gene expression analysis, RFLP and EST marker production, end-sequencing and others.

Our most significant findings are the following:

1. Improved direct selection M13 cloning vector
2. Rapid restriction mapping of cosmids using triple-helix affinity capture
3. High-throughput M13 template production using carboxylated beads
4. Sequencing of a cosmid encoding the Drosophila GABA receptor
5. Improved detection of sequencing clones by flow-cytometry
6. RSI-PCR, a strategy to obtain mapped and sequence-ready DNA directly from up to 0.5 kb regions of a complex genome using palindromic class II restriction enzymes; bypasses conventional cloning methodology (see previous section for applications).

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High-Speed DNA Sequence Analysis by Matrix-Assisted Laser Desorption Mass Spectrometry

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Our mass spec research has focused primarily on the possibility of utilizing Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS) as an alternative method to conventional gel electrophoresis for DNA sequence analysis. In this approach, extension fragments generated by the Sanger sequencing reactions are separated by size and detected in the mass spectrometer in one step.

Our group has shown fragmentation to be a major factor limiting accessible mass range, sensitivity, and mass resolution in the analysis of DNA by MALDI-MS. This DNA fragmentation was shown to be strongly dependent on both the MALDI matrix and the nucleic acid sequence employed. Fragmentation is proposed to follow a pathway in which nucleobase protonation leads to cleavage of the N-glycosidic bond with base loss, followed by cleavage of the phosphodiester backbone. Modifications of the deoxyribose sugar ring by replacing the 2' hydrogen with more electron-withdrawing groups such as the hydroxyl or fluoro group were shown to stabilize the N-glycosidic bond, partially or completely blocking fragmentation at the modified nucleosides. The stabilization provided by these chemical modifications was also shown to expand the range of matrices useful for nucleic acid analysis, yielding in some cases greatly improved performance.

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Relevant Publication

Zhu, L.; Parr, G. P.; Fitzgerald, M. C.; Nelson, C. M.; Smith, L. M. Oligodeoxynucleotide fragmentation in MALDI/TOF Mass spectrometry using 355 nm radiation. J. Am. Chem. Soc. 1995, 117, 6048-6056.

Analysis of Oligonucleotide Mixtures by Electrospray Ionization-Mass Spectrometry

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This project aims to develop electrospray ionization mass spectrometry (ESI-MS) methods for high speed DNA sequencing of oligonucleotide mixtures, that can be integrated into an effective overall sequencing strategy. A second goal is develop mass spectrometric methods that can be effective utilized in post genomic research in broad areas of DNA characterization, such as with polymerase chain reaction to rapidly and accurately identify single base polymorphisms. ESI produces intact molecular ions from DNA fragments of different size and sequence with high efficiency [1]. Our aim is to determine ESI mass spectrometry conditions that are compatible with biological sample preparation to allow efficient ionization of DNA and allowing for the analysis of complex mixtures (e.g., Sanger sequencing ladder). We have developed a novel on-line microdialysis method at PNNL to remove salts, detergents, and buffers from such biological preparations as PCR and dideoxy sequencing mixtures. This has allowed for rapid and efficient desalting (e.g., of samples having 0.25 M NaCl) allowing ESI mass spectral analysis without the typically problematic Na-adducts observed. Oligonucleotide ions are typically produced from ESI with