# Comparison between PMA-PCR and DNase-PCR methods for the discrimination of live and dead bacteria

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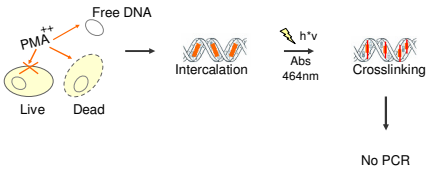
## Motivation

Due to the low biomass present in oligotrophic water habitats a sample concentration by filtration has to be done for subsequent DNA-based analyses. The use of propidium monoazide (PMA) and DNases have been here used to give us the possibility to distinguish the different physiological states of bacteria: viable cells with intact cell membrane and dead cells with harmed cell membrane. Our aim is to compare which of these treatments is better.

## Materials and Methods

**1. Live-dead differentiation:**

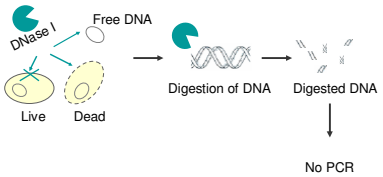
*-PMA treatment:* this substance intecalates DNA supressing later DNA amplification.




**PMA treatment**

1. Water concentration
2. Filter in plate + PMA
3. Incubate in dark at 25℃ x 15min
4. UV exposure 10min
5. Resuspension of filter content (throw filter, work with suspension)
6. Centrifuge, keep pellet
7. Wash pellet
8. Centrifuge, keep pellet
9. Resuspend pellet in water
10. DNA-based techniques

*- DNase I treatment:* this enzyme destroys free DNA and DNA from cells with harmed membrane.




**DNase I treatment**

1. Water concentration
2. Filter in reaction tube + DNase I
3. Incubate at 25℃ x 1h
4. Inactivate DNase at 75℃ x 10min
5. DNA-based techniques

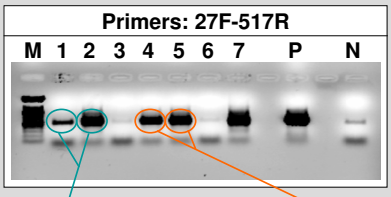
**2. Detection and characterization of bacteria:**

*- Polymerase Chain Reaction* (PCR)

*- Denaturing gradient gel electrophoresis* (DGGE)

## Results

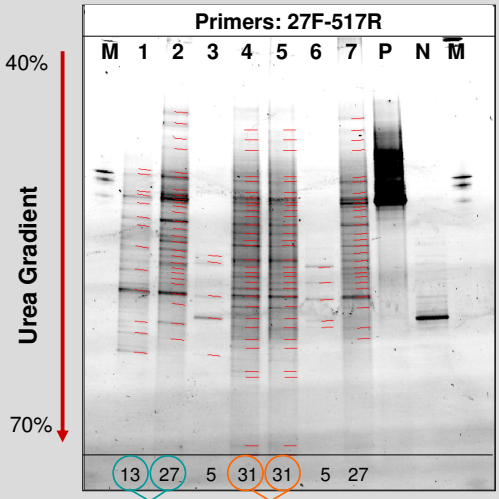
**Eubacterial - PCR**




No significant difference after PMA treatment

**A difference is seen between DNase I treated and untreated samples**

**DGGE**




**1.** Sample + DNase treatment
**2.** Sample + treatment without DNase
**3.** DNA + DNase treatment
**4.** Sample + PMA treatment
**5.** Sample + treatment without PMA
**6.** DNA + PMA treatment
**7.** Direct sample without treatment
**P:** Positive control (*E.faecium* DNA)
**N:** Negative template control (water)
**M:** 100bp Marker

**Live-Dead differentiation**
50% similarity *
A shift is present between the bacterial populations of the sample treated with DNases and the sample without this exposure.

**No live-dead differentiation**
100 % similarity *
No difference between the bacterial populations of the PMA treated and untreated samples

* Similarities were calculated using the Dice Coefficient

## Conclusions

DNase I treatment is more appropiate than PMA treatment for the detection of viable bacteria in oligotrophic water using DNA-based techniques after sample concentration. The DNase I approach is easier, faster, and needs no additional equipment. This enzymatic method has also a more homogeneous effect in the reaction tube and less procedure steps, therefore a subsequent less loss of valuable sample material is achieved.

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