Docket #: S07-358

Isotachophoretic Focusing of Nucleic Acids

An interdisciplinary team of Stanford researchers has developed a novel microfluidic technique to extract and purify RNA, DNA or proteins directly from cell lysate. This approach uses on- or off-chip isotachophoresis (ITP) for automated extraction of biomolecules from complex samples such as whole blood, tissues or cultured cells with simultaneous identification, and/or quantitation of specifically targeted macromolecules.

The inventors have combined the ITP purification technology with additional techniques to perform fast sequence identification of nucleic acids with sensitivity below 100 attomoles. This method can be used to extract and identify target nucleic acid sequences with high sensitivity without using PCR and has direct application to gene expression profiling, miRNA profiling, and RNA virus detection.

Applications

- **Microfluidic chips or capillary-based systems** for:
  - point-of-care diagnostics
  - biowarfare detection
  - research

Advantages

- **Fast:**
  - preconcentration, extraction, identification, and separation assays take 10's to 100's of seconds
  - assays can be performed directly from cell lysate (cultured cells, whole blood or other tissue)

- **High sensitivity** - detection in the 100 amol range
- **High volume throughput** - can process on the order of 100 ul volumes of DNA in 20 minutes (1 ml volume in 60 min is feasible)
- **Small sample volume** - can process as little as 1 nL of sample
- **High efficiency** - between 30 and 100% efficiency
- **Low cost** - less reagents are needed because there is limited sample preparation and small sample sizes needed for detection
- **Selective** - can selectively extract 22-base RNA from samples also containing >200-base RNA, >100bp DNA and proteins
- **High preconcentration factors** - million-fold for simple molecules; 10,000-fold for for proteins or nucleic acids from a complex mixture
- **Robust** - ITP zones are self-stabilizing and injection protocols are easily conducted with two pipetting dispensions
- **Automated** - no manual steps are needed between sample on-chip delivery and molecule identification
- **Parallelization** - miniaturization and automation allows for hugely parallel purification and identification of biomolecules.

Publications

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- Schoch, R.B., M. Ronaghi, and J.G. Santiago, "Rapid and selective extraction, separation, preconcentration, and detection of small RNAs from cell lysate using on-chip isotachophoresis," 9, 15, 2145 – 2152, *Lab on a Chip*, 2009.
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- Bercovici, M., S.K. Lele, and J.G. Santiago, "Open source simulation tool for electrophoretic stacking, focusing, and separation" *Journal of Chromatography*

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- Khurana, T., and J.G. Santiago, "Sample Zone Dynamics in Peak Mode Isotachophoresis," 80, 16, *Analytical Chemistry*, p. 6300-6307, 2008.

Patents

- Published Application: 20100224494
- Issued: 8,846,314 (USA)
- Issued: 9,753,007 (USA)

Innovators

- Rob Chambers
- Juan Santiago
- Alexandre Persat
- Mostafa Ronaghi
- Reto Schoch

Licensing Contact

Michael Spaid

Technology Licensing Associate 2

Email