG	6	five_prime	2	265, 1060
TthI III	GACNNGTC	6	five_prime	2	223, 445
AlfI	GCANNNNNTGC	6	three_prime	2	643, 677
BaeI	ACNNNNGTAYC	7	three_prime	2	788, 821
BcgI	CGANNNNNTGC	6	three_prime	2	420, 454
BpII	GAGNNNNNCTC	6	three_prime	2	1101, 1133
BseRI	GAGGAG	6	three_prime	2	143, 229
FalI	AAGNNNNNCTT	6	three_prime	2	754, 786
HaeIV	GAYNNNNNRTC	6	three_prime	2	641, 675
MmeI	TCCRAC	6	three_prime	2	985, 1051

Table 18: Counttue

TsoI	TARCCA	6	three_prime	2	261, 706
TspDTI	ATGAA	5	three_prime	2	161, 846
AccI	GTMKAC	6	five_prime	3	359, 440, 979
EcoRII	CCWGG	5	five_prime	3	536, 992, 1014
HgaI	GACGC	5	five_prime	3	4, 169, 435
SalI	GTCGAC	6	five_prime	3	358, 439, 978
AgsI	TTSAA	5	three_prime	3	579, 681, 734
BsrI	ACTGG	5	three_prime	3	369, 586, 678
HaeII	RGCGCY	6	three_prime	3	263, 332, 338
TspRI	CASTG	5	three_prime	3	187, 369, 1037
HindII	GTYRAC	6	blunt	4	360, 378, 441, 980
BccI	CCATC	5	five_prime	4	124, 691, 1053, 1094
CfrI	YGGCCR	6	five_prime	4	396, 450, 500, 1063
Tsp45I	GTSAC	5	five_prime	4	182, 914, 1133, 1172
BsaXI	ACNNNNNCTCC	6	three_prime	4	82, 112, 223, 253
Hin4I	GAYNNNNNVTC	6	three_prime	4	594, 626, 642, 674
Hpy99I	CGWCG	5	three_prime	4	20, 52, 388, 451
TspGWI	ACGGA	5	three_prime	4	470, 509, 513, 1079
EcoP15I	CAGCAG	6	five_prime	5	60, 664, 756, 1098, 1101
MboII	GAAGA	5	three_prime	5	72, 519, 525, 806, 977
TauI	GCSGC	5	three_prime	6	38, 88, 401, 424, 431, 485
SfaNI	GCATC	5	five_prime	8	30, 96, 377, 412, 641, 866, 1153, 1169
BbvI	GCAGC	5	five_prime	10	44, 96, 290, 573, 612, 678, 770, 911, 1115, 1163
TseI	GCWGC	5	five_prime	10	32, 109, 278, 586, 625, 691, 783, 924, 1128, 1151

Table 19: Restriction digestion of protease gene from MG867728.1 *Aspergillus sojae*

Name	Sequence	Site Length	Overhang	Frequency	Cut Positions
BsaAI	YACGTR	6	blunt	1	477
EcoRV	GATATC	6	blunt	1	990
StuI	AGGCTT	6	blunt	1	535
AccI	GTMKAC	6	five_prime	1	500
AgeI	ACCGGT	6	five_prime	1	211
BglII	AGATCT	6	five_prime	1	347
BsmAI	GTCTC	5	five_prime	1	67
BspMI	ACCTGC	6	five_prime	1	1193
Eco31I	GGTCTC	6	five_prime	1	67
HgaI	GACGC	5	five_prime	1	187
HindIII	AAGCTT	6	five_prime	1	94
NarI	GGCGCC	6	five_prime	1	206
PasI	CCCWGGG	7	five_prime	1	389
PfoI	TCCNGGA	6	five_prime	1	99
SalI	GTCGAC	6	five_prime	1	499
SgrAI	CRCCGGYG	8	five_prime	1	211
SmlI	CTYRAG	6	five_prime	1	1093
SpeI	ACTAGT	6	five_prime	1	448
TatI	WGTACW	6	five_prime	1	107
Tth111I	GACNNGTC	6	five_prime	1	1156
AatII	GACGTC	6	three_prime	1	1157
BfiI	ACTGGG	6	three_prime	1	714
BsgI	GTGCAG	6	three_prime	1	88
BsrDI	GCAATG	6	three_prime	1	599
Eco57MI	CTGRAG	6	three_prime	1	214
GsuI	CTGGAG	6	three_prime	1	214
HaeII	RGCGCY	6	three_prime	1	209
KpnI	GGTACC	6	three_prime	1	1043
MmeI	TCCRAC	6	three_prime	1	971
SacII	CCGCGG	6	three_prime	1	50
TspRI	CASTG	5	three_prime	1	909
BalI	TGGCCA	6	blunt	2	575, 1144
HindII	GTYRAC	6	blunt	2	501, 966
NaeI	GCCGGC	6	blunt	2	456, 867
AcyI	GRCGYC	6	five_prime	2	206, 1154
AvaII	GGWCC	5	five_prime	2	51, 342
EcoP15I	CAGCAG	6	five_prime	2	451, 1158
FokI	GGATG	5	five_prime	2	625, 1177
NcoI	CCATGG	6	five_prime	2	576, 1046
SapI	GCTCTTC	7	five_prime	2	916, 1017
TfiI	GAWTC	5	five_prime	2	147, 667
Tsp45I	GTSAC	5	five_prime	2	114, 1119
XhoII	RGATCY	6	five_prime	2	305, 347
BglI	GCCNNNNGGC	6	three_prime	2	532, 631
BseSI	GKGC MC	6	three_prime	2	63, 466
BsmI	GAATGC	6	three_prime	2	387, 811
BsrI	ACTGG	5	three_prime	2	709, 938
FalI	AAGNNNNNCTT	6	three_prime	2	977, 1009
TaqII	GACCGA	6	three_prime	2	636, 662
TsoI	TARCCA	6	three_prime	2	764, 1039

Table 19: Countinue

XmnI	GAANNNTTC	6	blunt	3	95, 405, 811
CfrI	YGGCCR	6	five_prime	3	573, 867, 1142
FauI	CCCGC	5	five_prime	3	54, 85, 1119
StyI	CCWWGG	6	five_prime	3	576, 1046, 1148
AgsI	TTSAA	5	three_prime	3	123, 702, 792
HphI	GGTGA	5	three_prime	3	172, 229, 676
SduI	GDGCHC	6	three_prime	3	63, 430, 466
BcgI	CGANNNNNTGC	6	three_prime	4	307, 341, 433, 467
Hpy99I	CGWCG	5	three_prime	4	203, 289, 337, 1159
Cfr10I	RCCGGY	6	five_prime	5	211, 454, 547, 844, 865
EcoRII	CCWGG	5	five_prime	5	99, 163, 388, 659, 1000
BseRI	GAGGAG	6	three_prime	5	16, 19, 316, 352, 523
TauI	GCSGC	5	three_prime	5	88, 268, 524, 913, 935
BccI	CCATC	5	five_prime	6	16, 304, 355, 691, 1039, 1099
SfaNI	GCATC	5	five_prime	6	279, 647, 786, 852, 885, 1139
MboII	GAAGA	5	three_prime	6	214, 331, 390, 933, 963, 1004
BbvI	GCAGC	5	five_prime	7	531, 686, 696, 735, 828, 1074, 1101
TseI	GCWGC	5	five_prime	7	544, 674, 709, 748, 841, 1087, 1114

Table 20: Restriction digestion of protease gene from XM_032073130.1 *Aspergillus caelatus*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsaAI	YACGTR	6	blunt	1	477
EcoRV	GATATC	6	blunt	1	990
HpaI	GTTAAC	6	blunt	1	1182
NaeI	GCCGGC	6	blunt	1	456
StuI	AGGCCT	6	blunt	1	535
XmnI	GAANNNTTC	6	blunt	1	405
AccI	GTMKAC	6	five_prime	1	500
BsmAI	GTCTC	5	five_prime	1	67
BspMI	ACCTGC	6	five_prime	1	1193
DraII	RGGNCCY	6	five_prime	1	393
Eco31I	GGTCTC	6	five_prime	1	67
EcoP15I	CAGCAG	6	five_prime	1	1084
HgaI	GACGC	5	five_prime	1	187
HindIII	AAGCTT	6	five_prime	1	94
NarI	GGCGCC	6	five_prime	1	206
NcoI	CCATGG	6	five_prime	1	1046
PasI	CCCWGGG	7	five_prime	1	389
PfoI	TCCNGGA	6	five_prime	1	99
SalI	GTCGAC	6	five_prime	1	499
SmlI	CTYRAG	6	five_prime	1	1093
TatI	WGTACW	6	five_prime	1	107
Tth111I	GACNNNGTC	6	five_prime	1	1156
XhoII	RGATCY	6	five_prime	1	305
AatII	GACGTC	6	three_prime	1	1157
BfiI	ACTGGG	6	three_prime	1	714
BsgI	GTGCAG	6	three_prime	1	88
BsmI	GAATGC	6	three_prime	1	387
BsrDI	GCAATG	6	three_prime	1	599
EciI	GGCGGA	6	three_prime	1	987
Eco57MI	CTGRAG	6	three_prime	1	214
GsuI	CTGGAG	6	three_prime	1	214
HaeII	RGCGCY	6	three_prime	1	209
KpnI	GGTACC	6	three_prime	1	1043
MmeI	TCCRAC	6	three_prime	1	971
NmeAIII	GCCGAG	6	three_prime	1	114
SacII	CCGCGG	6	three_prime	1	50
TsoI	TARCCA	6	three_prime	1	764
AcyI	GRCGYC	6	five_prime	2	206, 1154
AvaII	GGWCC	5	five_prime	2	51, 342
FokI	GGATG	5	five_prime	2	625, 1177
TfiI	GAWTC	5	five_prime	2	147, 667
Tsp45I	GTSAC	5	five_prime	2	114, 1119
BglI	GCCNNNNGGC	6	three_prime	2	532, 631
BseSI	GKGC MC	6	three_prime	2	63, 466
TspRI	CASTG	5	three_prime	2	456, 909
Ball	TGGCCA	6	blunt	3	869, 1051, 1144
HindII	GTyrAC	6	blunt	3	501, 966, 1182
Cfr10I	RCCGGY	6	five_prime	3	454, 547, 844
CfrI	YGGCCR	6	five_prime	3	867, 1049, 1142
FauI	CCCGC	5	five_prime	3	54, 85, 597
StyI	CCWWGG	6	five_prime	3	782, 1046, 1148
AgsI	TTSAA	5	three_prime	3	123, 702, 792
BsrI	ACTGG	5	three_prime	3	449, 709, 938
HphI	GGTGA	5	three_prime	3	172, 229, 676
SduI	GDGCHC	6	three_prime	3	63, 430, 466
TaqII	GACCGA	6	three_prime	4	562, 588, 636, 662

Table 20: Countiue

Hpy99I	CGWCG	5	three_prime	5	139, 203, 289, 337, 1159
MboII	GAAGA	5	three_prime	5	156, 214, 331, 390, 963
TauI	GCSGC	5	three_prime	5	88, 215, 268, 524, 913
BceI	CCATC	5	five_prime	6	16, 235, 355, 691, 1039, 1099
EcoRII	CCWGG	5	five_prime	6	99, 163, 388, 395, 659, 1000
BcgI	CGANNNNNTGC	6	three_prime	6	307, 341, 433, 467, 1093, 1127
SfaNI	GCATC	5	five_prime	7	140, 279, 578, 786, 852, 885, 1139
BseRI	GAGGAG	6	three_prime	7	16, 19, 316, 352, 523, 1002, 1005
BbvI	GCAGC	5	five_prime	8	531, 686, 696, 735, 828, 1074, 1101, 1187
TseI	GCWGC	5	five_prime	8	544, 674, 709, 748, 841, 1087, 1114, 1175

Table 21: Restriction digestion of protease gene from XM_001266851.1 *Aspergillus fischeri*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsrBI	CCGCTC	6	blunt	1	889
BtrI	CACGTC	6	blunt	1	507
Eco47III	AGCGCT	6	blunt	1	453
EcoRV	GATATC	6	blunt	1	990
NaeI	GCCGGC	6	blunt	1	276
PvuII	CAGCTG	6	blunt	1	1140
XmnI	GAANNNTTC	6	blunt	1	811
AscI	GGCGGCC	8	five_prime	1	59
AvaI	CYCGRG	6	five_prime	1	1093
AvaII	GGWCC	5	five_prime	1	342
Bpu10I	CCTNAGC	6	five_prime	1	1169
BsePI	GCCGCGC	6	five_prime	1	59
BtgZI	GCGATG	6	five_prime	1	512
Cfr10I	RCCGGY	6	five_prime	1	274
Eco31I	GGTCTC	6	five_prime	1	1096
FauI	CCCCG	5	five_prime	1	940
HindIII	AAGCTT	6	five_prime	1	1186
NarI	GGCGCC	6	five_prime	1	383
PfoI	TCCNGGA	6	five_prime	1	68
SmlI	CTYRAG	6	five_prime	1	1093
TatI	WGTACW	6	five_prime	1	107
TfiI	GAWTC	5	five_prime	1	96
Tth111I	GACNNNGTC	6	five_prime	1	568
XhoI	CTCGAG	6	five_prime	1	1093
BfiI	ACTGGG	6	three_prime	1	714
BglI	GCCNNNNNGGC	6	three_prime	1	1106
BseRI	GAGGAG	6	three_prime	1	352
BseSI	GKGC MC	6	three_prime	1	133
BsrDI	GCAATG	6	three_prime	1	1209
BtsI	GCAGTG	6	three_prime	1	1016
DrdI	GACNNNNNGTC	6	three_prime	1	823
Ecil	GGCGGA	6	three_prime	1	987
Eco57MI	CTGRAG	6	three_prime	1	214
GsuI	CTGGAG	6	three_prime	1	214
HphI	GGTGA	5	three_prime	1	1152
KpnI	GGTACC	6	three_prime	1	599
SduI	GDGCHC	6	three_prime	1	133
TsoI	TARCCA	6	three_prime	1	764
AccI	GTMKAC	6	five_prime	2	482, 965
AcyI	GRCGYC	6	five_prime	2	362, 383
BsmAI	GTCTC	5	five_prime	2	158, 1096
EcoP15I	CAGCAG	6	five_prime	2	814, 1084
NcoI	CCATGG	6	five_prime	2	388, 1046
SalI	GTCGAC	6	five_prime	2	481, 964
AlfI	GCANNNNNTGC	6	three_prime	2	6, 40
BcgI	CGANNNNNTGC	6	three_prime	2	93, 127
BpII	GAGNNNNNCTC	6	three_prime	2	1087, 1119
BseMII	CTCAG	5	three_prime	2	103, 1183
BsrI	ACTGG	5	three_prime	2	492, 709
FalI	AAGNNNNNCTT	6	three_prime	2	812, 844
Hin4I	GAYNNNNNVTC	6	three_prime	2	717, 749
MmeI	TCCRAC	6	three_prime	2	971, 1037
BalI	TGGCCA	6	blunt	3	625, 1051, 1144
HgaI	GACGC	5	five_prime	3	187, 370, 558
PleI	GAGTC	5	five_prime	3	159, 675, 770
StyI	CCWWGG	6	five_prime	3	30, 388, 1046
Tsp45I	GTSAC	5	five_prime	3	114, 1119, 1158
AgsI	TTSAA	5	three_prime	3	123, 702, 792
HaeII	RGCGCY	6	three_prime	3	386, 455, 461
HindII	GTYRAC	6	blunt	4	483, 501, 564, 966
CfrI	YGGCCR	6	five_prime	4	573, 623, 1049, 1142
BsaXI	ACNNNNNCTCC	6	three_prime	4	262, 292, 940, 970
TspRI	CASTG	5	three_prime	4	177, 492, 864, 1023
BccI	CCATC	5	five_prime	5	235, 304, 691, 1039, 1080

Table 21: Countiue

EcoRII	CCWGG	5	five_prime	5	68, 99, 191, 659, 1000
Hpy99I	CGWCG	5	three_prime	5	203, 511, 574, 820, 841
MboII	GAAGA	5	three_prime	5	105, 252, 642, 648, 963
TauI	GCSGC	5	three_prime	5	218, 268, 524, 547, 608
TspGWI	ACGGA	5	three_prime	5	593, 632, 636, 948, 1065
SfaNI	GCATC	5	five_prime	6	126, 195, 500, 535, 852, 1139
BbvI	GCAGC	5	five_prime	12	11, 72, 151, 413, 566, 696, 735, 828, 897, 1101, 1149, 1187
TseI	GCWGC	5	five_prime	12	24, 85, 139, 401, 554, 709, 748, 841, 910, 1114, 1137, 1175

Table 22: Restriction digestion of protease gene from >XM_024833434.1 *Aspergillus campestris*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BalI	TGGCCA	6	blunt	1	1048
BsrBI	CCGCTC	6	blunt	1	37
BtrI	CACGTC	6	blunt	1	81
Eco47III	AGCGCT	6	blunt	1	16
MslI	CAYNNNRTG	6	blunt	1	754
AclI	AACGTT	6	five_prime	1	494
AflIII	ACRYGT	6	five_prime	1	755
AgeI	ACCGGT	6	five_prime	1	484
BglII	AGATCT	6	five_prime	1	344
EcoP15I	CAGCAG	6	five_prime	1	514
Esp3I	CGTCTC	6	five_prime	1	1170
NarI	GGCGCC	6	five_prime	1	1199
NheI	GCTAGC	6	five_prime	1	631
SgrAI	CRCCGGYG	8	five_prime	1	1195
SgrDI	CGTCGACG	8	five_prime	1	139
TatI	WGTACW	6	five_prime	1	113
AatII	GACGTC	6	three_prime	1	1166
BfiI	ACTGGG	6	three_prime	1	711
BseSI	GKGC MC	6	three_prime	1	63
BsrI	ACTGG	5	three_prime	1	706
BstXI	CCANNNNNTGG	6	three_prime	1	1063
Eam1105I	GACNNNNNGTC	6	three_prime	1	990
MmeI	TCCRAC	6	three_prime	1	968
NspI	RCATGY	6	three_prime	1	759
StuI	AGGCCT	6	blunt	2	532, 745
AvaI	CYCGRG	6	five_prime	2	210, 1032
CfrI	YGGCCR	6	five_prime	2	47, 1046
PfoI	TCCNGGA	6	five_prime	2	686, 800
PleI	GAGTC	5	five_prime	2	672, 876
SalI	GTCGAC	6	five_prime	2	139, 961
StyI	CCWWGG	6	five_prime	2	385, 527
XhoII	RGATCY	6	five_prime	2	302, 344
BaeI	ACNNNNGTAYC	7	three_prime	2	569, 602
Eco57MI	CTGRAG	6	three_prime	2	654, 1111
GsuI	CTGGAG	6	three_prime	2	654, 1111
HaeII	RGCGCY	6	three_prime	2	18, 1202
HaeIV	GAYNNNNNRTC	6	three_prime	2	369, 1009
NmeAIII	GCCGAG	6	three_prime	2	446, 960
SacI	GAGCTC	6	three_prime	2	1100, 1139
TspGWI	ACGGA	5	three_prime	2	633, 669
HindII	GTYRAC	6	blunt	3	141, 498, 963
Tsp45I	GTSAC	5	five_prime	3	120, 1116, 1155
AgsI	TTSAA	5	three_prime	3	129, 699, 789
BglI	GCCNNNNNGGC	6	three_prime	3	92, 529, 628
EciI	GGCGGA	6	three_prime	3	73, 873, 984
KpnI	GGTACC	6	three_prime	3	581, 596, 1040
AccI	GTMKAC	6	five_prime	4	4, 140, 437, 962
BsmAI	GTCTC	5	five_prime	4	726, 764, 1076, 1170
FauI	CCCGC	5	five_prime	4	99, 211, 885, 937
FokI	GGATG	5	five_prime	4	37, 391, 670, 973
Thi111I	GACNNNGTC	6	five_prime	4	433, 718, 796, 1153
BsaXI	ACNNNNCTCC	6	three_prime	4	706, 736, 838, 868
AcyI	GRCGYC	6	five_prime	5	137, 794, 806, 1163, 1199
BseRI	GAGGAG	6	three_prime	5	16, 313, 520, 628, 1108
HphI	GGTGA	5	three_prime	5	114, 891, 991, 1110, 1149
MboII	GAAGA	5	three_prime	5	111, 346, 645, 960, 1137
SduI	GDGCHC	6	three_prime	5	63, 427, 557, 1100, 1139
BbvI	GCAGC	5	five_prime	6	132, 312, 410, 528, 1098, 1184
Cfr10I	RCCGGY	6	five_prime	6	86, 271, 484, 589, 841, 1195
EcoRII	CCWGG	5	five_prime	6	169, 260, 686, 800, 997, 1088
TseI	GCWGC	5	five_prime	6	145, 325, 398, 541, 1111, 1172
BcgI	CGANNNNNTGC	6	three_prime	6	99, 133, 502, 536, 942, 976
TauI	GCSGC	5	three_prime	6	160, 451, 551, 709, 841, 910
HgaI	GACGC	5	five_prime	7	126, 151, 171, 206, 213, 802, 814
Hin4I	GAYNNNNVTC	6	three_prime	8	336, 368, 649, 681, 706, 738, 976, 1008
SfaNI	GCATC	5	five_prime	9	215, 257, 276, 348, 369, 410, 435, 849, 882
Hpy99I	CGWCG	5	three_prime	11	142, 145, 202, 229, 445, 796, 838, 907, 964, 1153, 1165
BccI	CCATC	5	five_prime	12	16, 58, 301, 370, 604, 757, 838, 904, 919, 1036, 1038, 1096

Table 23: Restriction digestion of protease gene from XM_024825725.1 *Aspergillus novofumigatus*

Name	Sequence	Site Length	Overhang	Frequency	Cut Positions
BsrBI	CCGCTC	6	blunt	1	889
BtrI	CACGTC	6	blunt	1	507
EcoRV	GATATC	6	blunt	1	990
PvuII	CAGCTG	6	blunt	1	1140
XmnI	GAANNNTTC	6	blunt	1	811
AgeI	ACCGGT	6	five_prime	1	1162
AscI	GGCGGCC	8	five_prime	1	59
AsuII	TTCGAA	6	five_prime	1	868
AvaI	CYCGRG	6	five_prime	1	1093
AvaII	GGWCC	5	five_prime	1	342
BsePI	GCGCGC	6	five_prime	1	59
BtgZI	GCGATG	6	five_prime	1	512
Eco3II	GGTCTC	6	five_prime	1	1096
FauI	CCCGC	5	five_prime	1	940
HindIII	AAGCTT	6	five_prime	1	1186
NarI	GGCGCC	6	five_prime	1	383
PfoI	TCCNGGA	6	five_prime	1	68
SgrAI	CRCCGGYG	8	five_prime	1	454
SmlI	CTYRAG	6	five_prime	1	1093
TatI	WGTACW	6	five_prime	1	107
TfiI	GAWTC	5	five_prime	1	96
Tth111I	GACNNNGTC	6	five_prime	1	568
XhoI	CTCGAG	6	five_prime	1	1093
BfiI	ACTGGG	6	three_prime	1	714
BseRI	GAGGAG	6	three_prime	1	352
BseSI	GKGCMC	6	three_prime	1	133
BsmI	GAATGC	6	three_prime	1	3
BsrDI	GCAATG	6	three_prime	1	1209
BtsI	GCAGTG	6	three_prime	1	1016
Eco57MI	CTGRAG	6	three_prime	1	214
GsuI	CTGGAG	6	three_prime	1	214
KpnI	GGTACC	6	three_prime	1	599
MmeI	TCCRAC	6	three_prime	1	971
TsoI	TARCCA	6	three_prime	1	764
NaeI	GCCGGC	6	blunt	2	276, 456
AccI	GTMKAC	6	five_prime	2	482, 965
AcyI	GRCGYC	6	five_prime	2	362, 383
BsmAI	GTCTC	5	five_prime	2	158, 1096
EcoP15I	CAGCAG	6	five_prime	2	814, 1084
NcoI	CCATGG	6	five_prime	2	388, 1046
SalI	GTCGAC	6	five_prime	2	481, 964
AlfI	GCANNNNNTGC	6	three_prime	2	6, 40
BaeI	ACNNNNGTAYC	7	three_prime	2	212, 245
BcgI	CGANNNNNTGC	6	three_prime	2	93, 127
BpII	GAGNNNNNCTC	6	three_prime	2	1087, 1119
BseMII	CTCAG	5	three_prime	2	103, 1183
BstXI	CCANNNNNTGG	6	three_prime	2	592, 1066
Hin4I	GAYNNNNNVTC	6	three_prime	2	717, 749
HphI	GGTGA	5	three_prime	2	229, 1152
SduI	GDGCHC	6	three_prime	2	90, 133
BalI	TGGCCA	6	blunt	3	625, 1051, 1144
Cfr10I	RCCGGY	6	five_prime	3	274, 454, 1162
PleI	GAGTC	5	five_prime	3	159, 675, 770
StyI	CCWWGG	6	five_prime	3	30, 388, 1046
Tsp45I	GTSAC	5	five_prime	3	114, 1119, 1158
AgsI	TTSAA	5	three_prime	3	123, 702, 792
BsrI	ACTGG	5	three_prime	3	492, 709, 1148
HaeII	RGCGCY	6	three_prime	3	386, 455, 461
HindII	GTYRAC	6	blunt	4	483, 501, 564, 966
BccI	CCATC	5	five_prime	4	220, 304, 691, 1080
CfrI	YGGCCR	6	five_prime	4	573, 623, 1049, 1142
HgaI	GACGC	5	five_prime	4	145, 187, 370, 558
BsaXI	ACNNNNNCTCC	6	three_prime	4	262, 292, 940, 970
FalI	AAGNNNNCTT	6	three_prime	4	812, 844, 867, 899
EcoRII	CCWGG	5	five_prime	5	68, 99, 191, 659, 1000
MboII	GAAGA	5	three_prime	5	105, 252, 642, 648, 963
TauI	GCSGC	5	three_prime	5	268, 524, 547, 608, 751
TspGWI	ACGGA	5	three_prime	5	593, 632, 636, 948, 1065
SfaNI	GCATC	5	five_prime	6	195, 279, 500, 535, 852, 1139
Hpy99I	CGWCG	5	three_prime	6	83, 139, 203, 511, 574, 841
TspRI	CASTG	5	three_prime	6	177, 492, 822, 864, 1023, 1155
BbvI	GCAGC	5	five_prime	10	11, 151, 413, 566, 696, 828, 897, 1101, 1149, 1187
TseI	GCWGC	5	five_prime	10	24, 139, 401, 554, 709, 841, 910, 1114, 1137, 1175

Table 24: Restriction digestion of protease gene from XM_024813742.1 *Aspergillus candidus*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BalI	TGGCCA	6	blunt	1	1237
BsrBI	CCGCTC	6	blunt	1	226
BtrI	CACGTC	6	blunt	1	270
Eco47III	AGCGCT	6	blunt	1	205
EcoRV	GATATC	6	blunt	1	1155
MslI	CAYNNNNRTG	6	blunt	1	943
AflII	CTTAAG	6	five_prime	1	13
AflIII	ACRYGT	6	five_prime	1	944
AgeI	ACCGGT	6	five_prime	1	673
ApoI	RAATTY	6	five_prime	1	1126
BglII	AGATCT	6	five_prime	1	533
EcoP15I	CAGCAG	6	five_prime	1	610
EcoRI	GAATTC	6	five_prime	1	1126
Esp3I	CGTCTC	6	five_prime	1	1359
NarI	GGCGCC	6	five_prime	1	1388
NheI	GCTAGC	6	five_prime	1	820
PasI	CCCWGGG	7	five_prime	1	575
SalI	GTCGAC	6	five_prime	1	328
SgrAI	CRCCGGYG	8	five_prime	1	1384
SgrDI	CGTCGACG	8	five_prime	1	328
SmlI	CTYRAG	6	five_prime	1	13
StyI	CCWWGG	6	five_prime	1	716
TatI	WGTACW	6	five_prime	1	302
TfiI	GAWTC	5	five_prime	1	1545
AatII	GACGTC	6	three_prime	1	1355
BfiI	ACTGGG	6	three_prime	1	900
BseSI	GKGCMC	6	three_prime	1	252
BsgI	GTGCAG	6	three_prime	1	1516
BsrI	ACTGG	5	three_prime	1	895
BstXI	CCANNNNNNTGG	6	three_prime	1	1252
Eam1105I	GACNNNNNGTC	6	three_prime	1	1179
Eco57I	CTGAAG	6	three_prime	1	117
NspI	RCATGY	6	three_prime	1	948
TspRI	CASTG	5	three_prime	1	1540
AclI	AACGTT	6	five_prime	2	159, 683
AvaI	CYCGRG	6	five_prime	2	399, 1221
AvaII	GGWCC	5	five_prime	2	42, 84
CfrI	YGGCCR	6	five_prime	2	236, 1235
PfoI	TCCNGGA	6	five_prime	2	875, 989
PleI	GAGTC	5	five_prime	2	861, 1051
BaeI	ACNNNNGTAYC	7	three_prime	2	758, 791
BsaXI	ACNNNNCTCC	6	three_prime	2	895, 925
EciI	GGCGGA	6	three_prime	2	262, 1062
HaeII	RGCGCY	6	three_prime	2	207, 1391
SduI	GDGCHC	6	three_prime	2	252, 746
TspDTI	ATGAA	5	three_prime	2	1400, 1494
Tth111I	GACNNNGTC	6	five_prime	3	622, 907, 1342
BglI	GCCNNNNNGGC	6	three_prime	3	281, 718, 817
GsuI	CTGGAG	6	three_prime	3	843, 1044, 1300
HaeIV	GAYNNNNNRTC	6	three_prime	3	558, 1198, 1341
KpnI	GGTACC	6	three_prime	3	770, 785, 1229
MmeI	TCCRAC	6	three_prime	3	201, 1157, 1418
TspGWI	ACGGA	5	three_prime	3	822, 858, 1479
HindII	GTYRAC	6	blunt	4	157, 330, 687, 1462
StuI	AGGCCT	6	blunt	4	71, 721, 934, 973
AccI	GTMKAC	6	five_prime	4	193, 329, 626, 1520
AcyI	GRCGYC	6	five_prime	4	326, 995, 1352, 1388
BsmAI	GTCTC	5	five_prime	4	915, 953, 1265, 1359
FauI	CCCGC	5	five_prime	4	288, 400, 1074, 1126
Tsp45I	GTSAC	5	five_prime	4	3, 309, 1305, 1344
XhoII	RGATCY	6	five_prime	4	491, 533, 1284, 1323

Table 24: Countiue

BseRI	GAGGAG	6	three_prime	4	64, 502, 709, 817
Eco57MI	CTGRAG	6	three_prime	4	117, 843, 1044, 1300
HphI	GGTGA	5	three_prime	5	303, 1080, 1180, 1299, 1338
BbvI	GCAGC	5	five_prime	6	321, 501, 599, 624, 1287, 1373
Cfr10I	RCCGGY	6	five_prime	6	81, 275, 673, 778, 1030, 1384
HgaI	GACGC	5	five_prime	6	315, 340, 360, 395, 402, 1003
TseI	GCWGC	5	five_prime	6	334, 514, 587, 637, 1300, 1361
AgsI	TTSAA	5	three_prime	6	27, 318, 450, 888, 978, 1542
MboII	GAAGA	5	three_prime	6	96, 123, 300, 535, 1149, 1326
TauI	GCSGC	5	three_prime	6	349, 733, 740, 898, 1030, 1099
EcoRII	CCWGG	5	five_prime	7	358, 574, 875, 968, 989, 1186, 1277
SfaNI	GCATC	5	five_prime	10	404, 446, 465, 537, 558, 599, 624, 972, 1038, 1071
FokI	GGATG	5	five_prime	11	32, 95, 101, 217, 226, 580, 859, 1162, 1486, 1490, 1507
BcgI	CGANNNNNNTGC	6	three_prime	12	274, 288, 308, 322, 493, 527, 619, 653, 691, 725, 1131, 1165
Hin4I	GAYNNNNNVTC	6	three_prime	12	215, 247, 525, 557, 838, 870, 895, 927, 1165, 1197, 1308, 1340
Hpy99I	CGWCG	5	three_prime	12	331, 334, 391, 418, 634, 667, 1027, 1096, 1153, 1294, 1342, 1354
BccI	CCATC	5	five_prime	14	116, 205, 247, 490, 559, 793, 946, 1027, 1093, 1108, 1225, 1227, 1468, 1489

Table 25: Restriction digestion of protease gene from XM_025678012.1 *Aspergillus costaricensis*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BalI	TGGCCA	6	blunt	1	1051
BsaAI	YACGTR	6	blunt	1	333
Eco47III	AGCGCT	6	blunt	1	453
HindII	GTYRAC	6	blunt	1	966
OliI	CACNNNNGTG	6	blunt	1	416
PshAI	GACNNNNGTG	6	blunt	1	682
Smal	CCCGGG	6	blunt	1	101
AsuII	TTCGAA	6	five_prime	1	625
AvaI	CYCGRG	6	five_prime	1	99
BseYI	CCCAGC	6	five_prime	1	1110
BsmAI	GTCTC	5	five_prime	1	1131
BspMI	ACCTGC	6	five_prime	1	60
DraII	RGGNCCY	6	five_prime	1	72
MfeI	CAATTG	6	five_prime	1	702
NcoI	CCATGG	6	five_prime	1	1046
NheI	GCTAGC	6	five_prime	1	526
NotI	GCGGCCGC	8	five_prime	1	1115
PfoI	TCCNGGA	6	five_prime	1	978
PpuMI	RGGWCCY	7	five_prime	1	72
SmlI	CTYRAG	6	five_prime	1	1132
Tth111I	GACNNNGTC	6	five_prime	1	898
XhoII	RGATCY	6	five_prime	1	305
BfiI	ACTGGG	6	three_prime	1	406
BglI	GCCNNNNNGGC	6	three_prime	1	1123
BseRI	GAGGAG	6	three_prime	1	316
BsgI	GTGCAG	6	three_prime	1	872
BsrDI	GCAATG	6	three_prime	1	933
BstXI	CCANNNNNNTGG	6	three_prime	1	1188
HaeII	RGCGCY	6	three_prime	1	455
MmeI	TCCRAC	6	three_prime	1	1037
NmeAIII	GCCGAG	6	three_prime	1	997
TspDTI	ATGAA	5	three_prime	1	302
AflIII	ACRYGT	6	five_prime	2	558, 585
Cfr10I	RCCGGY	6	five_prime	2	487, 1171
PleI	GAGTC	5	five_prime	2	159, 770
SfaNI	GCATC	5	five_prime	2	500, 852
StyI	CCWWGG	6	five_prime	2	388, 1046
TfiI	GAWTC	5	five_prime	2	667, 732
AlfI	GCANNNNNNTGC	6	three_prime	2	400, 434
ArsI	GACNNNNNNNTYG	7	three_prime	2	940, 972
BaeI	ACNNNNGTAYC	7	three_prime	2	353, 386
BciVI	GTATCC	6	three_prime	2	477, 769
BseMII	CTCAG	5	three_prime	2	24, 1118
Hpy99I	CGWCG	5	three_prime	2	217, 898
KpnI	GGTACC	6	three_prime	2	357, 467
NspI	RCATGY	6	three_prime	2	562, 589
PstI	CTGCAG	6	three_prime	2	51, 549
TaqII	GACCGA	6	three_prime	2	672, 698
TspRI	CASTG	5	three_prime	2	63, 909
TstI	CACNNNNNTTCC	6	three_prime	2	628, 660
MslI	CAYNNNNRTG	6	blunt	3	320, 416, 563
CfrI	YGGCCR	6	five_prime	3	710, 1049, 1115
FauI	CCCGC	5	five_prime	3	32, 234, 267

Table 25: Countinue

FokI	GGATG	5	five_prime	3	73, 82, 706
TatI	WGTACW	6	five_prime	3	107, 205, 825
BsrI	ACTGG	5	three_prime	3	401, 1145, 1193
SacI	GAGCTC	6	three_prime	3	179, 387, 1133
SduI	GDGCHC	6	three_prime	3	179, 387, 1133
TsoI	TARCCA	6	three_prime	3	487, 1093, 1132
AvaII	GGWCC	5	five_prime	4	72, 596, 737, 956
BsmI	GAATGC	6	three_prime	4	4, 17, 813, 1158
HgaI	GACGC	5	five_prime	5	23, 194, 410, 904, 926
Tsp45I	GTSAC	5	five_prime	5	114, 574, 900, 1062, 1158
AgsI	TTSAA	5	three_prime	5	123, 238, 702, 792, 947
HphI	GGTGA	5	three_prime	5	565, 628, 676, 1152, 1237
TspGWI	ACGGA	5	three_prime	5	187, 636, 885, 948, 1214
BbvI	GCAGC	5	five_prime	6	14, 200, 252, 531, 760, 877
TseI	GCWGC	5	five_prime	6	27, 188, 265, 544, 748, 865
BsaXI	ACNNNNCTCC	6	three_prime	6	5, 35, 974, 1004, 1018, 1048
Hin4I	GAYNNNNVTC	6	three_prime	6	5, 37, 717, 749, 974, 1006
BccI	CCATC	5	five_prime	7	94, 103, 559, 688, 1039, 1080, 1228
TauI	GCSGC	5	three_prime	8	203, 608, 712, 844, 913, 1117, 1120, 1211

Table 26: Restriction digestion of protease gene from XM_026751684.1 *Aspergillus mulundensis*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
Ball	TGGCCA	6	blunt	1	1048
BsaAI	YACGTR	6	blunt	1	474
BsrBI	CCGCTC	6	blunt	1	1087
EcoRV	GATATC	6	blunt	1	987
ScaI	AGTACT	6	blunt	1	223
AccI	GTMKAC	6	five_prime	1	497
AgeI	ACCGGT	6	five_prime	1	484
ApoI	RAATTY	6	five_prime	1	508
AvaII	GGWCC	5	five_prime	1	339
EcoP15I	CAGCAG	6	five_prime	1	811
EcoRI	GAATTC	6	five_prime	1	508
NcoI	CCATGG	6	five_prime	1	1043
NheI	GCTAGC	6	five_prime	1	910
PspXI	VCTCGAGB	8	five_prime	1	67
SalI	GTCGAC	6	five_prime	1	496
SmlI	CTYRAG	6	five_prime	1	67
TfiI	GAWTC	5	five_prime	1	664
Tsp45I	GTSAC	5	five_prime	1	111
Tth111I	GACNNNGTC	6	five_prime	1	565
XhoI	CTCGAG	6	five_prime	1	67
XhoII	RGATCY	6	five_prime	1	302
BglI	GCCNNNNNGGC	6	three_prime	1	737
BseMII	CTCAG	5	three_prime	1	200
BseSI	GKGCMC	6	three_prime	1	463
BsgI	GTGCAG	6	three_prime	1	869
BsmI	GAATGC	6	three_prime	1	3
BstXI	CCANNNNNTGG	6	three_prime	1	589
DrdI	GACNNNNNGTC	6	three_prime	1	372
EciI	GGCGGA	6	three_prime	1	984
Eco57MI	CTGRAG	6	three_prime	1	211
GsuI	CTGGAG	6	three_prime	1	211
NspI	RCATGY	6	three_prime	1	559
TspRI	CASTG	5	three_prime	1	921
Eco47III	AGCGCT	6	blunt	2	16, 747
HindII	GTYRAC	6	blunt	2	498, 561
AcyI	GRCGYC	6	five_prime	2	149, 359
AvaI	CYCGRG	6	five_prime	2	67, 1032
Cfr10I	RCCGGY	6	five_prime	2	484, 736
StyI	CCWWGG	6	five_prime	2	385, 1043
TatI	WGTACW	6	five_prime	2	104, 221
BfiI	ACTGGG	6	three_prime	2	366, 711
BplI	GAGNNNNCTC	6	three_prime	2	26, 58
HphI	GGTGA	5	three_prime	2	105, 891
MboII	GAAGA	5	three_prime	2	249, 1094
TaqII	GACCGA	6	three_prime	2	125, 151
TspGWI	ACGGA	5	three_prime	2	633, 819
AcII	AACGTT	6	five_prime	3	329, 854, 1163
BccI	CCATC	5	five_prime	3	301, 688, 1036
CfrI	YGCCCR	6	five_prime	3	570, 1046, 1082
FokI	GGATG	5	five_prime	3	115, 817, 849
BseRI	GAGGAG	6	three_prime	3	31, 313, 349
KpnI	GGTACC	6	three_prime	3	354, 581, 1040
MmeI	TCCRAC	6	three_prime	3	194, 968, 1184
TsoI	TARCCA	6	three_prime	3	254, 761, 1129
FauI	CCCGC	5	five_prime	4	937, 1116, 1132, 1153

Table 26: Countinue

BaeI	ACNNNNGTAYC	7	three_prime	4	471, 504, 569, 602
BcgI	CGANNNNNTGC	6	three_prime	4	942, 952, 976, 986
BsrI	ACTGG	5	three_prime	4	219, 372, 417, 706
HaeII	RGCGCY	6	three_prime	4	18, 383, 452, 749
BbvI	GCAGC	5	five_prime	5	683, 693, 825, 894, 1184
HgaI	GACGC	5	five_prime	5	157, 184, 367, 555, 923
TseI	GCWGC	5	five_prime	5	671, 706, 838, 907, 1172
AgsI	TTSAA	5	three_prime	5	12, 120, 699, 789, 1093
SduI	GDGCHC	6	three_prime	5	39, 63, 74, 427, 463
EcoRII	CCWGG	5	five_prime	6	188, 656, 791, 975, 997, 1079
SfaNI	GCATC	5	five_prime	6	137, 276, 795, 849, 871, 1136
BsaXI	ACNNNNCTCC	6	three_prime	6	208, 238, 625, 655, 1141, 1171
Hin4I	GAYNNNNVTC	6	three_prime	6	208, 240, 850, 882, 976, 1008
TauI	GCSGC	5	three_prime	6	203, 521, 734, 1087, 1114, 1172
Hpy99I	CGWCG	5	three_prime	10	80, 151, 200, 286, 571, 694, 802, 961, 964, 1066

Table 27: Restriction digestion of protease gene from XM_025625888.1 *Aspergillus neoniger*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
Ball	TGGCCA	6	blunt	1	1063
BsaAI	YACGTR	6	blunt	1	345
Eco47III	AGCGCT	6	blunt	1	465
HindII	GTYRAC	6	blunt	1	978
NaeI	GCCGGC	6	blunt	1	243
OliI	CACNNNNGTG	6	blunt	1	428
PshAI	GACNNNNGTC	6	blunt	1	694
SmaI	CCCGGG	6	blunt	1	113
AsuII	TTCGAA	6	five_prime	1	637
AvaI	CYCGRG	6	five_prime	1	111
BsmAI	GTCTC	5	five_prime	1	1143
BspMI	ACCTGC	6	five_prime	1	72
DraII	RGGNCCY	6	five_prime	1	84
EcoRII	CCWGG	5	five_prime	1	1112
MfeI	CAATTG	6	five_prime	1	714
NcoI	CCATGG	6	five_prime	1	1058
NotI	GCGGCCGC	8	five_prime	1	1127
PfoI	TCCNGGA	6	five_prime	1	990
PpuMI	RGGWCCY	7	five_prime	1	84
SmlI	CTYRAG	6	five_prime	1	1144
Tth111I	GACNNNGTC	6	five_prime	1	910
XhoII	RGATCY	6	five_prime	1	317
BfiI	ACTGGG	6	three_prime	1	418
BglI	GCCNNNNNGGC	6	three_prime	1	1135
BseRI	GAGGAG	6	three_prime	1	328
BsgI	GTGACG	6	three_prime	1	884
BsrDI	GCAATG	6	three_prime	1	945
BstXI	CCANNNNNTGG	6	three_prime	1	1200
HaeII	RGCGCY	6	three_prime	1	467
MmeI	TCCRAC	6	three_prime	1	1049
NmeAIII	GCCGAG	6	three_prime	1	1009
TspDTI	ATGAA	5	three_prime	1	314
AflIII	ACRYGT	6	five_prime	2	570, 597
BseYI	CCCAGC	6	five_prime	2	277, 1122
PleI	GAGTC	5	five_prime	2	171, 782
StyI	CCWWGG	6	five_prime	2	400, 1058
TfiI	GAWTC	5	five_prime	2	679, 744
AlfI	GCANNNNNTGC	6	three_prime	2	412, 446
ArsI	GACNNNNNTTYG	7	three_prime	2	952, 984
BaeI	ACNNNNGTAYC	7	three_prime	2	365, 398
BciVI	GTATCC	6	three_prime	2	489, 781
BseMII	CTCAG	5	three_prime	2	36, 1130
Hpy99I	CGWCG	5	three_prime	2	229, 910
KpnI	GGTACC	6	three_prime	2	369, 479
NspI	RCATGY	6	three_prime	2	574, 601
PstI	CTGCAG	6	three_prime	2	63, 561
TaqII	GACCGA	6	three_prime	2	684, 710
TspRI	CASTG	5	three_prime	2	75, 921
TstI	CACNNNNNTCC	6	three_prime	2	640, 672
MslI	CAYNNNNRTG	6	blunt	3	332, 428, 575
Cfr10I	RCCGGY	6	five_prime	3	241, 499, 1183
CfrI	YGGCCR	6	five_prime	3	722, 1061, 1127
FauI	CCCGC	5	five_prime	3	44, 246, 279
FokI	GGATG	5	five_prime	3	85, 94, 718
SfaNI	GCATC	5	five_prime	3	254, 512, 864
TatI	WGTACW	6	five_prime	3	119, 217, 837
Tsp45I	GTSAC	5	five_prime	3	586, 912, 1074
BsrI	ACTGG	5	three_prime	3	413, 1157, 1205
SacI	GAGCTC	6	three_prime	3	191, 399, 1145

Table 27: Countiue

SduI	GDGCHC	6	three_prime	3	191, 399, 1145
TsoI	TARCCA	6	three_prime	3	499, 1105, 1144
AvaII	GGWCC	5	five_prime	4	84, 608, 749, 968
AgsI	TTSAA	5	three_prime	4	135, 714, 804, 959
BsmI	GAATGC	6	three_prime	4	16, 29, 825, 1170
BbvI	GCAGC	5	five_prime	5	26, 212, 543, 772, 889
HgaI	GACGC	5	five_prime	5	35, 206, 422, 916, 938
TseI	GCWGC	5	five_prime	5	39, 200, 556, 760, 877
HphI	GGTGA	5	three_prime	5	577, 640, 688, 1164, 1249
TspGWI	ACGGA	5	three_prime	5	199, 648, 897, 960, 1226
BsaXI	ACNNNNNCTCC	6	three_prime	6	17, 47, 986, 1016, 1030, 1060
Hin4I	GAYNNNNNVTC	6	three_prime	6	17, 49, 729, 761, 986, 1018
BccI	CCATC	5	five_prime	7	106, 115, 571, 700, 1051, 1092, 1240
TauI	GCSGC	5	three_prime	8	215, 620, 724, 856, 925, 1129, 1132, 1223

Table 28: Restriction digestion of protease gene from L19059.1 *Aspergillus niger*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsaAI	YACGTR	6	blunt	1	1165
BsaBI	GATNNNNATC	6	blunt	1	669
Eco47III	AGCGCT	6	blunt	1	1333
EcoRV	GATATC	6	blunt	1	1600
HpaI	GTTAAC	6	blunt	1	1683
PshAI	GACNNNGTC	6	blunt	1	1562
ScaI	AGTACT	6	blunt	1	1974
AarI	CACCTGC	7	five_prime	1	1684
AcyI	GRCGYC	6	five_prime	1	1878
AflIII	ACRYGT	6	five_prime	1	1465
ApoI	RAATY	6	five_prime	1	1818
AsuII	TTCGAA	6	five_prime	1	1505
AvrII	CCTAGG	6	five_prime	1	2790
BamHI	GGATCC	6	five_prime	1	2988
BspMI	ACCTGC	6	five_prime	1	1684
BtgZI	GCGATG	6	five_prime	1	2226
CfrI	YGGCCR	6	five_prime	1	2001
ClaI	ATCGAT	6	five_prime	1	427
Esp3I	CGTCTC	6	five_prime	1	2930
HindIII	AAGCTT	6	five_prime	1	1
NcoI	CCATGG	6	five_prime	1	2028
NheI	GCTAGC	6	five_prime	1	1406
PspXI	VCTCGAGB	8	five_prime	1	2863
RsrII	CGGWCCG	7	five_prime	1	2496
SpeI	ACTAGT	6	five_prime	1	2284
XbaI	TCTAGA	6	five_prime	1	2645
XhoI	CTCGAG	6	five_prime	1	2863
AlwNI	CAGNNNCTG	6	three_prime	1	2099
BciVI	GTATCC	6	three_prime	1	1177
BseRI	GAGGAG	6	three_prime	1	1145
BstXI	CCANNNNNTGG	6	three_prime	1	2170
BtsI	GCAGTG	6	three_prime	1	365
Eco57I	CTGAAG	6	three_prime	1	270
GsuI	CTGGAG	6	three_prime	1	2825
NmeAIII	GCCGAG	6	three_prime	1	1979
BtrI	CACGTC	6	blunt	2	2025, 2487
HindII	GTYRAC	6	blunt	2	1683, 1948
OliI	CACNNNGTG	6	blunt	2	1296, 2147
StuI	AGGCCT	6	blunt	2	718, 2685
AccI	GTMKAC	6	five_prime	2	785, 2281
AgeI	ACCGGT	6	five_prime	2	67, 2702
Bsp1407I	TGTACA	6	five_prime	2	313, 1198
DraII	RGGNCCY	6	five_prime	2	901, 2080
EcoNI	CCTNNNNNAGG	6	five_prime	2	161, 315
MfeI	CAATTG	6	five_prime	2	191, 1582
PpuMI	RGGWCCY	7	five_prime	2	901, 2080
Tth111I	GACNNNGTC	6	five_prime	2	28, 652
AgsI	TTSAA	5	three_prime	2	952, 1582
AlfI	GCANNNNNTGTC	6	three_prime	2	1826, 1860
BaeI	ACNNNNGTAYC	7	three_prime	2	1233, 1266
BfiI	ACTGGG	6	three_prime	2	577, 1286
BplI	GAGNNNNNCTC	6	three_prime	2	1273, 1305
BsgI	GTGCAG	6	three_prime	2	1854, 2435
Eco57MI	CTGRAG	6	three_prime	2	270, 2825
FalI	AAGNNNNNCTT	6	three_prime	2	1035, 1067
HaeII	RGCGCY	6	three_prime	2	1335, 2951
Hin4I	GAYNNNNNVTC	6	three_prime	2	2405, 2437
Hpy99I	CGWGC	5	three_prime	2	1880, 2488
KpnI	GGTACC	6	three_prime	2	1237, 2494
PstI	CTGCAG	6	three_prime	2	880, 1429

Table 28: Counttue

SphI	GCATGC	6	three_prime	2	534, 555
SmaI	CCCGGG	6	blunt	3	930, 1962, 2903
Cfr10I	RCCGGY	6	five_prime	3	67, 1988, 2702
EcoRII	CCWGG	5	five_prime	3	1170, 1410, 2319
PleI	GAGTC	5	five_prime	3	428, 988, 1650
SmlI	CTYRAG	6	five_prime	3	2114, 2379, 2863
StyI	CCWWGG	6	five_prime	3	1268, 2028, 2790
TfiI	GAWTC	5	five_prime	3	1547, 2642, 2878
BsrDI	GCAATG	6	three_prime	3	606, 1915, 2577
MmeI	TCCRAC	6	three_prime	3	935, 1432, 2615
BseYI	CCCAGC	6	five_prime	4	1094, 1334, 2092, 2396
EcoP15I	CAGCAG	6	five_prime	4	445, 829, 1166, 1765
XhoII	RGATCY	6	five_prime	4	747, 1134, 1669, 2988
ArsI	GACNNNNNTTYG	7	three_prime	4	965, 997, 1816, 1848
BdaI	TGANNNNNNTCA	6	three_prime	4	394, 428, 653, 687
BseMIII	CTCAG	5	three_prime	4	156, 475, 607, 2789
MboII	GAAGA	5	three_prime	4	263, 605, 1055, 2159
NspI	RCATGY	6	three_prime	4	534, 555, 1469, 2458
SacI	GAGCTC	6	three_prime	4	1008, 1267, 2115, 2799
TspGWI	ACGGA	5	three_prime	4	1016, 1516, 1867, 2719
TstI	CACNNNNNTTCC	6	three_prime	4	1468, 1500, 1508, 1540
AvaI	CYCGRG	6	five_prime	5	928, 1960, 2409, 2863, 2901
HgaI	GACGC	5	five_prime	5	605, 1290, 1886, 1908, 2947
Tsp45I	GTSAC	5	five_prime	5	603, 688, 2044, 2140, 2705
BsmI	GAATGC	6	three_prime	5	103, 776, 846, 1740, 2140
BsrI	ACTGG	5	three_prime	5	583, 1281, 1372, 1620, 2808
TsoI	TARCCA	6	three_prime	5	474, 1163, 1367, 2021, 2114
TspDTI	ATGAA	5	three_prime	5	360, 419, 2319, 2655, 2713
TspRI	CASTG	5	three_prime	5	365, 462, 892, 1372, 2629
MslI	CAYNNNNRTG	6	blunt	6	1149, 1155, 1296, 1443, 2027, 2147
BsmAI	GTCTC	5	five_prime	6	232, 729, 987, 2406, 2801, 2930
TaqII	GACCGA	6	three_prime	6	1552, 1578, 2097, 2123, 2487, 2513
FauI	CCCGC	5	five_prime	8	158, 315, 666, 861, 1063, 1096, 2070, 2146
SfaNI	GCATC	5	five_prime	8	136, 189, 244, 549, 1293, 1380, 1680, 1834
TatI	WGTACW	6	five_prime	8	121, 313, 936, 1034, 1198, 1972, 2601, 2767
BsaXI	ACNNNNNCTCC	6	three_prime	8	834, 864, 1956, 1986, 2000, 2030, 2532, 2562
FokI	GGATG	5	five_prime	9	288, 509, 894, 902, 911, 1586, 2414, 2449, 2772
HphI	GGTGA	5	three_prime	10	125, 174, 290, 694, 1283, 1445, 1508, 1556, 2134, 2717
TauI	GCSGC	5	three_prime	10	371, 992, 1032, 1631, 1771, 1895, 2099, 2193, 2468, 2474
BbvI	GCAGC	5	five_prime	11	45, 478, 843, 1029, 1411, 1576, 1859, 2086, 2455, 2594, 2937
TseI	GCWGC	5	five_prime	11	58, 491, 856, 1017, 1424, 1589, 1847, 2099, 2468, 2582, 2950
SduI	GDGCHC	6	three_prime	11	95, 462, 1008, 1267, 1440, 1757, 2115, 2550, 2789, 2799, 2970
AvaII	GGWCC	5	five_prime	13	44, 65, 209, 654, 704, 901, 1476, 1617, 1986, 2080, 2496, 2520, 2742
BccI	CCATC	5	five_prime	14	261, 309, 472, 530, 616, 923, 932, 1439, 1487, 1568, 2062, 2210, 2620, 2754

Table 29: Restriction digestion of protease gene from XM_025719167.1 *Aspergillus ibericus*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BalI	TGGCCA	6	blunt	1	1282
BsaBI	GATNNNNATC	6	blunt	1	356
NaeI	GCCGGC	6	blunt	1	414
NruI	TCGCGA	6	blunt	1	998
SmaI	CCCGGG	6	blunt	1	1251
SspI	AATATT	6	blunt	1	1128
AccI	GTMKAC	6	five_prime	1	1103
AvaI	CYCGRG	6	five_prime	1	1249
BsmAI	GTCTC	5	five_prime	1	1010
Clal	ATCGAT	6	five_prime	1	1183
EcoP15I	CAGCAG	6	five_prime	1	157
Esp3I	CGTCTC	6	five_prime	1	1010
FokI	GGATG	5	five_prime	1	844
NdeI	CATATG	6	five_prime	1	753
SalI	GTCGAC	6	five_prime	1	1102
SexAI	ACCWGGT	7	five_prime	1	482
SmlI	CTYRAG	6	five_prime	1	1270
StyI	CCWWGG	6	five_prime	1	526
TatI	WGTACW	6	five_prime	1	343
Tth111I	GACNNNGTC	6	five_prime	1	484
AatII	GACGTC	6	three_prime	1	820
BseMIII	CTCAG	5	three_prime	1	31
BseSI	GKGCMC	6	three_prime	1	604
BsmI	GAATGC	6	three_prime	1	774
BsrDI	GCAATG	6	three_prime	1	1071
DrdI	GACNNNNNGTC	6	three_prime	1	505
HaeII	RGCGCY	6	three_prime	1	593
SacI	GAGCTC	6	three_prime	1	814
TsoI	TARCCA	6	three_prime	1	113
TspGWI	ACGGA	5	three_prime	1	774

Table 29: Counttue

TspRI	CASTG	5	three_prime	1	573
HindII	GTyrAC	6	blunt	2	702, 1104
PshAI	GACNNNGTC	6	blunt	2	41, 820
AcyI	GRCGYC	6	five_prime	2	817, 1351
BglII	AGATCT	6	five_prime	2	357, 776
BtgZI	GCGATG	6	five_prime	2	1013, 1388
CfrI	YGGCCR	6	five_prime	2	657, 1280
DraII	RGGNCCY	6	five_prime	2	62, 531
FauI	CCCGC	5	five_prime	2	736, 975
HgaI	GACGC	5	five_prime	2	992, 1340
SgrAI	CRCCGGYG	8	five_prime	2	592, 685
BciVI	GTATCC	6	three_prime	2	615, 640
BfiI	ACTGGG	6	three_prime	2	16, 852
BglI	GCCNNNNNGGC	6	three_prime	2	335, 670
BseRI	GAGGAG	6	three_prime	2	454, 661
Hin4I	GAYNNNNNVTC	6	three_prime	2	1176, 1208
KpnI	GGTACC	6	three_prime	2	176, 495
NspI	RCATGY	6	three_prime	2	700, 727
TaqII	GACCGA	6	three_prime	2	810, 836
XcmI	CCANNNNNNNNTGG	6	three_prime	2	235, 649
MslI	CAYNNNNRTG	6	blunt	3	458, 895, 1183
Cfr10I	RCCGGY	6	five_prime	3	412, 592, 685
PleI	GAGTC	5	five_prime	3	50, 908, 1414
BsrI	ACTGG	5	three_prime	3	11, 207, 847
MmeI	TCCRAC	6	three_prime	3	348, 690, 1175
SduI	GDGCHC	6	three_prime	3	525, 604, 814
TauI	GCSGC	5	three_prime	3	685, 850, 1258
TspDTI	ATGAA	5	three_prime	3	368, 440, 1391
XhoII	RGATCY	6	five_prime	4	224, 357, 443, 776
BcgI	CGANNNNNNTGC	6	three_prime	4	1064, 1093, 1098, 1127
AvaII	GGWCC	5	five_prime	5	480, 734, 1010, 1093, 1148
Tsp45I	GTSAC	5	five_prime	5	507, 1038, 1200, 1296, 1366
AgsI	TTSAA	5	three_prime	5	150, 261, 289, 840, 930
HphI	GGTGA	5	three_prime	5	26, 208, 607, 1290, 1378
BbvI	GCAGC	5	five_prime	6	152, 319, 338, 873, 991, 1035
EcoRII	CCWGG	5	five_prime	6	64, 237, 482, 668, 1116, 1138
TfiI	GAWTC	5	five_prime	6	289, 371, 805, 836, 993, 1439
TseI	GCWGC	5	five_prime	6	165, 307, 326, 886, 979, 1048
Hpy99I	CGWCG	5	three_prime	6	370, 819, 822, 943, 1363, 1366
MboII	GAAGA	5	three_prime	6	183, 367, 487, 1315, 1390, 1442
SfaNI	GCATC	5	five_prime	8	267, 551, 568, 990, 1023, 1030, 1084, 1374
BccI	CCATC	5	five_prime	10	85, 132, 442, 745, 826, 898, 1177, 1218, 1418, 1427

Table 30: Restriction digestion of protease gene from XM_025536473.1 *Aspergillus eucaalypticola*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
Ball	TGGCCA	6	blunt	1	1051
BsaAI	YACGTR	6	blunt	1	333
Eco47III	AGCGCT	6	blunt	1	453
HindII	GTyrAC	6	blunt	1	966
OliI	CACNNNGTG	6	blunt	1	416
PshAI	GACNNNGTC	6	blunt	1	682
SmaI	CCCGGG	6	blunt	1	101
AsuII	TTCGAA	6	five_prime	1	625
AvaI	CYCGRG	6	five_prime	1	99
BseYI	CCCAGC	6	five_prime	1	265
BsmAI	GTCTC	5	five_prime	1	1131
BspMI	ACCTGC	6	five_prime	1	60
DraII	RGGNCCY	6	five_prime	1	72
MfeI	CAATTG	6	five_prime	1	702
NcoI	CCATGG	6	five_prime	1	1046
NheI	GCTAGC	6	five_prime	1	526
NotI	GCGGCCGC	8	five_prime	1	1115
PfoI	TCCNGGA	6	five_prime	1	978
PpuMI	RGGWCCY	7	five_prime	1	72
SmlI	CTYRAG	6	five_prime	1	1132
XhoII	RGATCY	6	five_prime	1	305
BfiI	ACTGGG	6	three_prime	1	406
BglI	GCCNNNNNGGC	6	three_prime	1	1123
BseRI	GAGGAG	6	three_prime	1	316
BsgI	GTGCAG	6	three_prime	1	872
BsrDI	GCAATG	6	three_prime	1	933
BstXI	CCANNNNNNTGG	6	three_prime	1	1188
Eco57MI	CTGRAG	6	three_prime	1	1001
GsuI	CTGGAG	6	three_prime	1	1001
HaeII	RGCGCY	6	three_prime	1	455
Hpy99I	CGWCG	5	three_prime	1	220
NmeAIII	GCCGAG	6	three_prime	1	997

Table 30: Countinue

NspI	RCATGY	6	three_prime	1	589
TspDTI	ATGAA	5	three_prime	1	302
NaeI	GCCGGC	6	blunt	2	129, 231
AflIII	ACRYGT	6	five_prime	2	558, 585
CfrI	YGGCCR	6	five_prime	2	1049, 1115
EcoRII	CCWGG	5	five_prime	2	978, 1100
HgaI	GACGC	5	five_prime	2	194, 410
PleI	GAGTC	5	five_prime	2	159, 770
StyI	CCWWGG	6	five_prime	2	388, 1046
TfiI	GAWTC	5	five_prime	2	667, 732
AlfI	GCANNNNNNTGC	6	three_prime	2	400, 434
BaeI	ACNNNGTAYC	7	three_prime	2	353, 386
BciVI	GTATCC	6	three_prime	2	477, 769
KpnI	GGTACC	6	three_prime	2	357, 467
PstI	CTGCAG	6	three_prime	2	51, 549
TaqII	GACCGA	6	three_prime	2	672, 698
TspRI	CASTG	5	three_prime	2	63, 909
TstI	CACNNNNNTCC	6	three_prime	2	628, 660
MslI	CAYNNNNRTG	6	blunt	3	320, 416, 563
FauI	CCCGC	5	five_prime	3	32, 234, 267
TatI	WGTACW	6	five_prime	3	107, 205, 825
Tsp45I	GTSAC	5	five_prime	3	900, 1062, 1158
BsmI	GAATGC	6	three_prime	3	17, 813, 1158
BsrI	ACTGG	5	three_prime	3	401, 1145, 1193
MmeI	TCCRAC	6	three_prime	3	106, 552, 1037
SacI	GAGCTC	6	three_prime	3	179, 387, 1133
SduI	GDGCHC	6	three_prime	3	179, 387, 1133
TsoI	TARCCA	6	three_prime	3	487, 1093, 1132
AvaII	GGWCC	5	five_prime	4	72, 596, 737, 956
Cfr10I	RCCGGY	6	five_prime	4	127, 229, 487, 1171
FokI	GGATG	5	five_prime	4	65, 73, 82, 706
SfaNI	GCATC	5	five_prime	4	242, 500, 852, 885
AgsI	TTSAA	5	three_prime	4	123, 702, 792, 947
ArsI	GACNNNNNTTYG	7	three_prime	4	136, 168, 940, 972
Hin4I	GAYNNNNNVTC	6	three_prime	4	717, 749, 974, 1006
HphI	GGTGA	5	three_prime	4	565, 628, 676, 1237
TspGWI	ACGGA	5	three_prime	4	187, 636, 885, 948
BbvI	GCAGC	5	five_prime	5	14, 531, 721, 760, 877
TseI	GCWGC	5	five_prime	5	27, 544, 709, 748, 865
BceI	CCATC	5	five_prime	6	94, 103, 559, 688, 1080, 1228
BsaXI	ACNNNNNTCC	6	three_prime	6	5, 35, 628, 658, 974, 1004
TauI	GCSGC	5	three_prime	7	203, 608, 844, 913, 1117, 1120, 1211

Table 31: Restriction digestion of protease gene from XM_025662057.1 *Aspergillus piperis*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsaAI	YACGTR	6	blunt	1	676
BsaBI	GATNNNNATC	6	blunt	1	186
Eco47III	AGCGCT	6	blunt	1	796
EcoRV	GATATC	6	blunt	1	1063
NaeI	GCCGGC	6	blunt	1	574
OliI	CACNNNGTG	6	blunt	1	759
PshAI	GACNNNGTC	6	blunt	1	1025
PvuII	CAGCTG	6	blunt	1	142
AccI	GTMKAC	6	five_prime	1	1640
ApoI	RAATTY	6	five_prime	1	283
AsuII	TTCGAA	6	five_prime	1	968
BglII	AGATCT	6	five_prime	1	262
BspMI	ACCTGC	6	five_prime	1	403
DraII	RGGNCCY	6	five_prime	1	415
EcoRI	GAATTC	6	five_prime	1	283
MfeI	CAATTG	6	five_prime	1	1045
NcoI	CCATGG	6	five_prime	1	1389
NheI	GCTAGC	6	five_prime	1	869
NotI	GCGGCCGC	8	five_prime	1	1458
PfoI	TCCNGGA	6	five_prime	1	1321
PpuMI	RGGWCCY	7	five_prime	1	415
SmlI	CTYRAG	6	five_prime	1	1475
SpeI	ACTAGT	6	five_prime	1	1643
BciVI	GTATCC	6	three_prime	1	820
BfiI	ACTGGG	6	three_prime	1	749
BglI	GCCNNNNNGGC	6	three_prime	1	1466
BseSI	GKGCMC	6	three_prime	1	176
BsgI	GTGCAG	6	three_prime	1	1215
BstXI	CCANNNNNNTGG	6	three_prime	1	1531
BtsI	GCAGTG	6	three_prime	1	25
HaeII	RGCGCY	6	three_prime	1	798
MboII	GAAGA	5	three_prime	1	46

Table 31: Countinue

NmeAIII	GCCGAG	6	three_prime	1	1340
HindII	GTyrAC	6	blunt	2	228, 1309
AflIII	ACRYGT	6	five_prime	2	901, 928
BseYI	CCCAGC	6	five_prime	2	608, 1453
BsmAI	GTCTC	5	five_prime	2	245, 1474
FauI	CCCGC	5	five_prime	2	375, 610
PleI	GAGTC	5	five_prime	2	502, 1113
StyI	CCWWGG	6	five_prime	2	731, 1389
XhoII	RGATCY	6	five_prime	2	262, 648
AlfI	GCANNNNNNTGC	6	three_prime	2	743, 777
BaeI	ACNNNGTAYC	7	three_prime	2	696, 729
BdaI	TGANNNNNNTCA	6	three_prime	2	170, 204
BseMII	CTCAG	5	three_prime	2	367, 1461
BseRI	GAGGAG	6	three_prime	2	307, 659
BsrDI	GCAATG	6	three_prime	2	270, 1276
Hpy99I	CGWCG	5	three_prime	2	105, 560
KpnI	GGTACC	6	three_prime	2	700, 810
NspI	RCATGY	6	three_prime	2	905, 932
PstI	CTGCAG	6	three_prime	2	394, 892
TaqII	GACCGA	6	three_prime	2	1015, 1041
TsoI	TARCCA	6	three_prime	2	830, 1436
TspDTI	ATGAA	5	three_prime	2	20, 645
TstI	CACNNNNNTCC	6	three_prime	2	971, 1003
Ball	TGGCCA	6	blunt	3	146, 1394, 1487
MslI	CAYNNNNRTG	6	blunt	3	663, 759, 906
AvaII	GGWCC	5	five_prime	3	415, 939, 1080
Cfr10I	RCCGGY	6	five_prime	3	572, 830, 1514
HgaI	GACGC	5	five_prime	3	366, 537, 1269
TfiI	GAWTC	5	five_prime	3	93, 1010, 1075
BsrI	ACTGG	5	three_prime	3	744, 1488, 1536
MmeI	TCCRAC	6	three_prime	3	449, 895, 1380
SacI	GAGCTC	6	three_prime	3	522, 730, 1476
TspRI	CASTG	5	three_prime	3	25, 406, 1252
EcoRII	CCWGG	5	five_prime	4	41, 442, 873, 1443
FokI	GGATG	5	five_prime	4	408, 416, 425, 1049
SfaNI	GCATC	5	five_prime	4	585, 843, 1195, 1584
TatI	WGTACW	6	five_prime	4	85, 450, 548, 1168
ArsI	GACNNNNNNTTYG	7	three_prime	4	225, 257, 479, 511
BsmI	GAATGC	6	three_prime	4	291, 360, 1156, 1501
SduI	GDGCHC	6	three_prime	4	176, 522, 730, 1476
CfrI	YGGCCR	6	five_prime	5	144, 949, 1392, 1458, 1485
Tsp45I	GTSAC	5	five_prime	5	205, 457, 1243, 1405, 1501
BsaXI	ACNNNNNTCC	6	three_prime	6	348, 378, 1317, 1347, 1361, 1391
Hin4I	GAYNNNNNVTCT	6	three_prime	6	348, 380, 1060, 1092, 1317, 1349
HphI	GGTGA	5	three_prime	6	211, 908, 971, 1019, 1495, 1580
TspGWI	ACGGA	5	three_prime	6	178, 530, 979, 1228, 1291, 1557
BccI	CCATC	5	five_prime	7	437, 446, 902, 1031, 1382, 1423, 1571
AgsI	TTSAA	5	three_prime	7	93, 288, 296, 466, 1045, 1135, 1290
BbvI	GCAGC	5	five_prime	8	138, 339, 357, 543, 874, 1064, 1103, 1220
TseI	GCWGC	5	five_prime	8	151, 327, 370, 531, 887, 1052, 1091, 1208
TauI	GCSGC	5	three_prime	8	31, 546, 951, 1187, 1256, 1460, 1463, 1554

Table 32: Restriction digestion of protease gene from XM_025711220.1 *Aspergillus vadensis*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
Ball	TGGCCA	6	blunt	1	1054
BsaAI	YACGTR	6	blunt	1	333
Eco47III	AGCGCT	6	blunt	1	453
HindII	GTyrAC	6	blunt	1	969
OliI	CACNNNGTG	6	blunt	1	416
PshAI	GACNNNGTC	6	blunt	1	682
PvuII	CAGCTG	6	blunt	1	1129
Smal	CCCGGG	6	blunt	1	101
AsuII	TTCGAA	6	five_prime	1	625
AvaI	CYCGRG	6	five_prime	1	99
BamHI	GGATCC	6	five_prime	1	958
BseYI	CCCAGC	6	five_prime	1	265
BsmAI	GTCTC	5	five_prime	1	1134
DraII	RGGNCCY	6	five_prime	1	72
EcoRII	CCWGG	5	five_prime	1	1103
MfeI	CAATTG	6	five_prime	1	702
NcoI	CCATGG	6	five_prime	1	1049
NheI	GCTAGC	6	five_prime	1	526
NotI	GCGGCCGC	8	five_prime	1	1118
PfoI	TCCNGGA	6	five_prime	1	981
PpuMI	RGGWCCY	7	five_prime	1	72
SmlI	CTYRAG	6	five_prime	1	1135
SpeI	ACTAGT	6	five_prime	1	877

Table 32: Countiue

AlwNI	CAGNNNCTG	6	three_prime	1	889
BfiI	ACTGGG	6	three_prime	1	406
BseMII	CTCAG	5	three_prime	1	1121
BseRI	GAGGAG	6	three_prime	1	316
BsgI	GTGCAG	6	three_prime	1	875
BsrDI	GCAATG	6	three_prime	1	936
BstXI	CCANNNNNNTGG	6	three_prime	1	1191
Hpy99I	CGWCG	5	three_prime	1	217
NmeAIII	GCCGAG	6	three_prime	1	1000
NspI	RCATGY	6	three_prime	1	589
TspDTI	ATGAA	5	three_prime	1	302
NaeI	GCCGGC	6	blunt	2	129, 231
AflIII	ACRYGT	6	five_prime	2	558, 585
AvaII	GGWCC	5	five_prime	2	72, 737
BspMI	ACCTGC	6	five_prime	2	60, 583
CfrI	YGGCCR	6	five_prime	2	1052, 1118
FokI	GGATG	5	five_prime	2	73, 706
PleI	GAGTC	5	five_prime	2	159, 770
StyI	CCWWGG	6	five_prime	2	388, 1049
TfiI	GAWTC	5	five_prime	2	667, 732
XhoII	RGATCY	6	five_prime	2	305, 958
AlfI	GCANNNNNNTGC	6	three_prime	2	400, 434
ArsI	GACNNNNNNTTYG	7	three_prime	2	136, 168
BaeI	ACNNNNGTAYC	7	three_prime	2	353, 386
BciVI	GTATCC	6	three_prime	2	477, 772
HaeII	RGCCGY	6	three_prime	2	388, 455
MmeI	TCCRAC	6	three_prime	2	552, 1040
PstI	CTGCAG	6	three_prime	2	51, 549
SacI	GAGCTC	6	three_prime	2	179, 1136
SduI	GDGCHC	6	three_prime	2	179, 1136
TaqII	GACCGA	6	three_prime	2	672, 698
TspRI	CASTG	5	three_prime	2	63, 912
TstI	CACNNNNNNTCC	6	three_prime	2	628, 660
MslI	CAYNNNNRTG	6	blunt	3	320, 416, 563
FauI	CCCCG	5	five_prime	3	32, 234, 267
HgaI	GACGC	5	five_prime	3	194, 410, 929
SfaNI	GCATC	5	five_prime	3	242, 500, 855
TatI	WGTACW	6	five_prime	3	107, 205, 828
BsmI	GAATGC	6	three_prime	3	17, 816, 1161
BsrI	ACTGG	5	three_prime	3	401, 1148, 1196
KpnI	GGTACC	6	three_prime	3	357, 467, 599
TsoI	TARCCA	6	three_prime	3	487, 1096, 1135
Cfr10I	RCCGGY	6	five_prime	4	127, 229, 487, 1174
Tsp45I	GTSAC	5	five_prime	4	114, 903, 1065, 1161
AgsI	TTSAA	5	three_prime	4	123, 702, 795, 950
Hin4I	GAYNNNNNVTC	6	three_prime	4	717, 749, 977, 1009
TspGWI	ACGGA	5	three_prime	5	187, 636, 888, 951, 1217
BbvI	GCAGC	5	five_prime	6	14, 200, 531, 721, 760, 880
BecI	CCATC	5	five_prime	6	94, 559, 688, 1042, 1083, 1231
TseI	CGWGC	5	five_prime	6	27, 188, 544, 709, 748, 868
BsaXI	ACNNNNNCTCC	6	three_prime	6	5, 35, 977, 1007, 1021, 1051
HphI	GGTGA	5	three_prime	6	70, 565, 628, 676, 1155, 1240
TauI	GCSGC	5	three_prime	7	203, 608, 847, 916, 1120, 1123, 1214

Table 33: Restriction digestion of protease gene from XM_024843524.1 *Aspergillus steynii*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BalI	TGGCCA	6	blunt	1	1078
BsrBI	CCGCTC	6	blunt	1	128
NaeI	GCCGGC	6	blunt	1	459
XmnI	GAANNNTTC	6	blunt	1	247
AclI	AACGTT	6	five_prime	1	500
AcyI	GRCGYC	6	five_prime	1	962
AgeI	ACCGGT	6	five_prime	1	382
AvaII	GGWCC	5	five_prime	1	345
BspMI	ACCTGC	6	five_prime	1	1094
BtgZI	GCGATG	6	five_prime	1	1133
ClaI	ATCGAT	6	five_prime	1	302
DraII	RGGNCCY	6	five_prime	1	396
EcoP15I	CAGCAG	6	five_prime	1	58
FokI	GGATG	5	five_prime	1	163
HgaI	GACGC	5	five_prime	1	561
NcoI	CCATGG	6	five_prime	1	1049
NheI	GCTAGC	6	five_prime	1	529
NotI	GCGGCCGC	8	five_prime	1	522
SalI	GTCGAC	6	five_prime	1	565
SexAI	ACCWGGT	7	five_prime	1	347
SgrAI	CRCCGGY	8	five_prime	1	457

Table 33: Countiue

StyI	CCWWGG	6	five_prime	1	1049
TatI	WGTACW	6	five_prime	1	107
AatII	GACGTC	6	three_prime	1	965
BfiI	ACTGGG	6	three_prime	1	717
BseMII	CTCAG	5	three_prime	1	80
BstXI	CCANNNNNNTGG	6	three_prime	1	1069
Eam1105I	GACNNNNNGTC	6	three_prime	1	996
Eco57MI	CTGRAG	6	three_prime	1	999
GsuI	CTGGAG	6	three_prime	1	999
HaeIV	GAYNNNNNRTC	6	three_prime	1	1015
MmeI	TCCRAC	6	three_prime	1	974
PstI	CTGCAG	6	three_prime	1	1091
SacI	GAGCTC	6	three_prime	1	1145
Sse8387I	CCTGCAGG	8	three_prime	1	1091
TsoI	TARCCA	6	three_prime	1	1042
TspRI	CASTG	5	three_prime	1	495
XcmI	CCANNNNNNNNTGG	6	three_prime	1	514
BtrI	CACGTC	6	blunt	2	564, 591
AccI	GTMKAC	6	five_prime	2	443, 566
Eco31I	GGTCTC	6	five_prime	2	868, 1108
SmlI	CTYRAG	6	five_prime	2	10, 1096
Tth111I	GACNNNGTC	6	five_prime	2	349, 571
XhoII	RGATCY	6	five_prime	2	95, 308
BaeI	ACNNNGTAYC	7	three_prime	2	477, 510
BciVI	GTATCC	6	three_prime	2	694, 772
BpII	GAGNNNNNCTC	6	three_prime	2	26, 58
BseRI	GAGGAG	6	three_prime	2	16, 19
BseSI	GKGC MC	6	three_prime	2	63, 469
Hin4I	GAYNNNNNVTC	6	three_prime	2	982, 1014
HindII	GTYRAC	6	blunt	3	486, 504, 567
FauI	CCCGC	5	five_prime	3	54, 205, 745
PleI	GAGTC	5	five_prime	3	225, 286, 678
Tsp45I	GTSAC	5	five_prime	3	114, 958, 1122
AgsI	TTSAA	5	three_prime	3	123, 252, 795
BsrI	ACTGG	5	three_prime	3	423, 495, 712
HaeII	RGCGCY	6	three_prime	3	323, 458, 1208
KpnI	GGTACC	6	three_prime	3	360, 602, 1046
SduI	GDGCHC	6	three_prime	3	63, 469, 1145
TspGWI	ACGGA	5	three_prime	3	596, 639, 713
BbvI	GCAGC	5	five_prime	4	72, 165, 900, 1190
BsmAI	GTCTC	5	five_prime	4	630, 770, 868, 1108
Cfr10I	RCCGGY	6	five_prime	4	382, 457, 595, 847
CfrI	YGGCCR	6	five_prime	4	522, 576, 984, 1076
TseI	GCWGC	5	five_prime	4	85, 153, 913, 1178
SfaNI	GCATC	5	five_prime	5	140, 195, 354, 855, 1142
HphI	GGTGA	5	three_prime	5	825, 970, 1098, 1116, 1155
EcoRII	CCWGG	5	five_prime	7	163, 347, 391, 398, 662, 986, 1003
BccI	CCATC	5	five_prime	8	58, 73, 238, 307, 415, 763, 1042, 1087
BsaXI	ACNNNNNCTCC	6	three_prime	8	265, 295, 631, 661, 844, 874, 1021, 1051
Hpy99I	CGWCG	5	three_prime	8	451, 568, 577, 595, 700, 808, 913, 967
MboII	GAAGA	5	three_prime	8	45, 105, 255, 352, 651, 665, 668, 966
TauI	GCSGC	5	three_prime	8	131, 271, 524, 527, 557, 715, 754, 847

Table 34: Restriction digestion of protease gene from XM_033554602.1 *Aspergillus lentulus*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsrBI	CCGCTC	6	blunt	1	889
BtrI	CACGTC	6	blunt	1	507
EcoRV	GATATC	6	blunt	1	990
NruI	TCGCGA	6	blunt	1	203
PvuII	CAGCTG	6	blunt	1	1140
XmnI	GAANNNTTC	6	blunt	1	811
AcyI	GRCGYC	6	five_prime	1	383
AscI	GGCGGCC	8	five_prime	1	59
AvaII	GGWCC	5	five_prime	1	342
BsePI	GCGCGC	6	five_prime	1	59
BtgZI	GCGATG	6	five_prime	1	512
Eco31I	GGTCTC	6	five_prime	1	1096
FauI	CCCGC	5	five_prime	1	940
HindIII	AAGCTT	6	five_prime	1	1186
NarI	GGCGCC	6	five_prime	1	383
PfoI	TCCNGGA	6	five_prime	1	68
SapI	GCTCTC	7	five_prime	1	235
SgrAI	CRCCGGYG	8	five_prime	1	454
SmlI	CTYRAG	6	five_prime	1	1093
TatI	WGTACW	6	five_prime	1	107
TfiI	GAWTC	5	five_prime	1	96
Tth111I	GACNNNGTC	6	five_prime	1	568

Table 34: Countiue

XhoI	CTCGAG	6	five_prime	1	1093
BfiI	ACTGGG	6	three_prime	1	714
BglI	GCCNNNNNGGC	6	three_prime	1	1106
BseMIII	CTCAG	5	three_prime	1	103
BseRI	GAGGAG	6	three_prime	1	352
BseSI	GKGC MC	6	three_prime	1	133
BsrDI	GCAATG	6	three_prime	1	1209
BtsI	GCAGTG	6	three_prime	1	1016
EciI	GGCGGA	6	three_prime	1	987
Eco57MI	CTGRAG	6	three_prime	1	214
GsuI	CTGGAG	6	three_prime	1	214
HphI	GGTGA	5	three_prime	1	1152
KpnI	GGTACC	6	three_prime	1	599
SduI	GDGCHC	6	three_prime	1	133
TsoI	TARCCA	6	three_prime	1	764
TspDTI	ATGAA	5	three_prime	1	833
NaeI	GCCGGC	6	blunt	2	276, 456
AccI	GTMKAC	6	five_prime	2	482, 965
AvaI	CYCGRG	6	five_prime	2	1093, 1169
BsmAI	GTCTC	5	five_prime	2	158, 1096
Cfr10I	RCCGGY	6	five_prime	2	274, 454
EcoP15I	CAGCAG	6	five_prime	2	814, 1084
HgaI	GACGC	5	five_prime	2	187, 558
NcoI	CCATGG	6	five_prime	2	388, 1046
SalI	GTCGAC	6	five_prime	2	481, 964
AlfI	GCANNNNNNTGC	6	three_prime	2	6, 40
ArsI	GACNNNNNNNTTYG	7	three_prime	2	212, 244
BplI	GAGNNNNNCTC	6	three_prime	2	1087, 1119
BsrI	ACTGG	5	three_prime	2	492, 709
Hin4I	GAYNNNNNVTC	6	three_prime	2	717, 749
MmeI	TCCRAC	6	three_prime	2	971, 1037
BalI	TGGCCA	6	blunt	3	625, 1051, 1144
BclI	CCATC	5	five_prime	3	304, 1039, 1080
PleI	GAGTC	5	five_prime	3	159, 675, 770
StyI	CCWWGG	6	five_prime	3	30, 388, 1046
Tsp45I	GTSAC	5	five_prime	3	114, 1119, 1158
AgsI	TTSAA	5	three_prime	3	123, 702, 792
HaeII	RGCGCY	6	three_prime	3	386, 455, 461
HindIII	GYRAC	6	blunt	4	483, 501, 564, 966
CfrI	YGGCCR	6	five_prime	4	573, 623, 1049, 1142
BcgI	CGANNNNNNTGC	6	three_prime	4	93, 127, 505, 539
BsaXI	ACNNNNNCTCC	6	three_prime	4	262, 292, 940, 970
FalI	AAGNNNNNCTT	6	three_prime	4	812, 844, 867, 899
TspRI	CASTG	5	three_prime	4	177, 492, 864, 1023
EcoRII	CCWGG	5	five_prime	5	68, 99, 191, 659, 1000
TauI	GCSGC	5	three_prime	5	218, 268, 547, 608, 913
TspGWI	ACGGA	5	three_prime	5	593, 632, 636, 948, 1065
Hpy99I	CGWCG	5	three_prime	6	83, 203, 232, 511, 574, 841
MboII	GAAGA	5	three_prime	6	105, 252, 642, 648, 662, 963
SfaNI	GCATC	5	five_prime	7	126, 276, 351, 500, 535, 852, 1139
BbvI	GCAGC	5	five_prime	12	11, 72, 151, 276, 413, 566, 696, 735, 828, 1101, 1149, 1187
TseI	GCWGC	5	five_prime	12	24, 85, 139, 289, 401, 554, 709, 748, 841, 1114, 1137, 1175

Table 35: Restriction digestion of protease gene from XM_001258144.1 *Aspergillus fischeri*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
BsaAI	YACGTR	6	blunt	1	15
EcoRV	GATATC	6	blunt	1	1806
FspAI	RTGCGCAY	8	blunt	1	1784
MslI	CAYNNNNRTG	6	blunt	1	657
NaeI	GCCGGC	6	blunt	1	336
ScaI	AGTACT	6	blunt	1	424
StuI	AGGCCT	6	blunt	1	139
AflIII	ACRYGT	6	five_prime	1	12
ApoI	RAATTY	6	five_prime	1	818
AvrII	CCTAGG	6	five_prime	1	21
BglII	AGATCT	6	five_prime	1	1052
BsePI	GCCGGC	6	five_prime	1	1473
PasI	CCCWGGG	7	five_prime	1	825
PfoI	TCCNGGA	6	five_prime	1	109
SgrAI	CRCCGGYG	8	five_prime	1	1692
SgrDI	CGTCGACG	8	five_prime	1	853
TatI	WGTACW	6	five_prime	1	422
AatII	GACGTC	6	three_prime	1	1616
BciVI	GTATCC	6	three_prime	1	935
BfiI	ACTGGG	6	three_prime	1	751
BglI	GCCNNNNNGGC	6	three_prime	1	1232
BseSI	GKGC MC	6	three_prime	1	832
BsgI	GTGCAG	6	three_prime	1	410
DraIII	CACNNNGTG	6	three_prime	1	685

Table 35: Countiue

Eco57I	CTGAAG	6	three_prime	1	865
MmeI	TCCRAC	6	three_prime	1	926
NspI	RCATGY	6	three_prime	1	1659
PfiMI	CCANNNNTGG	6	three_prime	1	935
SduI	GDGCHC	6	three_prime	1	832
SphI	GCATGC	6	three_prime	1	1659
Sse8387I	CCTGCAGG	8	three_prime	1	1232
BsrBI	CCGCTC	6	blunt	2	224, 1731
PvuII	CAGCTG	6	blunt	2	34, 1852
SmaI	CCCGGG	6	blunt	2	1527, 1826
BamHI	GGATCC	6	five_prime	2	1036, 1866
BseYI	CCCAGC	6	five_prime	2	642, 1344
BspHI	TCATGA	6	five_prime	2	192, 1919
BtgZI	GCGATG	6	five_prime	2	1167, 1608
Cfr10I	RCCGGY	6	five_prime	2	334, 1692
CfrI	YGGCCR	6	five_prime	2	937, 1703
DraII	RGGNCCY	6	five_prime	2	1140, 1379
Eco31I	GGTCTC	6	five_prime	2	169, 283
Esp3I	CGTCTC	6	five_prime	2	233, 1242
NarI	GGGCC	6	five_prime	2	1163, 1397
PpuMI	RGGWCCY	7	five_prime	2	1140, 1379
TfiI	GAWTC	5	five_prime	2	5, 898
Tth111I	GACNNNGTC	6	five_prime	2	73, 1633
AjuI	GAANNNNNNTTGG	7	three_prime	2	1003, 1035
BaeI	ACNNNGTAYC	7	three_prime	2	1588, 1621
BcgI	CGANNNNNNTGC	6	three_prime	2	70, 104
DrdI	GACNNNNNGTC	6	three_prime	2	1267, 1420
GsuI	CTGGAG	6	three_prime	2	56, 1096
KpnI	GGTACC	6	three_prime	2	1533, 1679
PstI	CTGCAG	6	three_prime	2	33, 1232
TsoI	TARCCA	6	three_prime	2	40, 658
TstI	CACNNNNNTCC	6	three_prime	2	29, 61
PleI	GAGTC	5	five_prime	3	327, 559, 1255
SmlI	CTYRAG	6	five_prime	3	316, 610, 863
StyI	CCWWGG	6	five_prime	3	21, 728, 1136
Tsp45I	GTSAC	5	five_prime	3	455, 1428, 1872
BseRI	GAGGAG	6	three_prime	3	133, 271, 1741
BsrDI	GCAATG	6	three_prime	3	461, 493, 741
Eco57MI	CTGRAG	6	three_prime	3	56, 865, 1096
HindII	GYRAC	6	blunt	4	855, 1032, 1551, 1751
AvaII	GGWCC	5	five_prime	4	261, 1065, 1140, 1379
EcoRII	CCWGG	5	five_prime	4	109, 284, 522, 824
SalI	GTCGAC	6	five_prime	4	853, 1030, 1549, 1749
HaeII	RGCGCY	6	three_prime	4	863, 1166, 1197, 1400
HaeIV	GAYNNNNNRTC	6	three_prime	4	281, 315, 953, 987
MboII	GAAGA	5	three_prime	4	135, 1320, 1429, 1635
NmeAIII	GCCGAG	6	three_prime	4	1191, 1202, 1233, 1373
TspRI	CASTG	5	three_prime	4	686, 1005, 1120, 1207
AccI	GTMKAC	6	five_prime	5	854, 1031, 1124, 1550, 1750
AvaI	CYCGRG	6	five_prime	5	1309, 1443, 1525, 1789, 1824
BsmAI	GTCTC	5	five_prime	5	120, 169, 233, 283, 1242
EcoP15I	CAGCAG	6	five_prime	5	612, 1516, 1587, 1654, 1657
XhoII	RGATCY	6	five_prime	5	756, 783, 1036, 1052, 1866
BsrI	ACTGG	5	three_prime	5	134, 746, 1005, 1113, 1200
HphI	GGTGA	5	three_prime	5	510, 1297, 1356, 1422, 1884
TspDTI	ATGAA	5	three_prime	5	98, 572, 752, 1231, 1908
TspGWI	ACGGA	5	three_prime	5	130, 240, 1049, 1230, 1330
AgsI	TTSAA	5	three_prime	6	403, 574, 803, 916, 1011, 1647
BsaXI	ACNNNNNTCC	6	three_prime	6	29, 59, 1039, 1069, 1495, 1525
BseMII	CTCAG	5	three_prime	6	184, 314, 364, 410, 1292, 1862
AcyI	GRCGYC	6	five_prime	7	1121, 1163, 1235, 1268, 1397, 1547, 1613
HgaI	GACGC	5	five_prime	7	391, 1110, 1224, 1257, 1536, 1607, 1671
SfaNI	GCATC	5	five_prime	7	353, 536, 755, 1014, 1261, 1589, 1794
FauI	CCCGC	5	five_prime	8	229, 602, 1076, 1092, 1369, 1414, 1444, 1522
BccI	CCATC	5	five_prime	9	198, 847, 937, 958, 1156, 1189, 1630, 1714, 1856
BbvI	GCAGC	5	five_prime	10	15, 43, 403, 626, 1500, 1571, 1630, 1671, 1674, 1839
FokI	GGATG	5	five_prime	10	778, 821, 826, 937, 1036, 1227, 1239, 1693, 1772, 1874
TseI	GCWGC	5	five_prime	10	28, 31, 391, 639, 1488, 1559, 1618, 1684, 1687, 1852
TauI	GCSGC	5	three_prime	11	618, 716, 942, 1097, 1121, 1163, 1199, 1451, 1580, 1705, 1734
Hin4I	GAYNNNNVTC	6	three_prime	16	124, 147, 156, 179, 208, 230, 240, 262, 282, 314, 331, 363, 954, 986, 1495, 1527
Hpy99I	CGWCG	5	three_prime	16	92, 256, 298, 856, 859, 1036, 1093, 1246, 1249, 1252, 1273, 1501, 1552, 1618, 1639, 1665

Table 36: Restriction digestion of protease gene from XM_033555156.1 *Aspergillus lentulus*

Name	Sequence	Site length	Overhang	Frequency	Cut positions
Eco47III	AGCGCT	6	blunt	1	734
HindII	GYRAC	6	blunt	1	1057
MslI	CAYNNNNRTG	6	blunt	1	287
NaeI	GCCGCG	6	blunt	1	387
PvuII	CAGCTG	6	blunt	1	790
AccI	GTMKAC	6	five_prime	1	328
AcII	AACGTT	6	five_prime	1	578
AcyI	GRCGYC	6	five_prime	1	587

Table 36: Countiue

AflIII	ACRYGT	6	five_prime	1	435
BglIII	AGATCT	6	five_prime	1	14
CfrI0I	RCCGGY	6	five_prime	1	385
ClaI	ATCGAT	6	five_prime	1	1124
HgaI	GACGC	5	five_prime	1	576
NdeI	CATATG	6	five_prime	1	405
PpuMI	RGGWCCY	7	five_prime	1	718
SmlI	CTYRAG	6	five_prime	1	646
XhoII	RGATCY	6	five_prime	1	14
AlwNI	CAGNNCTG	6	three_prime	1	616
ApaI	GGGCCC	6	three_prime	1	469
BseRI	GAGGAG	6	three_prime	1	1018
BseSI	GKGC MC	6	three_prime	1	469
BsmI	GAATGC	6	three_prime	1	1146
BsrDI	GCAATG	6	three_prime	1	294
BtsI	GCAGTG	6	three_prime	1	282
EciI	GGCGGA	6	three_prime	1	133
Eco57I	CTGAAG	6	three_prime	1	148
Eco57MI	CTGRAG	6	three_prime	1	148
HaeII	RGGCCY	6	three_prime	1	736
KpnI	GGTACC	6	three_prime	1	524
NmeAIII	GCCGAG	6	three_prime	1	491
NspI	RCATGY	6	three_prime	1	439
SduI	GDGCHC	6	three_prime	1	469
Ball	TGGCCA	6	blunt	2	4, 1063
ApoI	RAATTY	6	five_prime	2	205, 442
AvaII	GGWCC	5	five_prime	2	349, 718
Bpu10I	CCTNAGC	6	five_prime	2	83, 266
BseYI	CCCAGC	6	five_prime	2	50, 790
BsmAI	GTCTC	5	five_prime	2	672, 1030
FauI	CCCGC	5	five_prime	2	525, 831
NcoI	CCATGG	6	five_prime	2	516, 989
TatI	WGTACW	6	five_prime	2	90, 507
TfiI	GAWTC	5	five_prime	2	184, 1112
ArsI	GACNNNNNTTYG	7	three_prime	2	463, 495
BcgI	CGANNNNNNTGC	6	three_prime	2	820, 854
BciVI	GTATCC	6	three_prime	2	187, 233
BsaXI	ACNNNNNCTCC	6	three_prime	2	91, 121
MmeI	TCCRAC	6	three_prime	2	212, 831
TstI	CACNNNNNTCC	6	three_prime	2	890, 922
XcmI	CCANNNNNNNNTGG	6	three_prime	2	228, 305
EcoRV	GATATC	6	blunt	3	489, 633, 864
DraII	RGGNCCY	6	five_prime	3	465, 642, 718
EcoP15I	CAGCAG	6	five_prime	3	196, 715, 992
EcoRII	CCWGG	5	five_prime	3	176, 880, 921
FokI	GGATG	5	five_prime	3	193, 517, 972
PleI	GAGTC	5	five_prime	3	135, 716, 825
StyI	CCWWGG	6	five_prime	3	516, 989, 1043
HphI	GGTGA	5	three_prime	3	296, 613, 711
TsoI	TARCCA	6	three_prime	3	558, 982, 1036
TspGWI	ACGGA	5	three_prime	3	142, 812, 874
BbvI	GCAGC	5	five_prime	4	282, 401, 729, 799
CfrI	YGGCCR	6	five_prime	4	2, 513, 687, 1061
SfaNI	GCATC	5	five_prime	4	111, 729, 1116, 1164
TseI	GCWGC	5	five_prime	4	270, 389, 742, 787
AgsI	TTSAA	5	three_prime	4	188, 262, 442, 755
MboII	GAAGA	5	three_prime	4	142, 154, 238, 579
TauI	GCSGC	5	three_prime	4	323, 395, 689, 956
TspRI	CAS TG	5	three_prime	4	289, 402, 1038, 1151
BseMII	CTCAG	5	three_prime	5	74, 257, 357, 902, 1044
BsrI	ACTGG	5	three_prime	5	423, 545, 1064, 1151, 1163
Hin4I	GAYNNNNVTC	6	three_prime	6	91, 123, 365, 397, 922, 954

Amino Acid Sequences of *Asperigillus Protease*

>A CAA75805.1 *Asperigillus fumigatus*

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>XP_751651.1 *Asperigillus fumigatus*

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>CAA77666.1 *Aspergillus fumigatus*

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>XP_750914.1 *Aspergillus fumigatus*

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>EDP50543.1 *Aspergillus fumigatus*

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>1905286A1 *Aspergillus fumigatus*

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>XP_751651.1 *Aspergillus fumigatus*

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>B0Y708.1 *Aspergillus fumigatus*

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>Q4WH48 *Aspergillus fumigatus*

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>CAA75806.1 *Aspergillus fumigatus*

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>CAA75804.1 *Aspergillus fumigatus*

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>EAL89613.1 *Aspergillus fumigatus*

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>EAL86979.1 *Aspergillus fumigatus*

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>XP_749017.1 *Aspergillus fumigatus*

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>B0Y1V8.1 *Aspergillus fumigatus*

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>AAT85627.1 *Aspergillus viridinutans*

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>AAT85626.1 *Aspergillus viridinutans*

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>A1CIA7.1 *Aspergillus clavatus*

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>A1CBR4.1 *Aspergillus clavatus*

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>A1CBR4.1 *Aspergillus clavatus*

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>ACX47962.1 *Aspergillus clavatus*

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>EAW10612.1 *Aspergillus clavatus*

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>EAW10612.1 *Aspergillus clavatus*

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>XP_001272038 *Aspergillus clavatus*

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>AAA67705.1 *Aspergillus nidulans*

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>Q00208.1 *Aspergillus nidulans*

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>Q00204.2 *Aspergillus nidulans*

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>A1CWF3.1 *Aspergillus fischeri*

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>AAT85625.1 *Aspergillus sp.*

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>B8NLY9.1 *Aspergillus flavus*

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>P35211.1 *Aspergillus flavus*

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>P28296.2 *Aspergillus fumigatus*

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>P41748.2 *Aspergillus fumigatus*

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>B0XRVO.1 *Aspergillus fumigatus*

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>P0C959.1 *Aspergillus fumigatus*

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>O42630.1 *Aspergillus fumigatus*

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>A2R3L3.1 *Aspergillus niger*

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>XP_001391470.1 *Aspergillus niger*

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>P55325.1 *Aspergillus niger*

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>P24665.1 *Aspergillus niger*

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>A2Q7V4.1 *Aspergillus niger*

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>CAK44878.1 *Aspergillus niger*

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>Q00070 *Aspergillus niger*

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>P55325.1 *Aspergillus niger*

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>A2R2G1.1 *Aspergillus niger*

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>Q8NK92.2 *Aspergillus oryzae*

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>Q2U319 *Aspergillus oryzae*

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>Q2UDE1 *Aspergillus oryzae*

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>AAC60533.1 *Aspergillus oryzae*

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>Q2UW3 *Aspergillus oryzae*

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>Q8NKB2 *Aspergillus oryzae*

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>Q8NKB6.1 *Aspergillus oryzae*

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>AAB20819.1 *Aspergillus oryzae*

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>Q06902.2 *Aspergillus oryzae*

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>BAA00951.1 *Aspergillus oryzae*

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>Q9Y8E3.2 *Aspergillus oryzae*

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>P46076.2 *Aspergillus oryzae*

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>Q2TWA0 *Aspergillus oryzae*

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>Q8NKB5 *Aspergillus oryzae*

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>A1DDK1.1 *Aspergillus fischeri*

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