

# Comparison of PCR primers for analyzing denitrifying microorganisms in groundwater and stream water exchange zones (hyporheic zones)

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**Supplementary Table 1.** PCR primer information of denitrification-associated genes.

| Target gene | Primer set | Primer sequence (5'-3')                        | Product size (bp) | Reference |
|-------------|------------|------------------------------------------------|-------------------|-----------|
| <i>narG</i> | 1960m2f    | TAYGTSGGCCARGARAA                              | 110               | [26]      |
|             | 2050m2r    | CGTAGAAGAAGCTGGTGC<br>TGTT                     |                   |           |
|             | narG-f     | TCGCCSATYCCGGCSATGT<br>C                       | 173               | [27]      |
|             | narG-r     | GAGTTGTACCAGTCRGCSCG<br>AYTCSG                 |                   |           |
|             | narG1960F  | TAYGTSGGSCARGARAA                              | 644-653*          | [28]      |
|             | narG2650R  | TTYTCRTACCABGTBGC                              |                   |           |
|             | narG328f   | GACAAACTTCGCAGCGG                              | 170               | [29]      |
|             | narG497r   | TCACCCAGGACGCTGTTC<br>CAYGGNGTNAAYTGYACN<br>GG |                   |           |
|             | 1st        | T37                                            | 1,690             | [30]      |
|             |            | T39                                            |                   |           |
|             | 2n         | W9                                             | 500               | [30]      |
|             | d          | T38                                            |                   |           |
| <i>napA</i> | nap3F      | TGGACVATGGGYTTAAY<br>C                         | 152               | [27]      |
|             | napA4R     | ACYTCRCGHGCVGTRCCR<br>CA                       |                   |           |
|             | V16        | GCNCCNTGYMGNTTYTGY<br>GG                       | 126               | [31]      |
|             | V17        | RTGYTGRTRAANCCCATN<br>GTCCA                    |                   |           |
| <i>nirS</i> | nirS1F     | CCTAYTGGCCGCCRCART                             | 890               | [32]      |
|             | nirS6R     | CGTTGAACTTRCCGGT                               |                   |           |
|             | nirS4QF    | AACGYSAAGGARACSGG                              | 430               | [33]      |
|             | nirS6QR    | GASTTCGGRTGSGTCTTSAY<br>GAA                    |                   |           |
|             | nirS263F   | TGCGYAARGGGGCANCBG                             | 688               | [34]      |

|                                 |   |           |                             |     |      |
|---------------------------------|---|-----------|-----------------------------|-----|------|
|                                 |   |           | GCAA                        |     |      |
| <i>nirSpm</i>                   | 4 | nirS950R  | GCBACRCGSGGYTCGGAT<br>G     | 244 | [35] |
|                                 |   | nirSsh2F  | ACCGCCGCCAACAACCTCC<br>AACA |     |      |
|                                 |   | nirSsh4R  | CCGCCCTGGCCCTGGAGC          |     |      |
| <i>nirSps</i>                   | 5 | forward   | ACAAGGAGCACAACTGG<br>AAGGT  | 84  | [36] |
|                                 |   | reverse   | CGCGTCGGCCCAGA              |     |      |
| <i>nirK</i>                     | 1 | nirK1F    | GGMATGGTKCCSTGGCA           | 494 | [32] |
|                                 |   | nirK3R    | GAACCTGCCGGTVGKCCA<br>GAC   |     |      |
|                                 | 2 | nirK1F    | GGMATGGTKCCSTGGCA           | 514 |      |
|                                 |   | nirK5R    | GCCTCGATCAGRTRRTGG          |     |      |
|                                 | 3 | nirK876F  | ATYGGCGGVAYGGCGA            | 165 | [37] |
|                                 |   | nirK1040R | GCCTCGATCAGRTRRTGGT<br>T    |     |      |
|                                 | 4 | F1aCu     | ATCATGGTSCTGCCGCG           | 435 | [38] |
|                                 |   | R3Cu      | TTGGTGTRGACTAGCTCC<br>G     |     |      |
|                                 | 5 | nirK517F  | TTYGTSTAYCACTGCGCVC<br>C    | 539 | [39] |
|                                 |   | nirK1055R | GCYTCGATCAGRTRRTGGT<br>T    |     |      |
| <i>norB</i><br>( <i>cnorB</i> ) | 1 | cnorB1F   | GARTTYCTNGARCARCC           | 579 |      |
|                                 |   | cnorB6R   | GAANCCCCANACNCCNG<br>C      |     |      |
|                                 | 2 | cnorB2F   | GACAAGNNNTACTGGTG<br>GT     | 455 |      |
|                                 |   | cnorB7R   | TGNCCRTGNGCNGCNGT           |     |      |
|                                 | 3 | cnorB2F   | GACAAGNNNTACTGGTG<br>GT     | 390 | [11] |
|                                 |   | cnorB6R   | GAANCCCCANACNCCNG<br>C      |     |      |
| <i>norB</i><br>( <i>qnorB</i> ) | 4 | qnorB2F   | GGNCAYCARGGNTAYGA           | 263 |      |
|                                 |   | qnorB5R   | ACCCANAGRTGNACNAC<br>CCACCA |     |      |
|                                 | 5 | qnorB2F   | GGNCAYCARGGNTAYGA           | 638 |      |
|                                 |   | qnorB7R   | GGNGGRTTDTACADGAAN<br>CC    |     |      |
| <i>nosZ</i>                     | 1 | nosZ1840F | CGCRACGGCAASAAGGTS<br>MSSGT | 267 | [40] |
|                                 |   | nosZ2090R | CAKRTGCAKSGCRTGGCA<br>GAA   |     |      |
|                                 | 2 | nosZ1F    | WCSYTGTTCMTCGACAGC<br>CAG   | 259 | [35] |
|                                 |   | nosZ1R    | ATGTCGATCARCTGVKCRT<br>TYTC |     |      |
|                                 | 3 | Forward   | AGAACGACCAGCTGATCG<br>ACA   | 300 | [41] |
|                                 |   |           |                             |     |      |

|   |           |                           |     |      |
|---|-----------|---------------------------|-----|------|
|   | Reverse   | TCCATGGTGACGCCGTGG<br>TTG |     |      |
|   | nosZ-F-11 | CGCTGTTCTCGACAGYCA        |     |      |
|   | 81        | G                         |     |      |
| 4 | nosZ-R-18 | ATGTGCAKIGCRTGGCAGA       | 700 | [42] |
|   | 80        | A                         |     |      |
|   | nosLb     | CCCGCTGCACACCRCTTC        |     |      |
|   |           | GA                        |     |      |
| 5 | nosRb     | CGTCGCCSGAGATGTGGA        | 260 | [33] |
|   |           | TCA                       |     |      |

\*Range of PCR product size based on four sequences (AY113836.1–AY113839.1), 650 bp were reported [28].

Dentrificans spp. PCR result – 1. nirK set1

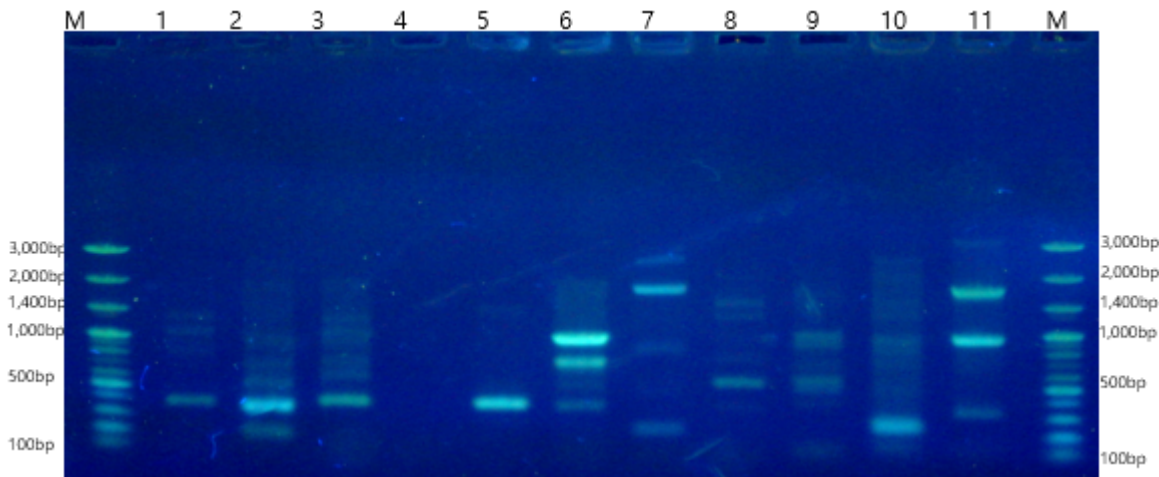


Fig. 1 M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |        |                     |     |                      |
|---|--------|---------------------|-----|----------------------|
| 1 | nirK1F | GGMATGGTKCCSTGGCA   | 392 | Ehrlich et al., 1998 |
|   | nirK3R | GAACCTGCGGTGKOCAGAC |     |                      |

PCR Condition

|             |       |  |
|-------------|-------|--|
| 95°C        | 5min  |  |
| 95°C        | 30sec |  |
| Touchdown°C | 40sec |  |
| 72°C        | 40sec |  |
| 72°C        | 10min |  |
| 4°C         | store |  |

1-11; PCR Product

Promega 2X green master Mix 5µl

M; Marker

Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µℓ |
| Primer -Forward(20pmol/µℓ) | 1µℓ    |
| Primer -Reverse(20pmol/µℓ) | 1µℓ    |
| Template (10ng)            | 1µℓ    |
| DW                         | 9.5µℓ  |
| -----                      |        |
| Total                      | 25µℓ   |

Dentirificans spp. PCR result – 1. nirK set 2

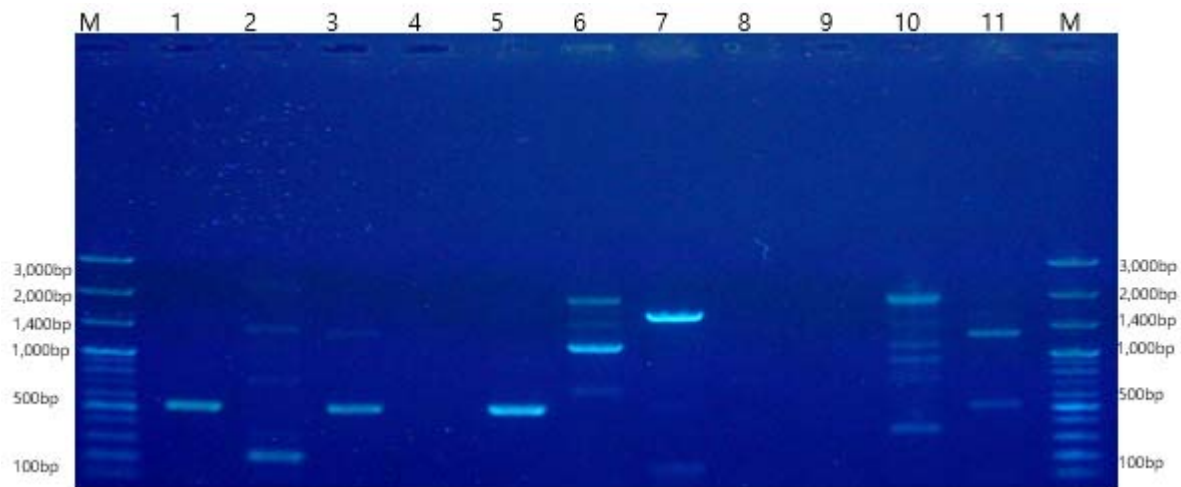


Fig. 2 M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*

|   |        |                    |     |                     |
|---|--------|--------------------|-----|---------------------|
| 2 | nirK1F | GGMATGGTKCCSTGGCA  | 514 | Braker et al., 1998 |
|   | nirK5R | GCCTCGATCAGRTTGTGG |     |                     |

PCR Condition

|             |       |                                                                            |
|-------------|-------|----------------------------------------------------------------------------|
| 95°C        | 5min  | 30 Cycle<br>Touchdown Condition (°C)<br>1-10cycle: 45→40<br>11-30cycle: 43 |
| 95°C        | 30sec |                                                                            |
| Touchdown°C | 40sec |                                                                            |
| 72°C        | 40sec |                                                                            |
| 72°C        | 10min |                                                                            |
| 4°C         | store |                                                                            |

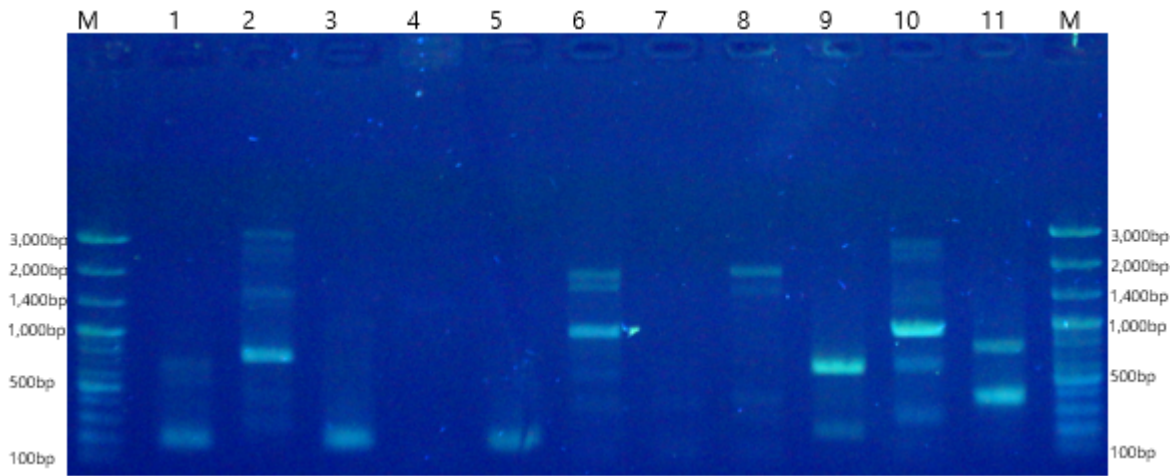
Loading composition (1% gel)

- 1-11; PCR Product
- Promega 2X green master Mix 5µℓ
- M; Marker
- Bionix 100bp step DNA ladder 4µℓ

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µℓ |
| Primer -Forward(20pmol/µℓ) | 1µℓ    |
| Primer -Reverse(20pmol/µℓ) | 1µℓ    |
| Template (10ng)            | 1µℓ    |
| DW                         | 9.5µℓ  |
| -----                      |        |
| Total                      | 25µℓ   |

Dentrificans spp. PCR result – 1. nirK set 3



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |           |                      |     |                            |
|---|-----------|----------------------|-----|----------------------------|
| 3 | nirK876F  | ATYGGCGGVAYGGCGA     | 173 | Henry <i>et al.</i> , 2004 |
|   | nirK1040R | GCCTCGATCAGRTTGTGGTT |     |                            |

PCR Condition

|             |       |                                                                             |
|-------------|-------|-----------------------------------------------------------------------------|
| 95°C        | 10min | } 41 Cycle<br>Touchdown Condition (°C)<br>1-6 cycle: 63→58<br>7-41cycle: 43 |
| 95°C        | 15sec |                                                                             |
| Touchdown°C | 30sec |                                                                             |
| 72°C        | 30sec |                                                                             |
| 72°C        | 10min |                                                                             |
| 4°C         | store |                                                                             |

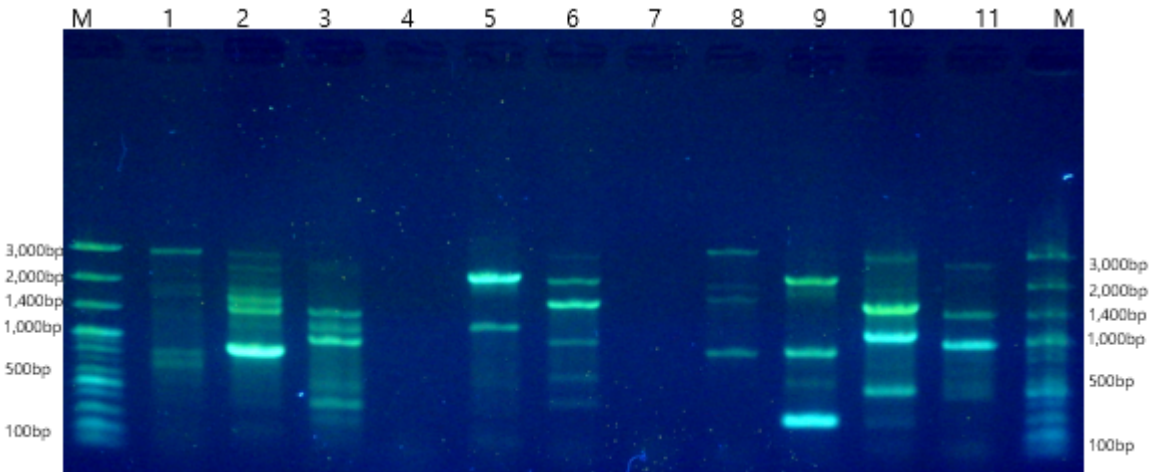
Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

Dentrificans spp. PCR result – 1. nirK set 4



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |       |                      |     |                           |
|---|-------|----------------------|-----|---------------------------|
| 4 | FlaCu | ATCATGGTISCTGCCGCG   | 454 | Hallin and Lindgren, 1999 |
|   | R3Cu  | TTGGTGTTRGACTAGCTCCG |     |                           |

PCR Condition

|             |       |            |
|-------------|-------|------------|
| 95°C        | 3min  | } 35 Cycle |
| 95°C        | 30sec |            |
| Touchdown°C | 60sec |            |
| 72°C        | 60sec |            |
| 72°C        | 10min |            |
| 4°C         | store |            |

Loading composition (1% gel)

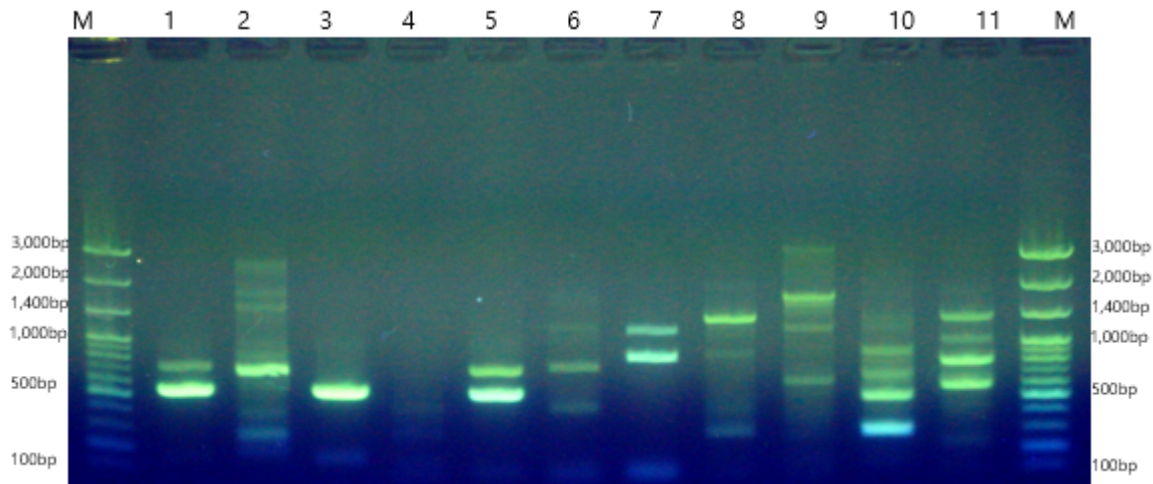
1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |



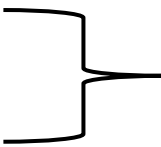
Dentrificans spp. PCR result – 1. nirK set 5



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |           |                      |   |                              |
|---|-----------|----------------------|---|------------------------------|
| 5 | nirK517F  | TTYGTSTAYCACTGCGCVCC | 5 | Baudoin <i>et al.</i> , 2011 |
|   | nirK1055R | GCYTCGATCAGTTTGTGGTT |   |                              |

PCR Condition

|             |       |                                                                                     |                                                                             |
|-------------|-------|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| 95°C        | 6min  |  | 40 Cycle<br>Touchdown Condition (°C)<br>1-15 cycle: 63→58<br>16-40cycle: 43 |
| 95°C        | 30sec |                                                                                     |                                                                             |
| Touchdown°C | 45sec |                                                                                     |                                                                             |
| 72°C        | 60sec |                                                                                     |                                                                             |
| 72°C        | 7min  |                                                                                     |                                                                             |
| 4°C         | store |                                                                                     |                                                                             |

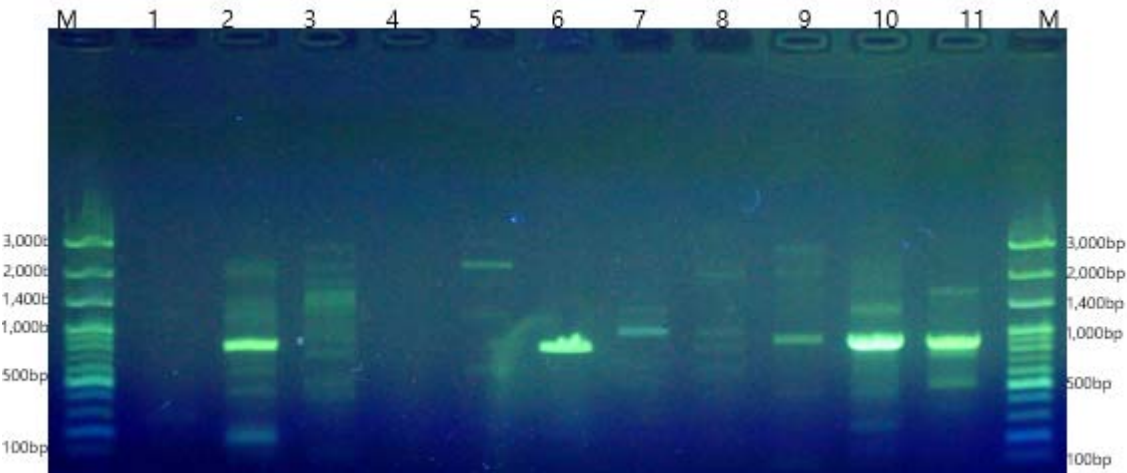
Loading composition (1% gel)

- 1-11; PCR Product
- Promega 2X green master Mix 5µl
- M; Marker
- Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| <hr/>                      |        |
| Total                      | 25µl   |

Dentrificans spp. PCR result – 2. nirS set 1



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |        |                   |     |                             |
|---|--------|-------------------|-----|-----------------------------|
| 1 | nirS1F | CCTAYTGCCGCCRCAAT | 890 | Braker <i>et al.</i> , 1998 |
|   | nirS6R | CGTTGAACCTTCCGGT  |     |                             |

PCR Condition

|             |       |                                   |
|-------------|-------|-----------------------------------|
| 95°C        | 5min  |                                   |
| 95°C        | 30sec |                                   |
| Touchdown°C | 40sec |                                   |
| 72°C        | 40sec |                                   |
| 72°C        | 10min |                                   |
| 4°C         | store |                                   |
|             |       | 30 Cycle Touchdown Condition (°C) |
|             |       | 1-10 cycle: 45→40                 |
|             |       | 11-30cycle: 43                    |

Loading composition (1% gel)

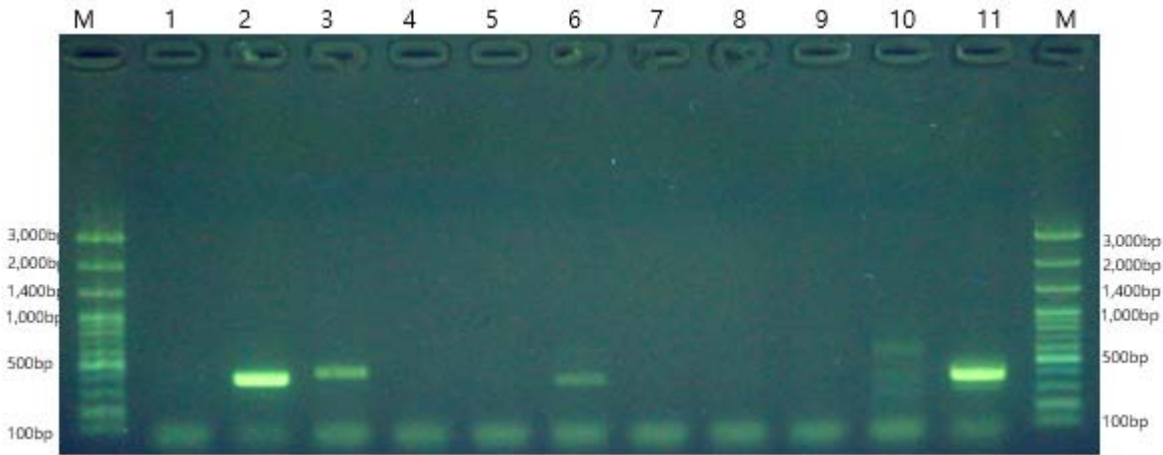
1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| <hr/>                      |        |
| Total                      | 25µl   |



Dentrificans spp. PCR result – 2. nirS set 2



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |         |                         |     |                       |
|---|---------|-------------------------|-----|-----------------------|
| 2 | nirS4QF | AACGYSAAGGARACSGG       | 425 | Throback et al., 2004 |
|   | nirS8QR | GASTTCGGRTGSGTCTTSAVGAA |     |                       |

PCR Condition

|             |       |  |                                                                           |
|-------------|-------|--|---------------------------------------------------------------------------|
| 95°C        | 10min |  | 41 Cycle<br>Touchdown Condition (°C)<br>1-6 cycle: 63→58<br>7-41cycle: 58 |
| 95°C        | 15sec |  |                                                                           |
| Touchdown°C | 30sec |  |                                                                           |
| 72°C        | 30sec |  |                                                                           |
| 72°C        | 10min |  |                                                                           |
| 4°C         | store |  |                                                                           |

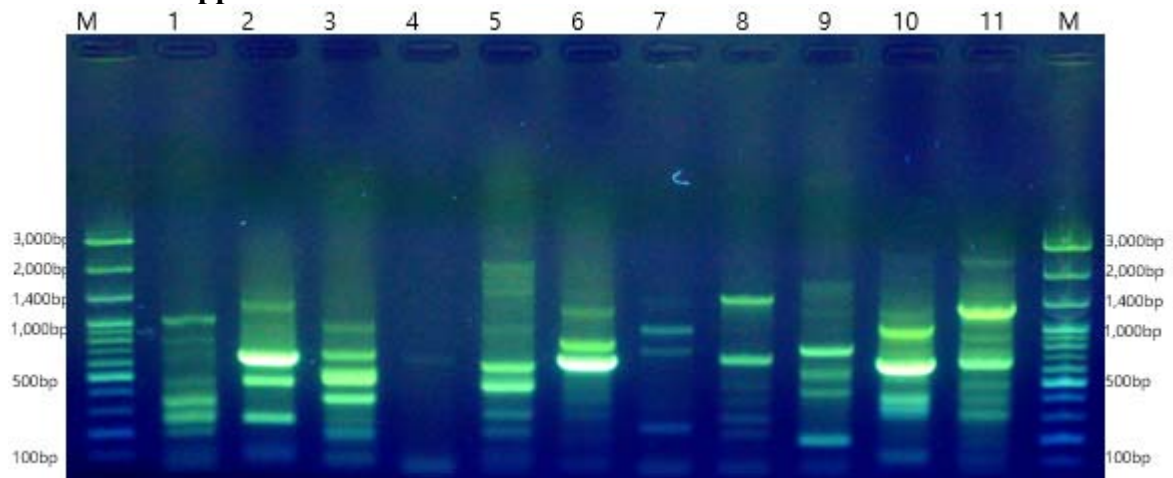
Loading composition (1% gel)

- 1-11; PCR Product
- Promega 2X green master Mix 5µl
- M; Marker
- Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

### Dentrificans spp. PCR result – 2. nirS set 3



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |          |                        |    |                           |
|---|----------|------------------------|----|---------------------------|
| 3 | nirS263F | TGCGYAARGGGGCANCBGGCAA | 16 | Chen <i>et al.</i> , 2010 |
|   | nirS950R | GCBACRCGGGGYTCSGGATG   |    |                           |

### PCR Condition

|             |       |
|-------------|-------|
| 95°C        | 5min  |
| 95°C        | 30sec |
| Touchdown°C | 45sec |
| 72°C        | 60sec |
| 72°C        | 7min  |
| 4°C         | store |

40 Cycle  
Touchdown Condition (°C)  
1-21 cycle: 60→40  
22-40cycle: 53

### Loading composition (1% gel)

1-11; PCR Product

Promega 2X green master Mix 5µl

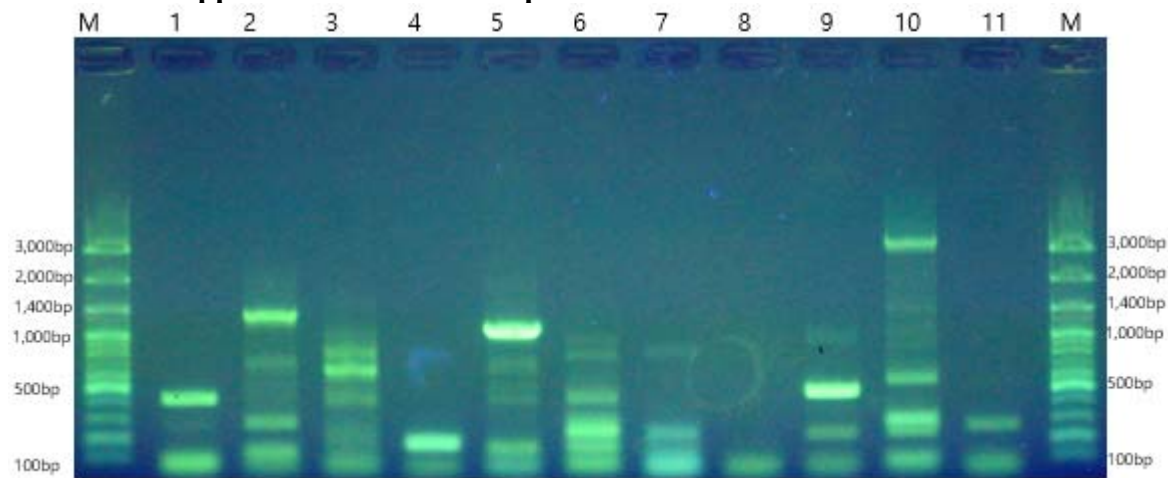
M; Marker

Bionix 100bp step DNA ladder 4µl

### PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| <hr/>                      |        |
| Total                      | 25µl   |

## Dentrificans spp. PCR result – 2. nirSpm set 4



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|                    |   |          |                         |     |                        |
|--------------------|---|----------|-------------------------|-----|------------------------|
| nirS <sub>pm</sub> | 4 | nirSsh2F | ACCGCCGCCAACAACCTCCAACA | 244 | Henderson et al., 2010 |
|                    |   | nirSsh4R | CGCCCTGGCCCTGGAGC       |     |                        |

## PCR Condition

|             |       |
|-------------|-------|
| 50°C        | 2min  |
| 95°C        | 10min |
| -----       |       |
| 95°C        | 15sec |
| Touchdown°C | 60sec |
| 72°C        | 45sec |
| -----       |       |
| 72°C        | 10min |
| 4°C         | store |

46 Cycle  
Touchdown Condition (°C)  
1-6 cycle: 68→62  
7-46cycle: 62

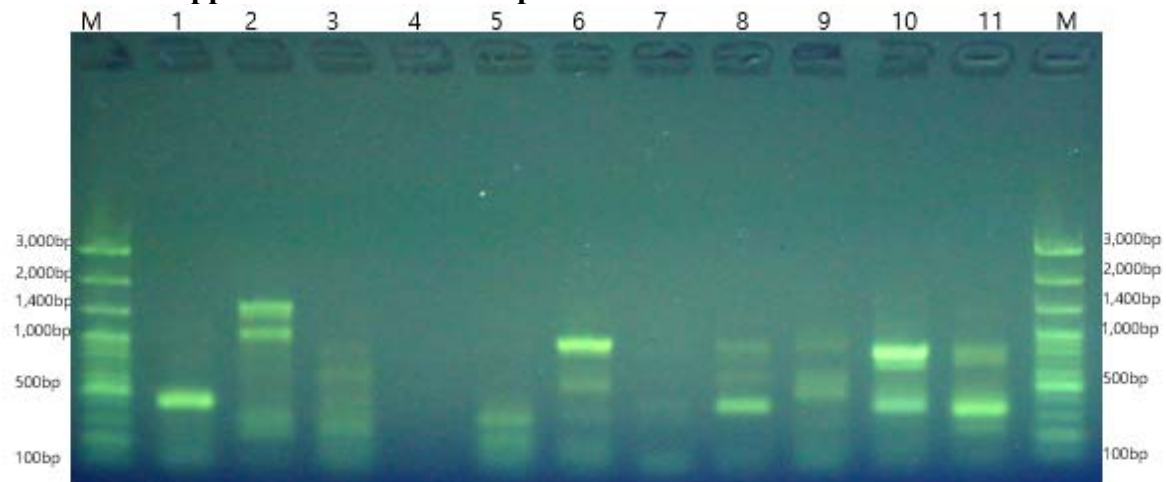
## Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

## PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

### Dentrificans spp. PCR result – 2. nirSps set 5



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|                    |   |         |                         |    |                      |
|--------------------|---|---------|-------------------------|----|----------------------|
| nirS <sub>ps</sub> | 5 | forward | ACAAGGAGCACAACCTGGAAGGT | 39 | Grüntig et al., 2001 |
|                    |   | reverse | CGCGTCGGCCGAGA          |    |                      |

### PCR Condition

|      |       |            |
|------|-------|------------|
| 50°C | 2min  | } 40 Cycle |
| 95°C | 10min |            |
| 95°C | 15sec |            |
| 60°C | 60sec |            |
| 4°C  | store |            |

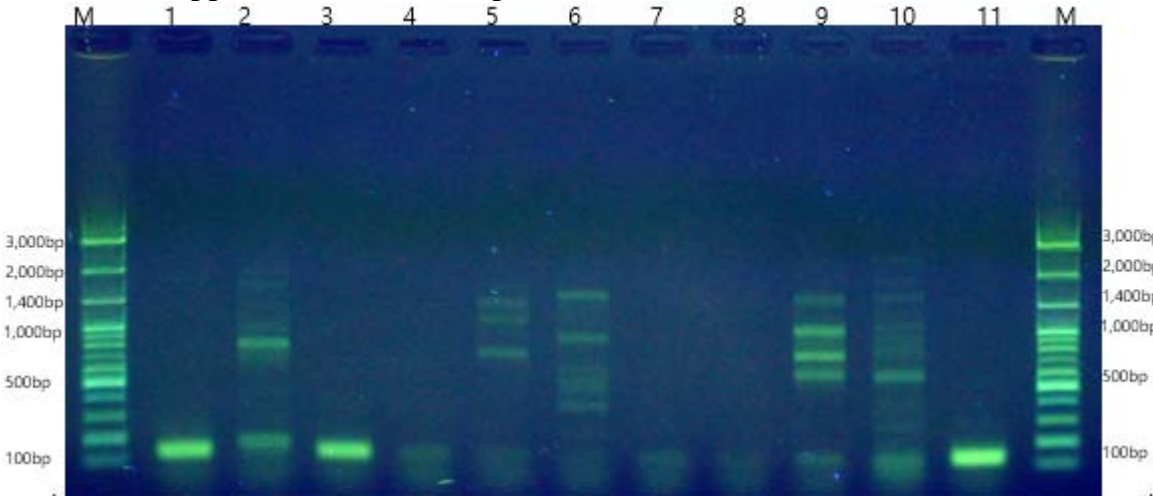
### Loading composition (1% gel)

1-11; PCR Product  
 Promega 2X green master Mix 5 µl  
 M; Marker  
 Bionix 100bp step DNA ladder 4 µl

### PCR Composition

|                            |         |
|----------------------------|---------|
| 2X Green Master Mix        | 12.5 µl |
| Primer -Forward(20pmol/µl) | 1 µl    |
| Primer -Reverse(20pmol/µl) | 1 µl    |
| Template (10ng)            | 1 µl    |
| DW                         | 9.5 µl  |
| <hr/>                      |         |
| Total                      | 25 µl   |

Dentrificans spp. PCR result – 3. napA set 1



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |                 |                                              |     |                  |
|---|-----------------|----------------------------------------------|-----|------------------|
| 1 | nap3F<br>napA4R | TGGACVATGGGYTTTAAAYC<br>ACYTCRCGHGCVGTGCCRCA | 152 | Bru et al., 2007 |
|---|-----------------|----------------------------------------------|-----|------------------|

PCR Condition

|             |       |                                                                           |
|-------------|-------|---------------------------------------------------------------------------|
| 95°C        | 10min | 41 Cycle<br>Touchdown Condition (°C)<br>1-6 cycle: 61→56<br>7-41cycle: 56 |
| 95°C        | 15sec |                                                                           |
| Touchdown°C | 30sec |                                                                           |
| 72°C        | 30sec |                                                                           |
| 72°C        | 10min |                                                                           |
| 4°C         | store |                                                                           |

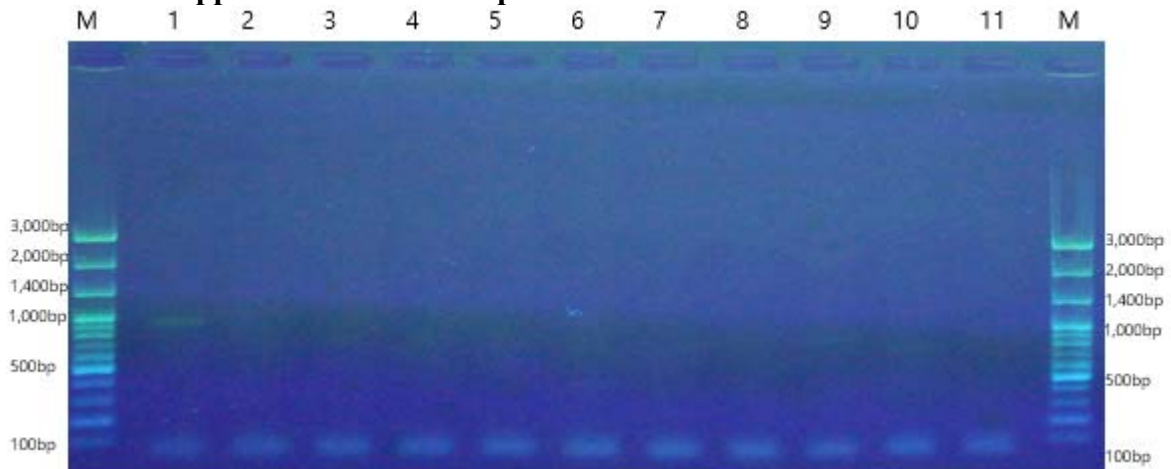
Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

### Dentrificans spp. PCR result – 3. napA set 2



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |     |                          |     |                      |
|---|-----|--------------------------|-----|----------------------|
| 2 | V16 | GCNCCNTGYMGNITTYTGYGG    | 128 | Wakelin et al., 2007 |
|   | V17 | RTGYTGRTTRAANCCCATNGTCCA |     |                      |

### PCR Condition

|      |        |            |
|------|--------|------------|
| 94°C | 15min  | } 30 Cycle |
| 94°C | 60sec  |            |
| 50°C | 60sec  |            |
| 72°C | 120sec |            |
| 72°C | 5min   |            |
| 4°C  | store  |            |

### Loading composition (1% gel)

1-11; PCR Product

Promega 2X green master Mix 5µl

M; Marker

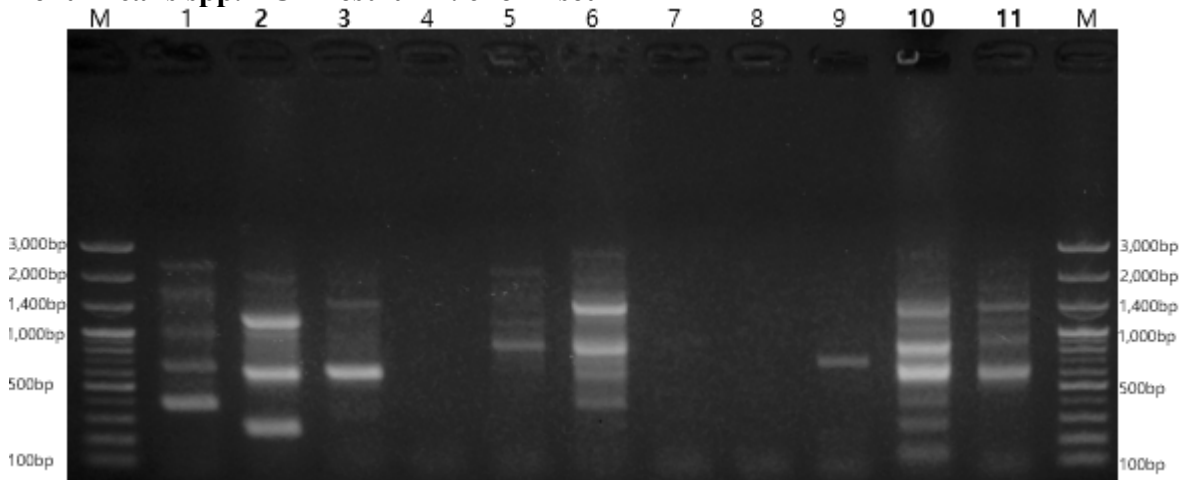
Bionix 100bp step DNA ladder 4µl

### PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |



# Dentrificans spp. PCR result – 4. cnorB set 1



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |         |                   |     |                         |
|---|---------|-------------------|-----|-------------------------|
| 1 | cnorB1F | GARTTYCTNGARCACCC | 578 | Braker and Tiedje, 2003 |
|   | cnorB8R | GAANCCCCANACNCNGC |     |                         |

## PCR Condition

|             |       |                                                                                 |
|-------------|-------|---------------------------------------------------------------------------------|
| 95°C        | 5min  | } 40 Cycle<br>Touchdown Condition (°C)<br>1-10 cycle: 57→52.5<br>11-40cycle: 55 |
| 95°C        | 30sec |                                                                                 |
| Touchdown°C | 40sec |                                                                                 |
| 72°C        | 30sec |                                                                                 |
| 72°C        | 10min |                                                                                 |
| 4°C         | store |                                                                                 |

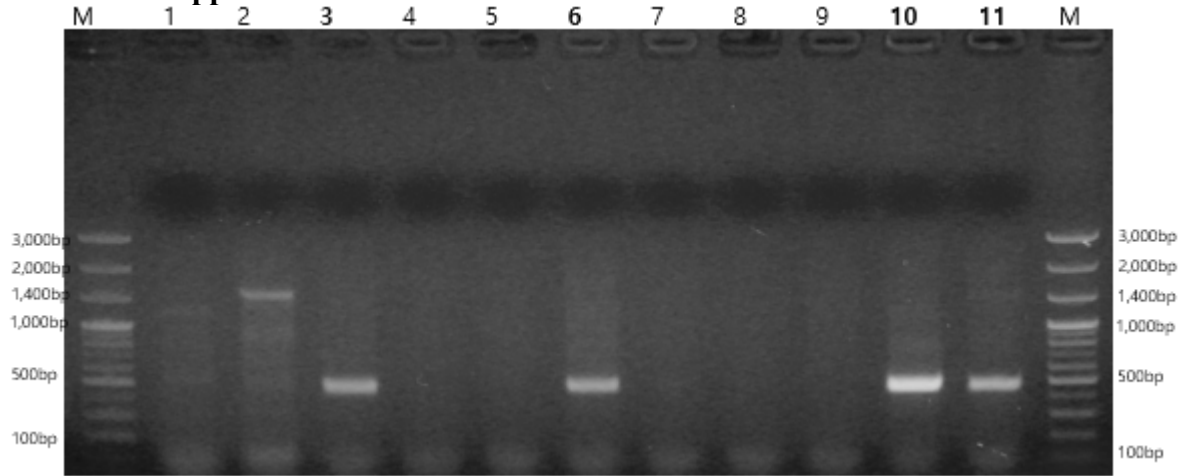
## Loading composition (1% gel)

1-11; PCR Product  
 Promega 2X green master Mix 5µl  
 M; Marker  
 Bionix 100bp step DNA ladder 4µl

## PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

Dentrificans spp. PCR result – 4. cnorB set 2



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |         |                      |     |                         |
|---|---------|----------------------|-----|-------------------------|
| 2 | cnorB2F | GACAAGNNINTACTGGTGGT | 454 | Bräler and Tiedje, 2003 |
|   | cnorB7R | TGNCERTGNGCNGCNGT    |     |                         |

PCR Condition

|             |       |  |                                                                               |
|-------------|-------|--|-------------------------------------------------------------------------------|
| 95°C        | 5min  |  | 40 Cycle<br>Touchdown Condition (°C)<br>1-10 cycle: 57→52.5<br>11-40cycle: 55 |
| 95°C        | 30sec |  |                                                                               |
| Touchdown°C | 40sec |  |                                                                               |
| 72°C        | 30sec |  |                                                                               |
| 72°C        | 10min |  |                                                                               |
| 4°C         | store |  |                                                                               |

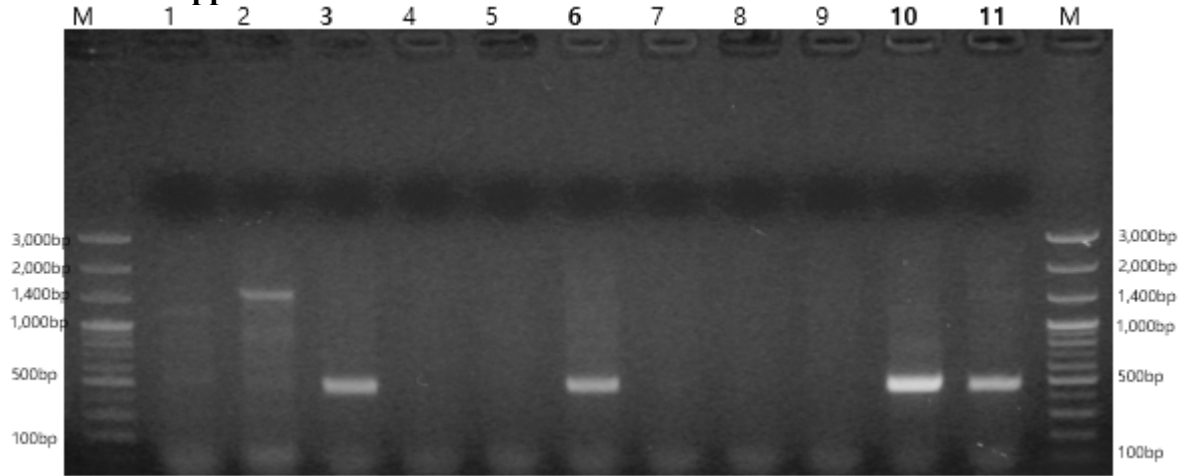
Loading composition (1% gel)

- 1-11; PCR Product
- Promega 2X green master Mix 5µl
- M; Marker
- Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

Dentrificans spp. PCR result – 4. cnorB set 3



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |         |                      |     |                         |
|---|---------|----------------------|-----|-------------------------|
| 3 | cnorB2F | GACAAGNNINTACTGGTGGT | 389 | Bräuer and Tiedje, 2003 |
|   | cnorB6R | GAANCCCCANACNCCNGC   |     |                         |

PCR Condition

|             |       |  |                                                                               |
|-------------|-------|--|-------------------------------------------------------------------------------|
| 95°C        | 5min  |  | 40 Cycle<br>Touchdown Condition (°C)<br>1-10 cycle: 57→52.5<br>11-40cycle: 55 |
| 95°C        | 30sec |  |                                                                               |
| Touchdown°C | 40sec |  |                                                                               |
| 72°C        | 30sec |  |                                                                               |
| 72°C        | 10min |  |                                                                               |
| 4°C         | store |  |                                                                               |

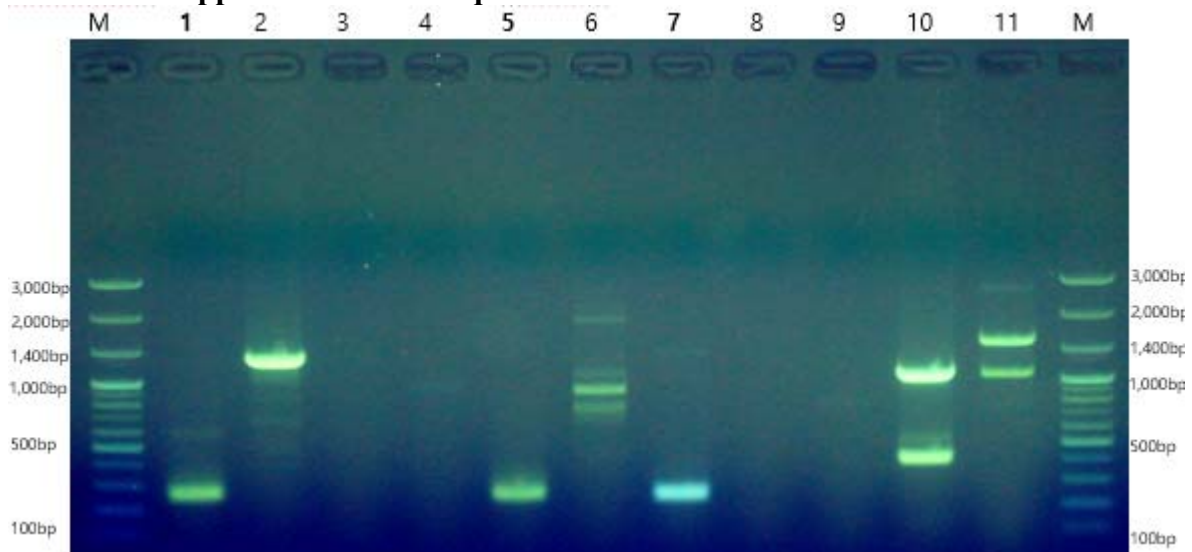
Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

# Dentrificans spp. PCR result – 5. qnorB set 1



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |         |                         |     |                         |
|---|---------|-------------------------|-----|-------------------------|
| 1 | qnorB2F | GGNCAYCARGGNTAYGA       | 262 | Braker and Tiedje, 2003 |
|   | qnorB5R | ACCCANAGRTGNACNACCCACCA |     |                         |

## PCR Condition

|             |       |                                                                                 |
|-------------|-------|---------------------------------------------------------------------------------|
| 95°C        | 5min  | } 40 Cycle<br>Touchdown Condition (°C)<br>1-10 cycle: 57→52.5<br>11-40cycle: 55 |
| 95°C        | 30sec |                                                                                 |
| Touchdown°C | 40sec |                                                                                 |
| 72°C        | 30sec |                                                                                 |
| 72°C        | 10min |                                                                                 |
| 4°C         | store |                                                                                 |

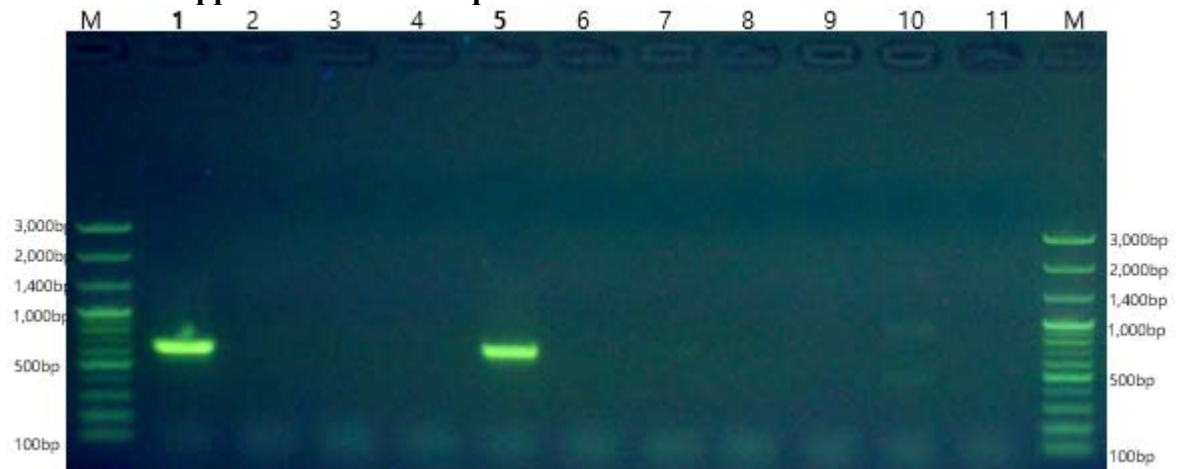
## Loading composition (1% gel)

1-11; PCR Product  
 Promega 2X green master Mix 5µl  
 M; Marker  
 Bionix 100bp step DNA ladder 4µl

## PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

# Dentrificans spp. PCR result – 5. qnorB set 2



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |         |                     |     |                         |
|---|---------|---------------------|-----|-------------------------|
| 2 | qnorB2F | GGNCAYCARGGNTAYGA   | 637 | Braker and Tiedje, 2003 |
|   | qnorB7R | GGNGGRTTDTACDGAANCC |     |                         |

## PCR Condition

|             |       |  |                                                                               |
|-------------|-------|--|-------------------------------------------------------------------------------|
| 95°C        | 5min  |  | 40 Cycle<br>Touchdown Condition (°C)<br>1-10 cycle: 57→52.5<br>11-40cycle: 55 |
| 95°C        | 30sec |  |                                                                               |
| Touchdown°C | 40sec |  |                                                                               |
| 72°C        | 30sec |  |                                                                               |
| 72°C        | 10min |  |                                                                               |
| 4°C         | store |  |                                                                               |

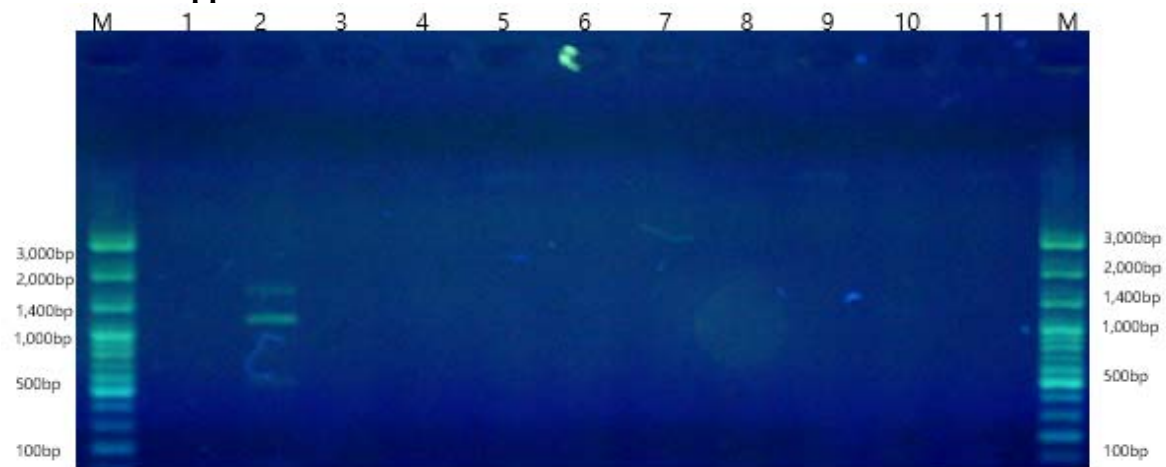
## Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

## PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

# Dentrificans spp. PCR result – 6. narG set 1



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |         |                        |    |                                      |
|---|---------|------------------------|----|--------------------------------------|
| 1 | 1960m2f | TAYGTSGGCCARGAAA       | 59 | López-Gutiérrez <i>et al.</i> , 2004 |
|   | 2050m2r | CGTAGAAGAAGCTGGTGCTGTT |    |                                      |

## PCR Condition

|             |       |  |                                                                               |
|-------------|-------|--|-------------------------------------------------------------------------------|
| 95°C        | 15min |  | 40 Cycle<br>Touchdown Condition (°C)<br>1-40 cycle: 63→58<br>(1°C down/cycle) |
| 94°C        | 15sec |  |                                                                               |
| Touchdown°C | 30sec |  |                                                                               |
| 72°C        | 30sec |  |                                                                               |
| 81°C        | 10min |  |                                                                               |
| 4°C         | store |  |                                                                               |

## Loading composition (1% gel)

1-11; PCR Product

Promega 2X green master Mix 5µl

M; Marker

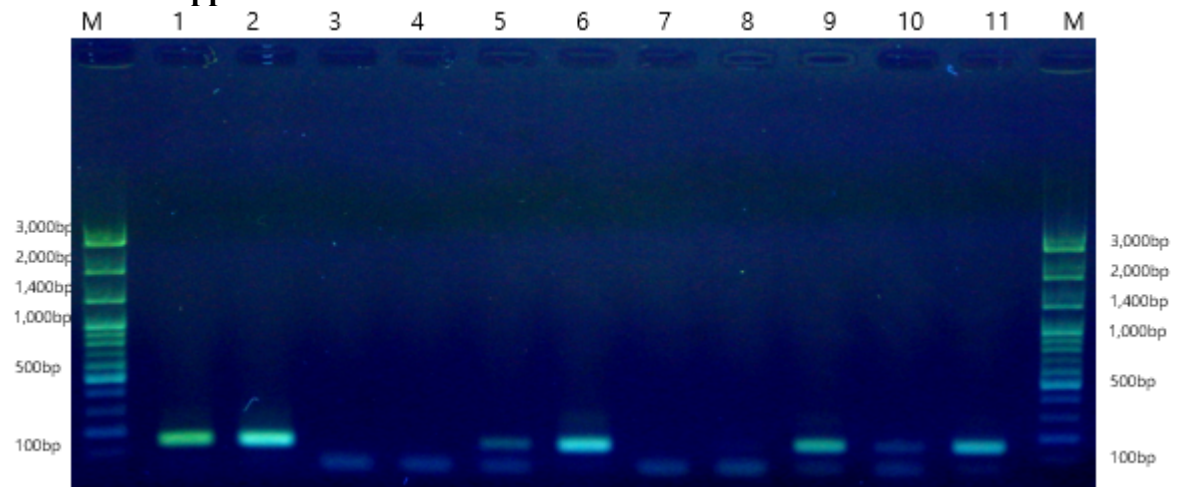
Bionix 100bp step DNA ladder 4µl

## PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| <hr/>                      |        |
| Total                      | 25µl   |



### Dentrificans spp. PCR result – 6. narG set 2



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |        |                             |     |                          |
|---|--------|-----------------------------|-----|--------------------------|
| 2 | narG-f | TCGCCSATYCCGGCSATGTC        | 174 | Bru <i>et al.</i> , 2007 |
|   | narG-r | GAGTTGTACCAGTCRGC SGAYTC SG |     |                          |

### PCR Condition

|             |       |                                                                            |
|-------------|-------|----------------------------------------------------------------------------|
| 95°C        | 10min |                                                                            |
| 94°C        | 15sec |                                                                            |
| Touchdown°C | 30sec |                                                                            |
| 72°C        | 30sec |                                                                            |
| 72°C        | 10min |                                                                            |
| 4°C         | store | 41 Cycle<br>Touchdown Condition (°C)<br>1-6 cycle: 63→58<br>7-41 cycle: 58 |

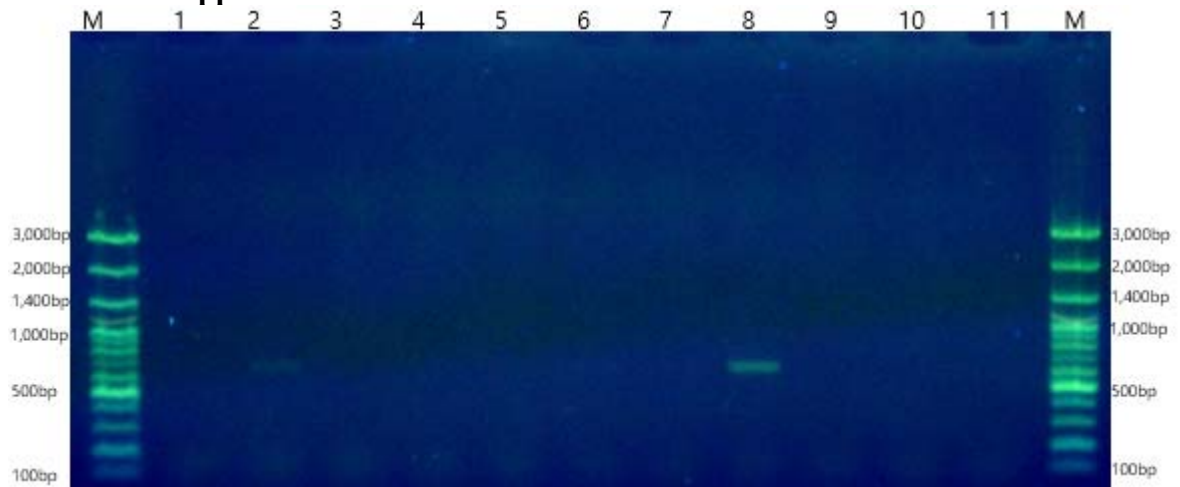
### Loading composition (1% gel)

1-11; PCR Product  
 Promega 2X green master Mix 5µl  
 M; Marker  
 Bionix 100bp step DNA ladder 4µl

### PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| <hr/>                      |        |
| Total                      | 25µl   |

### Dentrificans spp. PCR result – 6. narG set 3



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |                  |                   |     |                               |
|---|------------------|-------------------|-----|-------------------------------|
| 3 | <i>narG1960F</i> | TAYGTSGGSCARGARAA | 660 | Philippt <i>et al.</i> , 2002 |
|   | <i>narG2650R</i> | TTYTCRTACCABGTBGC |     |                               |

### PCR Condition

|             |       |   |                                                                            |
|-------------|-------|---|----------------------------------------------------------------------------|
| 95°C        | 5min  | } | 38 Cycle<br>Touchdown Condition (°C)<br>1-8 cycle: 59→55<br>9-38 cycle: 55 |
| 94°C        | 30sec |   |                                                                            |
| Touchdown°C | 30sec |   |                                                                            |
| 72°C        | 40sec |   |                                                                            |
| 72°C        | 10min |   |                                                                            |
| 4°C         | store |   |                                                                            |

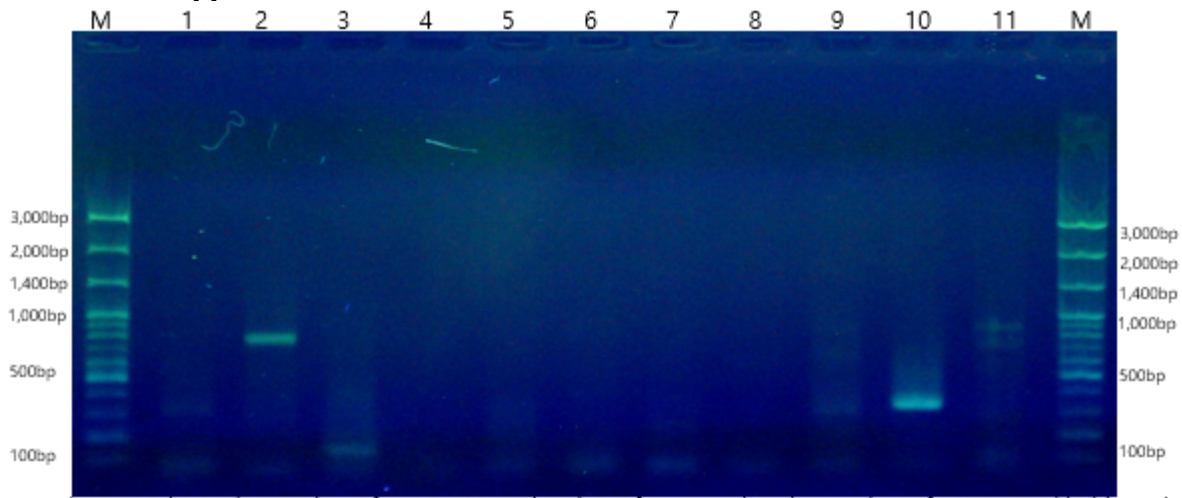
### Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5  $\mu\ell$   
M; Marker  
Bionix 100bp step DNA ladder 4  $\mu\ell$

### PCR Composition

|                                     |                |
|-------------------------------------|----------------|
| 2X Green Master Mix                 | 12.5 $\mu\ell$ |
| Primer -Forward(20pmol/ $\mu\ell$ ) | 1 $\mu\ell$    |
| Primer -Reverse(20pmol/ $\mu\ell$ ) | 1 $\mu\ell$    |
| Template (10ng)                     | 1 $\mu\ell$    |
| DW                                  | 9.5 $\mu\ell$  |
| -----                               |                |
| Total                               | 25 $\mu\ell$   |

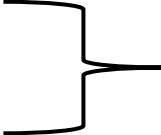
Dentrificans spp. PCR result – 6. narG set 4



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |          |                    |     |                    |
|---|----------|--------------------|-----|--------------------|
| 4 | narG32Ef | GACAAACTTCGCAGCGG  | 170 | Reyna et al., 2010 |
|   | narG497r | TCACCCAGGACGCTGTTC |     |                    |

PCR Condition

|      |       |                                                                                     |          |
|------|-------|-------------------------------------------------------------------------------------|----------|
| 50°C | 2min  |  | 40 Cycle |
| 95°C | 10min |                                                                                     |          |
| 95°C | 15sec |                                                                                     |          |
| 60°C | 960ec |                                                                                     |          |
| 4°C  | store |                                                                                     |          |

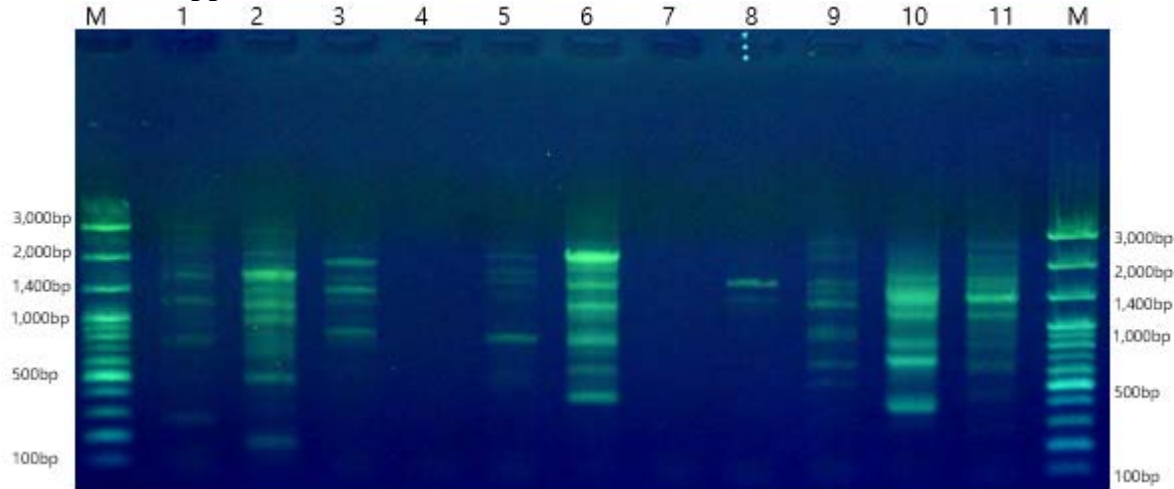
Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| <hr/>                      |        |
| Total                      | 25µl   |

Dentrificans spp. PCR result – 6. narG set 5



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|                     |     |                      |     |                      |
|---------------------|-----|----------------------|-----|----------------------|
| 5                   | T37 | CAYGGNGTNAAYTGYACNGG | 000 | Gregory et al., 2000 |
|                     | T39 | TARTGNGGCCANCCNCCNCC |     |                      |
| 5<br><i>revised</i> | W9  | MGNNGGTGYCCNMGNGGNGC | 000 | Gregory et al., 2000 |
|                     | T38 | ACRTQNGTYTGYTCNCCCCA |     |                      |

PCR Condition

|      |       |          |
|------|-------|----------|
| 94°C | 5min  | 30 Cycle |
| 94°C | 60sec |          |
| 58°C | 60sec |          |
| 72°C | 90sec |          |
| 72°C | 10min |          |
| 4°C  | store |          |

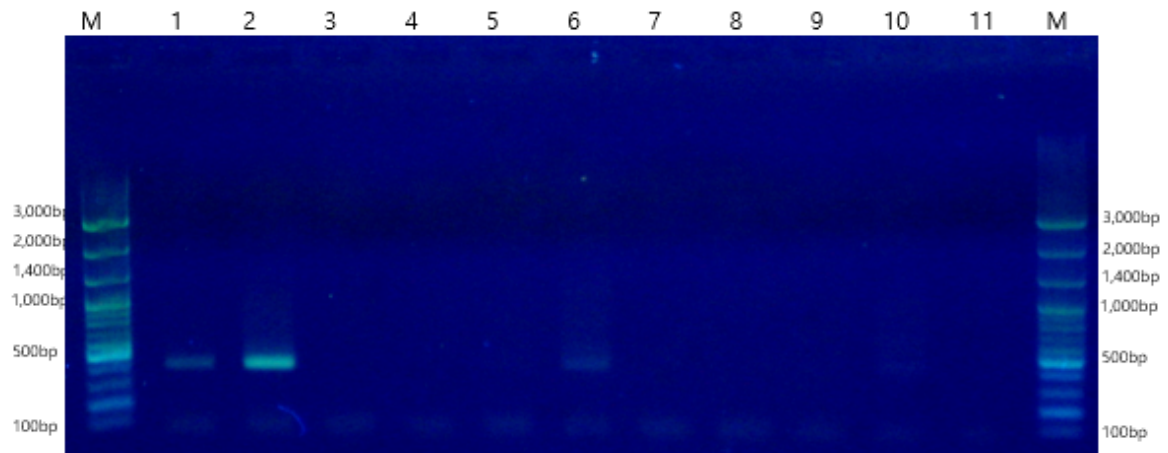
Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| Total                      | 25µl   |

# Dentrificans spp. PCR result – 6. narG set 5 nested



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|          |     |                       |     |                      |
|----------|-----|-----------------------|-----|----------------------|
| 5        | T37 | CAYGGNGTNAAYTGYACNGG  | 000 | Gregory et al., 2000 |
|          | T39 | TARTGNGGCCCANCCNCCNCC |     |                      |
| 5 nested | W9  | MGNGGNTGYCCNMGNGGNGC  | 000 | Gregory et al., 2000 |
|          | T38 | ACRTQNGTYTGYTCNCCCA   |     |                      |

## PCR Condition

|      |       |            |
|------|-------|------------|
| 94°C | 2min  | } 30 Cycle |
| 94°C | 60sec |            |
| 61°C | 30sec |            |
| 72°C | 60sec |            |
| 72°C | 10min |            |
| 4°C  | store |            |

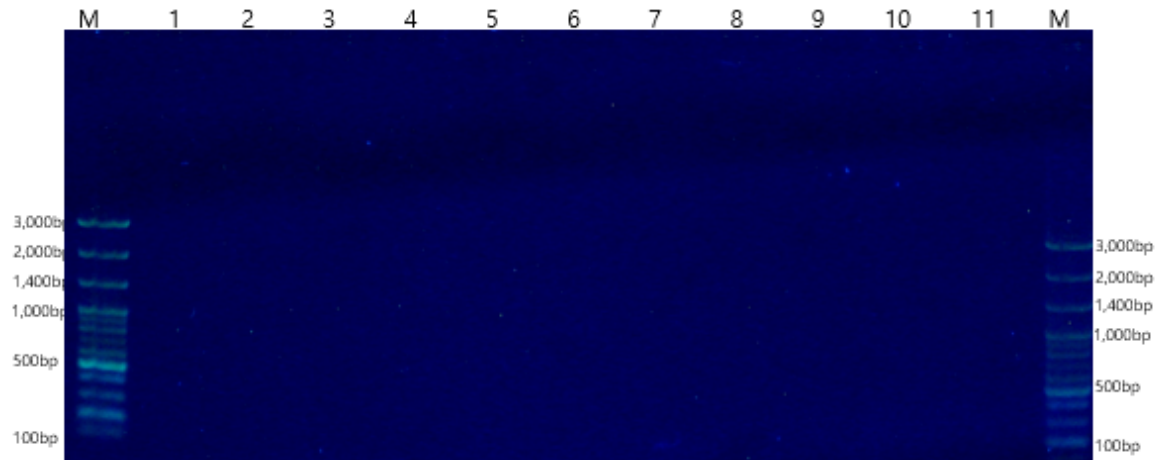
## Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

## PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

### Dentrificans spp. PCR result – 6. narG set 6



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |       |                   |     |                        |
|---|-------|-------------------|-----|------------------------|
| 6 | 1960F | TAYGTSGGCCARGARAA | 650 | Philippot et al., 2002 |
|   | 2659R | TTYTCRTACCABGTBGC |     |                        |

### PCR Condition

|      |       |            |
|------|-------|------------|
| 94°C | 5min  | } 30 Cycle |
| 94°C | 30sec |            |
| 61°C | 45sec |            |
| 72°C | 90sec |            |
| 72°C | 10min |            |
| 4°C  | store |            |

### Loading composition (1% gel)

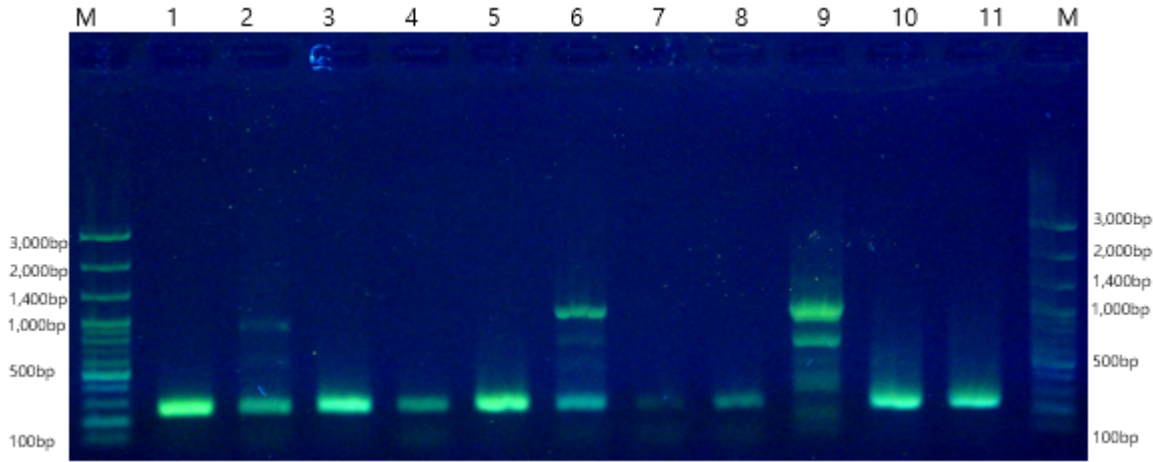
1-11; PCR Product  
 Promega 2X green master Mix 5µl  
 M; Marker  
 Bionix 100bp step DNA ladder 4µl

### PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |



Dentrificans spp. PCR result – 7. nosZ set 1



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |                  |                        |     |                            |
|---|------------------|------------------------|-----|----------------------------|
| 1 | <i>nosZ1840F</i> | CGCRACGGCAASAAGGTSMSST | 267 | Henry <i>et al.</i> , 2006 |
|   | <i>nosZ2090R</i> | CAKRTGCAKSGCCTGGCAGAA  |     |                            |

PCR Condition

|             |       |  |                                                                            |
|-------------|-------|--|----------------------------------------------------------------------------|
| 94°C        | 10min |  | 41 Cycle<br>Touchdown Condition (°C)<br>1-6 cycle: 65→60<br>7-41 cycle: 55 |
| 94°C        | 15sec |  |                                                                            |
| Touchdown°C | 30sec |  |                                                                            |
| 72°C        | 30sec |  |                                                                            |
| 72°C        | 10min |  |                                                                            |
| 4°C         | store |  |                                                                            |

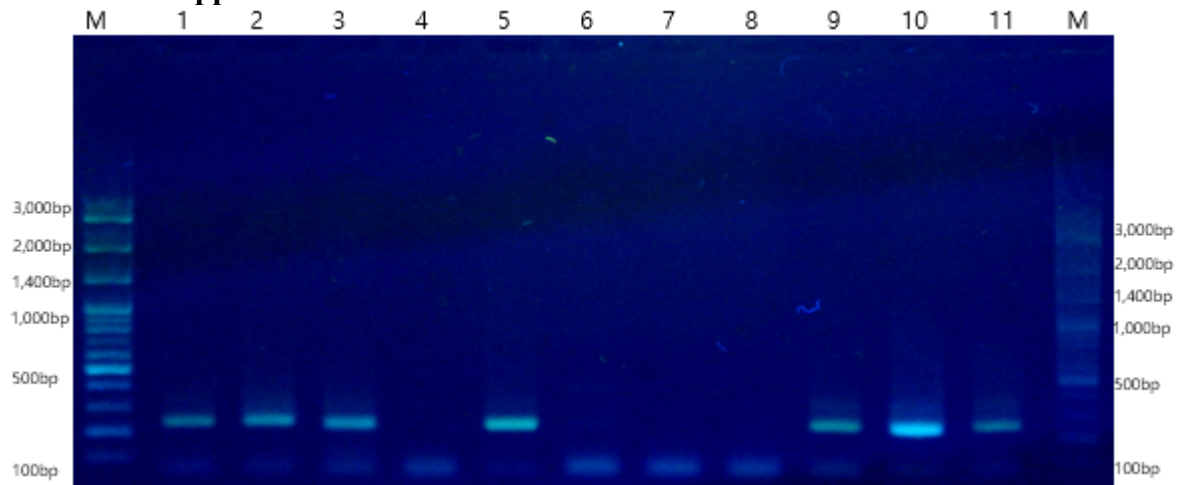
Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| <hr/>                      |        |
| Total                      | 25µl   |

### Dentrificans spp. PCR result – 7. nosZ set 2



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |        |                          |    |                        |
|---|--------|--------------------------|----|------------------------|
| 2 | nosZ1F | WCSYTGTTTCMTGACAGCCAG    | 44 | Henderson et al., 2010 |
|   | nosZ1R | ATGTCGATCARCTGVKCRITTYTC |    |                        |

### PCR Condition

|             |       |                                                                                     |                          |                  |
|-------------|-------|-------------------------------------------------------------------------------------|--------------------------|------------------|
| 50°C        | 2min  |  | 46 Cycle                 |                  |
| 95°C        | 10min |                                                                                     |                          |                  |
| 95°C        | 15sec |                                                                                     |                          |                  |
| Touchdown°C | 60sec |                                                                                     |                          |                  |
| 81.5°C      | 30sec |  | Touchdown Condition (°C) |                  |
| 81.5°C      | 10min |                                                                                     |                          |                  |
| 4°C         | store |                                                                                     |                          |                  |
|             |       |                                                                                     |                          | 1-6 cycle: 68→62 |
|             |       |                                                                                     |                          | 7-46 cycle: 62   |

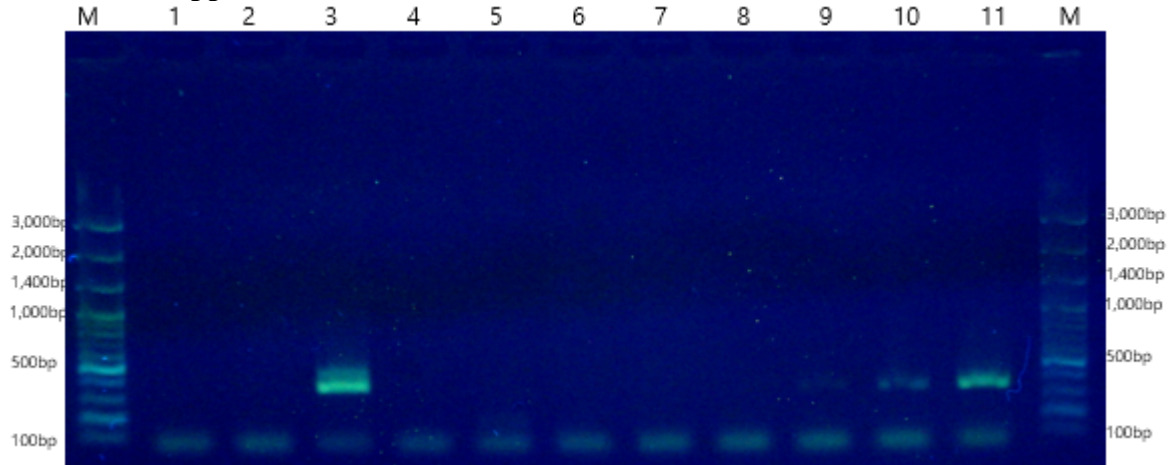
### Loading composition (1% gel)

1-11; PCR Product  
 Promega 2X green master Mix 5µl  
 M; Marker  
 Bionix 100bp step DNA ladder 4µl

### PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

Dentrificans spp. PCR result – 7. nosZ set 3



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |         |                       |    |                        |
|---|---------|-----------------------|----|------------------------|
| 3 | Forward | AGAACGACCAGCTGATCGACA | 19 | Sosa and Kerihof, 1998 |
|   | Reverse | TCCATGGTGACGCCGTGGTTG |    |                        |

PCR Condition

|      |       |          |
|------|-------|----------|
| 94°C | 5min  | 35 Cycle |
| 94°C | 60sec |          |
| 61°C | 60sec |          |
| 72°C | 75sec |          |
| 72°C | 5min  |          |
| 4°C  | store |          |

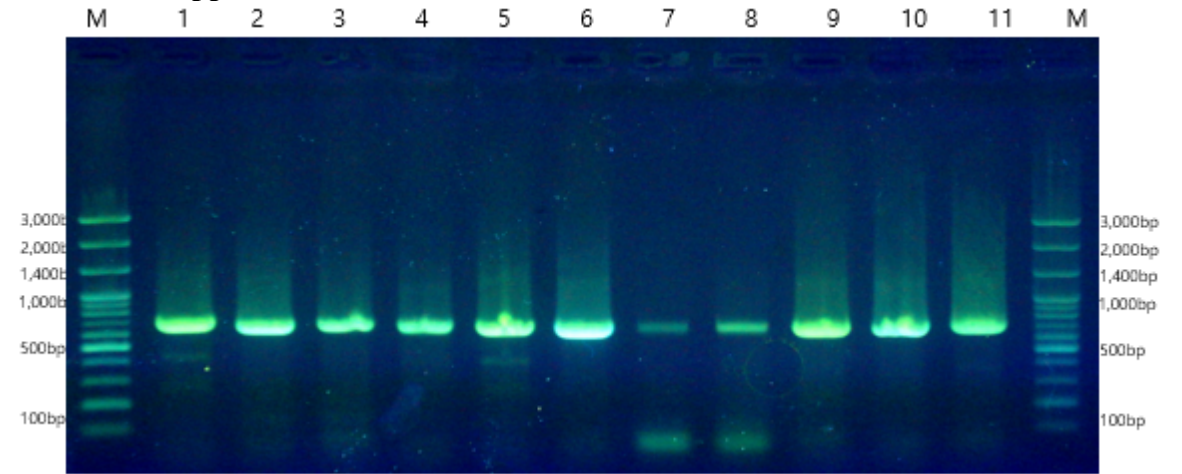
Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

Dentrificans spp. PCR result – 7. nosZ set 4



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |             |                      |    |                 |
|---|-------------|----------------------|----|-----------------|
| 4 | nosZ-F-1181 | CGCTGTTCTCGACAGYCAG  | 60 | Ma et al., 2008 |
|   | nosZ-R-1880 | ATGTGCAKIGCRTGGCAGAA |    |                 |

PCR Condition

|      |       |            |
|------|-------|------------|
| 97°C | 15min | } 45 Cycle |
| 94°C | 20sec |            |
| 54°C | 40sec |            |
| 72°C | 40sec |            |
| 72°C | 45sec |            |
| 4°C  | store |            |

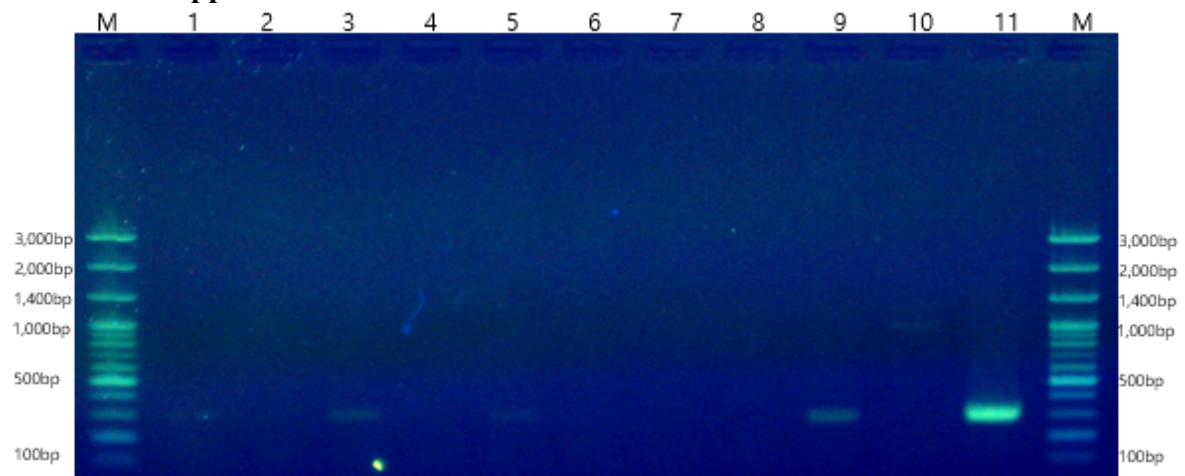
Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

# Dentrificans spp. PCR result – 7. nosZ set 5



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |       |                      |     |                               |
|---|-------|----------------------|-----|-------------------------------|
| 5 | nosLb | CCCGCTGCACACCCCTTCGA | 302 | Throbäck <i>et al.</i> , 2004 |
|   | nosRb | CGTCGCCGAGATGTGGATCA |     |                               |

## PCR Condition

|      |       |            |
|------|-------|------------|
| 94°C | 1min  | } 30 Cycle |
| 94°C | 60sec |            |
| 54°C | 60sec |            |
| 72°C | 60sec |            |
| 72°C | 5min  |            |
| 4°C  | store |            |

## Loading composition (1% gel)

1-11; PCR Product

Promega 2X green master Mix 5µl

M; Marker

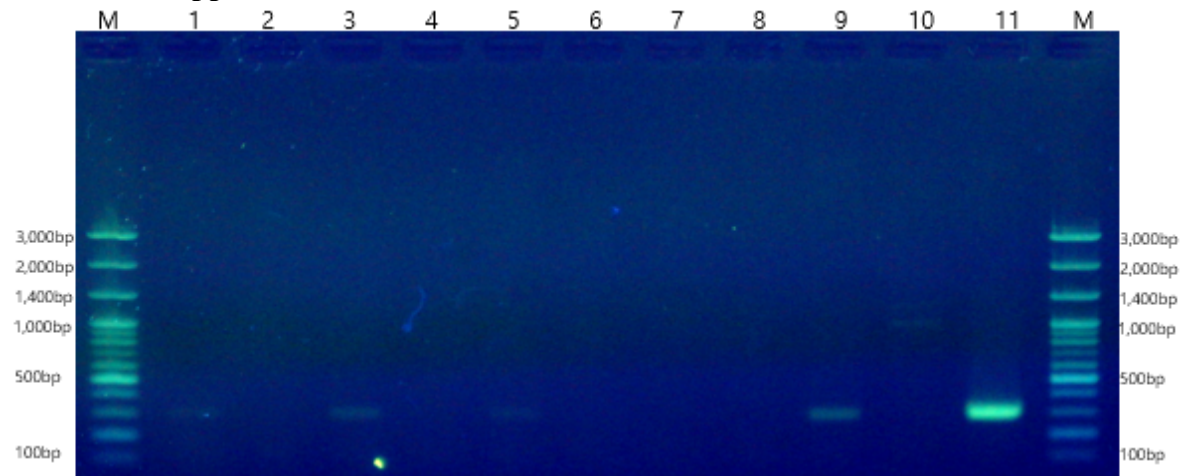
Bionix 100bp step DNA ladder 4µl

## PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |



Dentrificans spp. PCR result – 8. nrfA set 1



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |         |                   |     |                    |
|---|---------|-------------------|-----|--------------------|
| 1 | nrfAF1  | GCNTGYTGGWENTGYAA | 520 | Mohan et al., 2004 |
|   | nrfA7R1 | TWNGGCATRTGRCARTC |     |                    |

PCR Condition

|      |       |            |
|------|-------|------------|
| 94°C | 5min  | } 30 Cycle |
| 94°C | 30sec |            |
| 60°C | 45sec |            |
| 72°C | 90sec |            |
| 72°C | 10min |            |
| 4°C  | store |            |

Loading composition (1% gel)

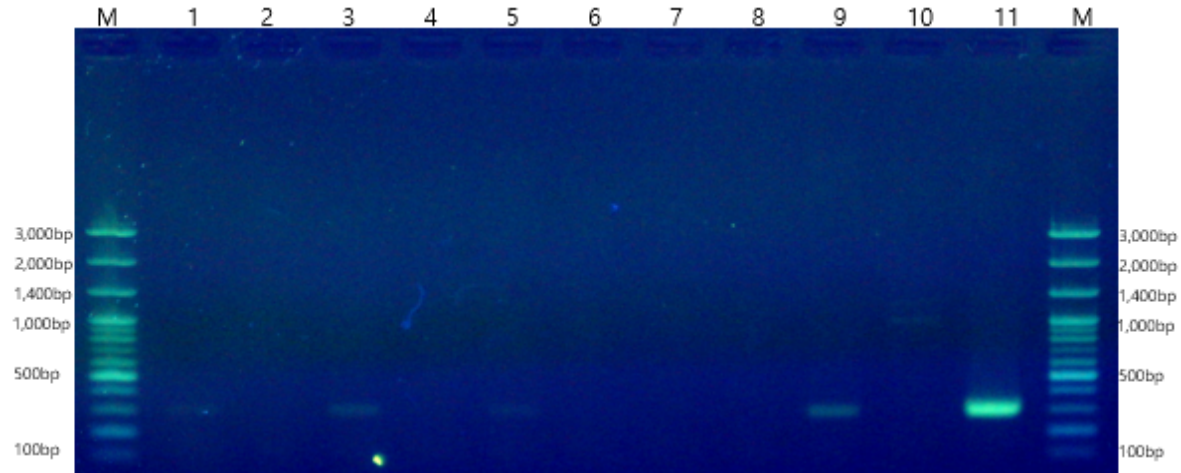
- 1-11; PCR Product
- Promega 2X green master Mix 5µl
- M; Marker
- Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |



Dentrificans spp. PCR result – 8. nrfA set 1



M, marker; 1, *Achromobacter denitrificans*; 2, *Aquitalea denitrificans*; 3, *Blastobacter denitrificans*; 4, *Burkholderia denitrificans*; 5, *Castellaniella denitrificans*; 6, *Comamonas denitrificans*; 7, *Flavobacterium denitrificans*; 8, *Leucobacter denitrificans*; 9, *Rhodanobacter denitrificans*; 10, *Roseobacter denitrificans*; 11, *Paracoccus denitrificans*.

|   |         |                   |     |                    |
|---|---------|-------------------|-----|--------------------|
| 1 | nrfAF1  | GCNTGYTGGWENTGYAA | 520 | Mohan et al., 2004 |
|   | nrfA7R1 | TWNGGCATRTGRCARTC |     |                    |

PCR Condition

|      |       |  |
|------|-------|--|
| 94°C | 5min  |  |
| 94°C | 30sec |  |
| 60°C | 45sec |  |
| 72°C | 90sec |  |
| 72°C | 10min |  |
| 4°C  | store |  |

Loading composition (1% gel)

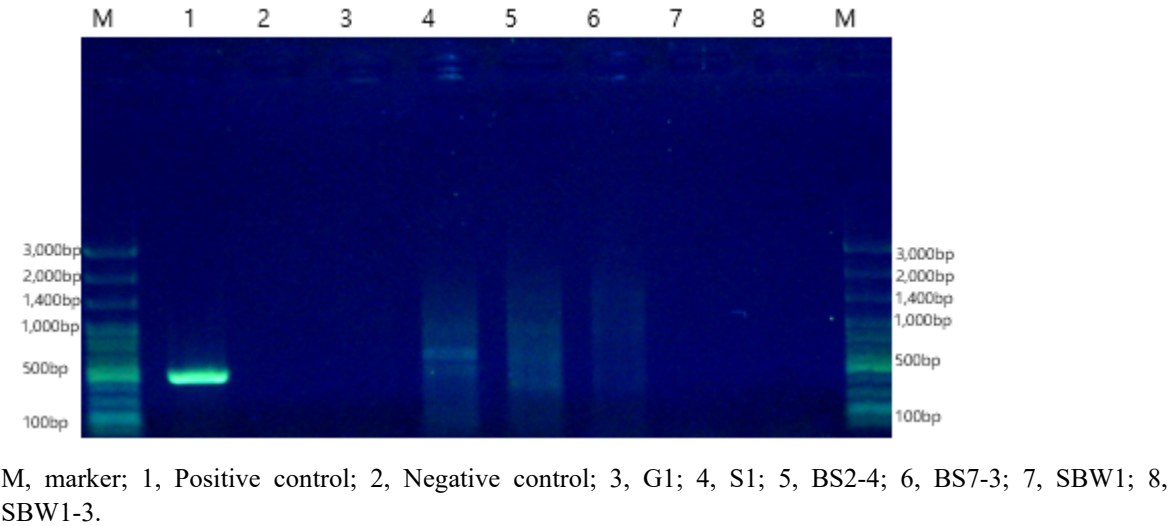
- 1-11; PCR Product
- Promega 2X green master Mix 5µl
- M; Marker
- Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| <hr/>                      |        |
| Total                      | 25µl   |

Groundwater, Streamwater and Hyporheic water Samples

PCR result – 1. nirK set 2



|   |        |                    |     |                             |
|---|--------|--------------------|-----|-----------------------------|
| 2 | nirK1F | GGMATGGTKCCSTGGCA  | 514 | Braker <i>et al.</i> , 1998 |
|   | nirK5R | GCCTCGATCAGRTTGTGG |     |                             |

PCR Condition

|             |       |  |
|-------------|-------|--|
| 95°C        | 5min  |  |
| 95°C        | 30sec |  |
| Touchdown°C | 40sec |  |
| 72°C        | 40sec |  |
| 72°C        | 10min |  |
| 4°C         | store |  |

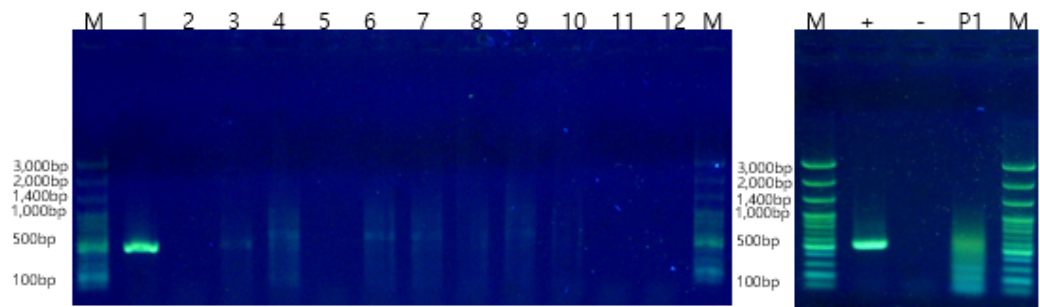
Loading composition (1% gel)

- 1-11; PCR Product
- Promega 2X green master Mix 5µl
- M; Marker
- Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| <hr/>                      |        |
| Total                      | 25µl   |

Water sample. PCR result – 1. nirK set 2



M, marker; 1, Positive control; 2, Negative control; 3-12, P1-P10.

|   |        |                    |     |                             |
|---|--------|--------------------|-----|-----------------------------|
| 2 | nirK1F | GGMATGGTKCCSTGGCA  | 514 | Braker <i>et al.</i> , 1998 |
|   | nirK5R | GCCTCGATCAGRTTGTGG |     |                             |

PCR Condition

|             |       |                                                                                |
|-------------|-------|--------------------------------------------------------------------------------|
| 95°C        | 5min  | } 30 Cycle<br>Touchdown Condition (°C)<br>1-10 cycle: 45→40<br>11-30 cycle: 43 |
| 95°C        | 30sec |                                                                                |
| Touchdown°C | 40sec |                                                                                |
| 72°C        | 40sec |                                                                                |
| 72°C        | 10min |                                                                                |
| 4°C         | store |                                                                                |

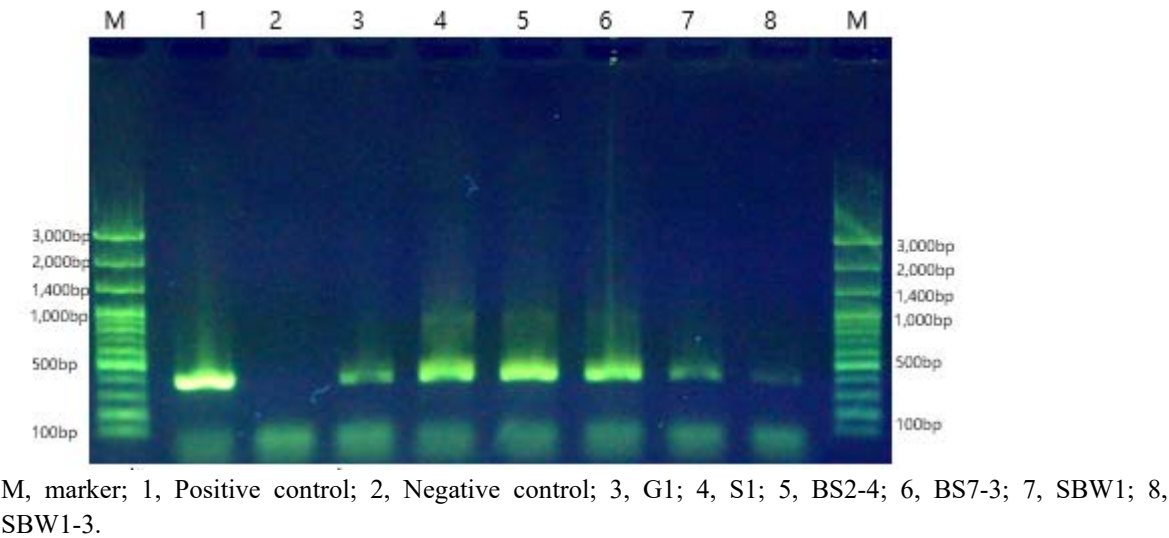
Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

Water sample. PCR result – 2. nirS set 2



|   |         |                         |     |                       |
|---|---------|-------------------------|-----|-----------------------|
| 2 | nirS4QF | AACGYSAAGGARACSGG       | 425 | Throback et al., 2004 |
|   | nirS8QR | GATTTCGGRTGSGTCTTSAYGAA |     |                       |

PCR Condition

|             |       |   |                                      |
|-------------|-------|---|--------------------------------------|
| 95°C        | 10min | } | 41 Cycle<br>Touchdown Condition (°C) |
| 95°C        | 15sec |   |                                      |
| Touchdown°C | 30sec |   |                                      |
| 72°C        | 30sec |   |                                      |
| 72°C        | 10min |   |                                      |
| 4°C         | store |   |                                      |
|             |       |   | 1-6 cycle: 63→58                     |
|             |       |   | 5-41 cycle: 58                       |

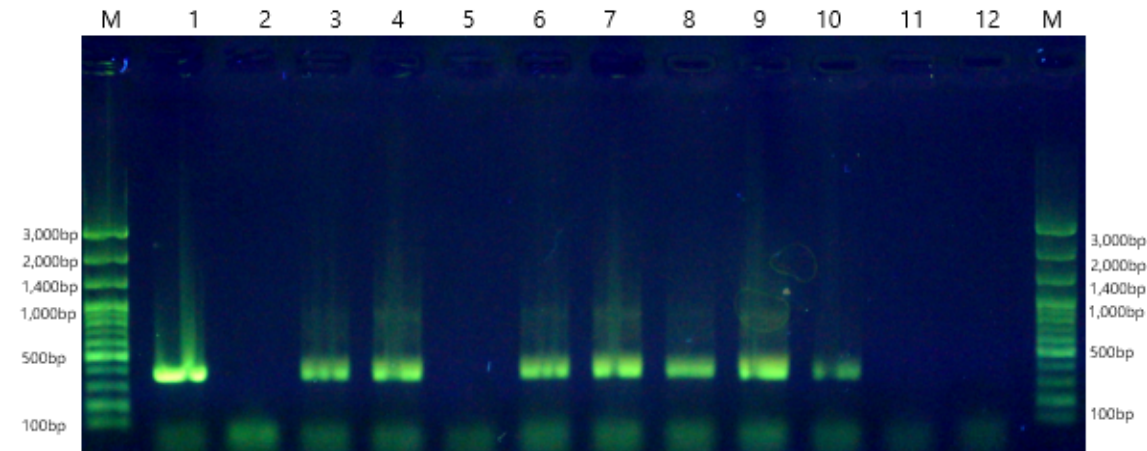
Loading composition (1% gel)

- 1-11; PCR Product
- Promega 2X green master Mix 5µl
- M; Marker
- Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

Water sample. PCR result – 2. nirS set 2



M, marker; 1, Positive control; 2, Negative control; 3-12, P1-P10.

|   |         |                          |     |                       |
|---|---------|--------------------------|-----|-----------------------|
| 2 | nirS4QF | AACGYSAAGGARACSGG        | 425 | Throback et al., 2004 |
|   | nirS8QR | GASTTCGGRTGSGTCITTSAYGAA |     |                       |

PCR Condition

|             |       |                                                                            |
|-------------|-------|----------------------------------------------------------------------------|
| 95°C        | 10min | 41 Cycle<br>Touchdown Condition (°C)<br>1-6 cycle: 63→58<br>5-41 cycle: 58 |
| 95°C        | 15sec |                                                                            |
| Touchdown°C | 30sec |                                                                            |
| 72°C        | 30sec |                                                                            |
| 72°C        | 10min |                                                                            |
| 4°C         | store |                                                                            |

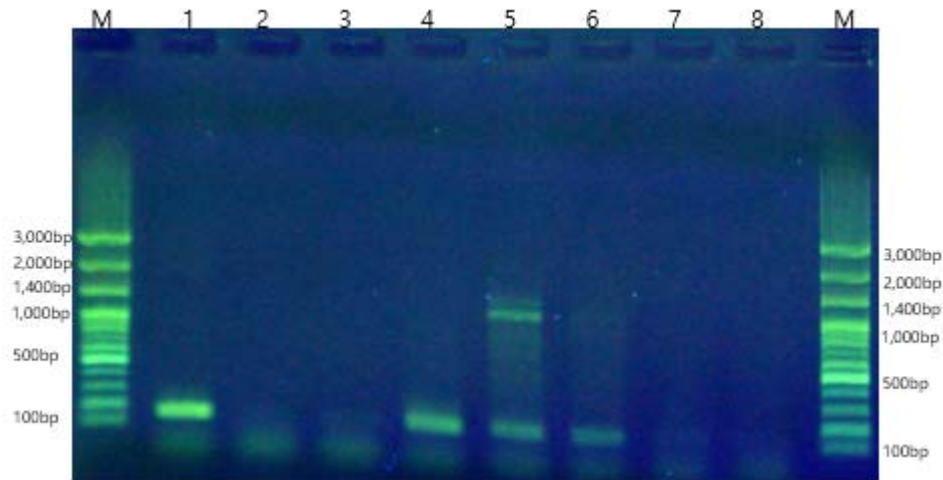
Loading composition (1% gel)

- 1-11; PCR Product
- Promega 2X green master Mix 5µl
- M; Marker
- Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

Water sample. PCR result – 3. napA set 1



M, marker; 1, Positive control; 2, Negative control; 3, G1; 4, S1; 5, BS2-4; 6, BS7-3; 7, SBW1; 8, SBW1-3

|   |        |                      |     |                          |
|---|--------|----------------------|-----|--------------------------|
| 1 | nap3F  | TGGACVATGGGYTTTAAAYC | 152 | Bru <i>et al.</i> , 2007 |
|   | napA4R | ACYTCRCGHGCGVTRCCRCA |     |                          |

PCR Condition

|             |       |  |                  |
|-------------|-------|--|------------------|
| 95°C        | 10min |  | 41 Cycle         |
| 95°C        | 15sec |  | Touchdown        |
| Touchdown°C | 30sec |  | Condition (°C)   |
| 72°C        | 30sec |  | 1-6 cycle: 63→58 |
| 72°C        | 10min |  | 5-41 cycle: 58   |
| 4°C         | store |  |                  |

Loading composition (1% gel)

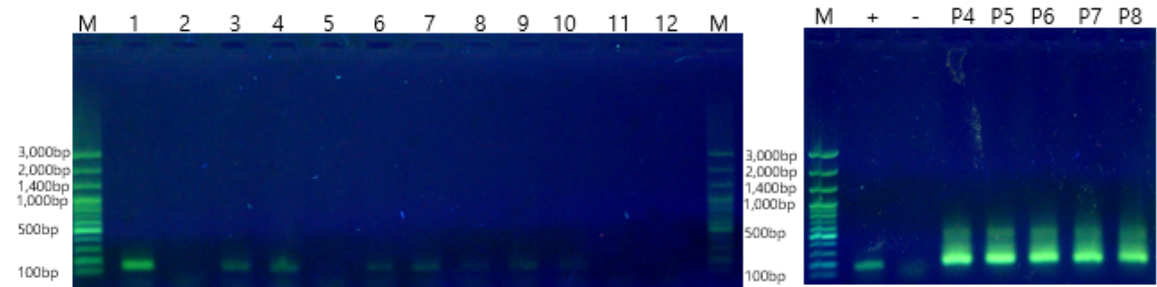
- 1-11; PCR Product
- Promega 2X green master Mix 5µl
- M; Marker
- Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| <hr/>                      |        |
| Total                      | 25µl   |



Water sample. PCR result – 3. napA set 1



M, marker; 1, Positive control; 2, Negative control; 3-12, P1-P10.

|   |        |                      |     |                  |
|---|--------|----------------------|-----|------------------|
| 1 | nap3F  | TGGACVATGGGYTTAAAYC  | 152 | Bru et al., 2007 |
|   | napA4R | ACYTCRCGHGCVGTGCCRCA |     |                  |

PCR Condition

|             |       |  |                                                                            |
|-------------|-------|--|----------------------------------------------------------------------------|
| 95°C        | 10min |  | 41 Cycle<br>Touchdown Condition (°C)<br>1-6 cycle: 63→58<br>5-41 cycle: 58 |
| 95°C        | 15sec |  |                                                                            |
| Touchdown°C | 30sec |  |                                                                            |
| 72°C        | 30sec |  |                                                                            |
| 72°C        | 10min |  |                                                                            |
| 4°C         | store |  |                                                                            |

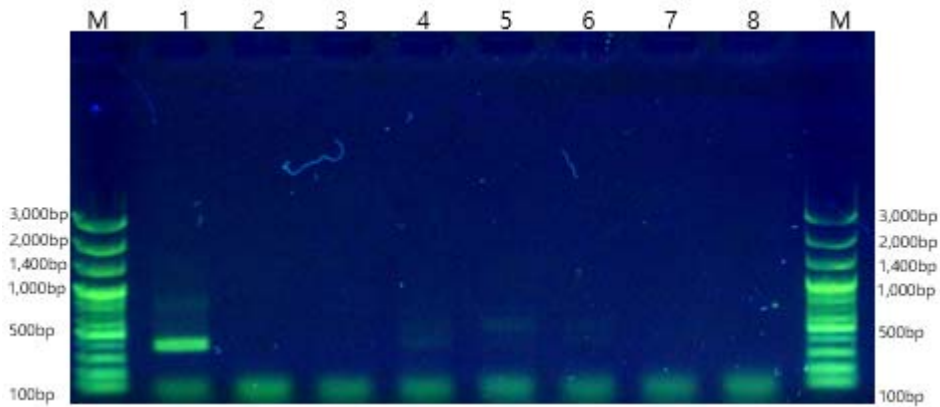
Loading composition (1% gel)

- 1-11; PCR Product
- Promega 2X green master Mix 5µl
- M; Marker
- Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| <hr/>                      |        |
| Total                      | 25µl   |

Water sample. PCR result – 4. cnorB set 3



M, marker; 1, Positive control; 2, Negative control; 3, G1; 4, S1; 5, BS2-4; 6, BS7-3; 7, SBW1; 8, SBW1-3.

|   |         |                     |     |                         |
|---|---------|---------------------|-----|-------------------------|
| 3 | cnorB2F | GACAAGNNNTACTGGTGGT | 389 | Bräler and Tiedje, 2003 |
|   | cnorB5R | GAANCCCCANACNCCNGC  |     |                         |

PCR Condition

|             |       |   |                                                                                |
|-------------|-------|---|--------------------------------------------------------------------------------|
| 95°C        | 5min  | } | 40 Cycle<br>Touchdown Condition (°C)<br>1-10 cycle: 57→52.5<br>11-40 cycle: 55 |
| 95°C        | 30sec |   |                                                                                |
| Touchdown°C | 40sec |   |                                                                                |
| 72°C        | 30sec |   |                                                                                |
| 72°C        | 10min |   |                                                                                |
| 4°C         | store |   |                                                                                |

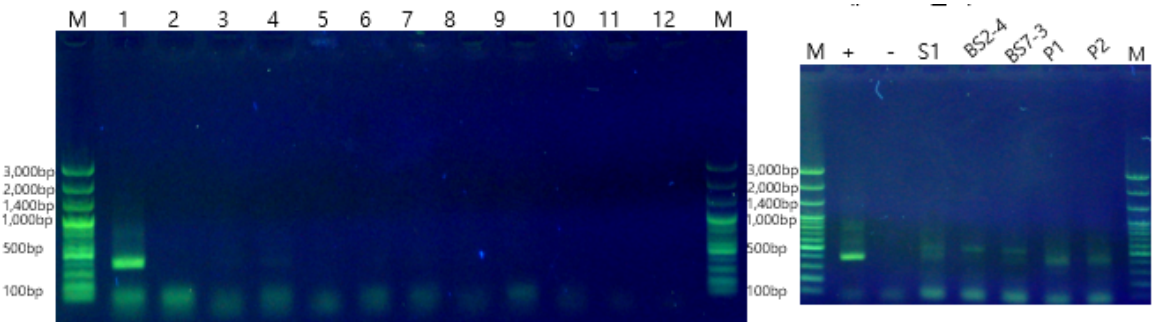
Loading composition (1% gel)

1-11; PCR Product  
Promega 2X green master Mix 5µl  
M; Marker  
Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

Water sample. PCR result – 4. cnorB set 3



M, marker; 1, Positive control; 2, Negative control; 3-12, P1-P10.

|   |         |                     |     |                         |
|---|---------|---------------------|-----|-------------------------|
| 3 | cnorB2F | GACAAGNNNTACTGGTGGT | 389 | Bräler and Tiedje, 2003 |
|   | cnorB6R | GAANCCCCANACNCCNGC  |     |                         |

PCR Condition

|             |       |   |                                                                                |
|-------------|-------|---|--------------------------------------------------------------------------------|
| 95°C        | 5min  | } | 40 Cycle<br>Touchdown Condition (°C)<br>1-10 cycle: 57→52.5<br>11-40 cycle: 55 |
| 95°C        | 30sec |   |                                                                                |
| Touchdown°C | 40sec |   |                                                                                |
| 72°C        | 30sec |   |                                                                                |
| 72°C        | 10min |   |                                                                                |
| 4°C         | store |   |                                                                                |

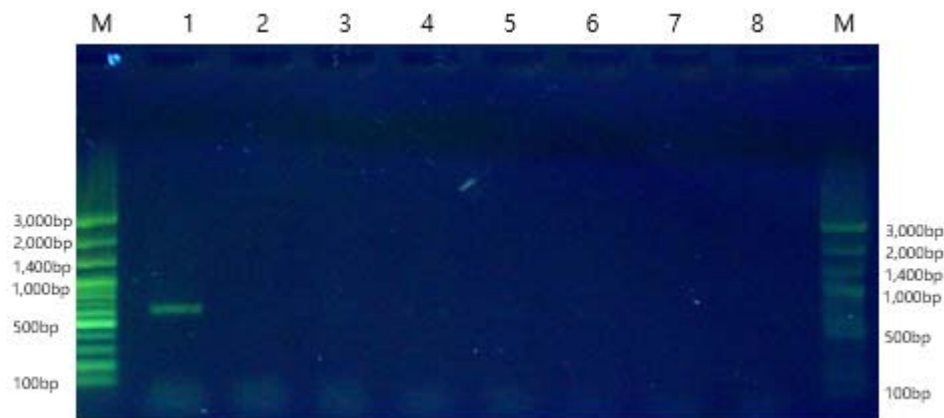
Loading composition (1% gel)

- 1-11; PCR Product
- Promega 2X green master Mix 5µl
- M; Marker
- Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| <hr/>                      |        |
| Total                      | 25µl   |

Water sample. PCR result – 5. qnorB set 2



M, marker; 1, Positive control; 2, Negative control; 3, G1; 4, S1; 5, BS2-4; 6, BS7-3; 7, SBW1; 8, SBW1-3.

|   |         |                    |     |                         |
|---|---------|--------------------|-----|-------------------------|
| 2 | qnorB2F | GGNCAYCARGGNTAYGA  | 637 | Braker and Tiedje, 2003 |
|   | qnorB7R | GGNGGRTTTCADGAANCC |     |                         |

### PCR Condition

|             |       |   |                                                                                |
|-------------|-------|---|--------------------------------------------------------------------------------|
| 95°C        | 5min  | } | 40 Cycle<br>Touchdown Condition (°C)<br>1-10 cycle: 57→52.5<br>11-40 cycle: 55 |
| 95°C        | 30sec |   |                                                                                |
| Touchdown°C | 40sec |   |                                                                                |
| 72°C        | 30sec |   |                                                                                |
| 72°C        | 10min |   |                                                                                |
| 4°C         | store |   |                                                                                |

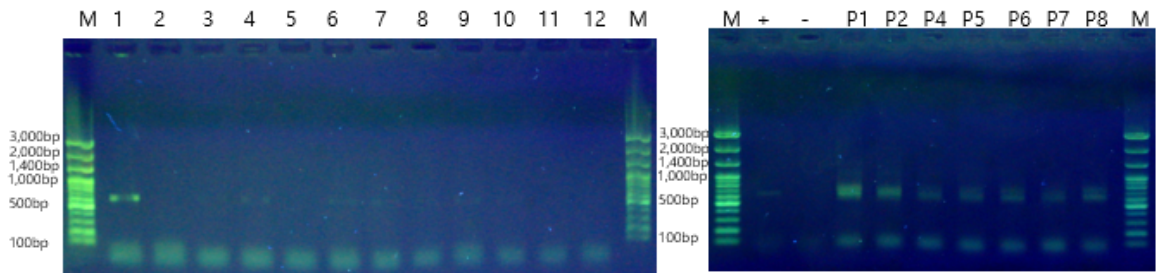
### Loading composition (1% gel)

1-11; PCR Product  
 Promega 2X green master Mix 5μl  
 M; Marker  
 Bionix 100bp step DNA ladder 4μl

### PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5μl |
| Primer -Forward(20pmol/μl) | 1μl    |
| Primer -Reverse(20pmol/μl) | 1μl    |
| Template (10ng)            | 1μl    |
| DW                         | 9.5μl  |
| -----                      |        |
| Total                      | 25μl   |

Water sample. PCR result – 5. qnorB set 2



M, marker; 1, Positive control; 2, Negative control; 3-12, P1-P10.

|   |         |                      |     |                         |
|---|---------|----------------------|-----|-------------------------|
| 2 | qnorB2F | GGNCAYCARGGNTAYGA    | 637 | Braier and Tiedje, 2003 |
|   | qnorB7R | GGNGGRTTDTACADGAANCC |     |                         |

PCR Condition

|             |       |   |                                                                                |
|-------------|-------|---|--------------------------------------------------------------------------------|
| 95°C        | 5min  | } | 40 Cycle<br>Touchdown Condition (°C)<br>1-10 cycle: 57→52.5<br>11-40 cycle: 55 |
| 95°C        | 30sec |   |                                                                                |
| Touchdown°C | 40sec |   |                                                                                |
| 72°C        | 30sec |   |                                                                                |
| 72°C        | 10min |   |                                                                                |
| 4°C         | store |   |                                                                                |

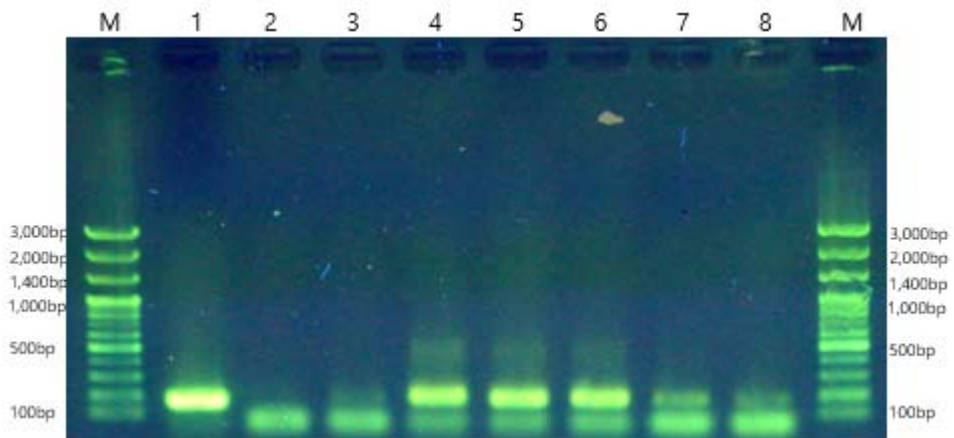
Loading composition (1% gel)

- 1-11; PCR Product
- Promega 2X green master Mix 5µl
- M; Marker
- Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

Water sample. PCR result – 5. qnorB set 2



M, marker; 1, Positive control; 2, Negative control; 3, G1; 4, S1; 5, BS2-4; 6, BS7-3; 7, SB W1; 8, SBW1-3.

|   |        |                           |     |                          |
|---|--------|---------------------------|-----|--------------------------|
| 2 | narG-f | TCGCCSATYCCGGCSATGTC      | 174 | Bru <i>et al.</i> , 2007 |
|   | narG-r | GAGTTGTACCACTCRGCSGAYTCSG |     |                          |

PCR Condition

|             |       |   |                                                                            |
|-------------|-------|---|----------------------------------------------------------------------------|
| 95°C        | 10min | } | 41 Cycle<br>Touchdown Condition (°C)<br>1-6 cycle: 63→58<br>7-41 cycle: 58 |
| 95°C        | 15sec |   |                                                                            |
| Touchdown°C | 30sec |   |                                                                            |
| 72°C        | 30sec |   |                                                                            |
| 72°C        | 10min |   |                                                                            |
| 4°C         | store |   |                                                                            |

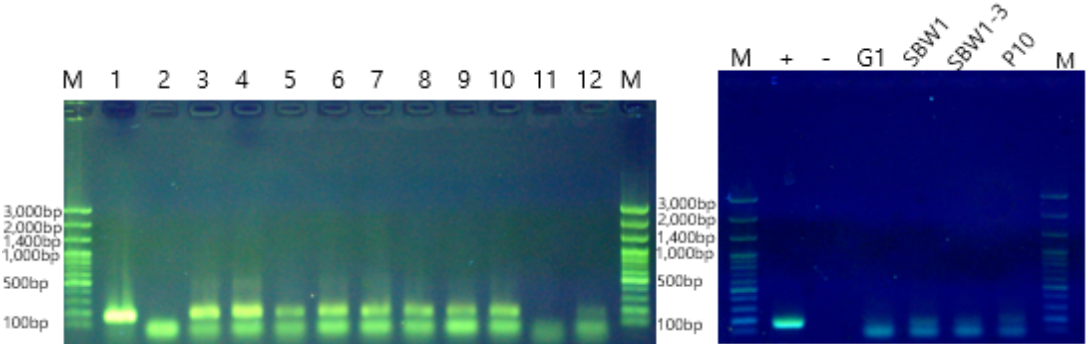
Loading composition (1% gel)

- 1-11; PCR Product
- Promega 2X green master Mix 5µℓ
- M; Marker
- Bionix 100bp step DNA ladder 4µℓ

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µℓ |
| Primer -Forward(20pmol/µℓ) | 1µℓ    |
| Primer -Reverse(20pmol/µℓ) | 1µℓ    |
| Template (10ng)            | 1µℓ    |
| DW                         | 9.5µℓ  |
| -----                      |        |
| Total                      | 25µℓ   |





M, marker; 1, Positive control; 2, Negative control; 3-12, P1-P10.

|   |        |                           |     |                          |
|---|--------|---------------------------|-----|--------------------------|
| 2 | narG-f | TCGCCSATYCCGGCSATGTC      | 174 | Bru <i>et al.</i> , 2007 |
|   | narG-r | GAGTTGTACCACTCRGCSGAYTCSG |     |                          |

PCR Condition

|             |       |   |                                                                            |
|-------------|-------|---|----------------------------------------------------------------------------|
| 95°C        | 10min | } | 41 Cycle<br>Touchdown Condition (°C)<br>1-6 cycle: 63→58<br>7-41 cycle: 58 |
| 95°C        | 15sec |   |                                                                            |
| Touchdown°C | 30sec |   |                                                                            |
| 72°C        | 30sec |   |                                                                            |
| 72°C        | 10min |   |                                                                            |
| 4°C         | store |   |                                                                            |

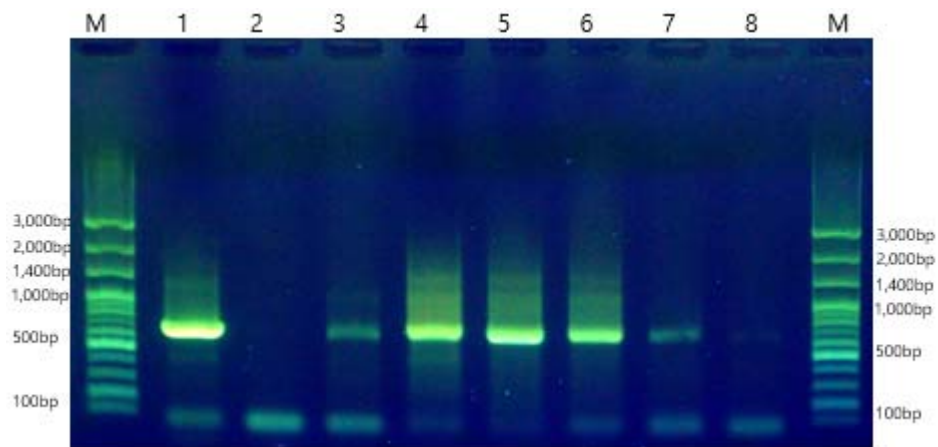
Loading composition (1% gel)

- 1-11; PCR Product
- Promega 2X green master Mix 5µℓ
- M; Marker
- Bionix 100bp step DNA ladder 4µℓ

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µℓ |
| Primer -Forward(20pmol/µℓ) | 1µℓ    |
| Primer -Reverse(20pmol/µℓ) | 1µℓ    |
| Template (10ng)            | 1µℓ    |
| DW                         | 9.5µℓ  |
| -----                      |        |
| Total                      | 25µℓ   |

Water sample. PCR result – 7. nosZ set 4



M, marker; 1, Positive control; 2, Negative control; 3, G1; 4, S1; 5, BS2-4; 6, BS7-3; 7, SB W1; 8, SBW1-3.

|   |            |                      |    |                         |
|---|------------|----------------------|----|-------------------------|
| 4 | msd-F-1181 | CGCTGTTCTTCGACAGYCAG | 60 | Ma <i>et al.</i> , 2008 |
|   | msd-R-1880 | ATGTGCAKIGCRTGGCAGAA |    |                         |

PCR Condition

|      |       |            |
|------|-------|------------|
| 97°C | 15min | } 45 Cycle |
| 94°C | 20sec |            |
| 54°C | 40sec |            |
| 72°C | 40sec |            |
| 72°C | 45sec |            |
| 4°C  | store |            |

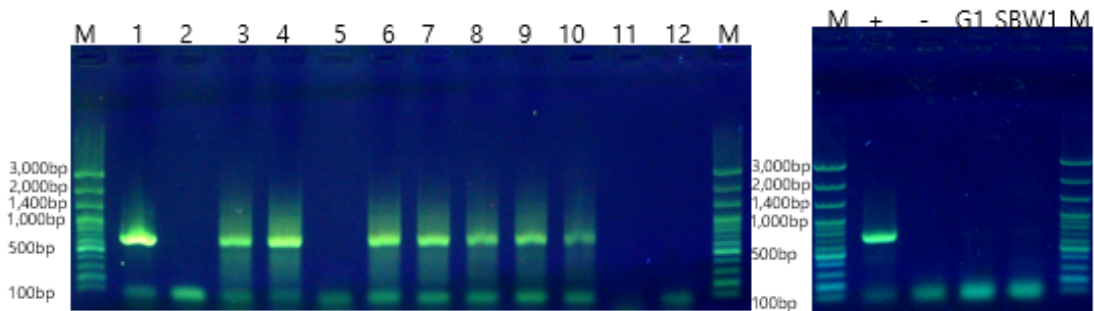
Loading composition (1% gel)

- 1-11; PCR Product
- Promega 2X green master Mix 5µl
- M; Marker
- Bionix 100bp step DNA ladder 4µl

PCR Composition

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| -----                      |        |
| Total                      | 25µl   |

1709     **Water sample. PCR result – 7. nosZ set 4**



1710  
1711     *M*, marker; 1, Positive control; 2, Negative control; 3-12, P1-P10.  
1712

|   |                    |                      |    |                        |
|---|--------------------|----------------------|----|------------------------|
| 4 | <i>nosZ-F-1181</i> | CGCTGTTCTTCGACAGYCAG | 60 | <i>Mn et al., 2008</i> |
|   | <i>nosZ-R-1880</i> | ATGTGCAKIGCRTGGCAGAA |    |                        |

1713  
1714  
1715     **PCR Condition**

|      |       |  |          |
|------|-------|--|----------|
| 97°C | 15min |  | 45 Cycle |
| 94°C | 20sec |  |          |
| 54°C | 40sec |  |          |
| 72°C | 40sec |  |          |
| 72°C | 45sec |  |          |
| 4°C  | store |  |          |

1722  
1723     **Loading composition (1% gel)**

- 1724     1-11; PCR Product  
1725     Promega 2X green master Mix 5µl  
1726     M; Marker  
1727     Bionix 100bp step DNA ladder 4µl

1728  
1729     **PCR Composition**

|                            |        |
|----------------------------|--------|
| 2X Green Master Mix        | 12.5µl |
| Primer -Forward(20pmol/µl) | 1µl    |
| Primer -Reverse(20pmol/µl) | 1µl    |
| Template (10ng)            | 1µl    |
| DW                         | 9.5µl  |
| <hr/>                      |        |
| Total                      | 25µl   |