

MK000060=chitinase=ASLSM3

Marker: =chitinase=ASLSM3

Type: PCR

Description: PCR marker designed from [M94106](#) *Allium sativum* chitinase mRNA, 3' end

Reference: 1. M. Ipek, A. Ipek, S. G. Almquist, P. W. Simon Demonstration of linkage and development of the first low-density genetic map of garlic, based on AFLP markers *Theor Appl Genet* (2005) 110: 228–236

2. submitted as "Rapid Characterization of Garlic Clones with Locus Specific DNA Markers" to the 5th International Symposium on Edible Alliaceae

Primers: As-Chtn-F (ASchitin-up).....5' -CAGCAACAGGCTATGCTGTAGC-3'

As-Chtn-R (AS-Chitin-SSR-1).....5' -GAATGAGT'TTGCAGCTGCTATGAAGG-3'

PCR Reaction: (from reference 1) 25 µl: [90 ng DNA; 0.8 µM each primer=20 pMol each; 0.03 U/µl Taq=0.75 U; 2? mM MgCl₂=50 nMol; 0.2 mM each dNTP=5 nMol]

(from reference 2): 25 µl: [60 ng DNA; 0.8 µM each primer=20 pMol each; 0.05 U/µl Taq=1.25 U; 2? mM MgCl₂=50 nMol; 0.2 mM each dNTP=5 nMol]

PCR Program: (from reference 1) 95°C 2:00; 35 cycles of {94°C 0:30; 58°C 1:00; 72°C 2:00}

(from reference 2) 95°C 2:00; 40 cycles of {94°C 0:30; 58°C 1:00; 72°C 2:00}; 72°C 5:00; 4°C hold.

Screening Method: Product size by 6% denaturing polyacrylamide sequencing gels containing 7.5 M urea in 1× Tris-Borate (TBE), silver staining

Product Sizes: 265 b.p. predicted

Example: Chitinase gene specific polymorphic allele amplifying only in Kast-Taskopru and other garlic clones (PI493112, PI282824, and PI493118) that were tightly clustered in AFLP dendrogram (Fig. 1). M is the PBR322-Msp I DNA molecular weight marker. Arrow indicates gene specific marker.

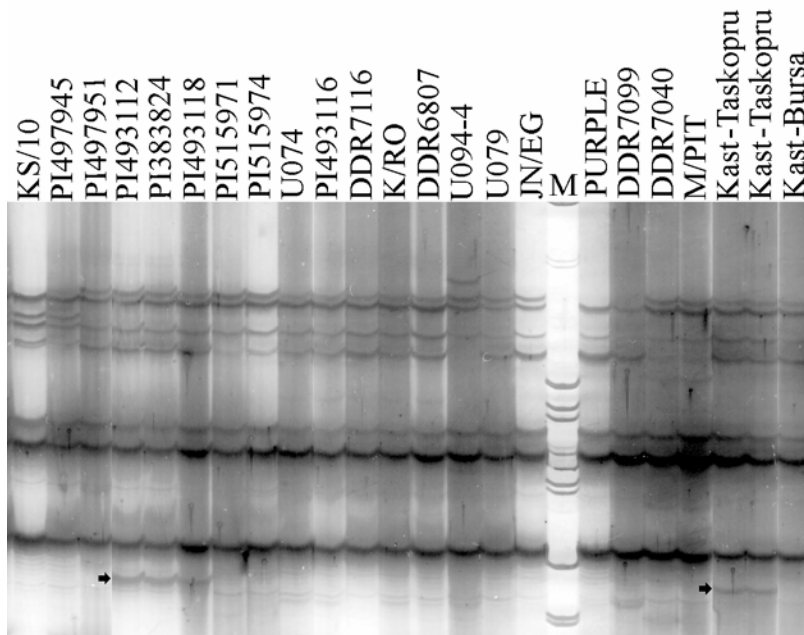


Diagram of how it works:

Genbank reference:

Sequence Information: >gi|166342|gb|M94106.1|ALCCHINTIA *Allium sativum* chitinase mRNA, 3' end

```
1 accgttctca ccttcgacct cttcacaaac tcttacgccc aacaatgcgg tagccaagga
61 agtggagcat tatgcagcaa cgggctatgc tgcagccaat acggttactg tgggaacggg
121 ggtccatact gtggaactgg atgccaaagc caatgtggtg gccccggagg agggtcatca
181 ggagtagctt ccatcataag ttcttctctg ttcaatcaaa tgctgctgca ccgcaacgat
241 gcggcattgcc ctgccaatgg attttacacc atcgatgcct ttatagctgc tgcaaattca
301 tttagtggct tcggaaccac tggagacact gatacacaaa agagagagct tgctgcttc
361 tttgctcaaa catctcatga aaccactggt ggggatgcaa gtgcacctga tgggccatat
421 gcatggggct actgcttcaa gcaagaacaa ggcaaccac cagattactg tcaagcaagt
```

<http://www.vcru.wisc.edu/simonlab/sdata/MK000060.pdf>

```
481 gccagtagc cctgtgcacc aggaaagaag tactatggga gaggacctat acagatatca
541 tacaactaca actacgggca gtgcgggaac gccataaatc aagacctgct aaacaacca
601 gatttggtagc cgtcagatcc tgctatttcc ttcaagacag cgatatggtt ctggatgacg
661 gcacagtccc caaaaccgtc gtgccatgca gtggccacag ggcagtggca gccatctgct
721 gcggatcagg cagcgggacag agttcctgga ttcggcgta taaccaacat catcaacggt
781 ggagttgagt gtggacatgg atcagaccct agtggtgagg atagaattgg attttataag
841 aggtactgag atttgtttca ggtgggatat ggagataact tggactgtgg aaatcaggct
901 catttttgat gcatggtgat ggtggtccgg tggagtatag tttttatgat atagtaata
961 agatctgctc tgataaagaa gtcctcatat gatacatgtg caataaa
```

Actually there was probably an error in the TAG publication, the primers appear to have been designed from this sequence, 265 b.p. product here:

```
>gi|166344|gb|M94105.1|ALCCHITIN Allium sativum chitinase mRNA, 3' end
TTCCTGGTAACAACCATTCTCGCCTTCGCCCTCTTCAAGAACTCCTACGCCCAACAATGTGGTAGCCAAGCT
GGTGGAGCATTATGCAGCAACAGGCTATGCTGTAGCAAATTCGGGTACTGTGGATCCACGGATCCATACTGT
GGAAGTGGATGCCAAAGCCAATGTGGGGGCGGAGGAGGTGGAGGAGGAGGCCGTGGAGGAGGAGGCCGGTGGT
GGAGGAGGATCAGGAGTAGCTTCCATCATAAATTCTTCACTGTTCAATCAAATGCTGCTGCACCGCAACGAT
GCGGCATGCCCTGCCAATGGATTTTACACCTACGATGCCTTCATAGCAGCTGCAAACCTCATTCCGGTGGCTTC
GGAACCACTGGAGACATCAATGCACAAAAGAGAGAGCTTGCTGCTTTCTTTGCTCAAACATCTCATGAAACC
ACTGGAGGGTATCCAAGTGCACCTGATGGGCCATATGCATGGGGATACTGCTTCAAGCAAGAACAAGGCAAT
CCATCCGATTACTGTCAAGCAAGTGCCAGTACCCCTGTGCACCAGGAAAGAAGTACTATGGGAGAGGACCT
ATACAAATATCATAACAATACTACGGGCAGTGCAGTAATGCTATAACATCAAGACCTGCTAAACAACCCA
GATTTGGTGGCGACGGATCCTACTATTTTCTTCAAGACAGCGATATGGTTCTGGATGACCGCGCAGTCTCCG
AAACCGTCGTGCCATGCAGTGGCCACAGGGCAGTGGCAGCCATCTGCTGCGGATCAGGCAGCGGGAAGAGTT
CCTGGATTCCGGCGTTATAACCAACATCATCAACGGTGGAGTGCAGTGTGGACATGGATCAGACACTCATGTT
GCGGATAGAATTGGGTATTATAAGAGGTATTGCGATTTGTTGCAGGTGGGCTATGGGGATAACTTGGACTGT
GGTAATCAGGCTCATTTTTTGTATGCATGGTGTGTAAGTATAGTTTTTAAAGATATATGTAATAAGATCTGC
CTCGATAGCGAAGTCGTATATGATATATATGCAATAAAATATATACGCCGTGAATAAAAAGTTTGCCTATGA
GAAAA
```

Map Location: Map: MP1; [Linkage Group: vii](#); Position: 0 cm

Published Reference:

Other Information:

Primer Location (lab specific): Aschitin-up@Bx6:H4; AS-Chitin-SSR-1@Bx6:G4

PCR Program Name (lab specific):