# Detection of five bacterial pathogens of rice by multiplex PCR

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1 Works for me

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ABSTRACT

This multiplex PCR protocol is used for the detection of five bacterial pathogens of rice : *Pseudomonas fuscovaginae, Burkholderia glumae and gladioli, Pantoea spp, Xanthomonas oryzae, Sphingomonas spp.*

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

M. Bangratz, I. Wonni, K. Kini, M. Sondo, C. Brugidou, G. Béna, F. Gnacko, M. Barro, R. Koebnik, D. Silué, C. Tollenaere (2020) Design of a new multiplex PCR assay for rice pathogenic bacteria detection and its application to infer disease incidence and detect co-infection in rice fields in Burkina Faso. PLoS One.

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Agarose | | Sigma |
| 5X Hot Firepol Multiplex Mix Ready to load | | |
| (NH4)2SO4 160 mM | | |
| TBE buffer | | |
| ethidium bromide | | |

MATERIALS TEXT

- Applied Biosystems Veriti 96-Well Thermal Cycler

- Oligonucleotides :

*Pseudomonas fuscovaginae* (amplicon size: 710 pb)
Pfs207-F : CAGTTCGATGGTCTGGGAAT
Pfs207-R : GGGACTGGTAAAGCACGGTA

*Burkholderia glumae and B.gladioli* (amplicon size: 508 pb)
toxB_F : GCATTTGAAACCGAGATGGT
toxB_Rd : TCGCATGCAGATAACCRAAG

*Sphingomonas* spp.(amplicon size: 435 pb)
Sphingo_KK_F1: CGGCTGCTAATACCGGATGAT
Sphingo_KK_R1: AGGCAGTTCTGGAGTTGAGC

*Xanthomonas oryzae* (amplicon size: 331 pb)
Xo3756F : CATCGTTAGGACTGCCAGAAG
Xo3756R : GTGAGAACCACCGCCATCT



**Citation:** Martine Bangratz, Charlotte Tollenaere (04/15/2020). Detection of five bacterial pathogens of rice by multiplex PCR. https://dx.doi.org/10.17504/protocols.io.bcpaivie

*Pantoea* spp (amplicon size: 263 pb)
PAN_KK263F : GCGAGCCAATCGACATTA
PAN_KK263R : CGAGTAACCTGAGTGTTCAG

BEFORE STARTING
- Wear clean gloves
- Clean and disinfect the PCR cabinet
- Defreeze DNA samples and reagents
- Gently mix the DNA samples and the 5X Hot Firepol master mix
- Vortex the (NH4)2SO4 and the oligonucleotides
- Spin down the DNA sample and all reagents and keep them on ice
- Mark the 1.5 ml "Mix" tube and the 0.2 ml tubes for the PCR . Keep them on ice

1 The amounts described in this protocol are for one sample. Calculate the quantity you need based on your number of sample. Always prepare 10 % more mix.

2

Prepare the PCR reaction mixture following the specifications below

| Component | Volume (µl) |
|---|---|
| Template | 2 |
| H20 | 14,55 |
| Pfs207-F (5 µM) | 0,2 |
| Pfs207-R (5 µM) | 0,2 |
| ToxB_F (100 µM) | 0,2 |
| ToxB_Rd (100 µM) | 0,2 |
| Sphingo_KK_F1 (10 µM) | 0,1 |
| Sphingo_KK_R1 (10 µM) | 0,1 |
| Xo3756F (10 µM) | 0,3 |
| Xo3756R (10 µM) | 0,3 |
| PAN_KK263F (100 µM) | 0,3 |
| PAN_KK263R (100 µM) | 0,3 |
| $(NH_4)_2SO_4$ | 1,25 |
| Hot Firepol Multiplex Mix RTL | 5 |



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3 Perform the amplification in a thermocycler in the following conditions

| | | |
|---|---|---|
| Hot start | 95°C | 12 min |
| | | |
| Amplification cycles | | |
| Denaturation | 94°C | 30 sec |
| Annealing | 58°C | 30 sec |
| Extension | 72°C | 45 sec |
| | | |
| Final extension | 72°C | 7 min |
| Hold | 4°C | |


4 Prepare a 2% agarose gel. Load 10µl PCR product and perform the electrophoresis at 100 Volts for 90min

5 Observe the gel under UV light after a ethidium bromide bath





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