

Fig. S2. Deletion of the *nox-1* gene. (A) The *nox-1* deletion plasmid pNCKA-8 containing the *mat-a1* gene, as counter selection marker, and the hygromycin resistance marker *Hyg^r* was used to transform strain RPNCR3A. A double recombination event results in replacement of the wild type *nox-1* gene. B indicates *Bam*HI sites. (B) DNA from the WT and hygromycin resistance transformants was digested with *Bam*HI and used for Southern blot analysis with the indicated probe. The wild type *Bam*HI pattern corresponds to bands of 14.3 and 1.9 kb, while the *nox-1* deletion pattern corresponds to bands of 14.3 and 0.8 kb (strains TCNB2 and TCND10).

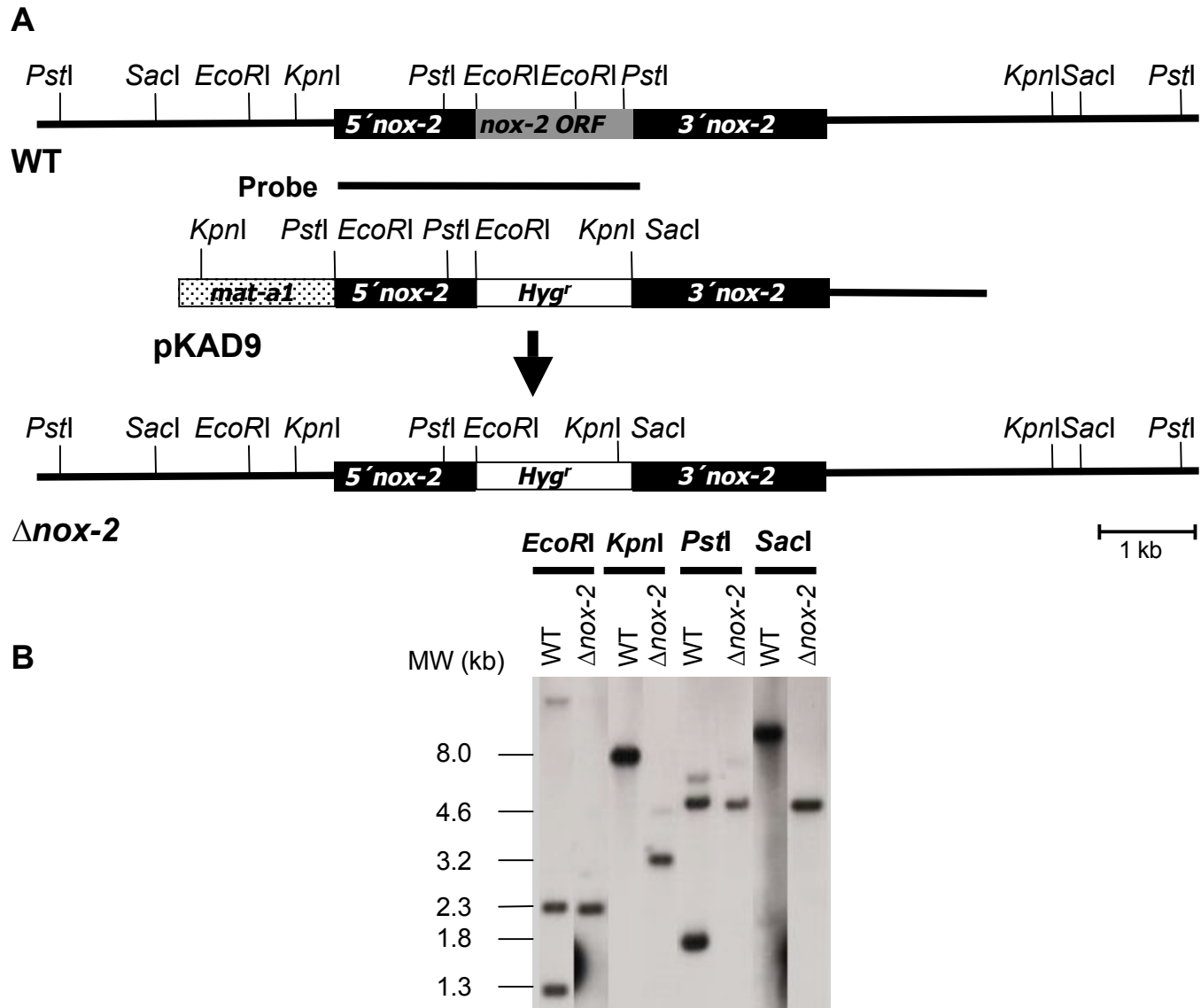
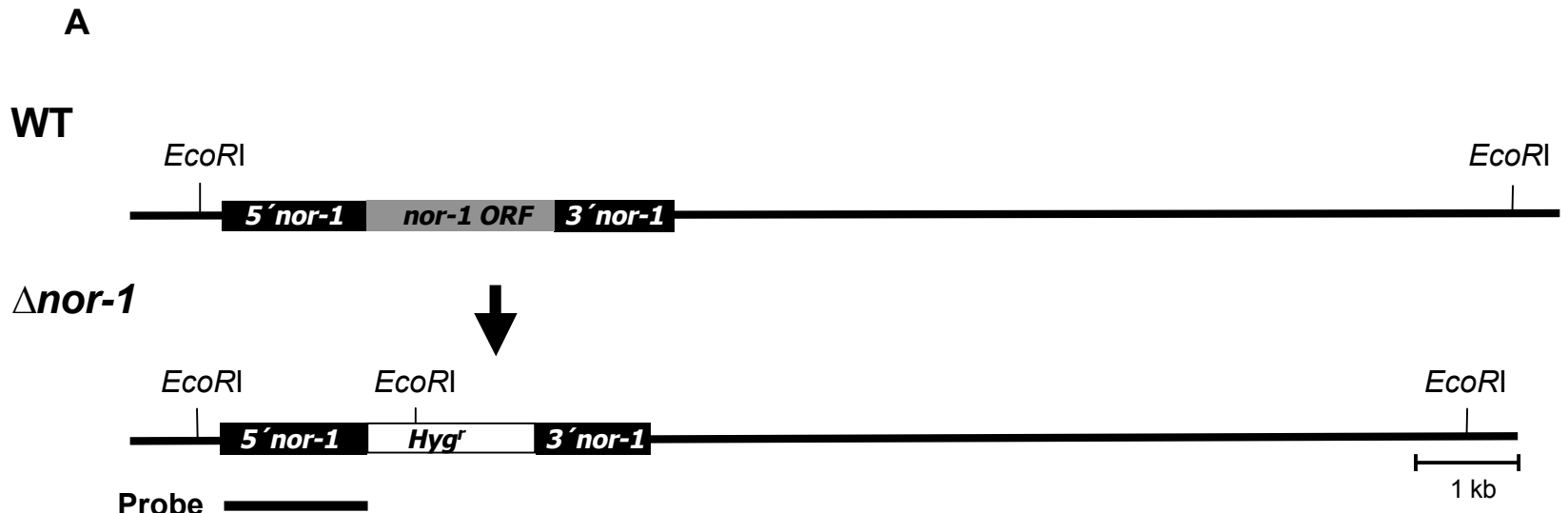


Fig. S3. Deletion of the *nox-2* gene. (A) In plasmid pKAD9 85% of *nox-2* ORF was replaced by the hygromycin resistance marker *Hyg^r*. (B) To verify *nox-2* deletion, DNA from WT and hygromycin resistance transformants was digested with *PstI*, *SacI*, *EcoRI* and *KpnI* and used for Southern blot analysis with the probe indicated in (A).

		helix A	TRP1	helix B	helix A			
<i>H. sapiens</i> NP_000424.2	526 aa	1				50		
<i>E. festucae</i> BAF36501	519 aa	1				49		
<i>N. crassa</i> NOX-R	517 aa	1				49		
<i>A. nidulans</i> XP_6636501	517 aa	1				49		
<i>M. grisea</i> XP_359497.1	525 aa	1				49		
			TRP2	helix B	helix A	TRP3	helix B	
<i>H. sapiens</i> NP_000424.2	526 aa	51						100
<i>E. festucae</i> BAF36501	519 aa	50						99
<i>N. crassa</i> NOX-R	517 aa	50						99
<i>A. nidulans</i> XP_6636501	517 aa	50						99
<i>M. grisea</i> XP_359497.1	525 aa	50						99
			Helix A	TRP4	helix B			
<i>H. sapiens</i> NP_000424.2	526 aa	101						150
<i>E. festucae</i> BAF36501	519 aa	100						149
<i>N. crassa</i> NOX-R	517 aa	100						149
<i>A. nidulans</i> XP_6636501	517 aa	100						149
<i>M. grisea</i> XP_359497.1	525 aa	100						149
<i>H. sapiens</i> NP_000424.2	526 aa	151						200
<i>E. festucae</i> BAF36501	519 aa	150						197
<i>N. crassa</i> NOX-R	517 aa	150						197
<i>A. nidulans</i> XP_6636501	517 aa	150						189
<i>M. grisea</i> XP_359497.1	525 aa	150						197
			Activation domain	P-rich	SH3 domain			
<i>H. sapiens</i> NP_000424.2	526 aa	201						250
<i>E. festucae</i> BAF36501	519 aa	198						247
<i>N. crassa</i> NOX-R	517 aa	198						247
<i>A. nidulans</i> XP_6636501	517 aa	190						224
<i>M. grisea</i> XP_359497.1	525 aa	198						247
<i>H. sapiens</i> NP_000424.2	526 aa	251						298
<i>E. festucae</i> BAF36501	519 aa	248						289
<i>N. crassa</i> NOX-R	517 aa	248						291
<i>A. nidulans</i> XP_6636501	517 aa	225						274
<i>M. grisea</i> XP_359497.1	525 aa	248						292
<i>H. sapiens</i> NP_000424.2	526 aa	299						348
<i>E. festucae</i> BAF36501	519 aa	290						335
<i>N. crassa</i> NOX-R	517 aa	292						339
<i>A. nidulans</i> XP_6636501	517 aa	275						324
<i>M. grisea</i> XP_359497.1	525 aa	293						339
			PB1 domain					
<i>H. sapiens</i> NP_000424.2	526 aa	349						398
<i>E. festucae</i> BAF36501	519 aa	336						380
<i>N. crassa</i> NOX-R	517 aa	340						378
<i>A. nidulans</i> XP_6636501	517 aa	325						374
<i>M. grisea</i> XP_359497.1	525 aa	340						380
<i>H. sapiens</i> NP_000424.2	526 aa	399						444
<i>E. festucae</i> BAF36501	519 aa	381						425
<i>N. crassa</i> NOX-R	517 aa	378						423
<i>A. nidulans</i> XP_6636501	517 aa	375						423
<i>M. grisea</i> XP_359497.1	525 aa	381						427
			SH3 domain					
<i>H. sapiens</i> NP_000424.2	526 aa	445						489
<i>E. festucae</i> BAF36501	519 aa	426						470
<i>N. crassa</i> NOX-R	517 aa	424						468
<i>A. nidulans</i> XP_6636501	517 aa	424						469
<i>M. grisea</i> XP_359497.1	525 aa	428						477
<i>H. sapiens</i> NP_000424.2	526 aa	489						526
<i>E. festucae</i> BAF36501	519 aa	471						519
<i>N. crassa</i> NOX-R	517 aa	469						517
<i>A. nidulans</i> XP_6636501	517 aa	470						515
<i>M. grisea</i> XP_359497.1	525 aa	478						525
<i>H. sapiens</i> NP_000424.2	526 aa	526	--	526				
<i>E. festucae</i> BAF36501	519 aa	519	--	519				
<i>N. crassa</i> NOX-R	517 aa	517	--	517				
<i>A. nidulans</i> XP_6636501	517 aa	516	MI	517				
<i>M. grisea</i> XP_359497.1	525 aa	525	--	525				

Fig. S4. The amino acid sequence of *N. crassa* NOR-1 is aligned with human p67^{phox}, *E. festucae* NoxR and orthologs from *A. nidulans* and *M. grisea*. Sequences are identified by species name, GenBank accession and protein size. Top red bar indicates the active domain. Top black bars show the helices between tetratricopetide domains (TRP). Proline rich region, SH3 and PB1 domains are indicated by pink, black and blue rectangles, respectively.



B

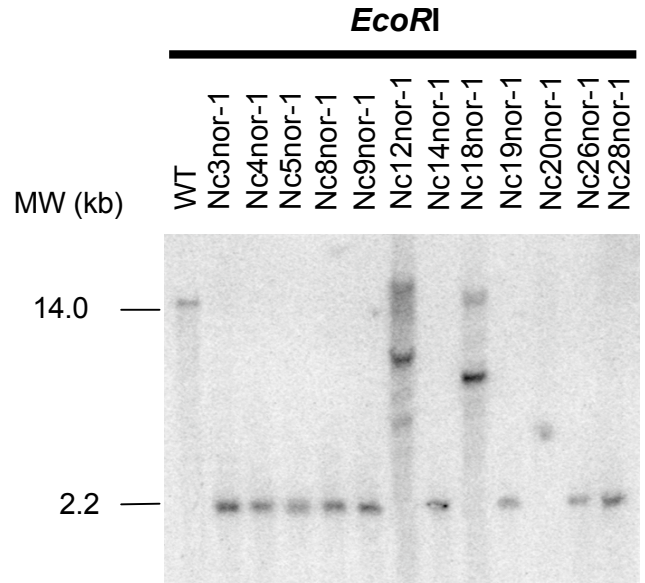


Fig. S5. Deletion of the *nor-1* gene. (A) The *nor-1* deletion construct, containing the hygromycin resistance gene, was generated by double-joint PCR and used to transform strain $\Delta mus-51$. A double recombination event results in replacement of the wild type locus by the deletion construct. (B) To verify *nor-1* deletion, DNA from WT and hygromycin resistance transformants was digested with *EcoRI* and used for Southern blot analysis with the indicated probe. The wild type *EcoRI* pattern corresponds to a single band of 14.0 kb, while the $\Delta nor-1$ pattern corresponds to one band of 2.2 kp.

<i>M. musculus</i> P12815 191 aa	1	MAAY-SYRPG-----	9
<i>D. discoideum</i> AAM00240 205 aa	1	-MYGYTPAVVAP-----	13
<i>N. crassa</i> XP_965123 311 aa	1	MAYNRSYNPDELPRFAEPEPRQGTSPAPPQQAPTARYETKPPPPRPVEH	50
<i>M. grisea</i> XP_362372 256 aa	1	MAYNRPYNPDELPRFAEPESK-GSN-----QKLFSP----	30
<i>G. zeae</i> XP_380620 286 aa	1	MAYNRPYDEDA LPRFAEPEQKPGQSRTPAP-----QQHPPPPQ---	39
<i>M. musculus</i> P12815 191 aa	9	-----	9
<i>D. discoideum</i> AAM00240 205 aa	13	-----	13
<i>N. crassa</i> XP_965123 311 aa	51	RNTGFQRQNSYDPQRQNTYNQAYHQAYTQNHYASGSADPHRLSPRMQ	100
<i>M. grisea</i> XP_362372 256 aa	30	-----SQASPO-----FHQPQHPQSRYEN-----	49
<i>G. zeae</i> XP_380620 286 aa	39	-----QQYQHAPP-----QQYHQQSRYDKPLPTQRDARSHSL	74
<i>M. musculus</i> P12815 191 aa	9	-----PGGGPGPAAGAALP-----DQSF L	28
<i>D. discoideum</i> AAM00240 205 aa	13	---TVMSFSFVFPQAFQQCWFYS-----LYTQIQQAQLYEM	46
<i>N. crassa</i> XP_965123 311 aa	101	QGPPPDYRGMSPPPSATGSRPAHNNLPPVSSRPPPS---PTTRDGADPTL	147
<i>M. grisea</i> XP_362372 256 aa	49	-KPVPPRPDQRLG SATQQR-----PPVTSRPPSPAPPETKGDNDPTL	92
<i>G. zeae</i> XP_380620 286 aa	75	QQGPASHGYMSPPPNTGGARPQAHNRPA PNSRPPSP--ALDGN GSDPTL	122
		EF-1	EF-2
<i>M. musculus</i> P12815 191 aa	29	WNVFRVVDKDRSGVITSDNELQQALNNGTWTFFNPVTVRSTIISMFDRENKA	78
<i>D. discoideum</i> AAM00240 205 aa	47	QSWFMRYDANRSGTISSGELQYLNIGG--IPLGIETATKLIKVF DHNKNG	94
<i>N. crassa</i> XP_965123 311 aa	148	LPLFRAVDKDG TGQLSERELSAALVNGDWTAFDPHTVRRMIRMFDSDRSG	197
<i>M. grisea</i> XP_362372 256 aa	93	RPLFRAVDKDG TGQLSEKELSAALVNGDWTAFDPHTVRRMIRMF DADRSG	142
<i>G. zeae</i> XP_380620 286 aa	123	LPLFRAVDKDG TGHL SERELSAALVNGDWTAFDPHTVRRMIRMFDSDRSG	172
		EF-3	
<i>M. musculus</i> P12815 191 aa	79	GVNRFSEFTGVVKYITDQNVFRTYDRNNSGMIDKNELKQALSGFGYRLSD	128
<i>D. discoideum</i> AAM00240 205 aa	95	QIDFYEAALHOFINNLYRCFVANDRNFSGCTIDANEIYNALITSGFQLPF	144
<i>N. crassa</i> XP_965123 311 aa	198	TIGFEFCGLWSFLASWRTLFD RFDMDKSGNISLDEFNNALVAFRYRLSP	247
<i>M. grisea</i> XP_362372 256 aa	143	TIGFEFCGLWSFLASWRTLFD RFDMDKSGNISLDEFNNALVAFRYRLSD	192
<i>G. zeae</i> XP_380620 286 aa	173	TIGFEFCGLWSFLASWRTLFD RFDADRSGNISLSDEFNNALVAFRYRLSP	222
		EF-4	EF-5
<i>M. musculus</i> P12815 191 aa	129	QFHDILLRKFDRCGRQIAFDDFIQGCIVLQRLTDFRRYDTDQDGWIQV	178
<i>D. discoideum</i> AAM00240 205 aa	145	PTVNYLFLKLSPSGYG-LLFTQFLNLCA TVALTRSLFEWNDPMRTGVVHL	193
<i>N. crassa</i> XP_965123 311 aa	248	RFVELLERTYDKRGGVMSFDL FVQACISLKRMTDVFKRYDDDRDGYITL	297
<i>M. grisea</i> XP_362372 256 aa	193	RFTPELLERTYDKRGGVMSFDL FVQSCISLKRMTDVFKRYDDDRDGYITL	242
<i>G. zeae</i> XP_380620 286 aa	223	QFVELIFNTYDKRNEGVM SFDL FVQSCISLKRMTDVFKRYDDDRDGYITL	272
<i>M. musculus</i> P12815 191 aa	179	SYEQYLSMVFSTV- 191	
<i>D. discoideum</i> AAM00240 205 aa	194	NLAQLYDIHALV-- 205	
<i>N. crassa</i> XP_965123 311 aa	298	SFEDFLTELLRQLK 311	
<i>M. grisea</i> XP_362372 256 aa	243	SFEDFLTELLKQLK 256	
<i>G. zeae</i> XP_380620 286 aa	273	SFEDFLTELLKQLK 286	

Fig. S6. *N. crassa* predicted PEF-1 protein is aligned with *Mus musculus* (mouse) Alg-2, *Dictyostelium discoideum* Alg-2b and hypothetical orthologs from *Magnaporthe grisea* and *Giberella zeae*. Sequences are identified by species name, followed by GeneBank accession or contig number and protein size. Top bars indicate EF-hand domains as deduced from ExPASy-Prosite database (<http://www.expasy.org/prosite/>).

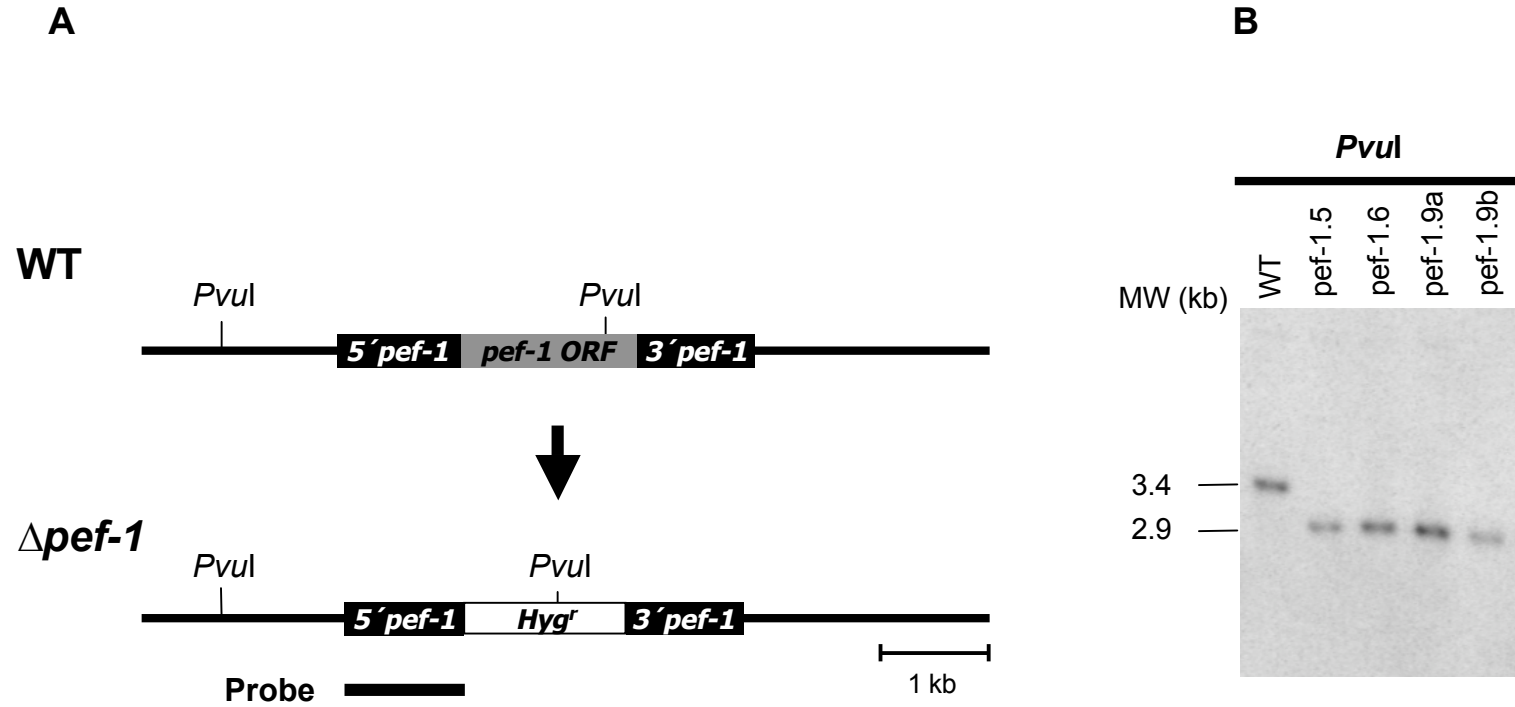


Fig. S7. Deletion of the *pef-1* gene. (A) A *pef-1* deletion construct based on the hygromycin resistance gene was generated by double-joint PCR and used to transform strain Δ *mus-51*. Double recombination results in replacement of the wild type *pef-1* locus. (B) To verify *pef-1* deletion, DNA from WT and hygromycin resistance transformants was digested with *PvuI* and used for Southern blot analysis with the probe indicated in (A). The wild type *PvuI* pattern corresponds to one band of 3.4 kb; the Δ *pef-1* pattern corresponds to a band of 2.9 kb.