a broad distribution of net charge states for each molecular species, and thus leading to difficulties in analysis of complex mixtures [1]. To make identification of each component in a sequencing mixture possible, the charge states of molecular ions can be reduced using gas-phase reactions. The charge-state reduction methods being examined include: (1) reactions with organic acids and bases (in the solution to be electrosprayed and the ESI-MS interface or the gas phase); (2) the labeling of the oligonucleotides with a designed functional group for production of molecular ions of very low charge states; and (3) the shielding of potential charge sites on the oligonucleotide phosphate/phosphodiester groups with polyamines (and the subsequent gas-phase removal of the neutral amines). In initial studies two methods for charge state reduction of gas phase oligonucleotide negative ions have been tested: (1) the addition of acids and bases to the oligonucleotide solution and (2) the formation of diamine adducts followed by dissociation in the interface region [2,3]. Several methods show promise for charge state reduction and results have been demonstrated for series of smaller oligonucleotides. We have recently demonstrated for the first time that PCR products can be rapidly detected using ESI-MS with significant improvements projected [4,5]. Finally, new mass spectrometric methods have been developed to provide the dynamic range expansion necessary for addressing DNA sequencing mixtures [6]. Our overall aim is to provide a foundation for the development of an overall approach to high speed sequencing (including the rapid and precise PCR product characterization) using cost effective high-throughput instrumentation.

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High-Speed Sequencing of Single DNA Molecules in the Gas Phase by FTICR-MS

Richard D. Smith, David C. Muddiman, S. A. Hofstadler, and J. E. Bruce
Environmental Molecular Sciences Laboratory; Pacific Northwest National Laboratory; Richland, WA 99352
509/376-0723, Fax: -5824, *rd_smith@pnl.gov*
http://www.emsl.pnl.gov:2080/docs/msd/fticr/advmasspec.html

This project is aimed at the development of a totally new concept for high speed DNA sequencing based upon the analysis of single (i.e., individual)large DNA fragments using electrospray ionization (ESI) combined with Fourier transform ion cyclotron resonance (FTICR) mass spectrometry. In our approach, large single-stranded DNA segments extending to as much as 25 kilobases (and possibly much larger), are transferred to the gas phase using ESI. The multiply-charged molecular ions are trapped in the cell of an FTICR mass spectrometer, where one or more single ion(s) are then selected for analysis in which its mass-to-charge ratio (m/z) is measured both rapidly and non-destructively. Single ion detection is achievable due to the high charge state of the electrosprayed ions and the unique sensitivity of new FTICR detection methodologies.

Initial efforts under this project have demonstrated the capability for the formation, extended trapping, isolation, and monitoring of sequential reactions of highly charged DNA molecular ions with molecular weights well into the megadalton range [1-6]. We have shown that large multiply-charged individual ions of both single and double-stranded DNA anions can also be efficiently trapped in an FTICR cell, and their mass-to-charge ratios measured with very high accuracy. Thus, it is feasible to quickly determine the mass of each lost unit as the DNA is subjected to rapid reactive degradation steps. One approach is to develop methods based upon the use of ion-molecule or photochemical processes that can promote a stepwise reactive degradation of gas-phase DNA anions. Successful development of one of these approaches could greatly reduce the cost and enhance the speed of DNA sequencing, potentially allowing for sequencing DNA segments of more than 25 kilobase in length, on a time scale of minutes with negligible error rates with the added potential for conducting many such measurements in parallel. Instrumentation optimized for these purposes is currently being introduced and promises to greatly advance the methodology. The techniques being developed promise to lead to a host of new methods for DNA characterization, potentially extending to the size of much larger DNA restriction fragments (>500 kilobases).

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(abstract continued)