Monitoring Genetic and Metabolic Potential for *in situ* Bioremediation: Mass Spectrometry

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EM Need Addressed

- More rapid characterization and monitoring of microbial populations for *in-situ* bioremediation

Introduction

- DNAPL's (dense non-aqueous phase liquids) and other pollutants in groundwater can be degraded by microbial action (bioremediation)
- In-situ bioremediation requires characterization of native or augmented microbial populations
- MALDI-MS (matrix-assisted laser desorption/ionization mass spectrometry) detection of specially-designed bacterial gene probes (PCR products) shows potential as a rapid alternative to conventional microbial characterization methods.

Potential advantages of characterizing microbial populations by MALDI-MS detection of PCR products

- *Speed*: MALDI is much faster than gel electrophoresis of PCR products
- *Accuracy*: PCR size measurement in MALDI is a direct measurement of mass
- *Generality*: Any genomic region for which a suitable PCR can be designed can be interrogated
  - functional (enzymes that degrade pollutants)
  - phylogenetic (16S rRNA genes)
- *Sensitivity*: No need for bacterial culturing

To demonstrate the feasibility of MALDI detection of bacterial PCR products, we have developed a functional gene probe assay for the particulate methane monooxygenase gene[4-6] (*pmoA)*. Special PCR primers were designed for *pmoA* in two species of methanotrophic bacteria that co-metabolize trichloroethylene:

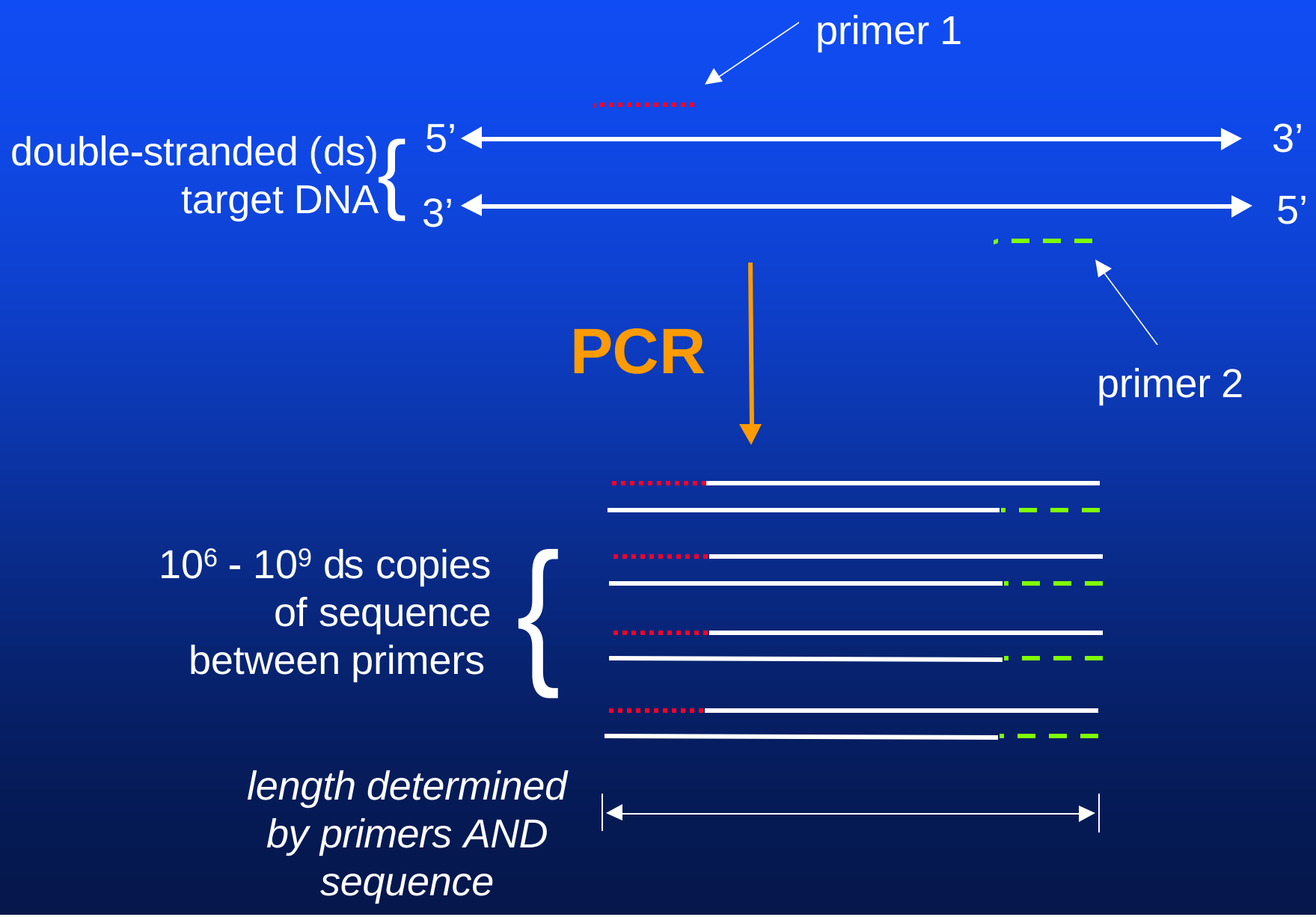
- ***Methylosinus trichosporium* OB3b (type II)**
- ***Methylomicrobium albus* BG8 (type I)**

Two technologies are combined in this work[1] for rapid "gene probe" analysis of microbial populations:

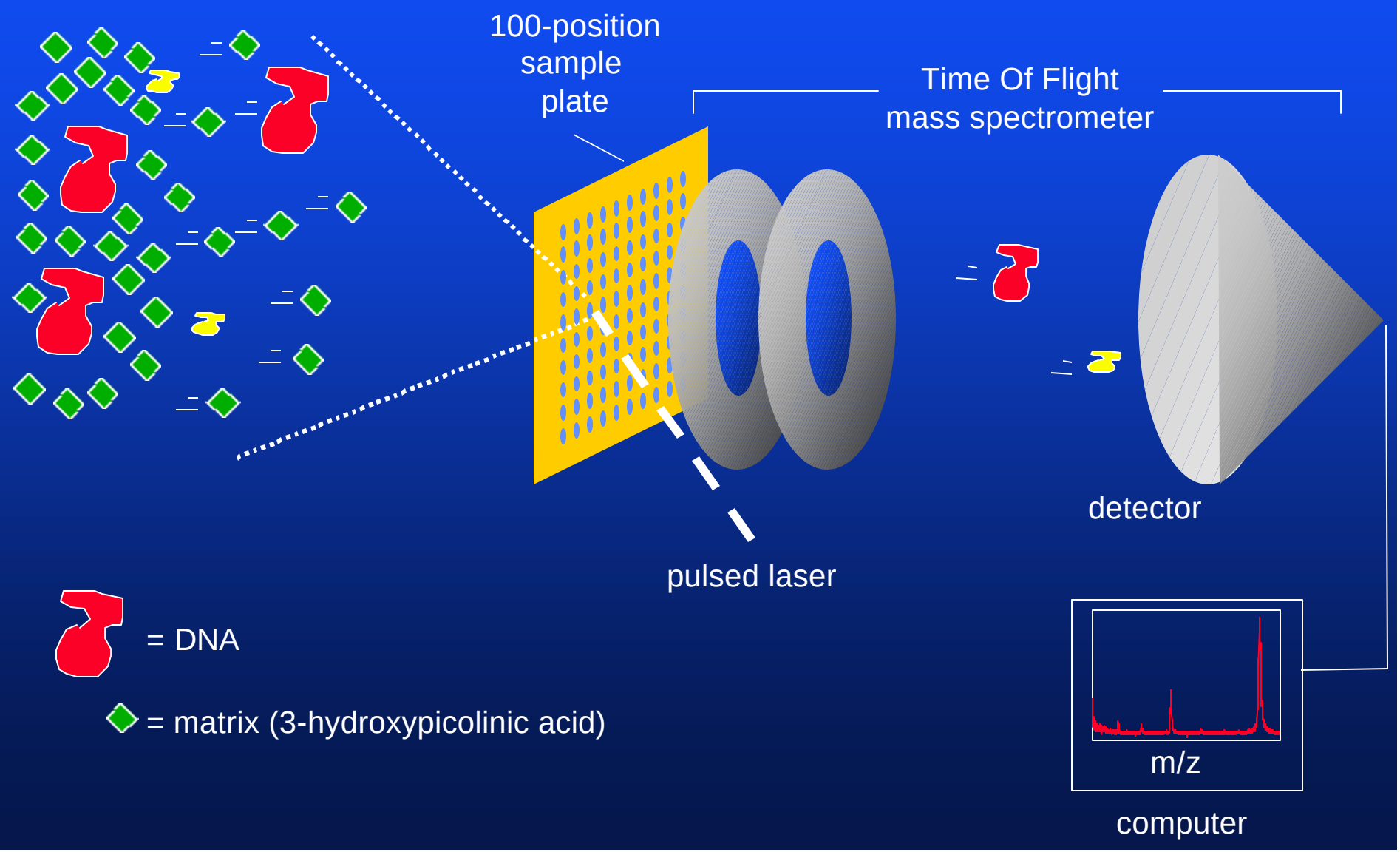
- **PCR (Polymerase Chain Reaction): creates many copies of a selected region from an organism's genetic material (DNA).**

- **MALDI-MS[2] (Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry): measures molecular mass of biomolecules (DNA[3] in this case).**

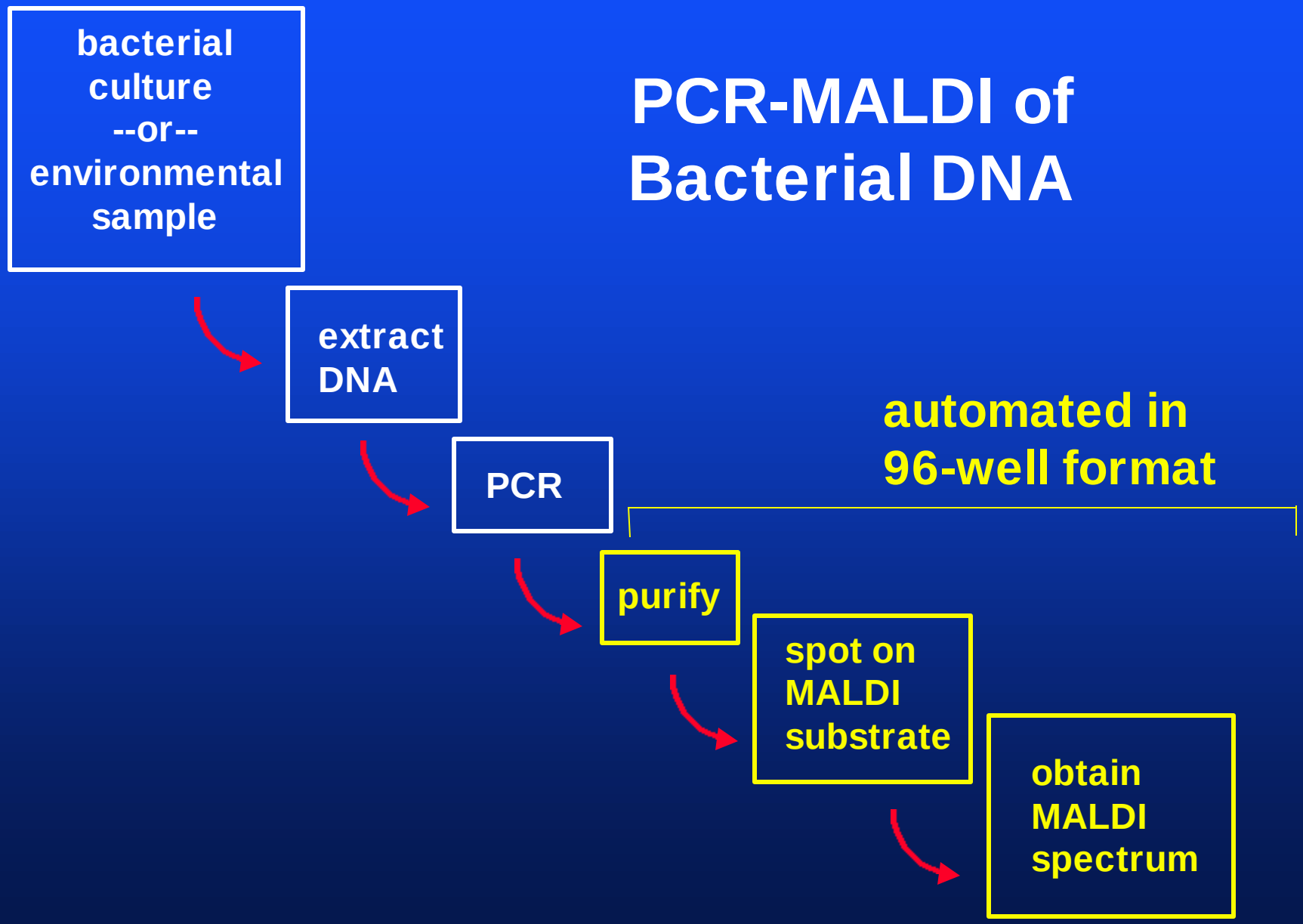
Polymerase Chain Reaction



MALDI Mass Spectrometry
(Matrix-Assisted Laser Desorption/Ionization)



PCR-MALDI of Bacterial DNA



MALDI-MS of Type II *pmoA* PCR Products

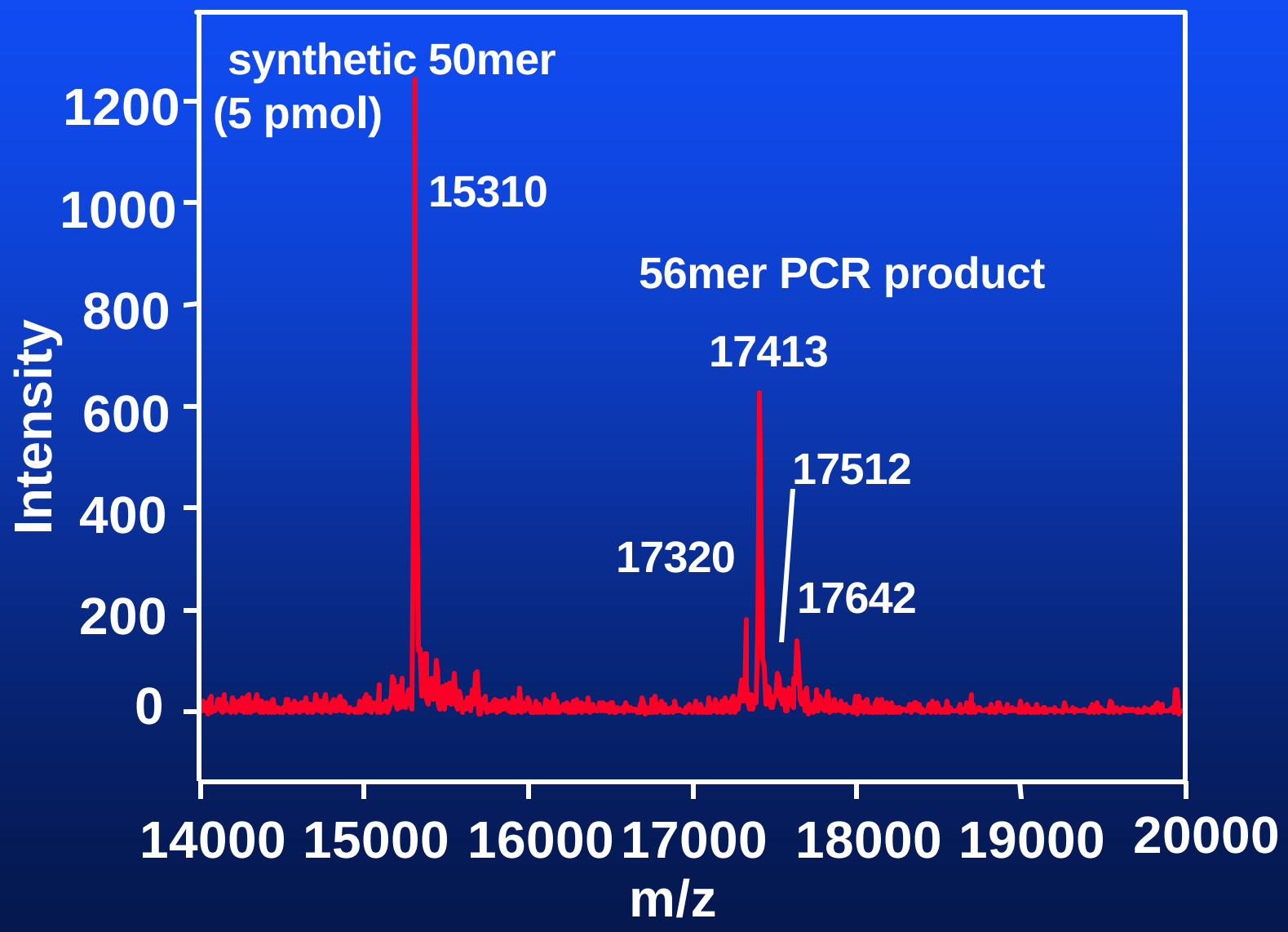
MALDI-TOF spectrum of the 56-base PCR product obtained from type II methanotroph DNA, mixed with 5 picomoles of a synthetic DNA 50-mer.

The appearance of the 50-mer peak indicates that MALDI artifacts, such as fragmentation and cation adduction, are minor under the conditions used.

The four peaks in the vicinity of the 56-mer in Figure 2 demonstrate that multiple species may arise from the PCR. Possible sources of "extra" peaks are:

- non-specific inosine bases in the primers
- complementary strands of the ds PCR product
  - calculated m/z values from published sequence: 17282 Da and 17202 Da.)
- non-templated enzymatic incorporation of an extra adenosine nucleotide.

MALDI-MS of Type II *pmoA* PCR Product



Blind Analysis of Different DNA Types

MALDI-MS spectra from eight separate PCR amplifications using type II primers with

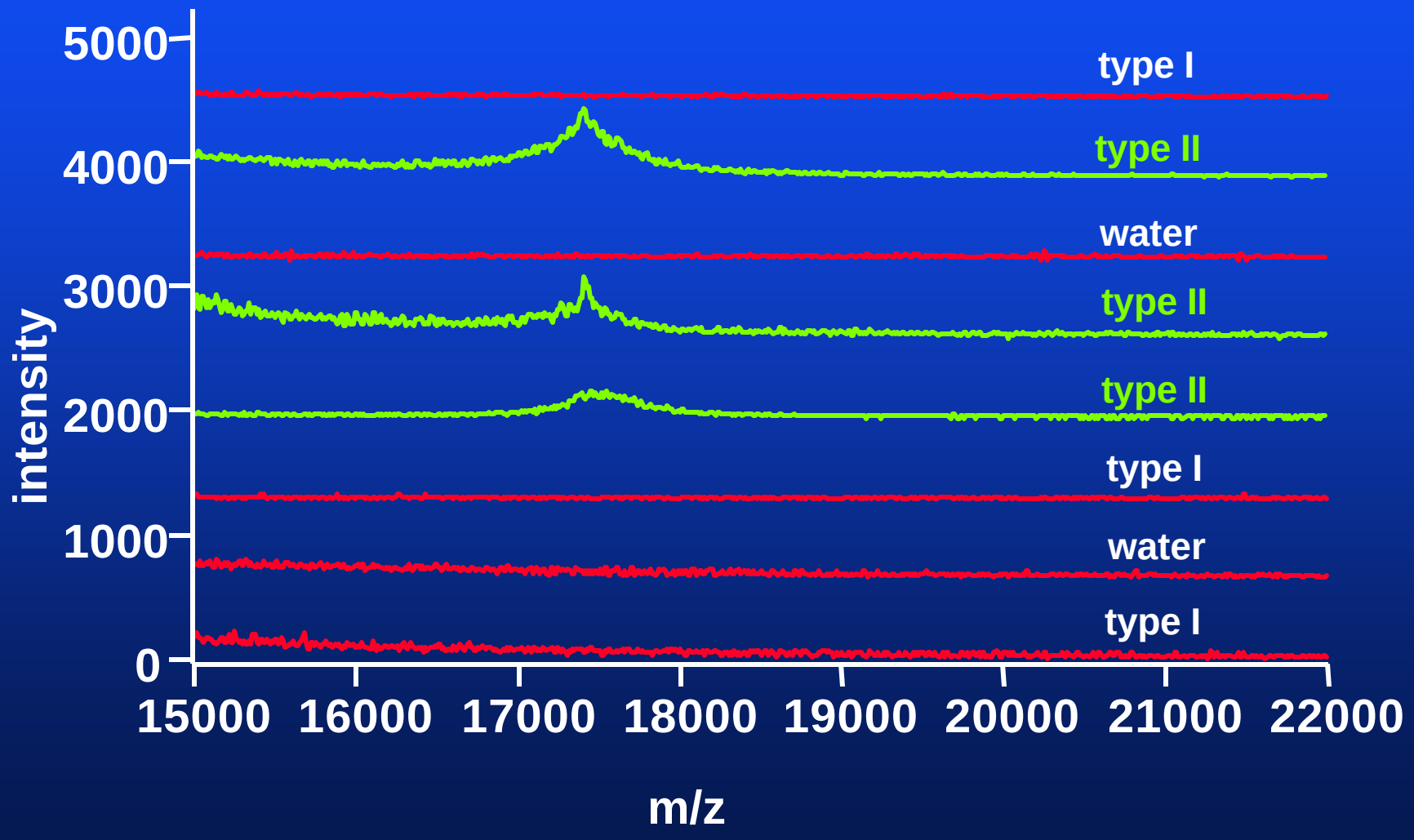
- Type II target DNA (should be amplified)
- Type I target DNA (should NOT be amplified)
- no DNA (blank)

These amplifications and MALDI analyses were performed "blind"--target DNA was aliquoted by one experimenter, with all subsequent steps performed by a second.

Signal due to the type II product is observed only in those reactions containing type II target DNA, demonstrating the specificity of the primers in distinguishing between the same gene (*pmoA*) in the two methanotroph types.

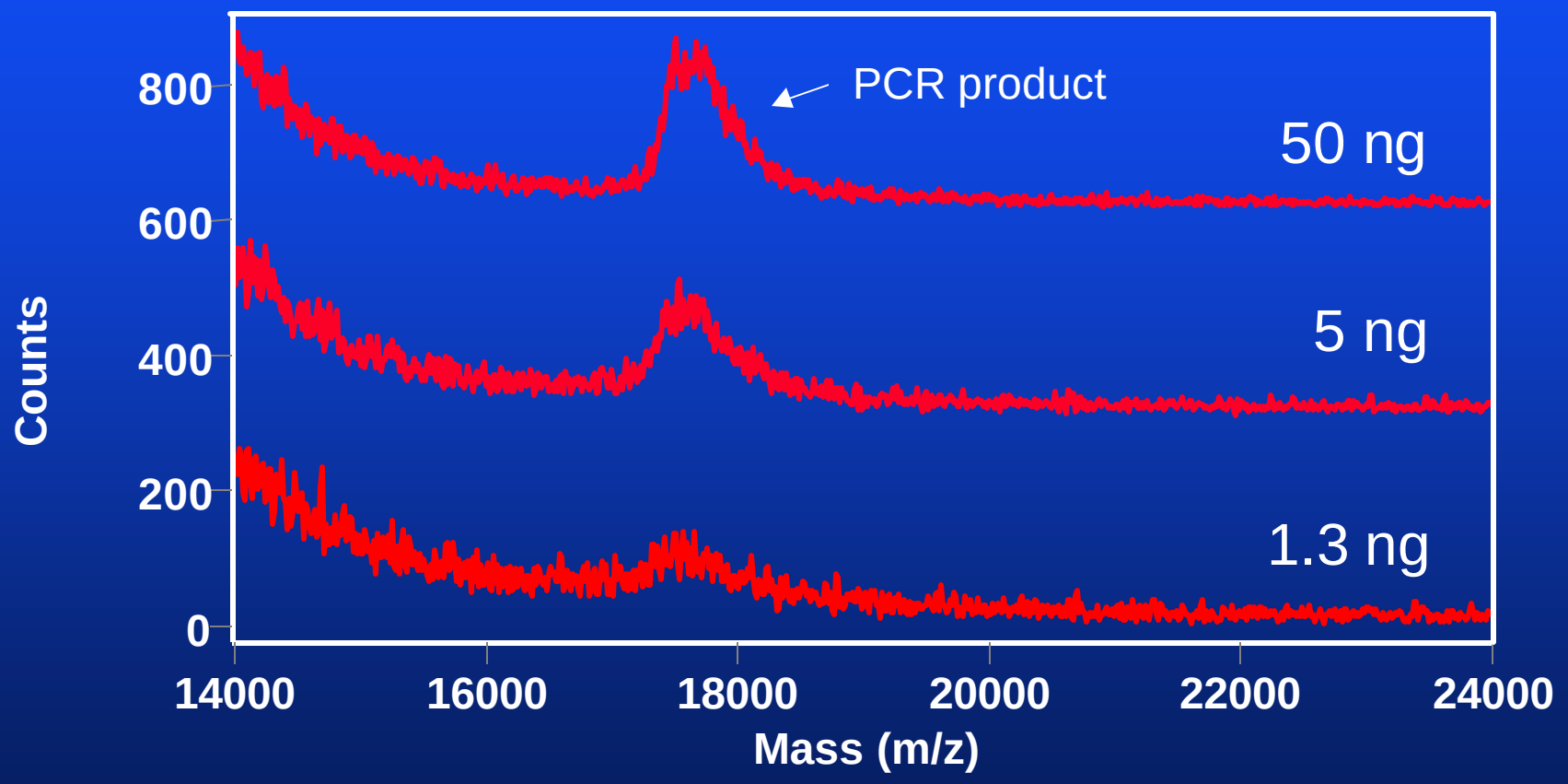
Blind Analysis of Different DNA Types

PCR with Type II Primers



Effect of Target DNA Amount

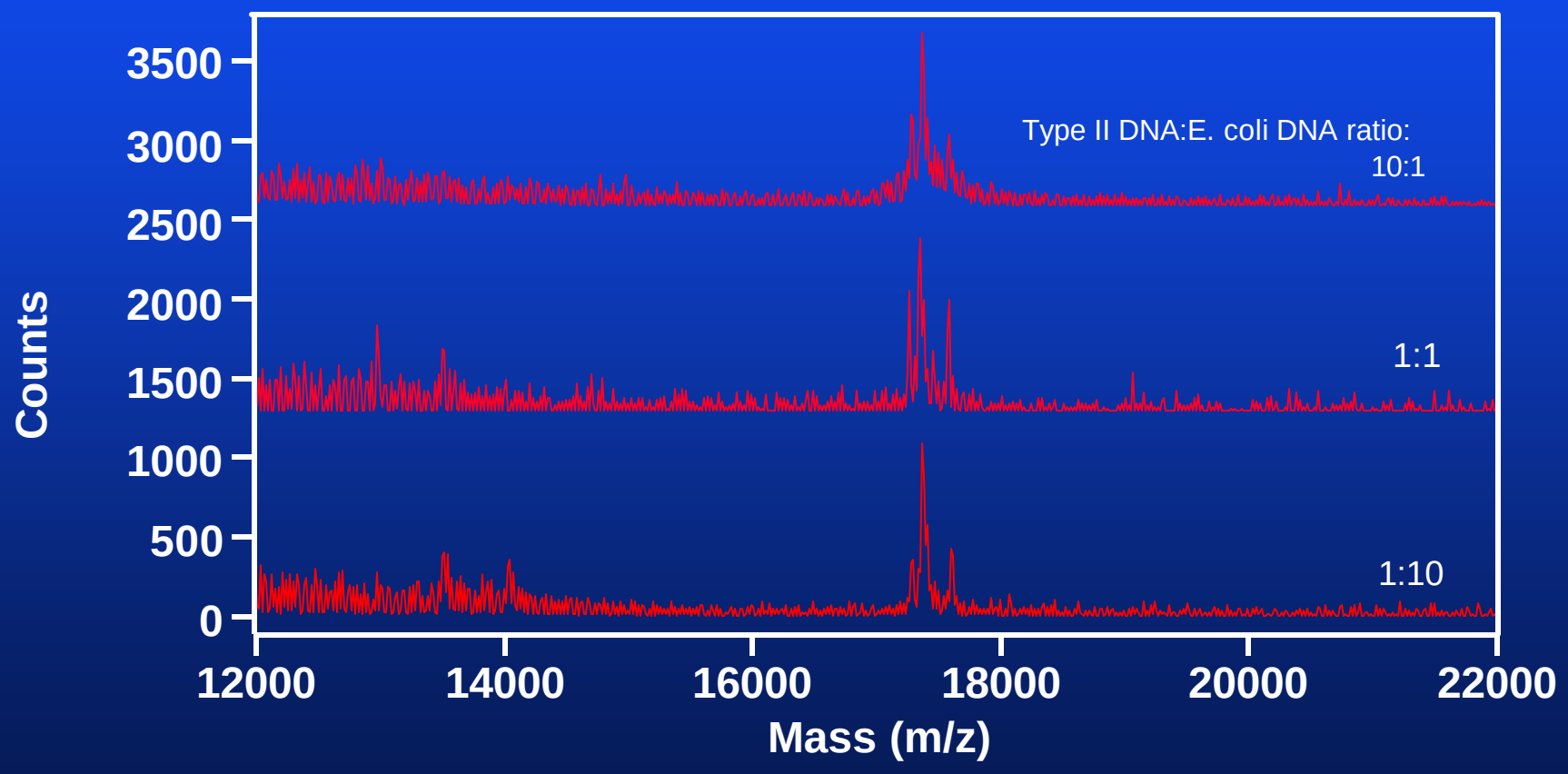
PCR of Type II Methanotroph



Original Filename: c:\voyager\1dec\kris\1201_028.ms

Type II Assay Works in Presence of “interfering” DNA

Type II DNA with *E. coli* DNA



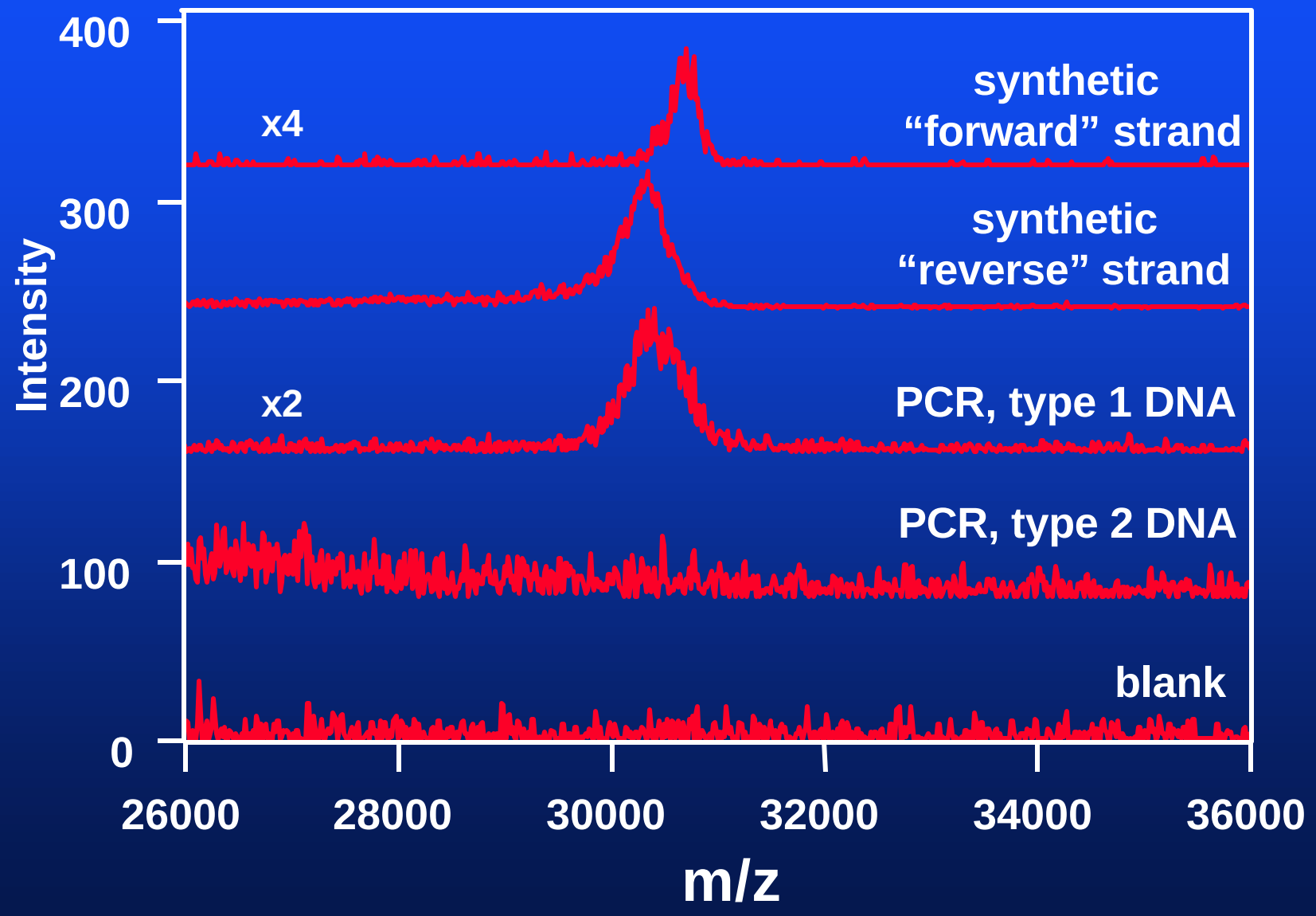
MALDI-MS of Type I pmoA PCR Products

MALDI-TOF mass spectra of PCR products using the *pmoA* primer pair for type I methanotrophs.

- "forward" and "reverse"--single-stranded, synthetic 99-mers corresponding to the two PCR product strands
- "type I"--PCR using type I primers with type I target DNA
- "type II"--PCR using type I primers with type II target DNA (negative control)
- "water"--PCR using type I primers with no target DNA (blank).

Again, only the correct sequence is amplified. Resolution is poorer for this larger product than for the type II product.

MALDI-MS of Type I *pmoA* PCR Product

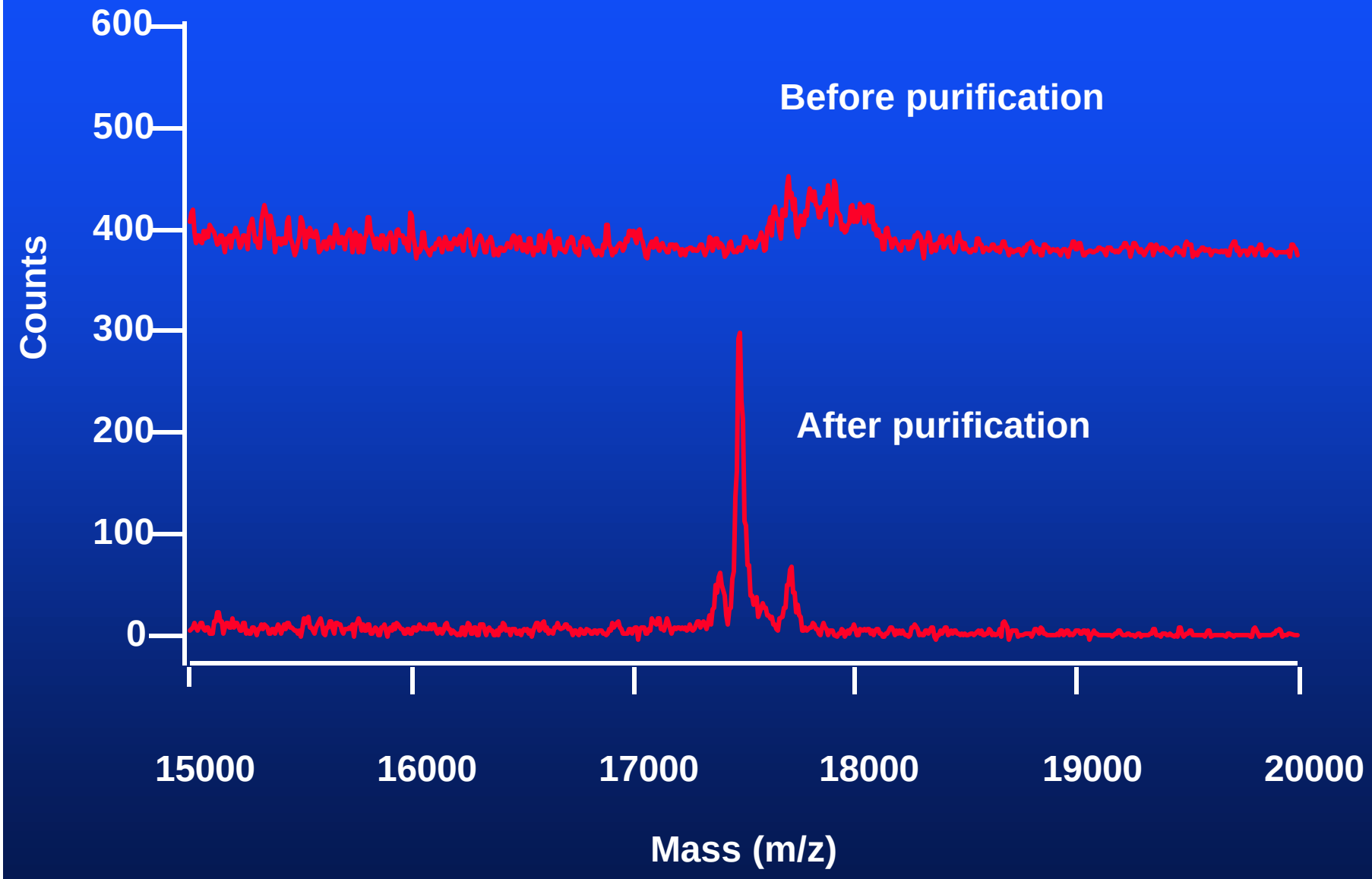


Remaining Issues for MALDI-MS Detection of PCR Products

- **Resolution**--ability to distinguish products of similar molecular mass
  - baseline resolved up to ~60 bases
  - Sample preparation: removal of salts, dNTP's, etc.
  - Instrumentation: Delayed extraction
- **Sensitivity**
  - for small (< 100 bp) products, can detect DNA from a fraction of a single 25 μL PCR.
  - above 100 bp, need more DNA
- **Mass Range:** MALDI with a UV laser is limited to PCR products < ca. 500 bases, but recent work with IR lasers[6] goes up to >2000 bases.
- **Reproducibility and Convenience**
  - "sweet spots": non-uniform DNA/matrix mixture

The following results summarize our work addressing some of these issues.

Reverse-Phase Purification Improves MALDI of PCR Products



MALDI-MS of synthetic pdA_{40-60}

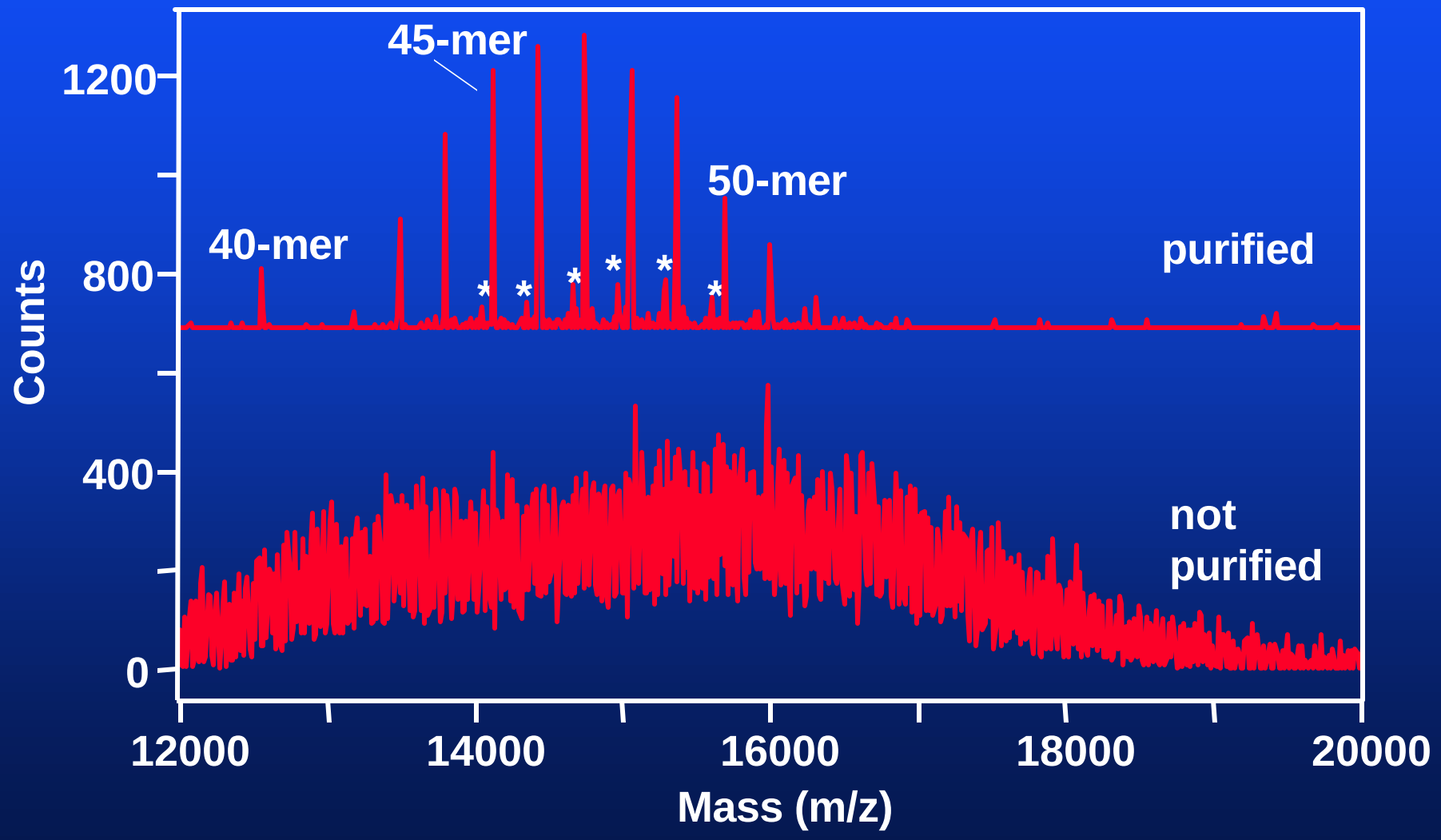
Before reverse-phase purification (bottom trace), no distinct peaks were observed in a broad feature encompassing the m/z range of the sample (mixture of synthetic polynucleotides, pdA_{40-60}.)

After purification (top trace), distinct peaks ranging from 10 to 12 Da in width appear for the 40-mer through the 52-mer ($M/\Delta M$ 1000-1500).

The mass accuracy (0.03%) and resolution illustrated here demonstrate the utility of MALDI-TOF in this size range for multiplexed PCR or amplification of a polymorphic region, if suitable primers can be chosen.

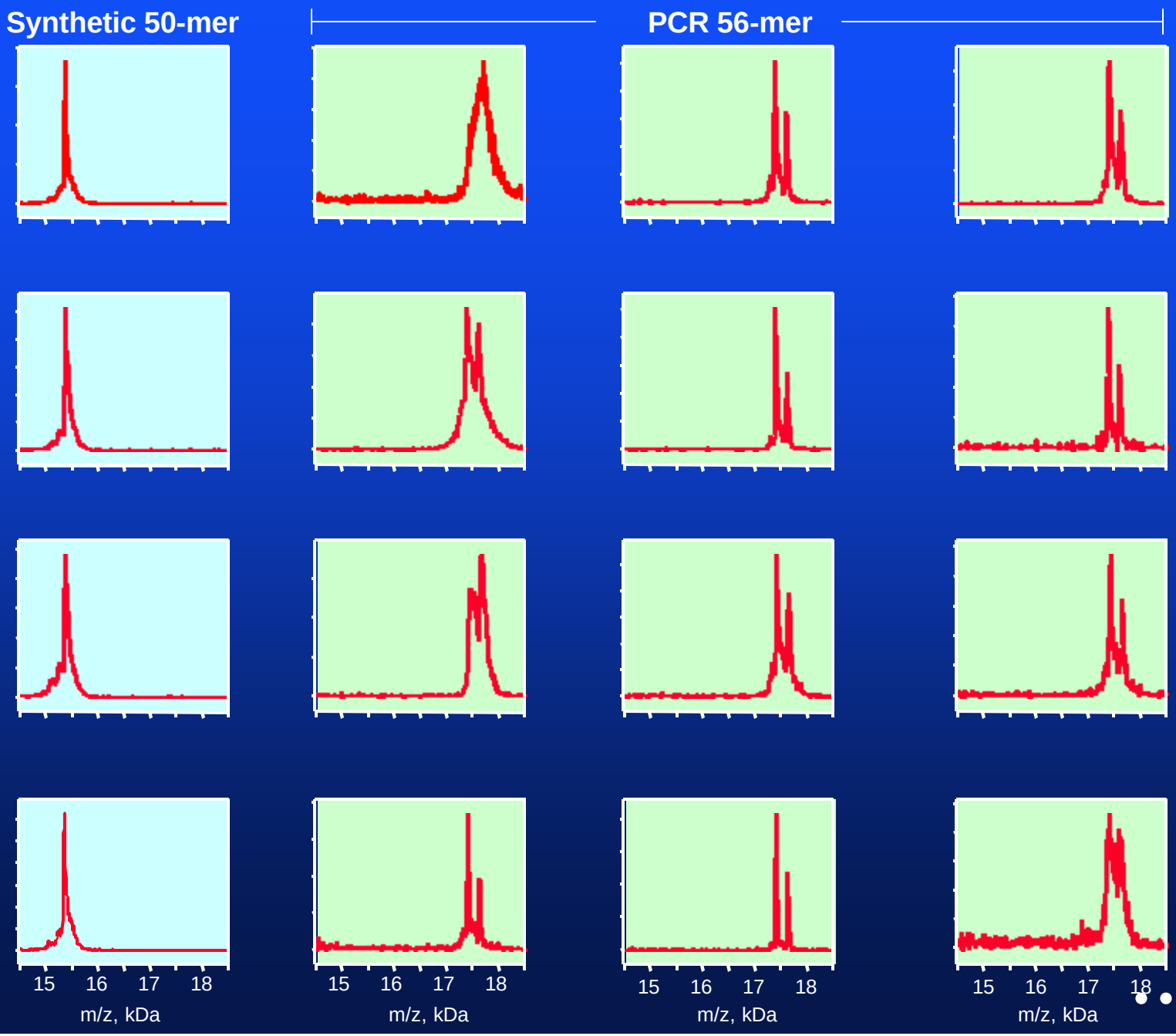
Effect of Rapid Purification of DNA

Sample: synthetic pdA_{40-60}



Rapid MALDI Analysis of PCR Products[7]

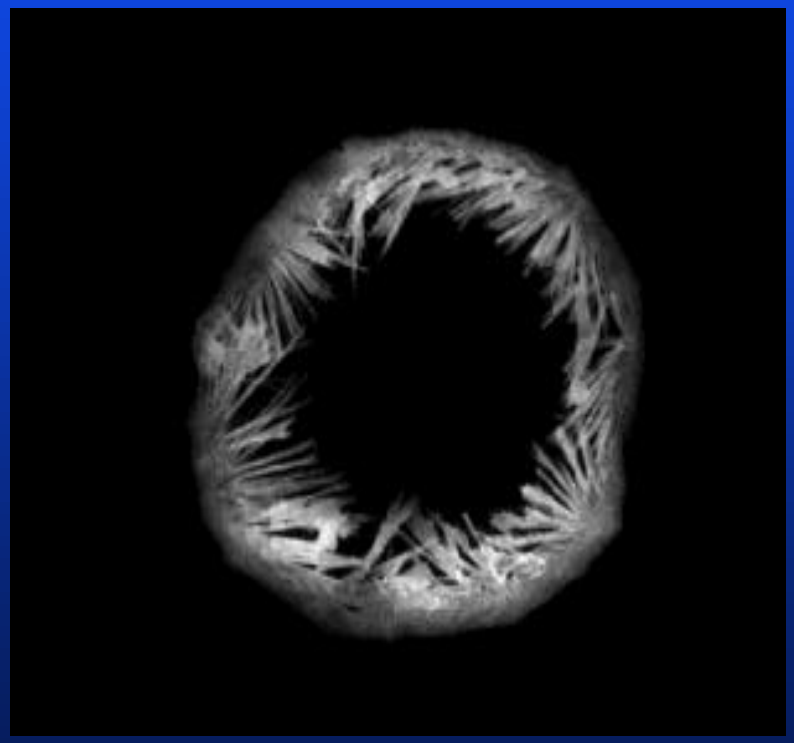
- 96-well format, parallel purification
- Automated MALDI analysis
- 16 of 96 total spectra shown
- Potential for high-throughput microbial characteri-zation



Polymer Substrates Improve Spot Homogeneity[8]

⇒ Increased Analytical Throughput

Bare Metal Substrate

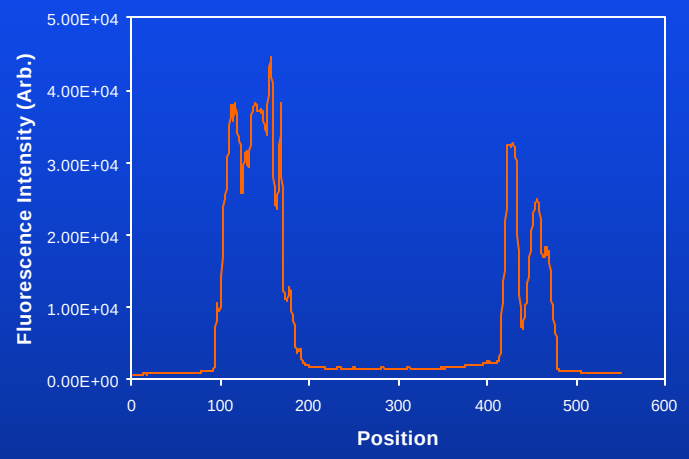


Polymer Substrate

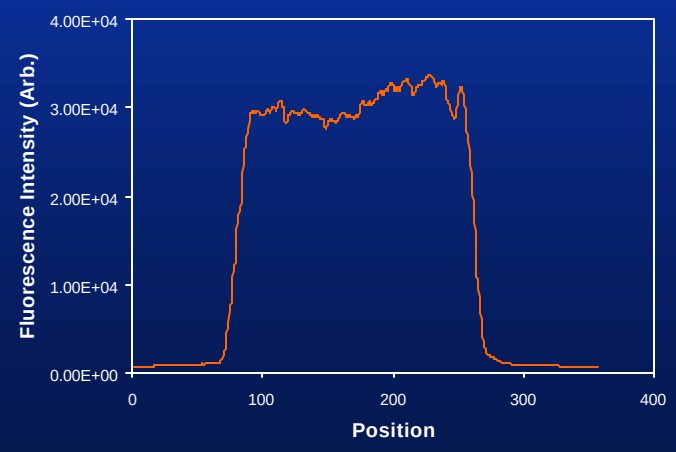
Fluorescence micrographs of HEX-labeled DNA in MALDI matrix (3-hydroxypicolinic acid)

Polymer Substrates Improve Spot Homogeneity

Cross-sectional views of DNA fluorescence in MALDI spots:



Bare Metal Substrate



Polymer Substrate

Polymer Substrates Improve Spot Homogeneity[8]

Success Rate for Obtaining MALDI Signal from DNA/matrix Spots on Various Substrates[a]

Substrate	Bare Gold Plate	LPA	PEO	MC	PDA	PAA	PEI
Polymer molecular weight (Da)		1 000 000	1 000 000	86 000	NA	450 000	1 000 000
Initial polymer concentration (w/w in water)		1.0%	1.0%	2.0%	10.0%	2.0%	2.0%
Success Rate	52±16% (n=6)[b]	84± 3% (n=12)	87± 2% (n=12)	71± 3% (n=8)	12± 4% (n=5)	-[c] (n=5)	- (n=5)

a: Nafion was added on top of the dried polymer layer
b: n is the number of the spots investigated
c: "-" indicates no MALDI signal was obtained from the spot prepared on this substrate.

Summary

- **MALDI-MS detection of PCR-amplified bacterial targets offers promise as a rapid method for assessing the genetic potential of microbial populations for bioremediation.**
- **Type I and type II methanotrophic bacteria can be distinguished based on MALDI-MS detection of PCR-amplified regions of their *pmoA* genes.**
- **The design of shorter PCR products facilitates MALDI-MS detection.**
- **A rapid reverse-phase purification can be used to interface PCR with MALDI-MS.**
- **Polymer substrates enhance reproducibility of MALDI for small oligonucleotides**

References

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