Supplementary Information

Rapid and Visual Detection of *Pantoea ananatis* in Rice Using an RPA–CRISPR/Cas12a Lateral-Flow Assay

Supplementary Tables S1, S1b, S2 and S3 present in silico target mapping and comparative sequence analysis.

Table S1. Target gene and predicted assay recognition sites in *P. ananatis* HQ01.

Feature	**Chromosomal position (CP158663.1)**	**Annotation / sequence**
Hypothetical protein CDS (339 bp)	4,254,477–4,254,815 (+)	Locus ABTQ13_19615; protein XYH26110.1
Predicted RPA amplicon	4,254,522–4,254,770	249 bp
Candidate PAM	4,254,601–4,254,604	5′-TTTC-3′
20-nt protospacer	4,254,605–4,254,624	GACAGCCGACCAGTATGTGG
Genome assembly	CP158663.1–CP158666.1	5,060,023 bp total

Coordinates refer to the HQ01 genomic reference and are 1-based and inclusive. The amplicon and PAM are predicted from sequence mapping.

Table S1b. Primer, predicted amplicon and crRNA mapping in the *original P. ananatis* PA13 design reference.

Feature (PA13)	Coordinates (NC_017554.1; 1-based inclusive)	Sequence / interpretation
Original design reference target	286,446–286,784	339-bp reference region
Predicted RPA amplicon	286,491–286,739	249 bp
Candidate PAM	286,570–286,573	TTTC (plus strand)
Protospacer	286,574–286,593	GACAGCCGACCAGTATGTGG (20 nt)

Coordinates are 1-based and inclusive. The amplicon and PAM are predicted from sequence mapping.

Table S2. Representative P. ananatis BLASTN alignments and recognition-site comparison.

Strain	Accession	Identity %	PAM	Protospacer mismatches	Pa-F/Pa-R mismatches vs primer
Z6-6	JBSPZU010000009	100.00	TTTC	0	1/4
JT6-7	JANFWD010000008	100.00	TTTC	0	1/4
ARC272	PUGD01000029	99.70	TTTC	0	1/3
LA2-28	JANFVY010000008	99.11	TTTC	1	1/2
PANS_99_4	JABDZP010000011	98.53	TTTC	0	1/1
UBA12293	DPYY01000049	98.53	TTTC	0	1/1

Query: 339-bp HQ01 target (CP158663.1:4,254,477–4,254,815). The BLASTN search returned 100 records with full query coverage (98.53–100.00% identity); 99 matched both the candidate PAM and protospacer.

Table S3. Representative non-target BLASTN alignments and recognition-site comparison.

Taxon/strain	Accession	Identity %	PAM	Protospacer mismatches	Pa-F/Pa-R mismatches
Pantoea sp. RSPAM1	CP046137	99.41	TTTC	0	1/3
Pantoea sp. nfd-35	CP122403	98.23	TTTC	0	1/0
Pantoea allii strain DOAB1051	CP193924	90.86	TTTC	2	4/5
Pantoea stewartii subsp. indologenes	CP186715	86.14	TATC	2	6/8
Pantoea agglomerans strain HJS002	CP090207	74.62	TCGC	5	8/10
Pantoea vagans strain GM1	CP171471	71.94	TCGC	7	7/11

The BLASTN search excluding P. ananatis returned 100 records, 85 with full query coverage (71.64–99.41% identity).