Rapid Mass Spectrometric DNA Diagnostics for Assessing Microbial Activity During Bioremediation

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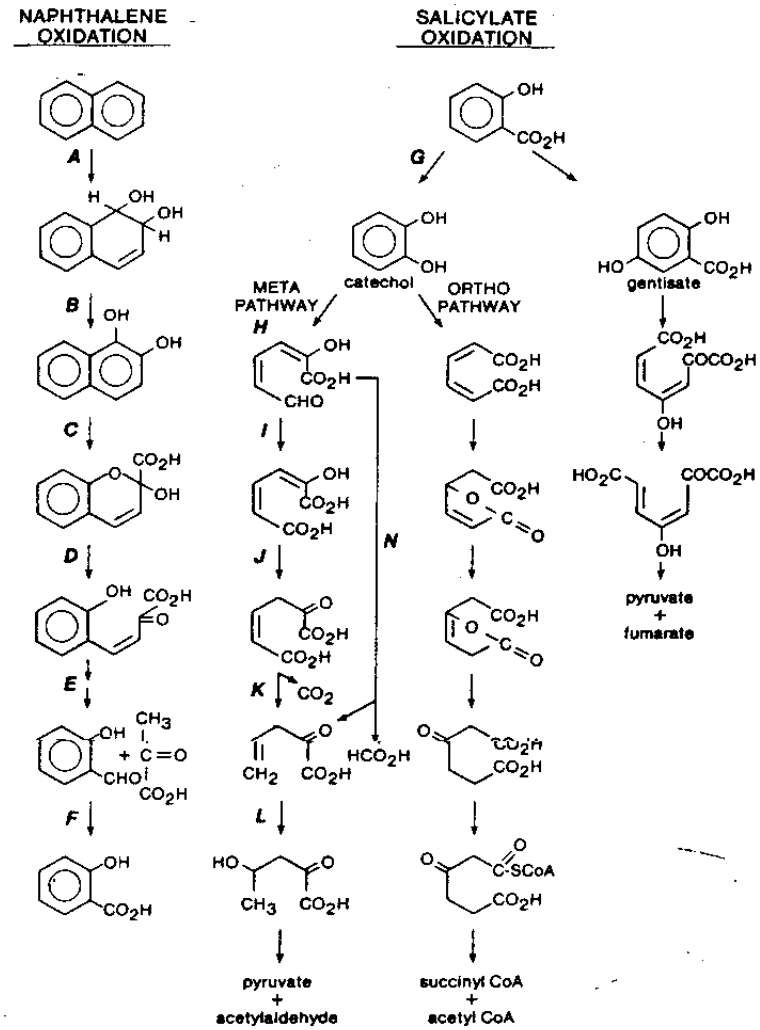
Overview

- Identify a region of a naphthalene-degrading bacterial gene for use as a tracer during bioremediation
- develop a Peptide Nucleic Acid (PNA) diagnostic probe
- Optimize Matrix-Assisted-Laser-Desorption Ionization for detecting the PNA probe via mass spectrometry
- Investigate the relationship between the measured PNA ion intensity and the amount of PNA probe using two different target DNA denaturing conditions
- Determine analytical sensitivity by varying the concentration of target DNA in the PCR procedure
- Compare mass spectrometry results to gel electrophoresis identification

Introduction

Biological activity has often been attributed to changes in pollutant profiles found in contaminated soils when abiotic processes actually caused pollutant removal . Our work is directed at evaluating a monitoring strategy that relies on the combined use of DNA diagnostic procedures and mass spectrometry as the detection scheme. The intent is to track bioremediation by measuring the occurrence of genes in soil samples that are known to code for enzymes capable of degrading specific pollutants. This type of test is commonly performed with PCR and gel electrophoresis but matrix-assisted-laser-desorption ionization mass spectrometry offers the possibility for automation and high throughput as is needed to track the course of bioremediation over large polluted areas.

Importance of dioxygenase gene

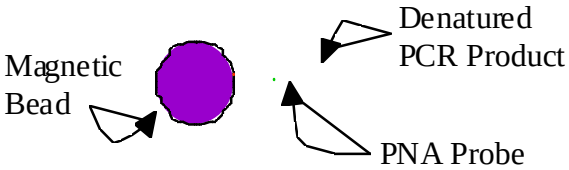


Identification of Bacterial DNA

Case 1: Bacterial DNA contains gene of interest

1. PCR amplify region of bacterial DNA

2. Perform PNA affinity capture assay (PNA binds to PCR amplicon)

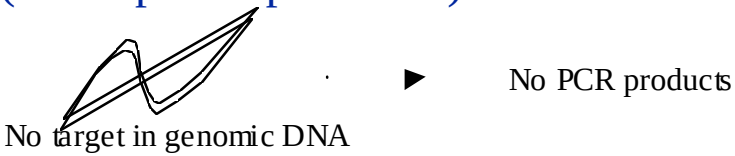


3. Analyze by MALDI-TOF MS

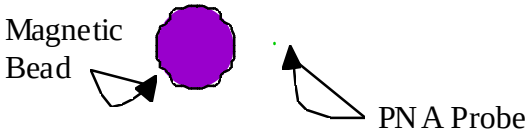
4. Spectrum shows PNA peak; test scored positive

Case 2: Bacterial DNA does not contain gene of interest

1. Attempt PCR of bacterial DNA (no amplicon produced)

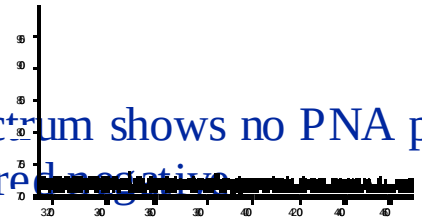


2. Perform PNA affinity capture assay (PNA does not bind and is washed away)

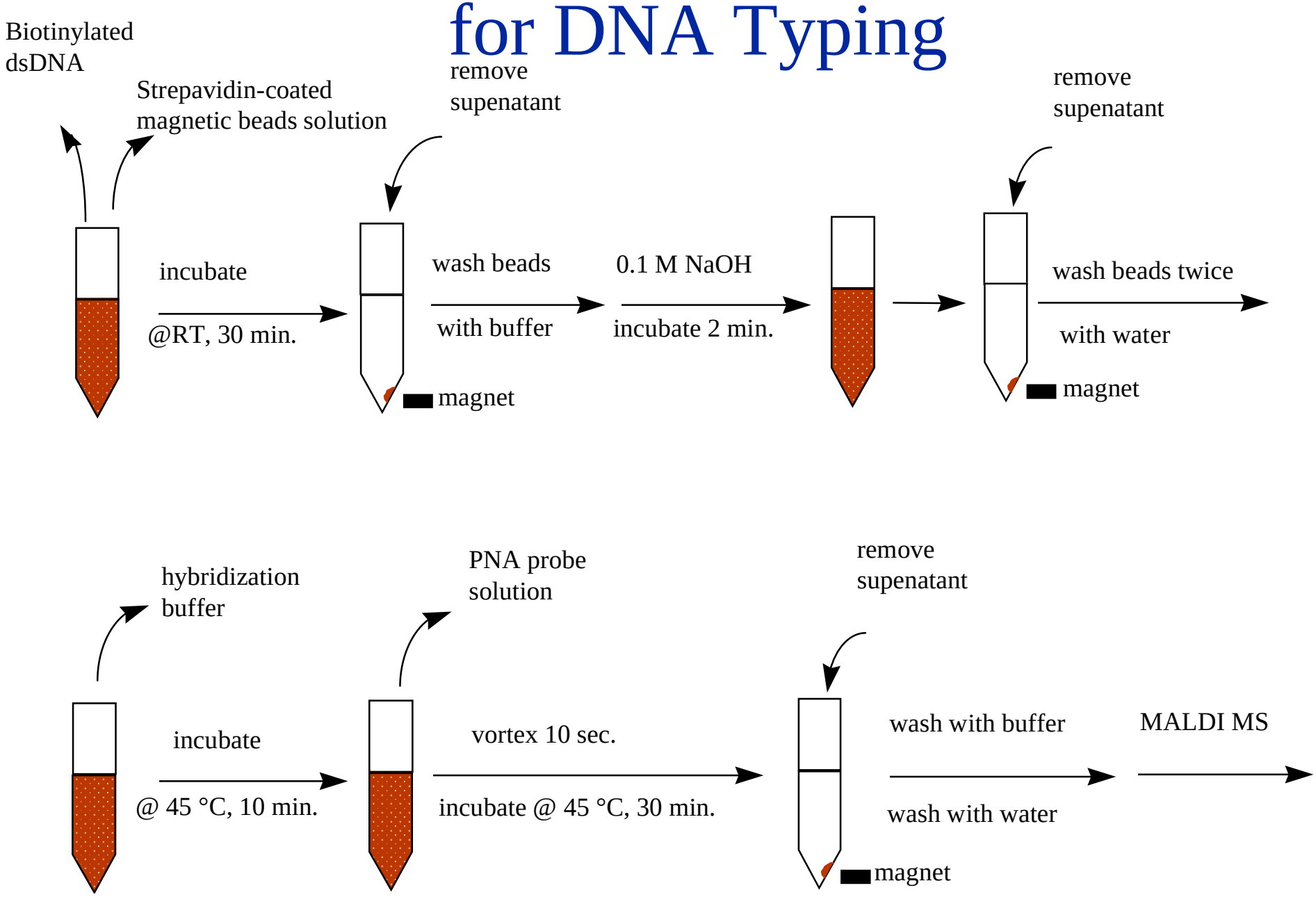


3. Analyze by MALDI-TOF MS

4. Spectrum shows no PNA peak; test scored negative



PNA Affinity Capture Assay for DNA Typing



Mass Spectrometry Detection Scheme

- A laboratory-built delayed extraction time-of-flight mass spectrometer equipped with a 337 nm nitrogen laser was used in positive mode for all of these studies. The acceleration voltage is + 23 kV, the pulsed voltage is - 7.6 kV with 1 μs delay time. An interleaved gate and a pulsed microchannel plate (MCP) detector were also used to avoid the saturation of the MCP by matrix ions.
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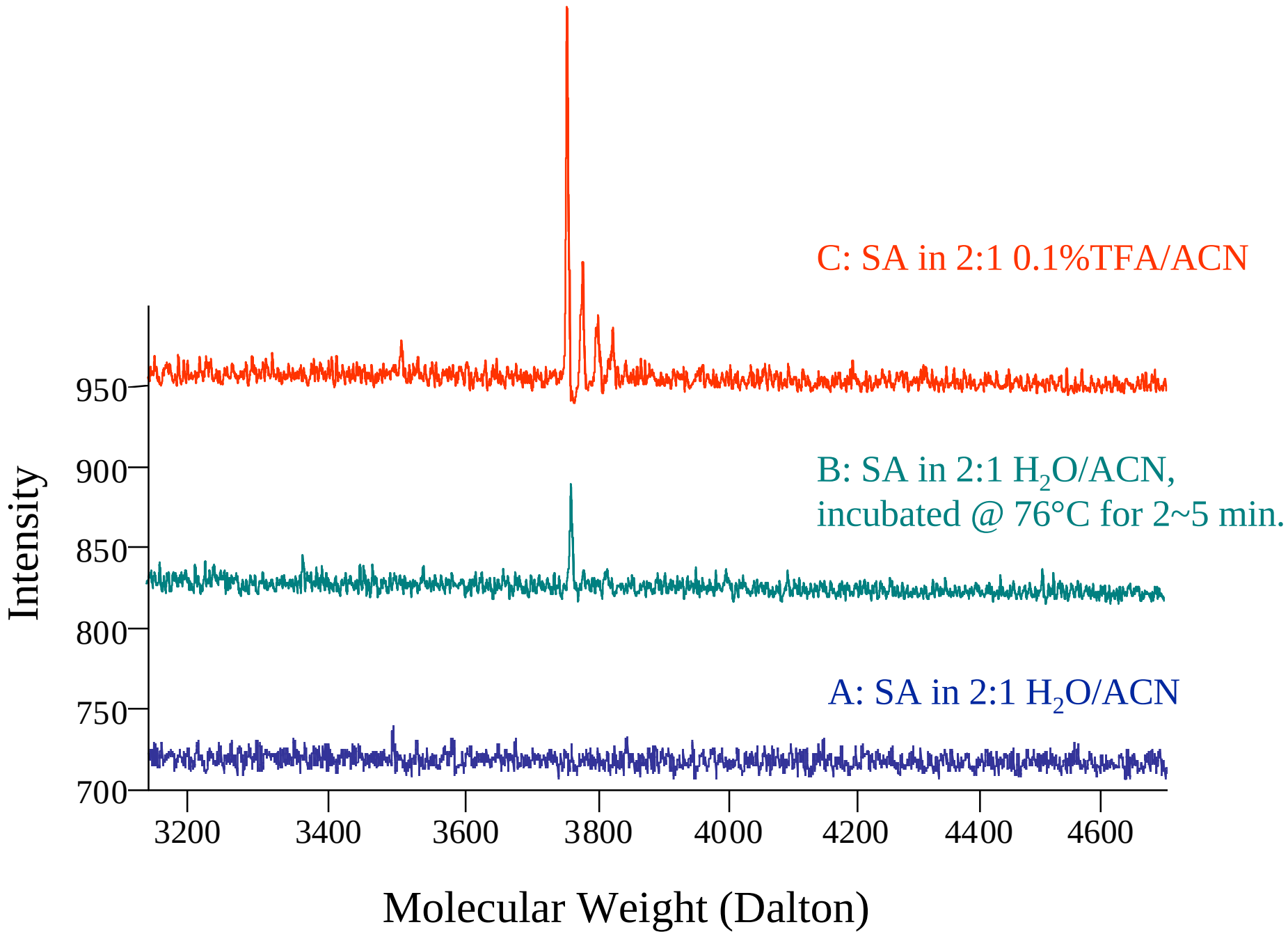
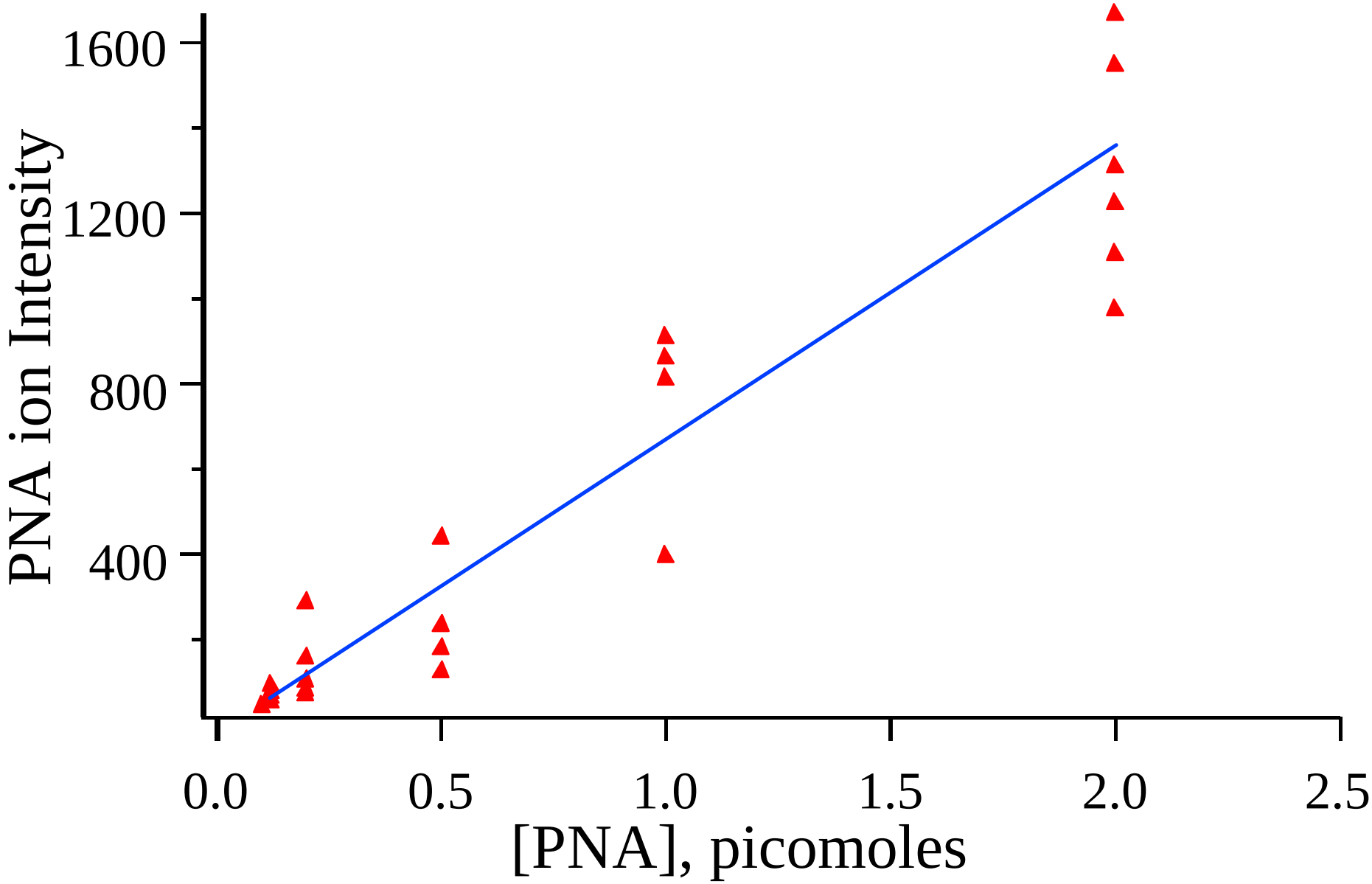
- MALDI matrix solution was prepared by dissolving sinapinic acid in 2:1 0.1% trifluoroacetic acid/actonitrile to a concentration of 10 mg/mL. A MALDI sample was prepared by mixing 1 μL PNA solution or PNA-DNA-Bead complex with 6 μL matrix solution on a sample probe, then dried under a stream of N_2.

MALDI Sample Preparation for Bead-DNA-PNA Complex

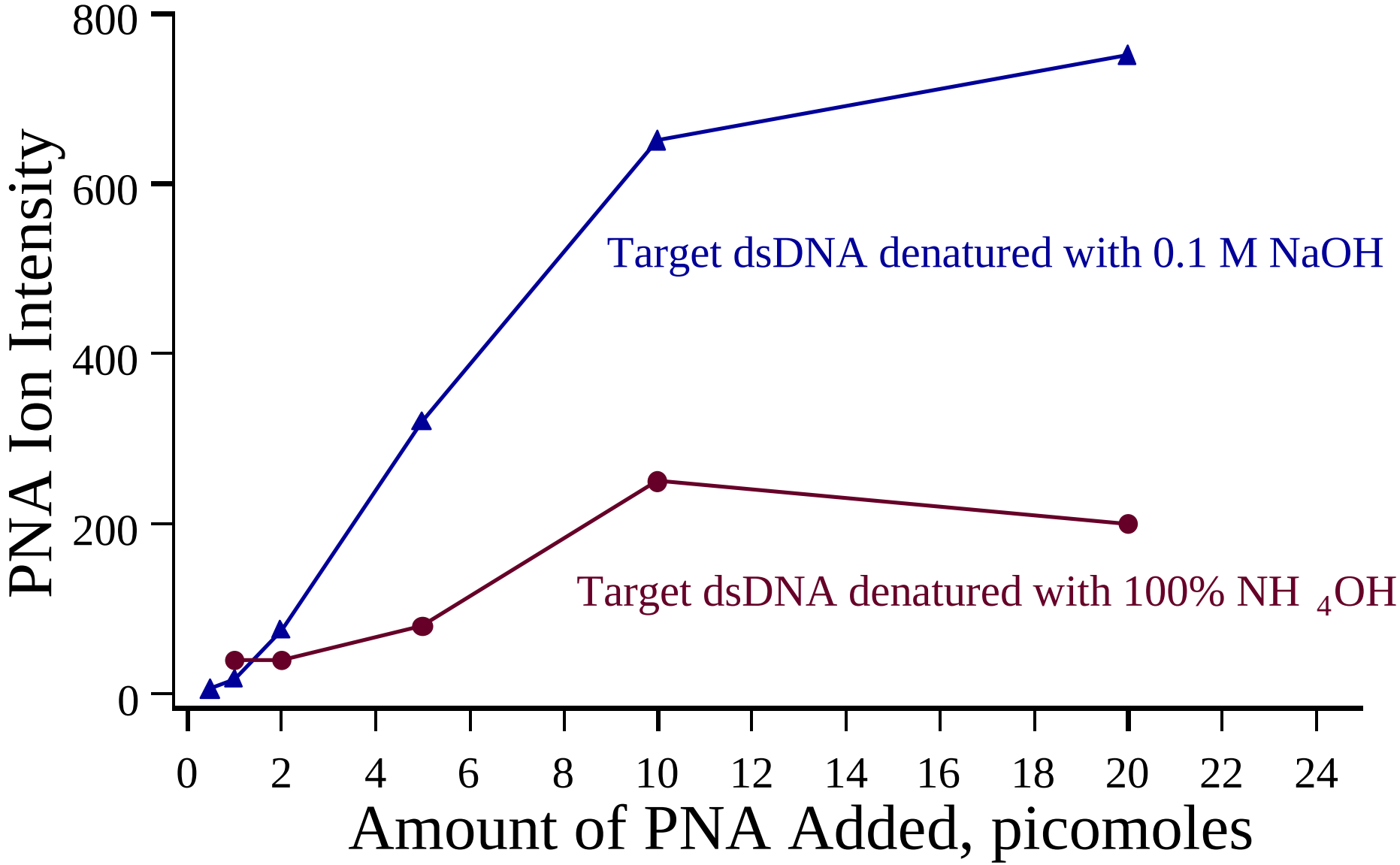
Spect. #	Matrix Solution	PH of Matrix Solution	Sample Prep. Temperture	PNA^+ Intensity
A	SA in 2:1 H_2O/ACN	3 ~ 4	Room Temperture	0
B	SA in 2:1 H_2O/ACN	3 ~ 4	incubate @ 76°C for 2 min.	50
C	SA in 2:1 0.1% TFA/ACN	> 1	Room Temperture	200


C: SA in 2:1 0.1%TFA/ACN
B: SA in 2:1 H2O/ACN,
incubated @ 76°C for 2~5 min.
A: SA in 2:1 H2O/ACN
Intensity
950
900
850
800
750
700
3200
3400
3600
3800
4000
4200
4400
4600
Molecular Weight (Dalton)

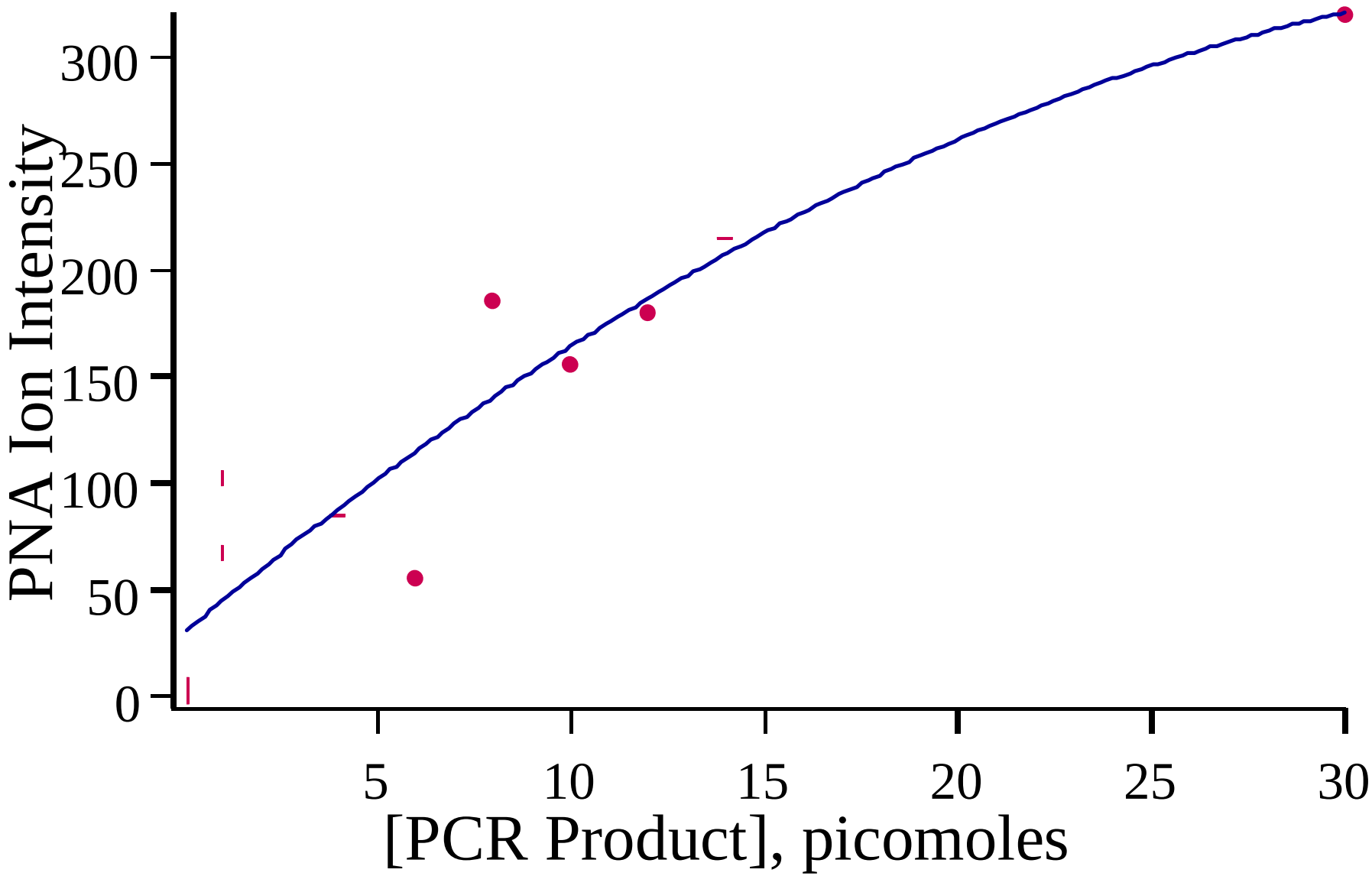
PNA Ion Intensity vs. [PNA]



PNA Ion Intensity vs. [PNA] for Constant [Target DNA]



PNA Ion Intensity vs. [PCR Product] for Constant [PNA]



MALDI-MS vs. Gel-Based Methods for Identifying Genes

- The MALDI procedures are more easily automated
- MALDI produces analysis data directly in digital form, thus easily managed by computer
- Gel-based analysis equipment is cheaper but tedious and manual

Future

- Develop robotic technique for MALDI sample preparation
- Develop collaborations to identify presence of dioxygenase gene in soil samples
- Perform high though-put monitoring

Conclusion

- PNA molecules denature from PNA-DNA-Bead complex in acidic matrix solution, and are easily detected by MALDI MS. The presence of magnetic beads sometimes interferes with this cocrystalization process.
- Detection of 100 fmol of pure 14-mer PNA is readily achieved with DE-MALDI TOF MS and the intensity of the PNA^+ signal increases dramatically with increasing [PNA].
- PNA^+ signal intensity increases with the amount of PCR product when PNA probe is in excess
- Detection limit for PNA-DNA-bead complexes not only depends on the amount of PCR product and PNA probe but also on the affinity capture assay efficiency. At optimum affinity capture conditions, as little as 500 fmol of PNA probe was detected.

Acknowledgement

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