# Detecting ice- nucleating bacteria in environmental samples using PCR of the gene conferring ice nucleation activity


Caroline Guilbaud — INRA- Avignon, France

Helen Ahern — University of East London, UK

Hélène Dominguez — University of Avignon, IUT, France

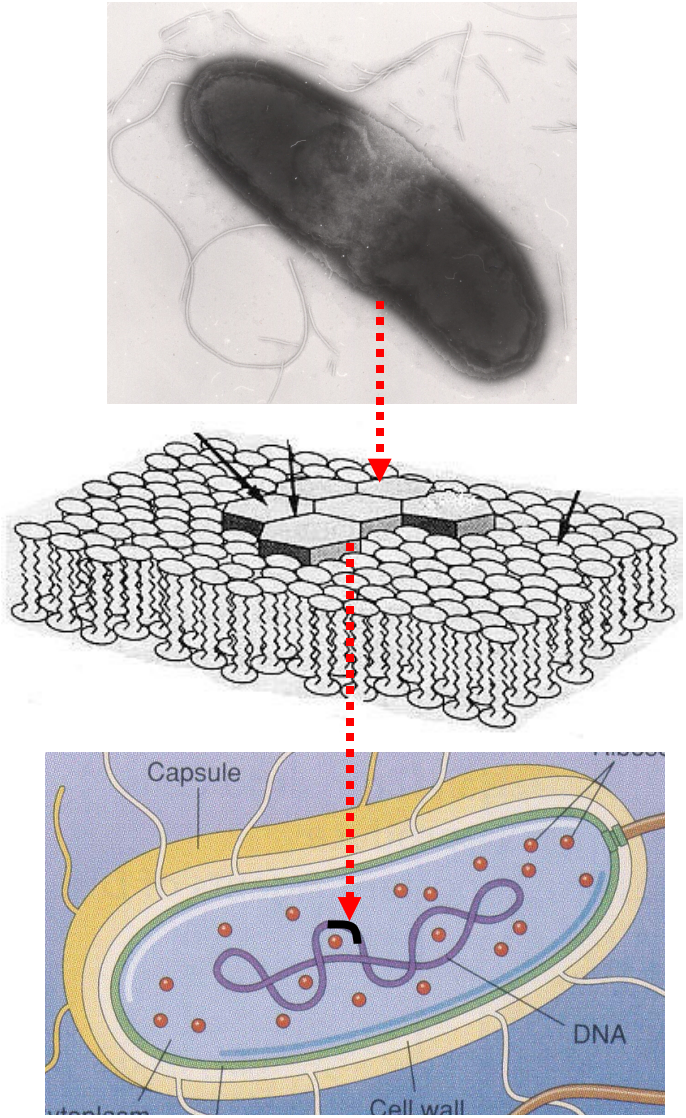
Catherine Glaux

Bruce Moffett

Tom Hill

Cindy E. Morris

# Detection targets




http://morayeel.louisiana.edu/SeaweedsLab/Gavio/bacterial%20cell%20copy

- **Whole, viable cells (culture on nutrient medi**
- **INA protein (serology)**
- **Gene for the INA protein (polymerase chain reactio PCR)**

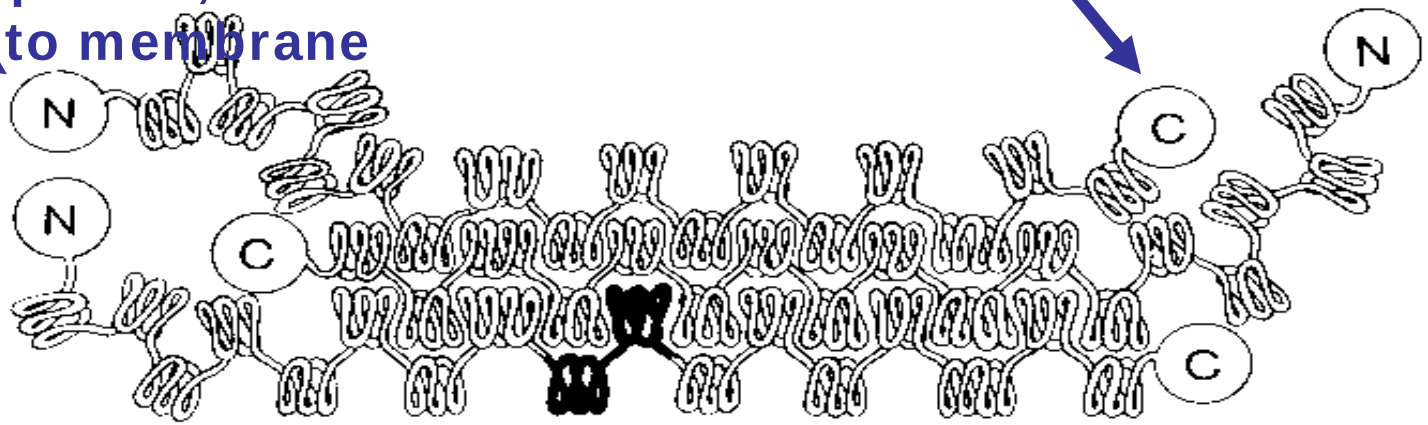
Click here to see an animated description of the PCR process.

If the link isn't working, you can go directly to:
<http://www.biomultimedia.net/archiv/pcr/pcr.htm>

# INA protein

N- terminal: hydrophobic, insertion into membrane

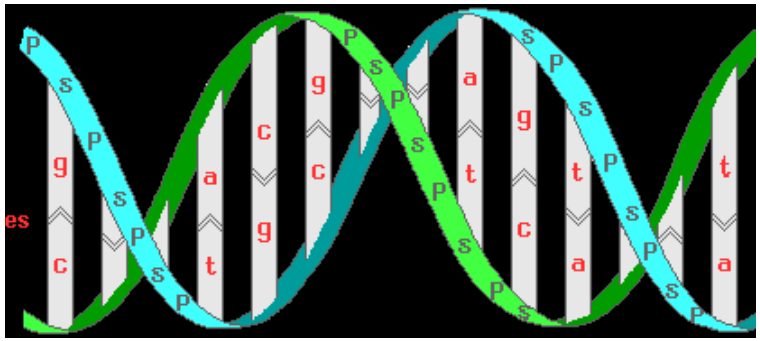
C- terminal: hydrophilic




Core: central domain, repeating units, template for binding water

(Kajava and Lindow 1993)

~4000 pairs of bases (A T G C) constitute the code in the DNA (gene) for this protein.



# INA gene

**The sequence of bases in this gene has been determined for several strains of INA bacteria. These sequences (for a single strand) are available at an open data base (GenBank).**

**Example**

**5' end of sequence, corresponding to N- terminal of protein**

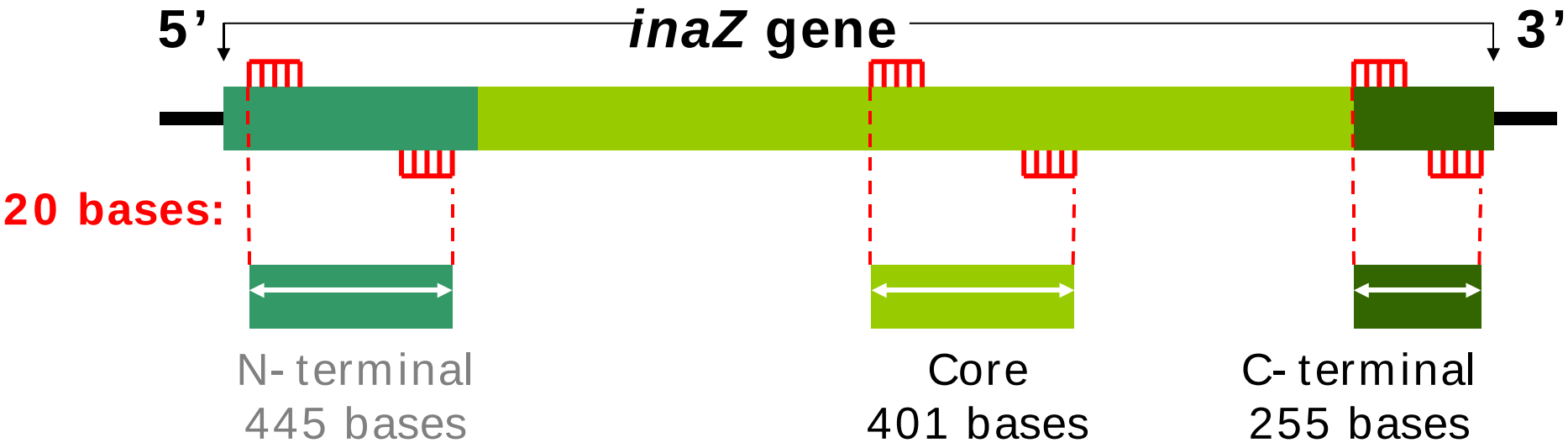
```
agatctgtcg cgcgcgacgg atcgatcagc gtctggtgct gtatgtcgag cactacctga
 gaccggagtt gttccccggc attctcgaac acgacctgaa cgaatcgctc agtgaactct
 acgcccgccg ctatgacatt cattacggtc gggtgcgctt cgacatggtc cccacggcgc
 tgcacagcga agcagccgct gcactgaagg tttcactggg tagcccgggc ctgcgcattg
 cacgtgtcaa ttatgatcgg aaagatcggt tgatcgactg tgacctcgaa tactggcgtc
 atgatgctat tcatgtgcgc gcagaggtga acggcgaatg aatgccggtg tggcaaaccc
 acttcccaag cgcggtctgt gccaccgtta gttcgctgtt ttctataaat aaatactcat
 tttgttgata agaatgtgtg caatgtacag cctgtttaat aggttttgtg taatgcatgc
 atttgattaa caaaaaaaat taatgagcat tattctattg ttgctggcgg ggtggcgtta
aattagttgg ttttttaatg gaatttccca gagtaattgt tctagttttc agcggattta
. . . . . ggcgcgtcga tggaattc
```

**4458 bases**

**3' end of sequence, corresponding to C- terminal of protein**

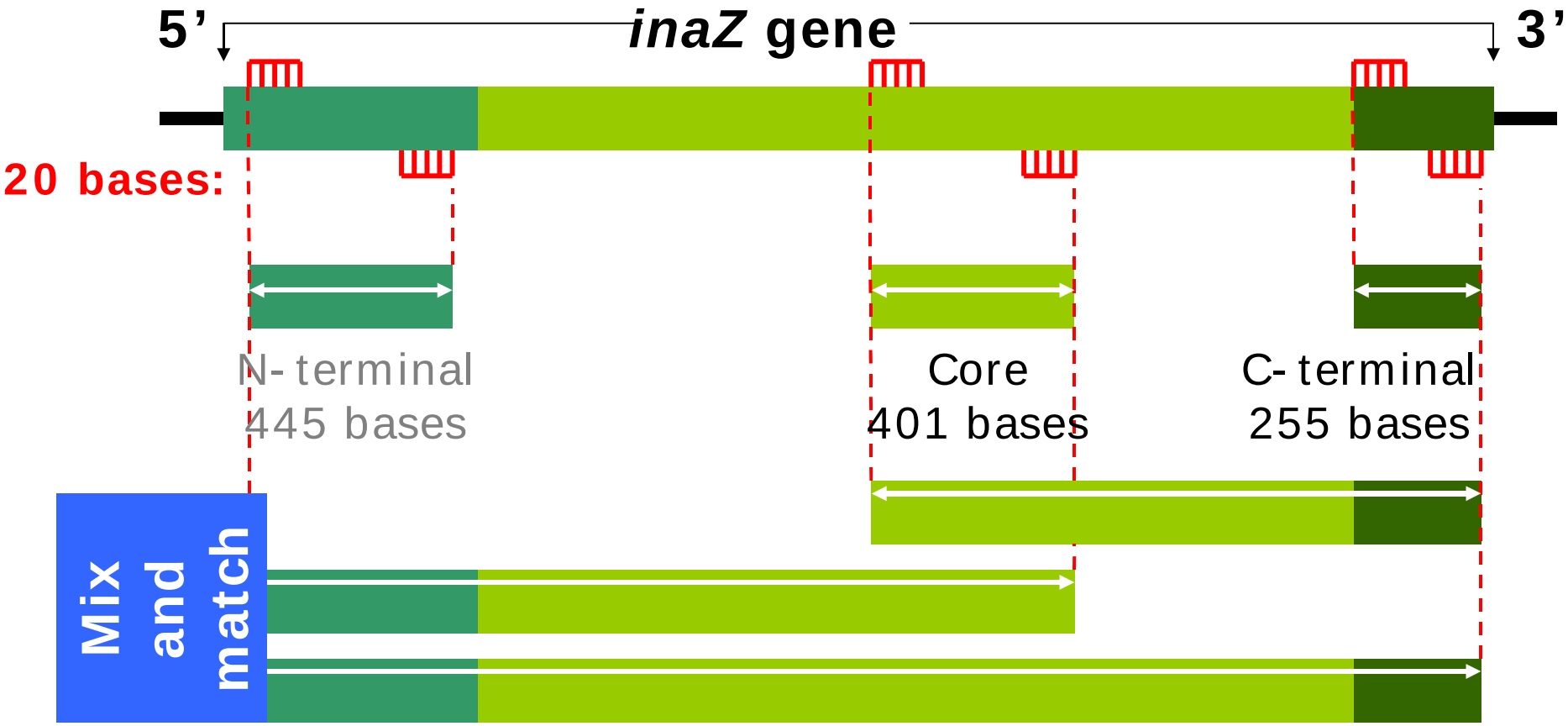
# Primers

**Ideal primers target unique sequences that are identical among alleles of the gene.**



# Primers

**Ideal primers target unique sequences that are identical among alleles of the gene.**



# Primers : based on the *inaZ* sequence

## C- terminal

GGG AGA CCA AAG CAG ATT GA

5'→

→3'

CTG CTC CTG CTA CTG ACC TA

---

## Core

ACC GCG AGT TAC AGA AGC AT

5'→

→3'

CTG GAA TAG CCC GCA GT**A** GA

CTG GAA TAG CCC GCA GT**C** GA

CTG GAA TAG CCC GCA GT**G** GA

CTG GAA TAG CCC GCA GT**T** GA

# INA bacteria

## Species reported to have active strains

***Pseudomonas syringae*** ♣

***P. fluorescens***

***P. viridiflava*** ♣

***Pantoea agglomerans (Erwinia herbicola)*** ♣

***Xanthomonas campestris* pv. *translucens*** ♣

**Gram – negative**
**non spore- forming**
**♣ plant pathogens**

# INA bacteria

## Species reported to have active strains

***Pseudomonas syringae*** ♣

***P. fluorescens***

***P. viridiflava*** ♣

***Pantoea agglomerans (Erwinia herbicola)*** ♣

***Xanthomonas campestris* pv. *translucens*** ♣

**Gram – negative**
**non spore- forming**
**♣ plant pathogens**

## Test strains

**> 50 from the above species**
**plants, water, snow, rain**
**US, Europe, Asia, Antarctica**

**10 'negative' strains: no INA gene or other species**

# Primer specificity

## Strains without an ice nucleation gene

| # strains | | C- terminal | Core A | Core C | Core G | Core T |
|---|---|---|---|---|---|---|
| 1 | *Agrobacterium radiobacter* | - | - | - | - | - |
| 1 | *Escherichia coli* | - | - | - | - | - |
| 2 | *Erwinia carotovora* | + (2 bands) | - | - | - | - |
| 1 | *Erwina chrysanthemi* | + | - | - | - | - |
| 1 | *Flavobacterium* sp. | + | - | - | - | - |
| 1 | *Aureobacterium* sp. | - | - | - | - | - |
| 1 | *Micrococcus* sp. | + | - | - | - | - |
| 1 | *P. syringae* 1448A | - | - | - | - | - |
| 1 | *P. syringae* DC3000 | - | - | - | - | - |

# Primer specificity

## Ice nucleation active strains (- 2° to - 8°C)

| # strains | | C- terminal | Core A | Core C | Core G | Core T |
|---|---|---|---|---|---|---|
| 1 | *P. viridiflava* (China) | - | - | - | - | - |
| 2 | *P. viridiflava* (Montana) | + | - | + | - | - |
| 2 | *P. fluorescens* (Antarctica) | - | - | - | - | - |
| 2 | *Pantoea agglomerans* | + | - | - | - | - |
| 1 | *Pantoea agglomerans* | + | + | + | + | + |
| 1 | *Pantoea agglomerans* | + | + | + | - | - |
| 1 | *Pantoea agglomerans* | + | + | + | + | + |
| 33 | *P. syringae* (diverse) | + | - | + | - | - |
| 1 | *P. syringae* (irrigation lake) | - | + | + | + | + |
| 1 | *P. syringae* (pv. *pisi*) | - | - | + | + | + |
| 1 | *P. syringae* (pv. *melea*) | - | - | - | + | - |
| 2 | *X. campestris* pv. *transluscens* | + | + | + | - | - |
| 1 | *X. campestris* pv. *transluscens* | + | + | - | - | - |
| 1 | *X. campestris* pv. *transluscens* | + | - | + | - | + |

# Primer specificity

## Inactive strains of ice nucleation species (< - 8°C)

| # strains | | C- terminal | Core A | Core C | Core G | Core T |
|---|---|---|---|---|---|---|
| 1 | *P. fluorescens* | - | - | - | - | - |
| 3 | *P. fluorescens* | + | - | - | - | - |
| 2 | *P. syringae* | - | - | - | - | - |
| 6 | *P. syringae* | + | - | - | - | - |
| 1 | *P. syringae* | + | - | - | + | - |
| 1 | *P. syringae* | + | - | + | + | - |
| 1 | *P. syringae* | + | + | + | + | - |
| 7 | *P. syringae* | + | + | + | + | + |

# Applications

## Limits of detections, and direct detection of INA bacteria in environmental samples

### Validation

**1 L of 'test water' filtered on 0.22 µm membrane**

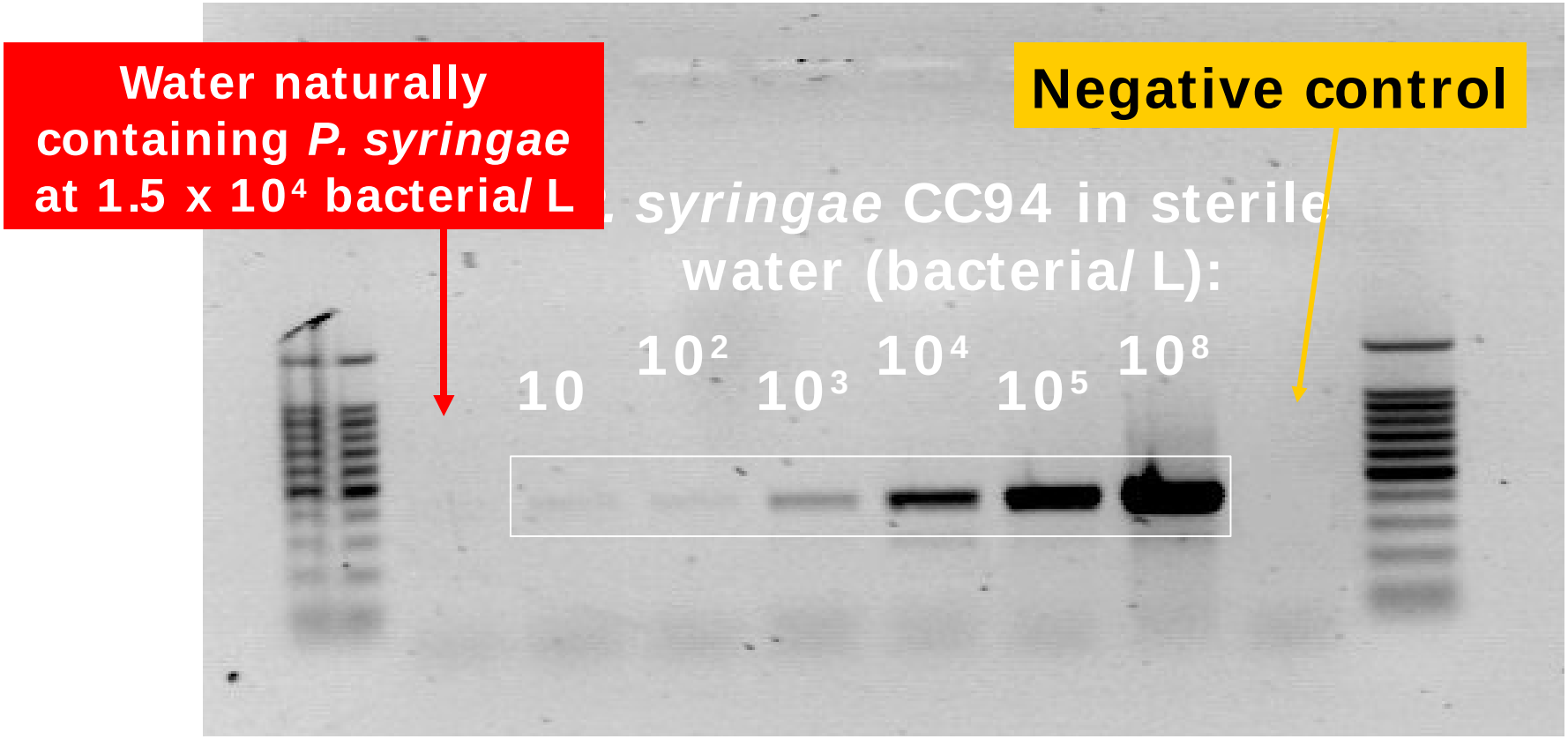
**DNA extracted in 50 µl solvents**

**PCR (core) with 2 µl of extracted total DNA**

**'test water' =**

- ***P. syringae* (CC94) at $10^6$ and $10^7$ bacteria/ L in river water**
- ***P. syringae* (CC94) in sterile distilled water at 10- $10^8$ bacteria/ L**
- **River sample from which *P. syringae* was isolated at 1.5 x $10^4$ bacteria/ L**

# Applications




***P. syringae*** **seeded into river water at $10^7$ and $10^6$ bacteria/ L was detected (lower concentrations not yet tested).**

# Applications

## Perspectives

**Pursue development of methodology for detection in environmental samples – snow, rain, cloud water, lake and river water, ice cores**
**(qualitative measures).**

**Concentrations of INA *P. syringae* in environmental samples are generally above the limit of detection by PCR**

# *P. syringae in* non- agricultural substrates

| Substrate | *Ps* bacteria / g or / L |
|---|---|
| Snow, fresh | 150 – $10^5$ |
| Snow, firn | 100 – 5000 |
| Lakes and streamheads | 100 – $10^4$ |
| Rain | $10^4$ |
| Cloud water | ca. $10^4$ |

# Applications

## Perspectives

**Develop quantitative- PCR method for use with environmental samples.**

- **quantify**
- **enhance sensitivity of detection**

# Applications

## Perspectives

**Use primers to amplify large regions of the genes to determine the sequence of bases:**

- **gene evolution**
- **relationship between gene sequence and expression of ice nucleation activity.**

# Summary

## PCR detection of INA bacteria in environmental samples

**Development of this tool in progress:**

- **Primers available for 'core' region of gene for diverse INA bacteria.**
- **Additional primers needed for *P. fluorescens*, etc.**
- **Fine-tuning of sample preparation and PCR conditions for environmental samples in progress.**