



**ENABLING GENOMIC ANALYSIS OF
YOUR MOST CHALLENGING SAMPLES**

Boreal provides nucleic acid purification and enrichment solutions for environmental, forensic, and clinical applications



Aurora - Nucleic Acid Purification

Eliminate PCR inhibitors from heavily inhibited samples and recover trace nucleic acids from low biomass or low abundance samples, with our novel electrophoretic technology:

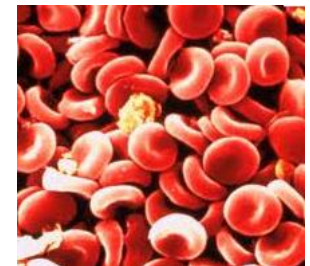
- Up to 100x more efficient at recovering small amounts of DNA and RNA from volumes up to 5mL
- Up to 1000x more efficient at rejecting contaminants



OnTarget™ - Allele Enrichment

Sensitive detection of rare nucleic acid sequences in samples with overwhelming amounts of background or wild-type DNA:

- Can enrich for targets with specific sequence at SNP resolution
- Can provide rejection of background > 1,000,000 fold for sequence mismatch



Early access program is ongoing in anticipation of commercial launch in 2013

Remainder of presentation focuses exclusively on the Aurora nucleic acid purification system



Proven Applications

Forensics

- Extraction of DNA from intractable casework samples
- Produced STR profiles from samples that failed conventional analysis due to low abundance nucleic acids or residual PCR inhibition



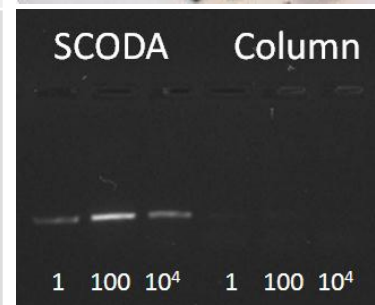
Metagenomics

- Extraction of DNA from challenging environmental samples including Athabasca tar sands, Atacama desert soil, sea sediments, tundra, ice, various soils
- Low biomass and heavily inhibited samples are not an issue with electrophoretic purification



Ag Bio

- DNA extraction from plant materials including leaves, pericarp and contaminated samples
- Extracted amplifiable DNA from archived samples including 60 year old leaf material





Boreal History

Technology invented at the University of British Columbia

Boreal Genomics founded by Dr. Andre Marziali and team

Technology licensed from UBC and prototype Aurora instruments placed at customer and collaborator sites

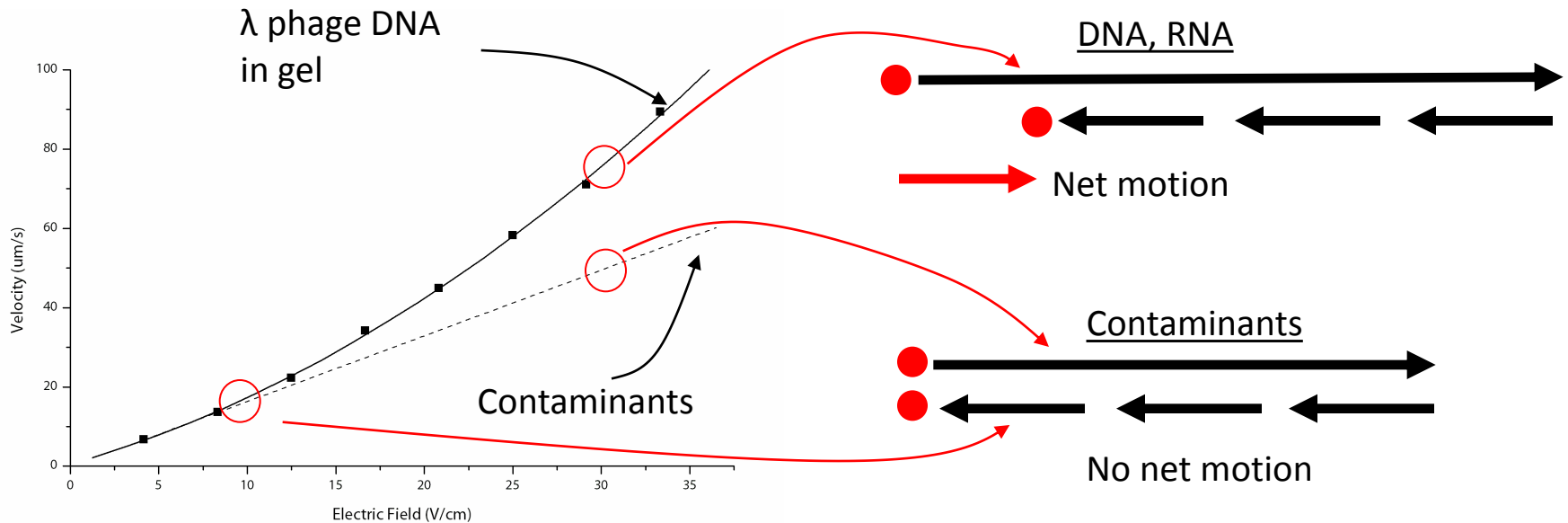
Commercial launch of Aurora instrument

US corporate and sales office opened in Los Altos, CA

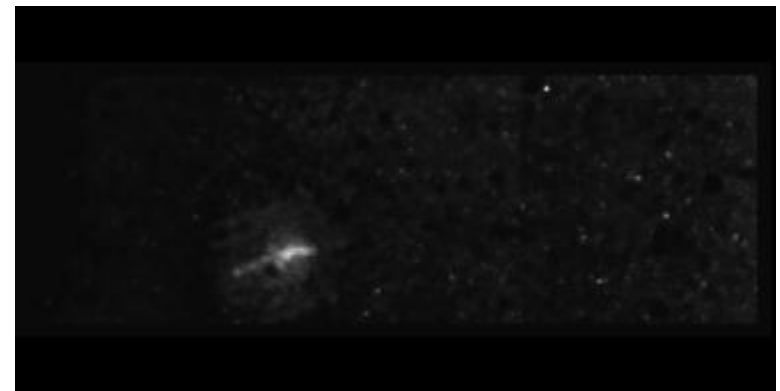
International focus for Aurora sales



Our Core Technology: Non-Linear Electrophoresis



Non-linear response to electric fields can be used to select nucleic acids for motion under a net AC field, leaving contaminants stationary.



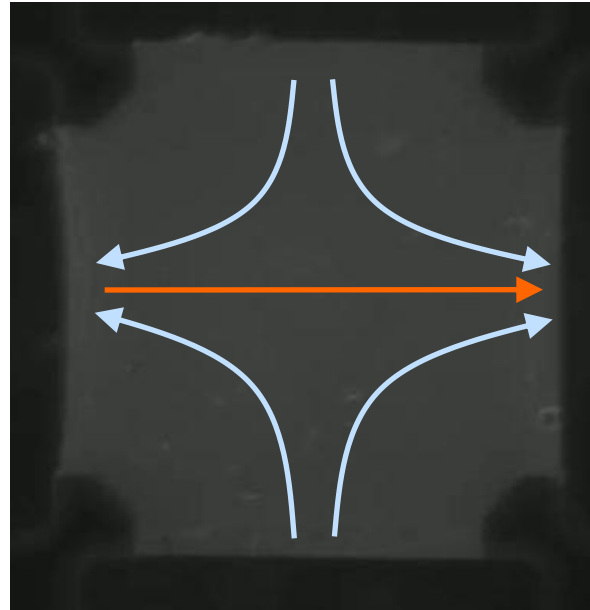
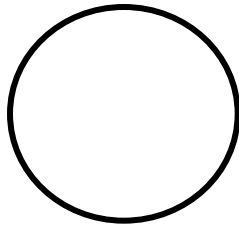
<http://www.umich.edu/~morgroup/hsvm.html>



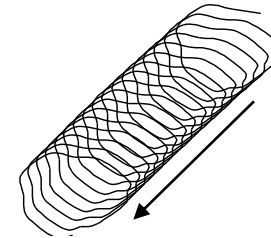
SCODA: 2D Non-Linear Electrophoresis

Synchronous Coefficient of Drag Alteration (SCODA)

Most molecules:



DNA and RNA:



- A new molecular separation parameter based on physical properties of DNA/RNA to separate them from contaminants
- Rotating electric fields selectively impart a drift velocity to nucleic acids
- DNA and RNA are driven to the center of the electric field pattern for easy extraction



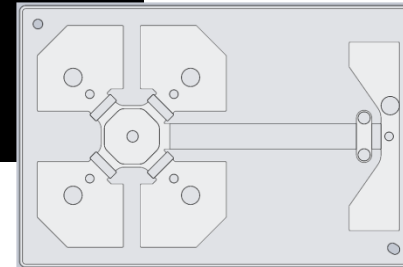
Aurora Nucleic Acid Purification System

- Up to 5mL sample input
- 60 uL output volume
- Completely unattended operation
- Lysate to purified sample in one step
- 2-4 hr run time
- Large dynamic range in molecular weight recovery
 - dsDNA 300 bp up to 1 Mb
 - ssDNA >500 nt
 - RNA >500 nt



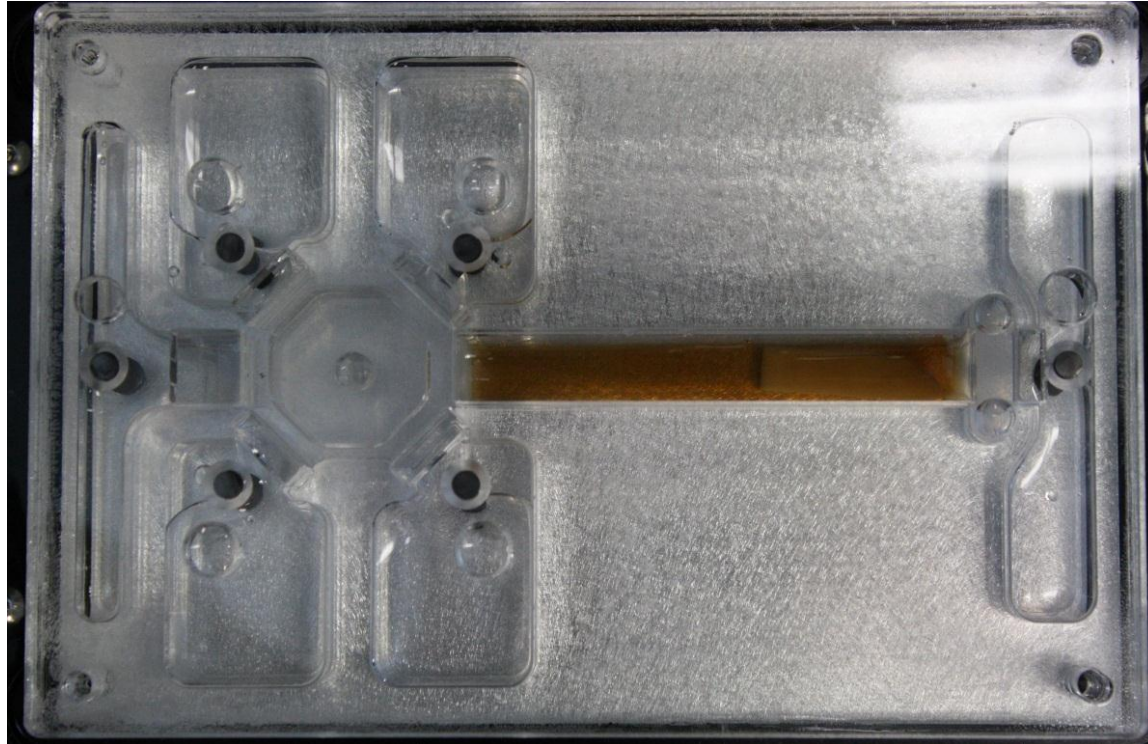


Aurora Cartridge



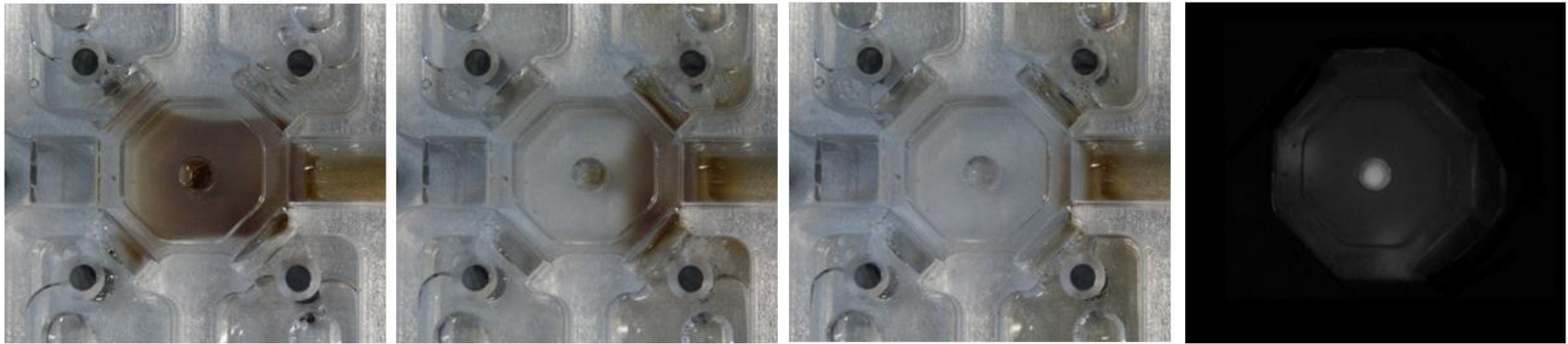
- Single sample, up to 5 mL input volume
- 60 uL output volume
- Disposable or reusable cartridge formats
- **New reusable** cartridge – minimizes per-sample cost of DNA/RNA extraction

Sample Injection



Up to 5 mL of lysate is placed next to the gel: nucleic acids are electrophoretically injected into the gel and focused by the rotating fields.

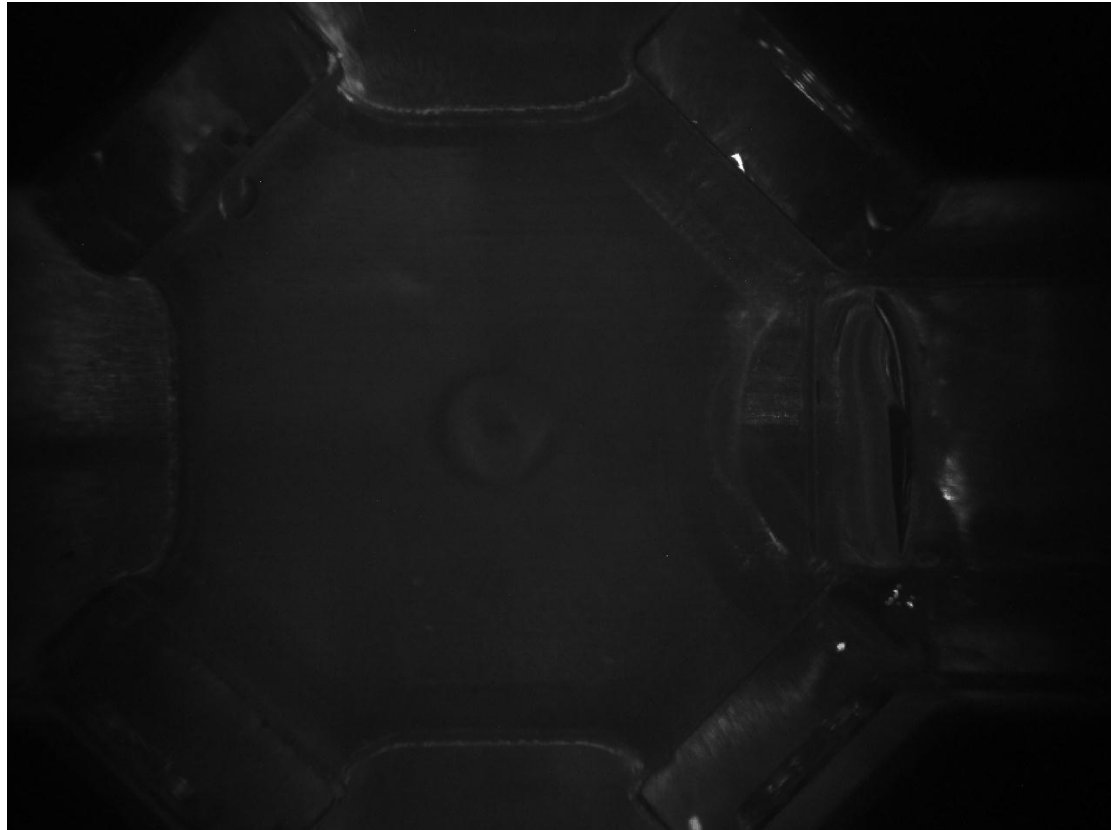
Contaminant Rejection



- Both nucleic acids and contaminants are injected into the gel.
- Contaminants are electrophoretically removed while the nucleic acids remain trapped by SCODA fields



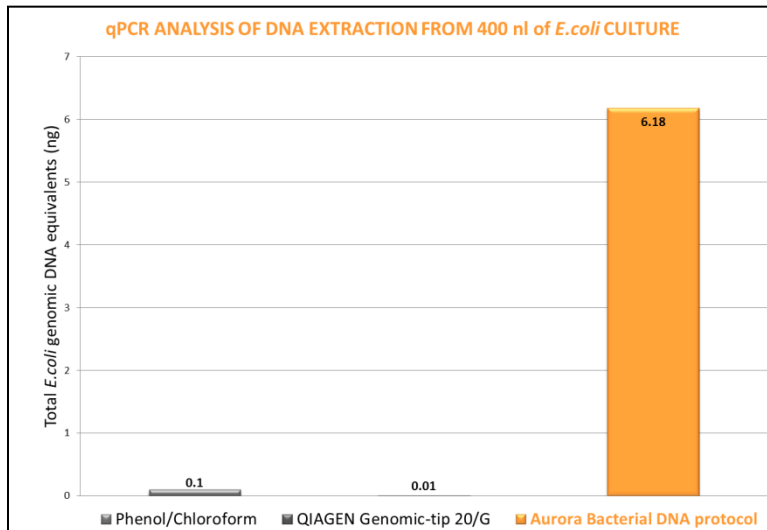
Sample Injection and Concentration



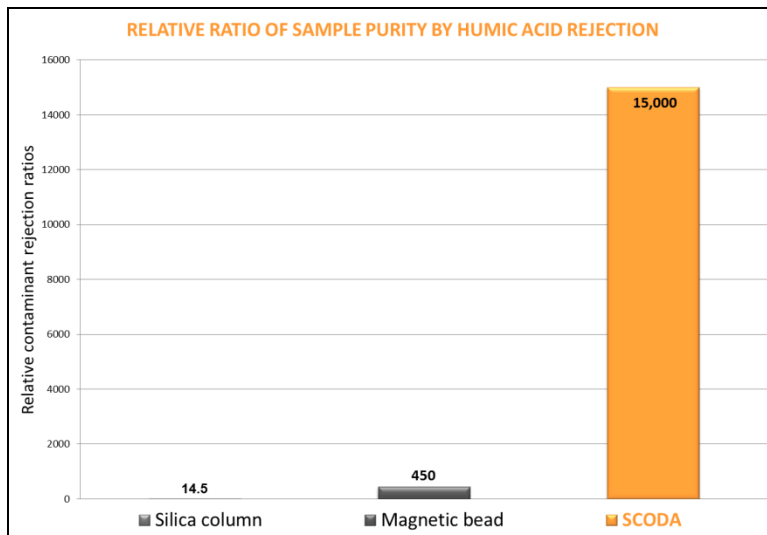
Up to 5 mL of lysate is placed next to the gel: nucleic acids are electrophoretically injected into the gel and focused by the rotating fields.



SCODA vs. Conventional Purification Methods



- Unlike columns, SCODA does not suffer from strong binding sites and dead volumes that limit yield at low target concentrations



- While columns and beads co-purify contaminants by non-specific binding, SCODA operates in a low surface area system, allowing up to 1000X improved contaminant rejection

J.Pel et al. *PNAS* 2009

Customer Application



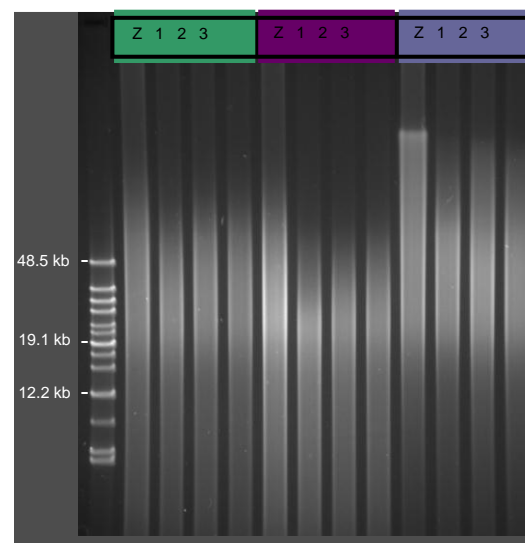
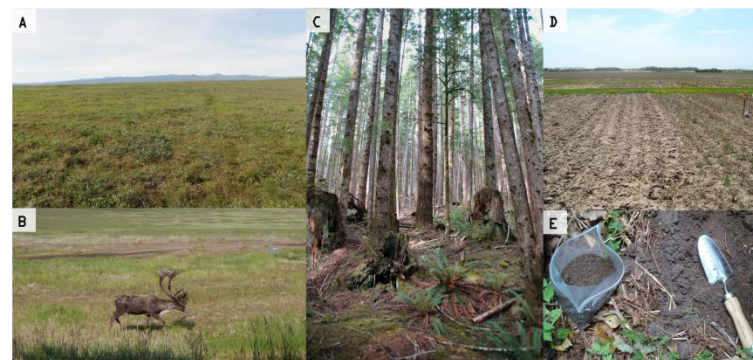
Environmental DNA extraction from soil for large-insert (30-50 kb) library construction and metagenomic studies¹.

- Arctic tundra
- Temperate rain forest
- Agricultural soil

Methods

- Anion exchange chromatography
- Gel filtration on Sephadex-G50 columns
- Zhou lysis² + 2x successive purifications
Wizard® DNA Clean-up System (Promega)
- PowerSoil® DNA Isolation Kit (MO BIO)
- Zhou lysis² + Boreal's SCODA purification

DNA purified using SCODA was the most suitable for large-insert library construction.



BOREAL SCODA

Pulsed field gel showing DNA prepared using SCODA (1,2,3) along with DNA extracted using the Zhou protocol⁶ (Z). SCODA provides higher molecular weight DNA, ideal for large-insert library construction.

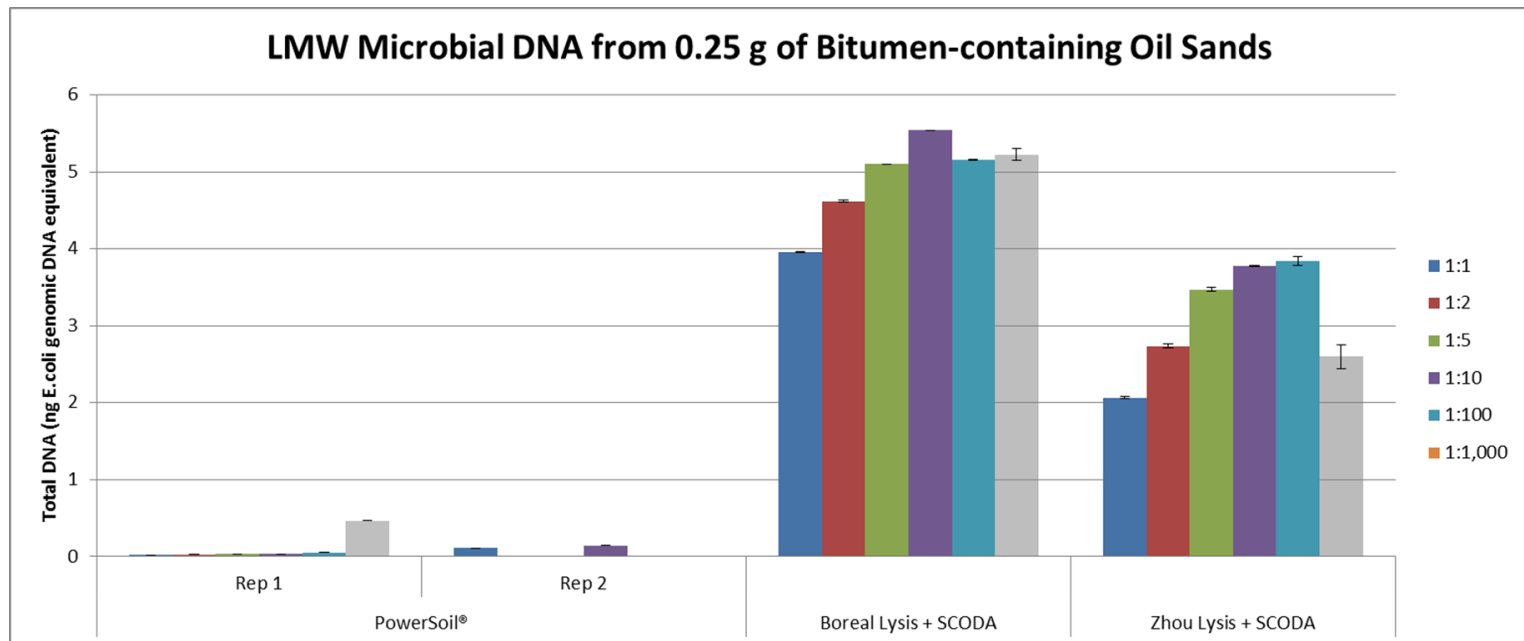
¹ K. Engel, L. Pinnell, J. Cheng, T.C. Charles and J.D. Neufeld. University of Waterloo, presented at ASM 2011 (New Orleans, LA, 05/11) and Argonne Soil Metagenomics Workshop (Chicago, IL, 10/11)

² Zhou et al. *Applied and Environmental Biology* 1996, 62(2):316-22



Customer Application

Microbial DNA recovery from extremely low abundance and heavily inhibited bitumen-rich oil sands.



Our technology excels where all other column and manual methods fail

- Environmental – soil, sediment, water
- LSR – Low copy, clean-up, agarose recovery
- Clinical – stool, blood
- Agricultural – seeds, plant tissues

Application Note: Optical Mapping and HMW analysis

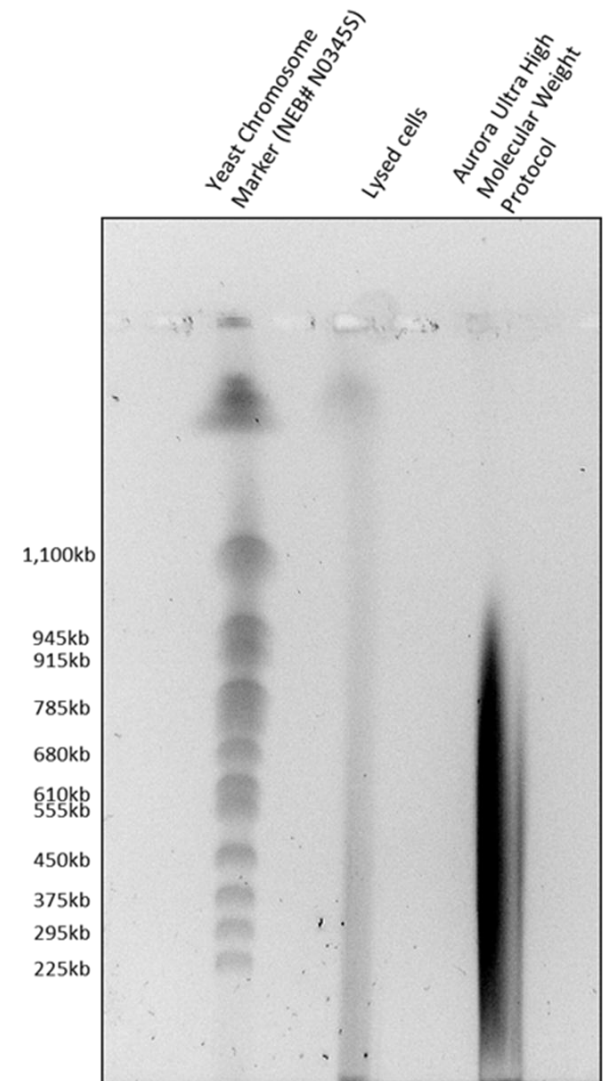


High molecular weight DNA recovery from 50 kb up to 1 Mb

Applications include:

- Recovery of DNA from agarose
- Large insert library construction
- Sample prep for long-read sequencing or optical mapping

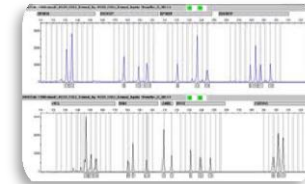
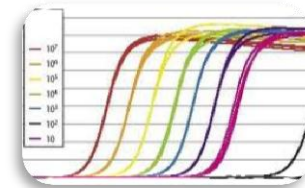
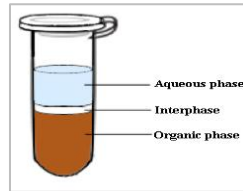
Purified DNA can be provided in buffer or an agarose plug to avoid mechanical shearing





Forensic Applications: DNA Clean-Up workflow

- Boreal's technology is recognized as the world leader in removal of PCR inhibitors. We can rescue inhibited samples that fail analysis using conventional DNA extraction methods alone.



Input sample

Conventional
DNA extraction

Re-purification

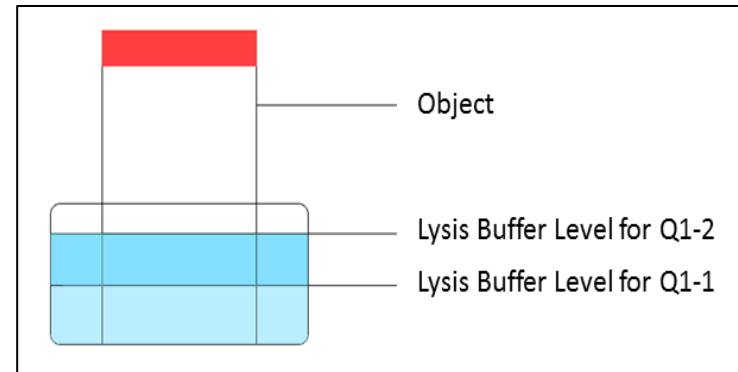
Successful
analysis



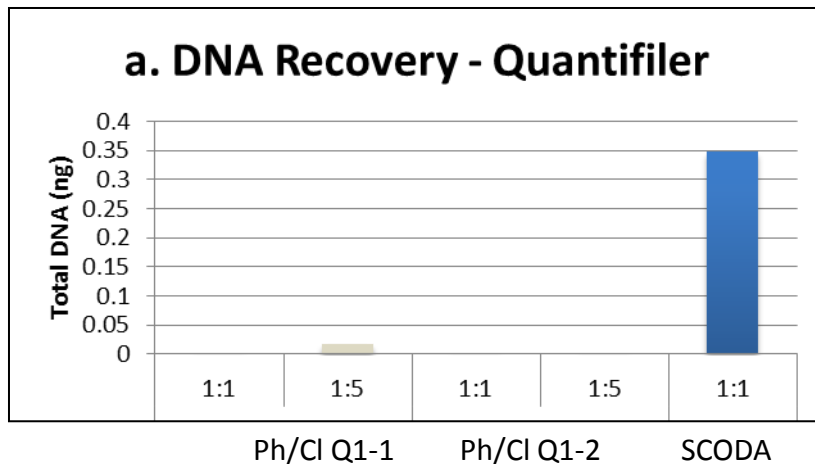
Application Note: Forensic Casework

Low template DNA recovery from large surface area casework samples

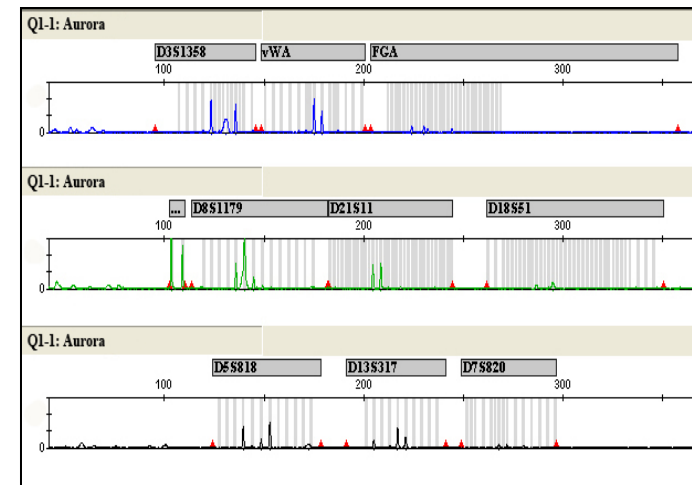
Touch DNA was recovered where manual purification failed due to PCR inhibition.



Large volume extraction of touch DNA from a presumed murder weapon



Successful quantification of recovered DNA without PCR inhibition

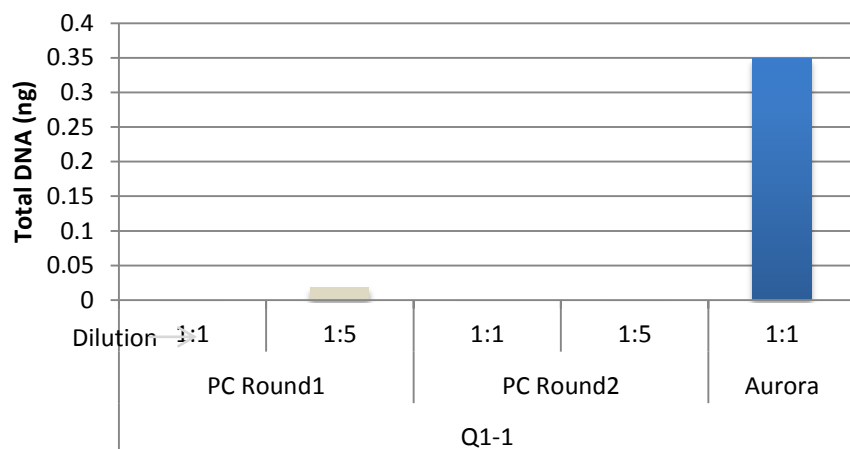


STR Profile Obtained from SCODA extraction on Aurora instrument

Application Note: DNA recovered from murder weapon



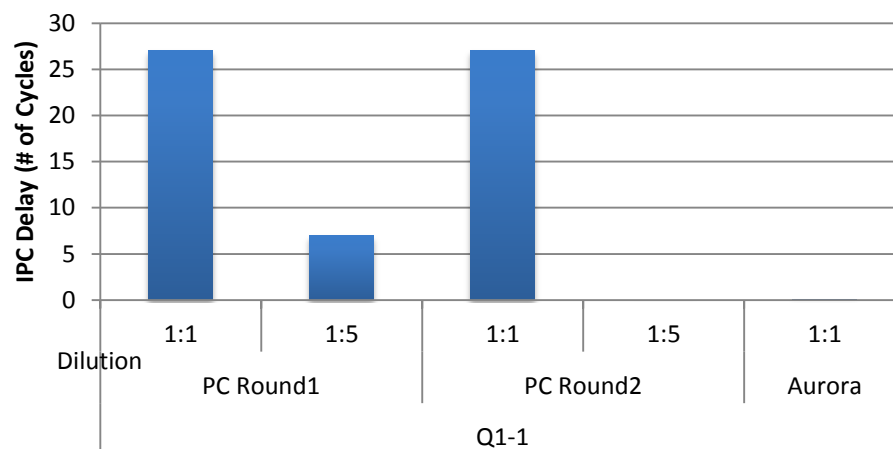
a. DNA Recovery - Quantifiler



Quantifiler results before and after Aurora Clean-up

1. Undiluted Phenol/Chloroform (PC) extracts are too inhibited to quantify.
2. 2nd round of PC purification does not improve purity (inhibitors persist)
3. Aurora clean-up removes inhibitors.

b. PCR Inhibition - IPC



Delayed C_T Values for DNA samples before and after Aurora Clean-up

1. Inhibition is reduced after dilution, but the resulting decrease in DNA concentration prohibits STR amplification.
2. 2nd PC extraction does not reduce inhibition, and results in DNA losses.
3. Aurora output is uninhibited, concentrated, and allows STR profiling.



Forensic Technology Evaluation: SCODA vs. Qiaquick

S. Schmedes, P. Marshall, J. King, and B. Budowle

Institute of Applied Genetics, Department of Forensic and Investigative Genetics, University of North Texas Health Sciences Center

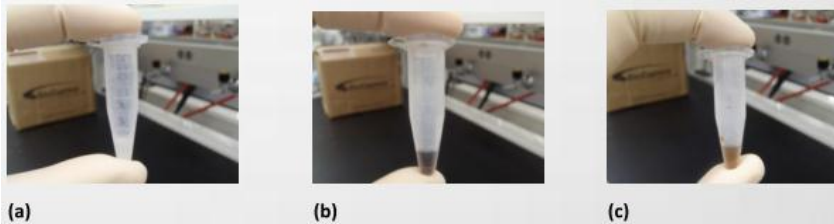


Figure 4. Images of purified SCODA and QIAquick® products. (a) Post-SCODA purified DNA originally spiked with 220µg melanin. All SCODA purified product for each inhibitor was clear (i.e., no color). (b) Post-QIAquick® purified DNA originally spiked with 220µg melanin. (c) Post-QIAquick® purified DNA originally spiked with 1mg humic acid.

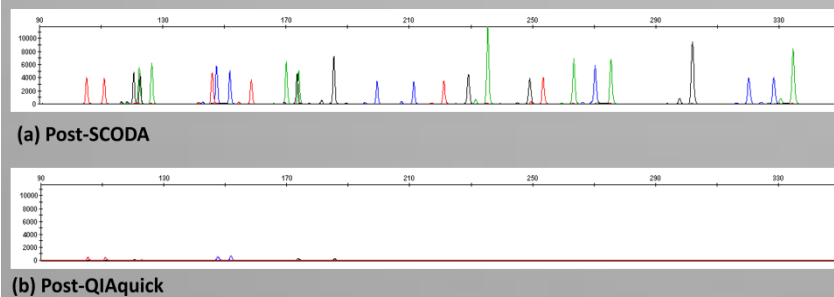


Figure 3. STR profile comparison of post-SCODA and post-QIAquick® DNA product originally contaminated with 600µg humic acid. Electropherogram comparison depicting (a) post-SCODA and (b) post-QIAquick® purified DNA amplified using the AmpFℓSTR® Identifier® Plus PCR Amplification Kit. Each purification method had an input of 10ng DNA and 600µg humic acid.

Results: SCODA purification yielded overall higher efficiency of purification of contaminated samples. The IPC was delayed for humic acid and melanin purified products produced by QIAquick® purification. SCODA products yielded no IPC shifts, suggesting successful purification of the inhibitors. Allele percentage recovery and higher relative fluorescence unit (RFU) values were greater for the SCODA method as compared with the QIAquick® purification method for each inhibitor tested.

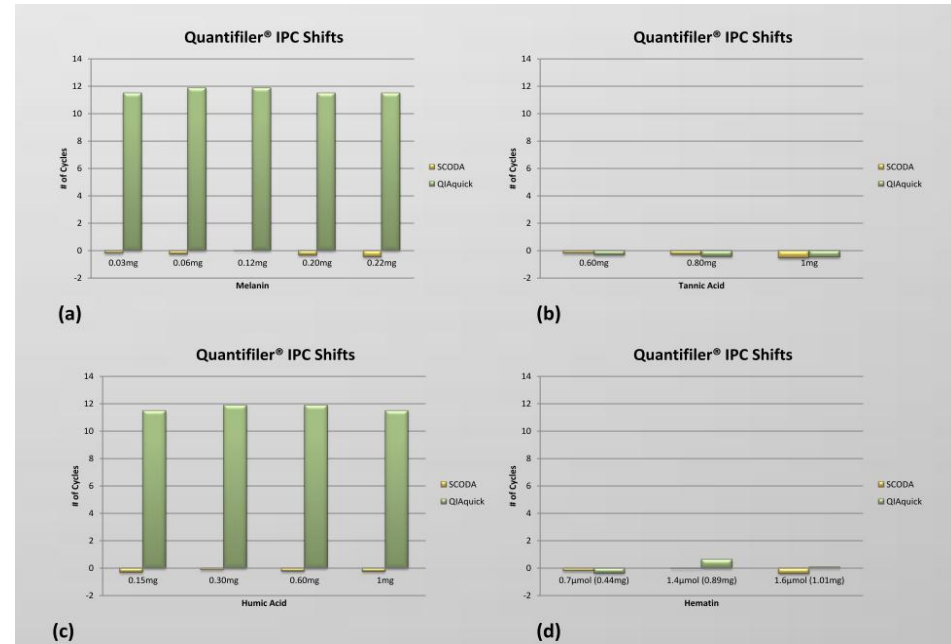


Figure 5. Quantifiler® IPC Shifts. Real-time amplification using Quantifiler® for (a) melanin, (b) tannic acid, (c) humic acid, and (d) hematin purified samples using SCODA and QIAquick®. Inhibition is indicated for any IPC shift value >1 cycle number. IPC shifts >11 cycles indicate complete failure of IPC amplification. QIAquick® purification failed to remove inhibitory activity from all concentrations of melanin and humic acid tested.

Conclusions: The SCODA technology provides an automated, minimal-step approach to successfully remove inhibitors and concentrate DNA from challenged forensic samples and medically-relevant tissue samples. The technology potentially provides a platform for the extraction of nucleic acids from any tissue source or solid-support containing biological materials and will accommodate different medical diagnostic applications.



Our Team

Management



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R&D and Commercial Teams

- Cross-functional R&D teams specializing in molecular biology, cancer genomics, engineering and physics are based in Vancouver, BC
- Commercial team based in San Francisco bay area with access to R&D facilities at Stanford University

Please contact us for more information

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Thank you