Table S1 Oligonucleotide primers used in this study.

Name	Sequence
r^{ef} fragment amplification	
Eco-RSP-F	CAGAATTCAGTCGAGGACAGAACGCAGCA
Eco-RSP-R	TTGAATTCTTGGACCTCTTCCGCAGTTTCC
hph marker amplification	
APAI-HPH-F	AAGGGCCCAACTGATATTGAAGGAGCAT
APAI-HPH-R	AAGGGCCCAACTGGTTCCCGGTCGGCAT
r^{ef}-*hph* amplification (center fragment for DJ-PCR)	
Rsp-center-A	AGGACAGAACGCAGCAGCAGAGC
Rsp-center-B	ACAGCGAACGAAACCCCTGAAAC
r^{ef1}-*hph* insertion between *ncu09443* and *ncu09444*	
Rsp-040613-C	TAGTGGAGGGGCTTGGGATGGT
Rsp-040613-D	AGAGAAGCTCTGCTGCTGCGTTCTGTCCTCTGCTGAACGAACACCCCTGCT
Rsp-040613-E	TAACGGGTTTCAGGGGTTTCGTTCGCTGTCGTCCACTGATCTTCGCTAGAATTT
Rsp-040613-F	TCACCGCCCGTCCCTACTATCA
Rsp-040613-G	GCCTTGGACTGGTGATGGTGCT
Rsp-040613-H	GGAGGAGTCGGTTTGCTTTGGTG
r^{ef2}-*hph* insertion between *ncu09444* and *ncu09445*	
Rsp-040613-I	ATGAGGGAGGTGCCGTGTCC
Rsp-040613-J	AGAGAAGCTCTGCTGCTGCGTTCTGTCCTCCATTCTGCCATTTCCCATGC
Rsp-040613-K	TAACGGGTTTCAGGGGTTTCGTTCGCTGTCCGCACACTTTCTCCACCCATC
Rsp-040613-L	GCAATCCACCTCTGGCATCGAC
Rsp-040613-M	AGCCAATCCTTTACCGACTCCAACA
Rsp-040613-N	GTGGTTCTCGCCCGCTTTCAAC
r^{ef3}-*hph* insertion between *ncu09449* and *ncu09450*	
RSP-042613-A	CGAGGGCCGAGTCTGGTGGTTA
RSP-042613-B	AGAGAAGCTCTGCTGCTGCGTTCTGTCCTGTACTAGCGTTTGCGCGGGACA
RSP-042613-C	TAACGGGTTTCAGGGGTTTCGTTCGCTGTAGGTGGGAAAGTGTTAGTGGTGGA
RSP-042613-D	GTTGAGGGTCTTGAGGGCGAAG
RSP-042613-E	TCTCACACGTTGCTTCGGCTGT
RSP-042613-F	GAGGTTCTGGTTGGCTGGTTGG
r^{ef4}-*hph* insertion between *ncu09451* and *ncu17161*	
RSP-042613-A	AAGTGGGCGTTGAAGGAGGATG
RSP-042613-B	AGAGAAGCTCTGCTGCTGCGTTCTGTCCTCGGAGGTCGGAGACGAGATG
RSP-042613-C	TAACGGGTTTCAGGGGTTTCGTTCGCTGTCCAAGTCCTCCATCCGTCCATC
RSP-042613-D	TTCATCCAGCAATCCACCACCA
RSP-042613-E	CCTCTTCACCCTCTACCCAAACGA
RSP-042613-F	AGCGACCATCCCAAACCAACAA
r^{ef5}-*hph* insertion between *ncu09455* and *ncu09456*	
RSP-050213-A	CAGACAGTGGTGGGAAGGTGGTC
RSP-050213-B	AGAGAAGCTCTGCTGCTGCGTTCTGTCCTCAGTGCGGAAATGGAAGGGAGAG
RSP-050213-C	TAACGGGTTTCAGGGGTTTCGTTCGCTGTCGGCCATCACGGTCAAAGAAAC
RSP-050213-D	ATGGTGCCGACGCTAAAGGAGA
RSP-050213-E	CGTTCCGTCATTCGGGTATTGC
RSP-050213-F	ACGCAGGGAGGGAGATTGCCTA
r^{ef6}-*hph* insertion between *ncu06068* and *ncu06067*	
RSP-061813Y	AGCTCGTTTGGGTATCAGCAGTCC
RSP-061813Z	AGAGAAGCTCTGCTGCTGCGTTCTGTCCTGGATGCAAGGGCGAGAGTCAA

RSP-061813AA	TAACGGGTTTCAGGGGTTTCGTTCGCTGTGCGAAACCCTGGAGATAACGGAAG
RSP-061813AB	GGCGTCGGCAACTGAAGGAC
RSP-061813AC	CGTGGGAAGCGAGGTGAGAGG
RSP-061813AD	CGTGGTCTGTGTGTGTGTGGTCTG

Construction of the *gfp-sad-6 gfp* tagging vector

NCU06190-E	TTGAAAATGCGAGGATAAGACGAAGA
NCU06190-NGFP1	GCAGCCTGAATGGCGAATGGACGCGCCAAGTGTGAAAGCAATCTGTGTTGGA
NCU06190-NGFP2	CAGGAGCGGGTGCGGGTGCTGGAGCGATGGCCGAACTCAACGAAAATGAACC
NCU06190-F	CCTCTCCAAAGTCCAAGACGACCT
NCU06190-G	TGCCCAACAGATAACGTGACTTCG
NCU06190-H	TTGGCATCGGAAAGAAAGGTGCT

Construction of the *mCherryNC-spo76* tagging vector

NCU00424-E	ACTCGCAAGCAAGGCACTGAA
NCU00424-X1	GCAGCCTGAATGGCGAATGGACGCGCCCTGCGTATGATCTTGAGGACGAG
NCU00424-X2	CAGGAGCGGGTGCGGGTGCTGGAGCGATGGCGCCACGTCGAAGCGCTC
NCU00424-F	TCACTTGGGGTTCCGCTCTTTCT
NCU00424-G	CAAAGGCCCCCATCCAGTACGA
NCU00424-H	TCTTTTCCAACCTGCTCTCCCTTG

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r^{ef} fragment amplification primers were used to amplify a fragment of the r^{+} gene.

hph marker amplification primers were used to amplify the *hph* fragment.

r^{ef}-*hph* amplification primers. The r^{ef} fragment and the *hph* marker were placed next to each other in a plasmid. These primers amplified the r^{ef} fragment and the *hph* marker from the plasmid to serve as the center product in DJ-PCR (Yu *et al*. 2004).

$r^{ef1\text{-}6}$-*hph* insertions: For each set of r^{ef}-*hph* insertion primers, the first two primers were used to amplify the left flank, the middle two primers were used to amplify the right flank, and the last two primers were used for nested amplification of the final vector.

The *gfp-sad-6* and *mCherryNC-spo76* primers were used to make tagging vectors for *sad-6* and *spo76* by DJ-PCR as previously described (Hammond *et al*. 2011b). The center product for g*fp* tagging was obtained from plasmid pTH1117.12 (Hammond *et al*. 2011b). The center product for *mCherryNC* tagging was obtained from plasmid pEBTH1252.3, which is similar to pTH1117.12 except that *gfp* sequences are replaced with *mCherryNC* sequences.
