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

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# 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.

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## 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

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**To demonstrate the feasibility of MALDI detection of bacterial PCR products, we describe 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)**

4 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).

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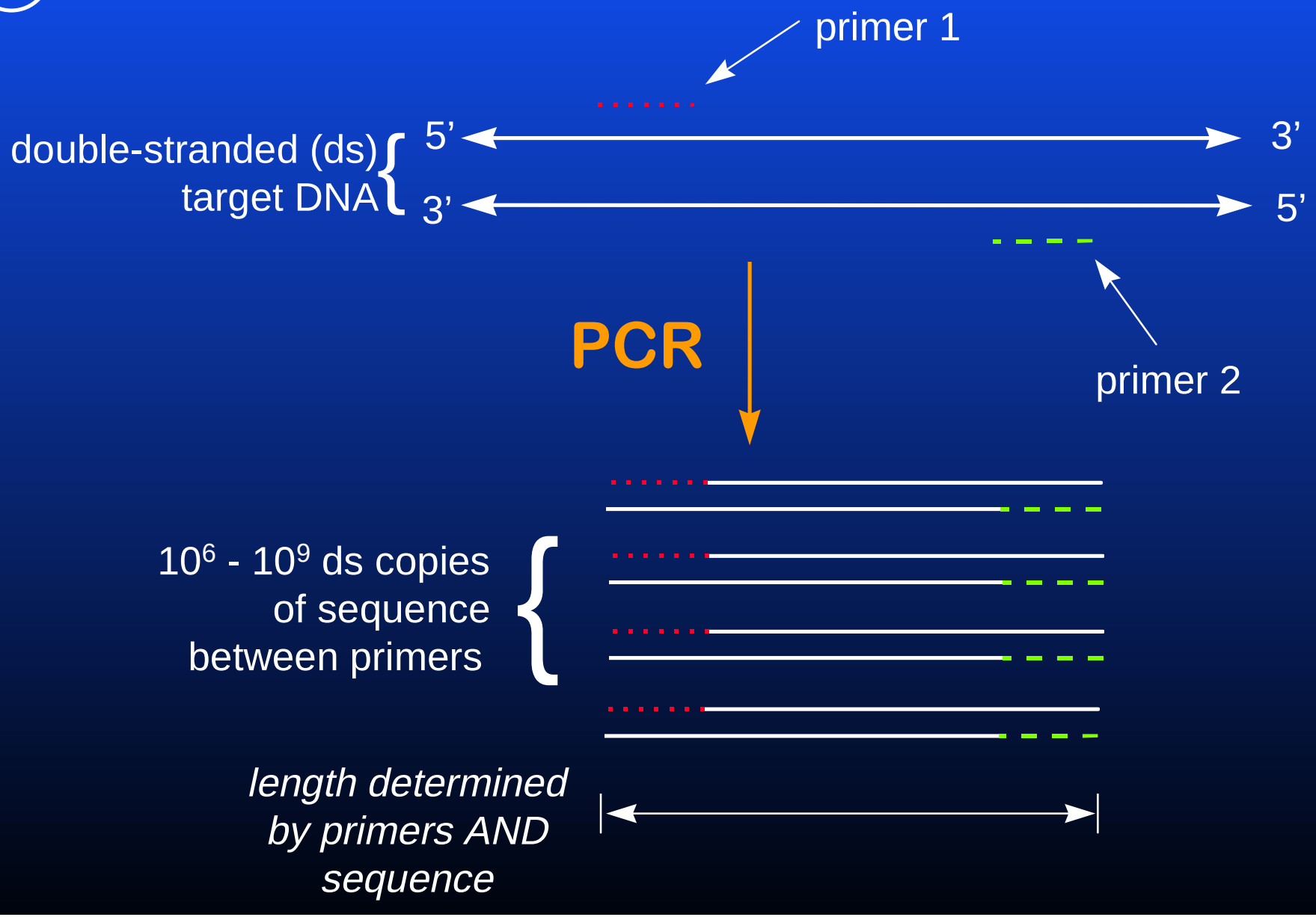
# Polymerase Chain Reaction



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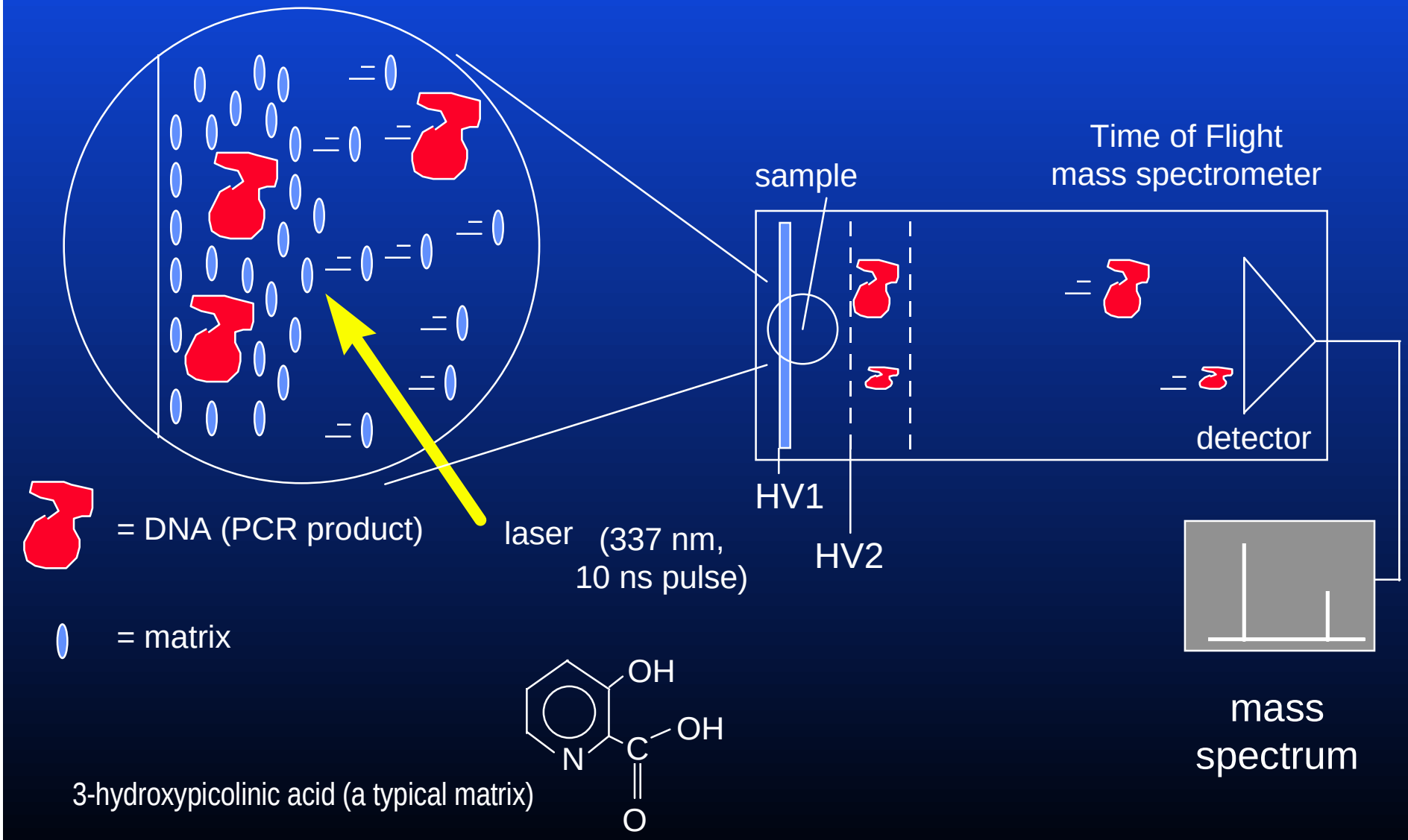
# MALDI-TOF Mass Spectrometry for DNA Mass Measurement



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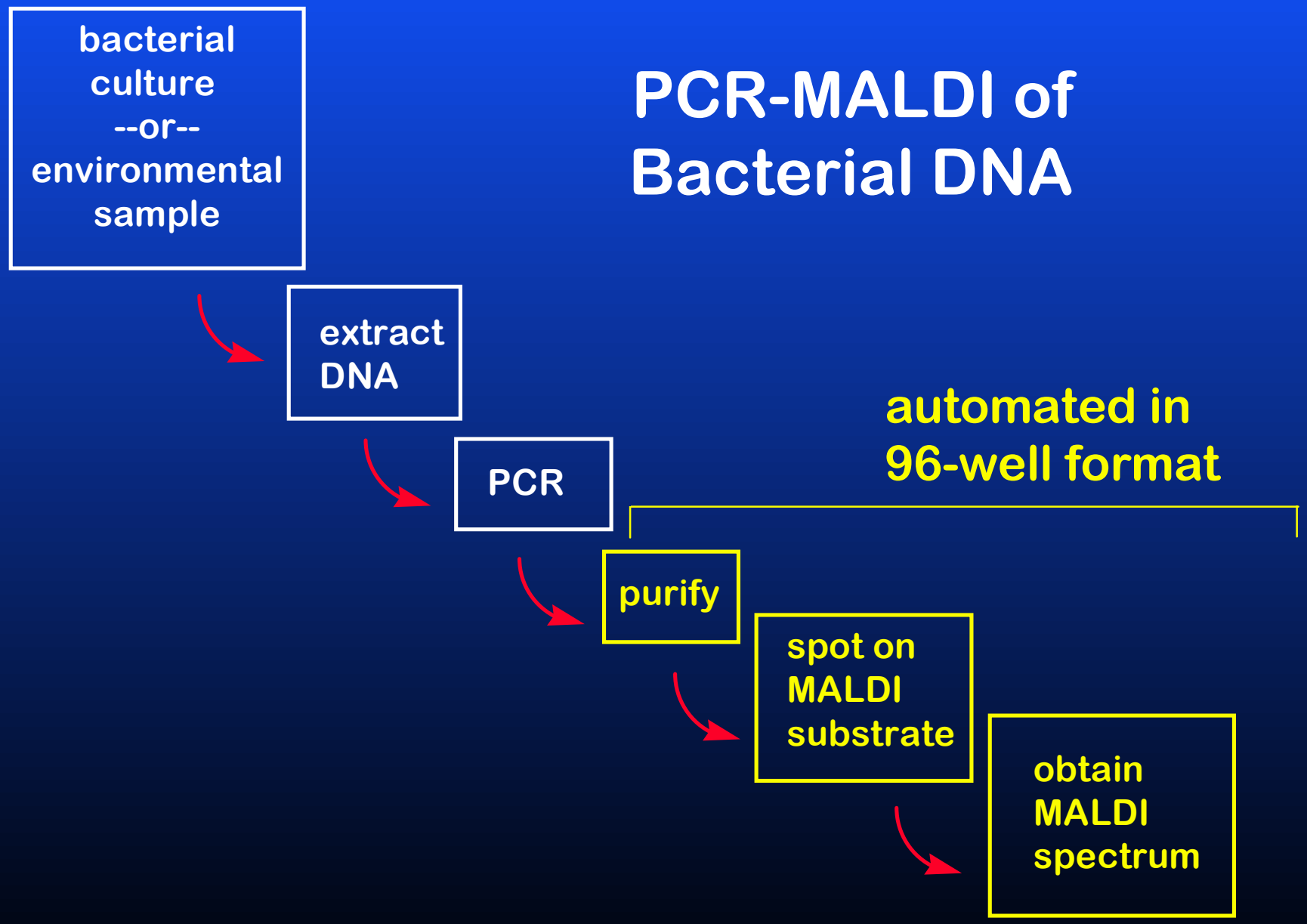
# Issues for MALDI-MS Detection of PCR Products

- **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

# Experimental

- Isolate bacterial DNA
- PCR
  - specially-designed primers[1] yield "short" products amenable to MALDI
    - Type I methanotrophs → 99-mer product
    - Type II methanotrophs → 56-mer product
- MALDI Sample Preparation
  - Rapid (5-minute) reverse-phase purification of PCR products
  - Mix purified PCR product with MALDI matrix solution.
- MALDI Analysis
  - PerSeptive Biosystems Voyager-DE™
    negative ions, delayed extraction

# PCR-MALDI of Bacterial DNA



# ⑩ MALDI-TOF 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 ($pdA_{40-60}$, obtained from Pharmacia.)

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).

Mass accuracy is ~ 0.03% (5 Da).

The mass accuracy 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.

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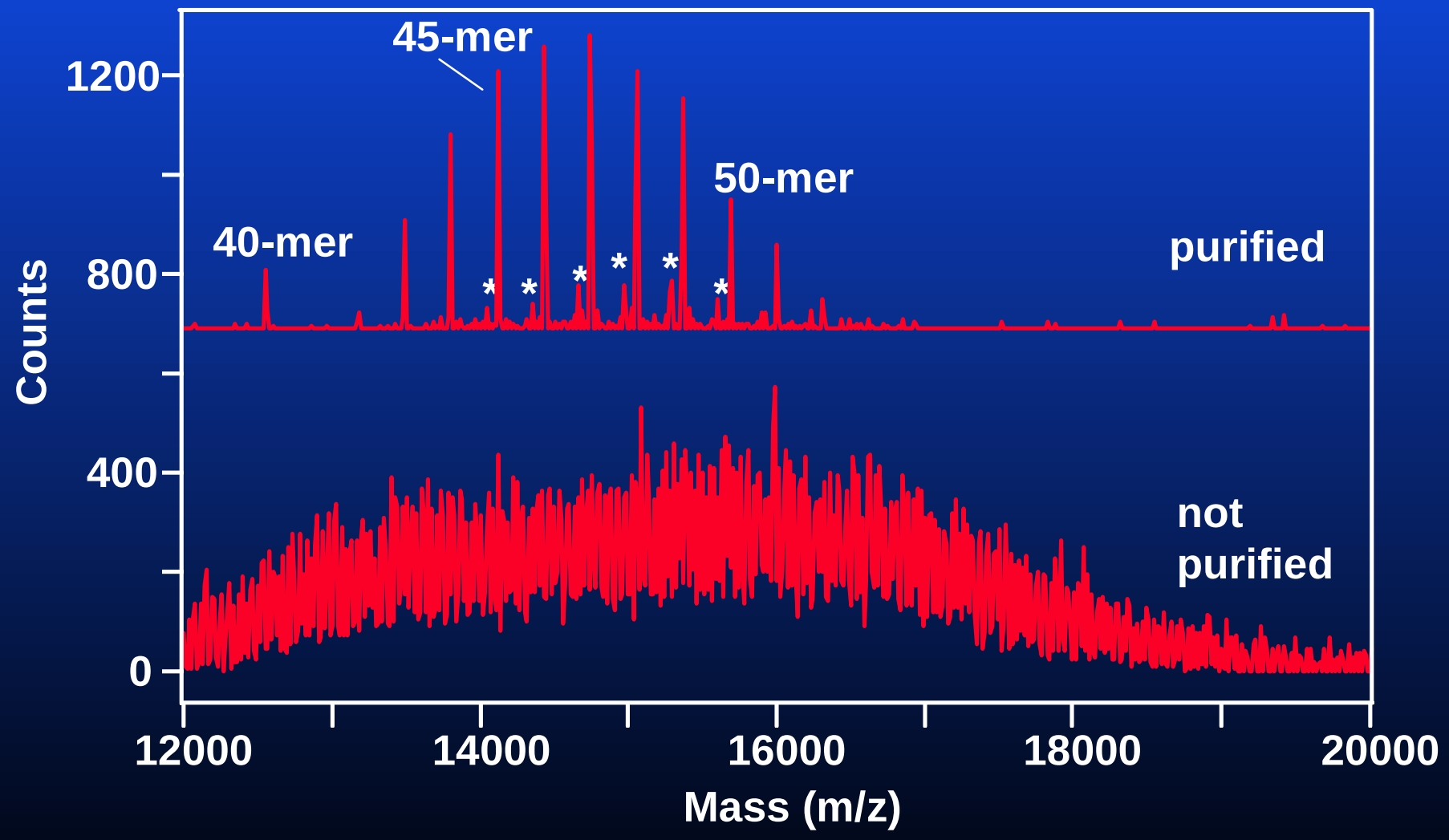
# Effect of Rapid Purification of DNA

Sample: synthetic $pdA_{40-60}$



# MALDI-TOF 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.

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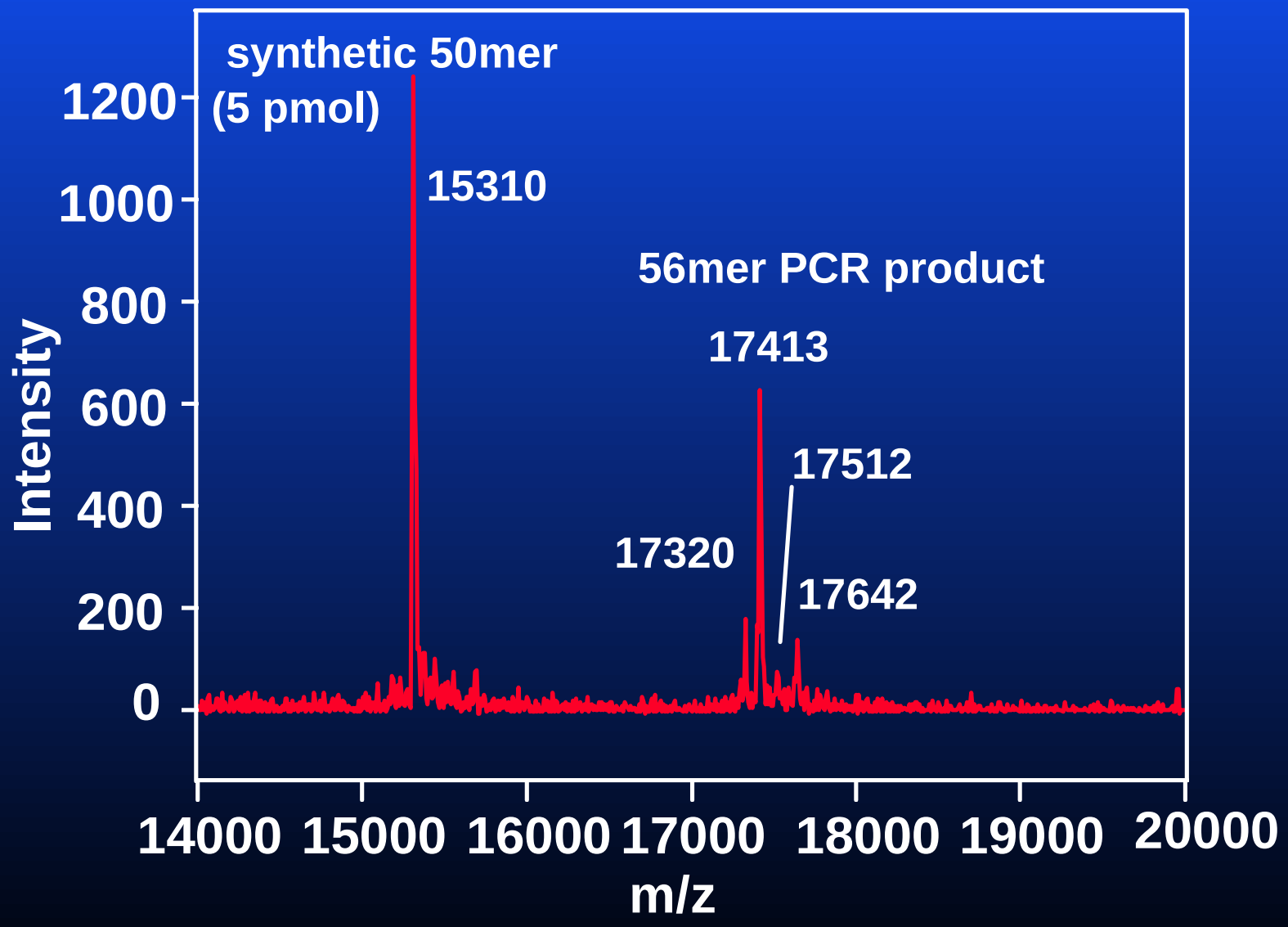
# MALDI-TOF of Type II *pmoA* PCR Product



# Blind Analysis of Different DNA Types

MALDI-TOF 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.

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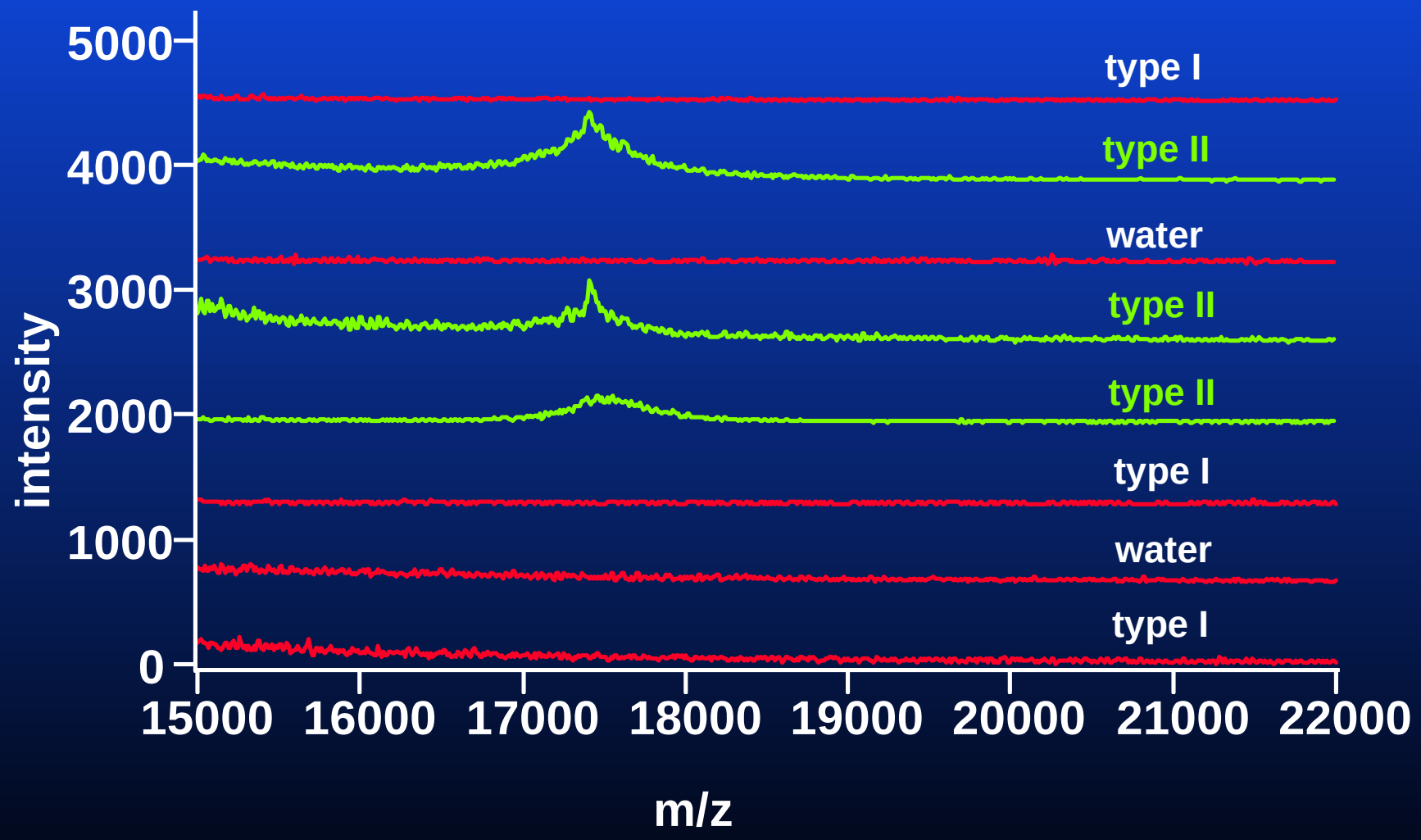
# Blind Analysis of Different DNA Types

## PCR with Type II Primers



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# MALDI-TOF 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.

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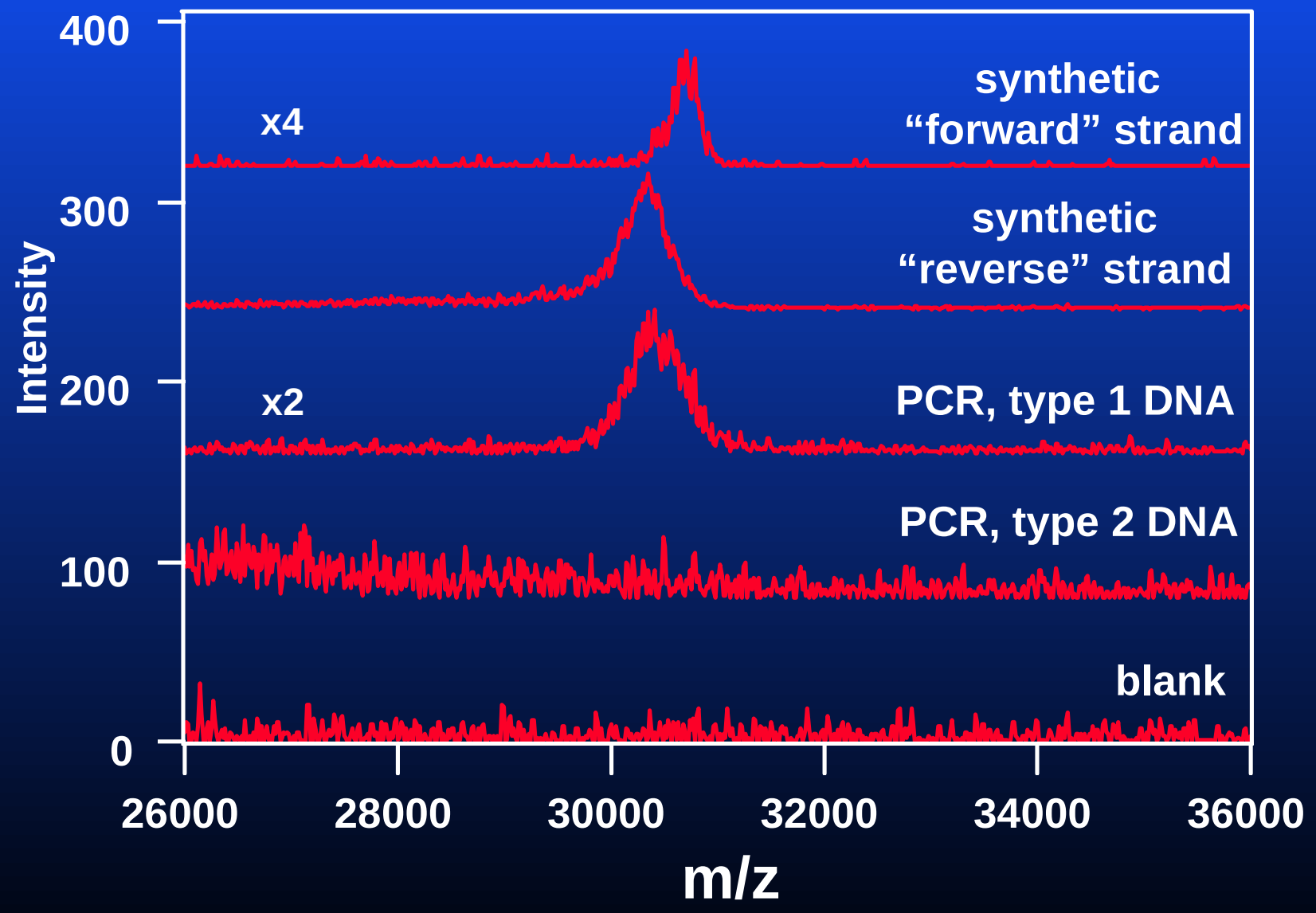
# MALDI-TOF of Type I *pmoA* PCR Product



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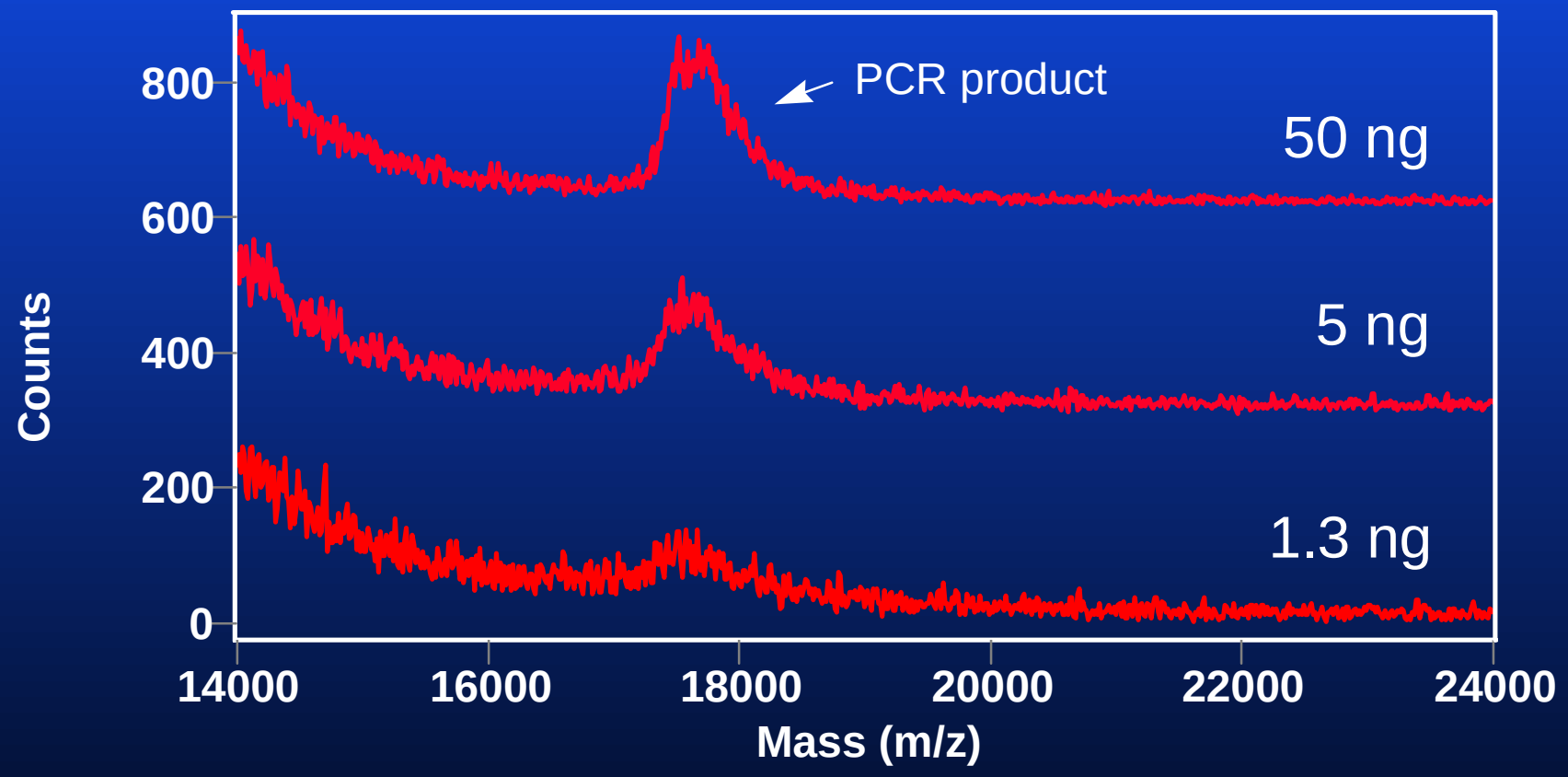
# Effect of Target DNA Amount

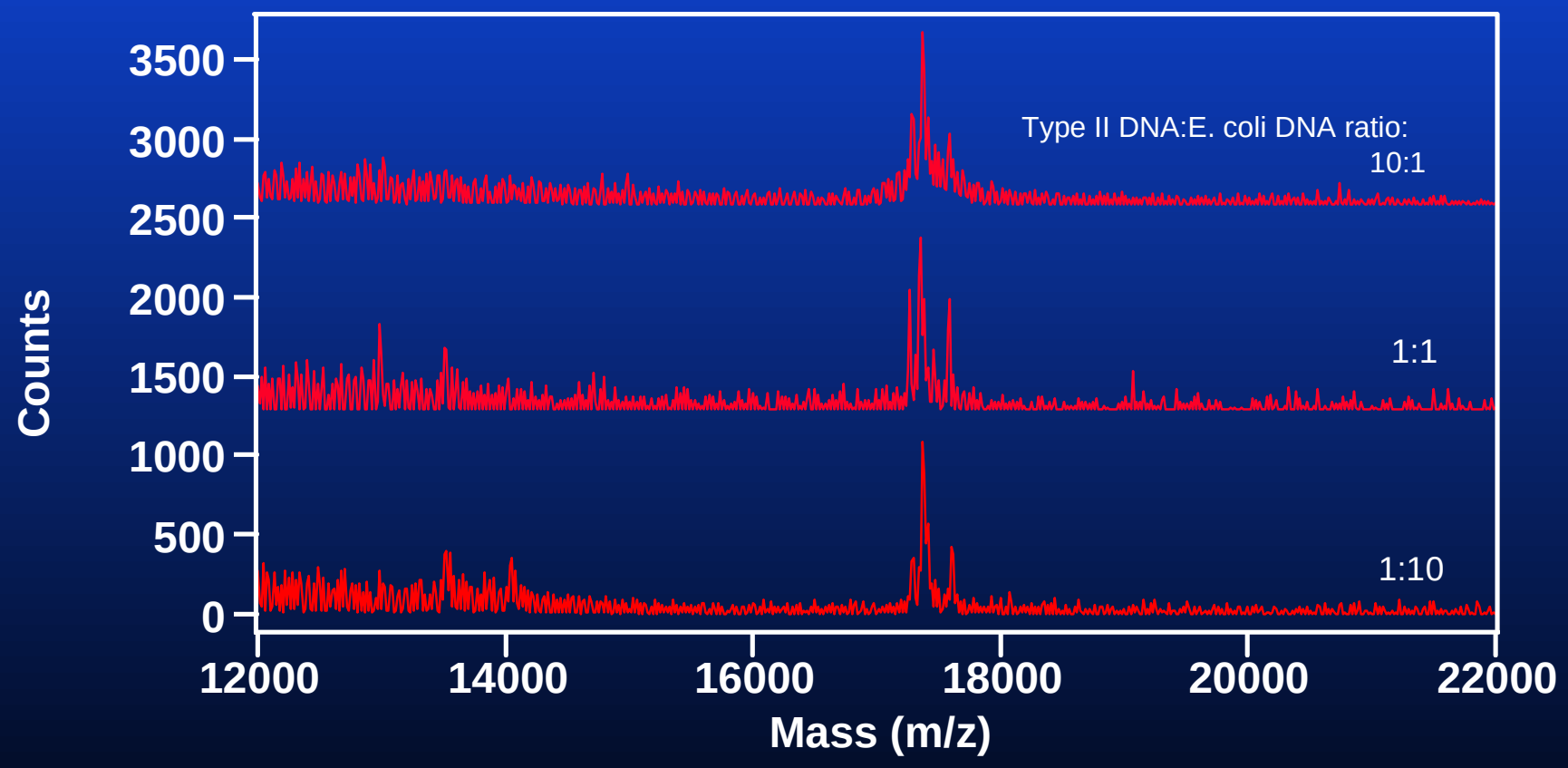
## PCR of Type II Methanotroph




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



# Summary

- MALDI-TOF 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-TOF detection of PCR-amplified regions of their *pmoA* genes.
- The design of shorter PCR products facilitates MALDI-TOF detection.

# Future Plans

- Characterize detection limits and interferences more fully
- Evaluate utility for environmental samples from DOE sites
- Extend gene probe/MALDI to other bacterial types/species
- Implement MALDI improvements:
  - better MALDI sample homogeneity
  - IR laser for larger PCR products

# References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4. A.J. Holmes, A. Costello, M.E. Lidstrom and J.C. Murrell, *FEMS Microbiol. Lett.* **1995**, *132*, 203.

5. A.J. Holmes, N.J.P. Owens and J.C. Murrell, *Microbiology UK* **1995**, *141*, 1947.

6. S. Berkenkamp, F. Kirpekar and F. Hillenkamp, *Science* **1998**, *281*, 260.

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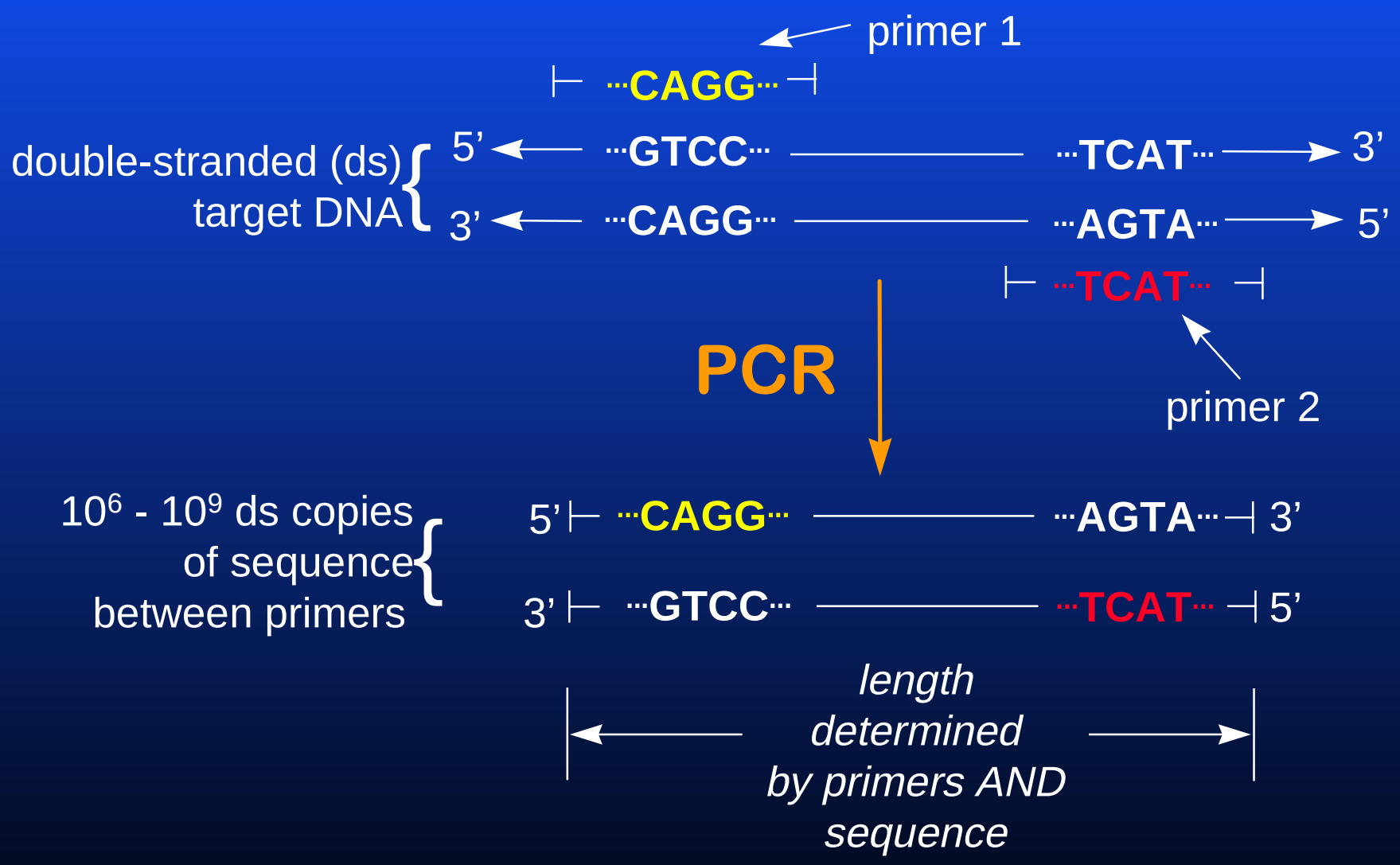
# Polymerase Chain Reaction (PCR)

- Amplifies a defined DNA region
- Presence/absence of:
  - gene
  - organism
- Size of product
  - alleles
  - mutations
- Detection of products
  - gel electrophoresis
  - hybridization
  - mass spectrometry

*•slow*
*•inaccurate*
*•no size limit*
*•widely accepted*

*•faster*
*•better size accuracy*
*•limited size range*
*•under development*

# Polymerase Chain Reaction



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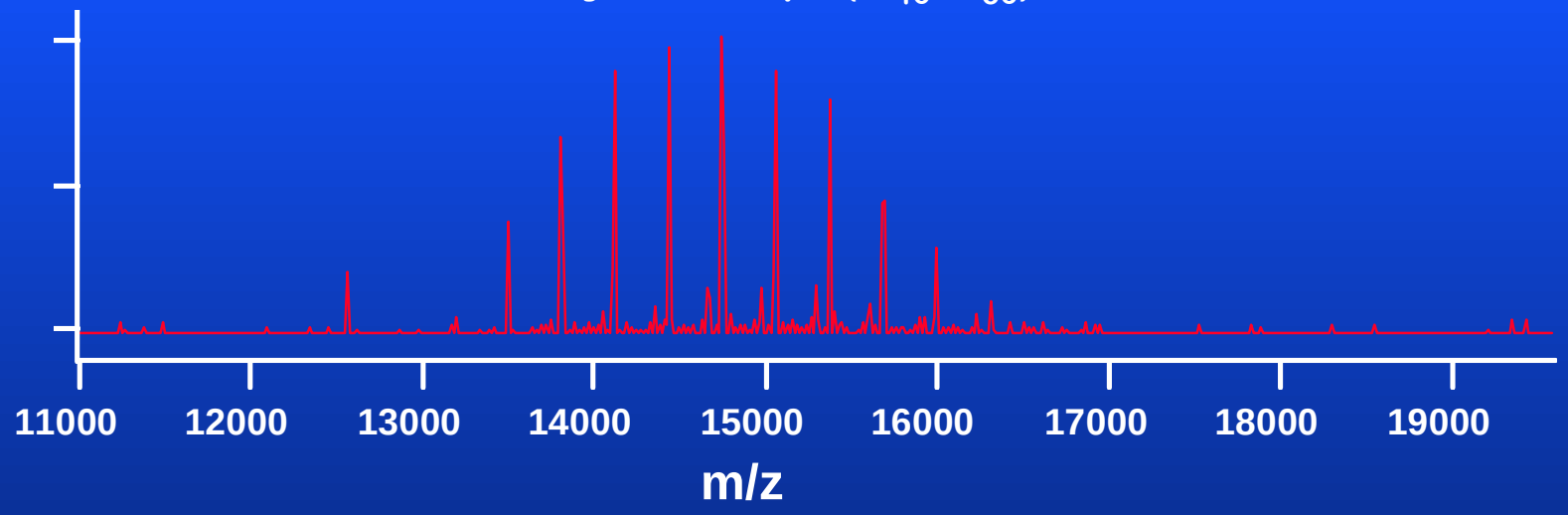
Improved screening of microbial populations for ability to destroy pollutants by combining:

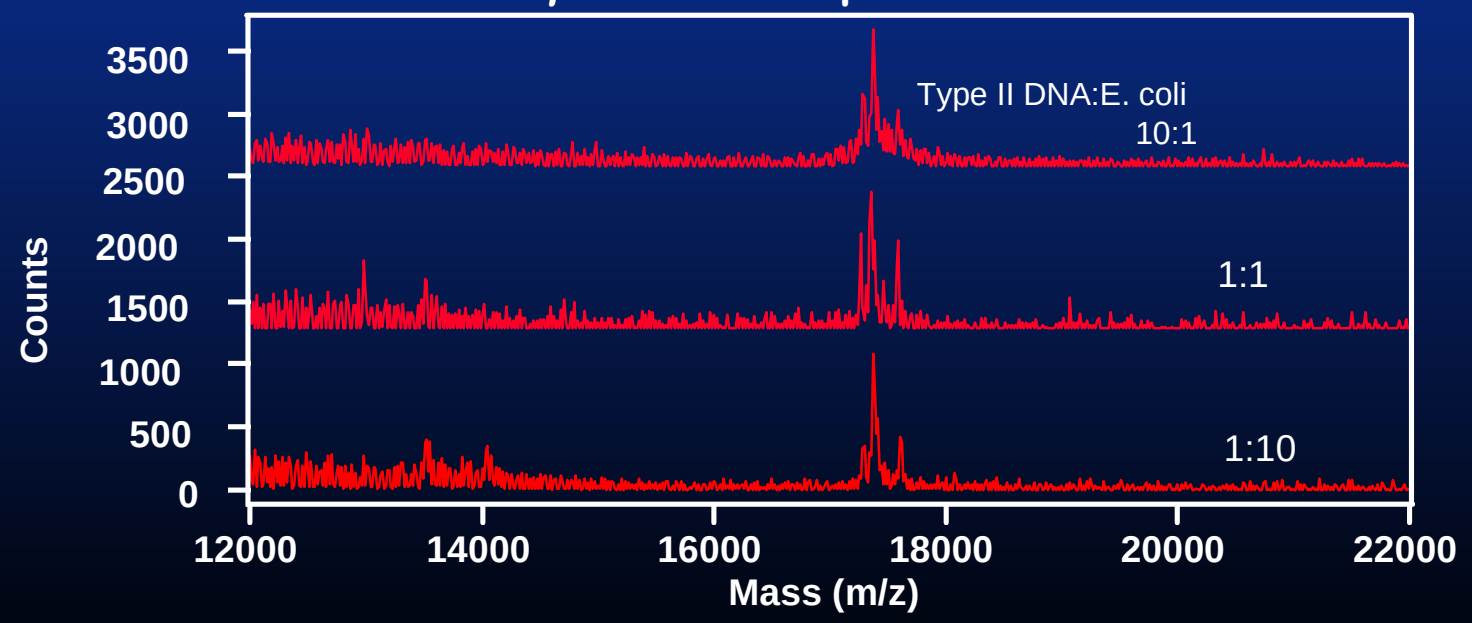
- PCR (Polymerase Chain Reaction): creates many copies of a selected region from an organism's genetic material (DNA).

+

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

## MALDI-TOF of Synthetic pd($A_{40}$-$A_{60}$)




## *Methylosinus trichosporium* Ob3b DNA with *E. coli* DNA *pmoA* "shortprobes"