

FIGURE 1

Whole Genome Sequencing: Sample/Library Prep (example Roche 454)

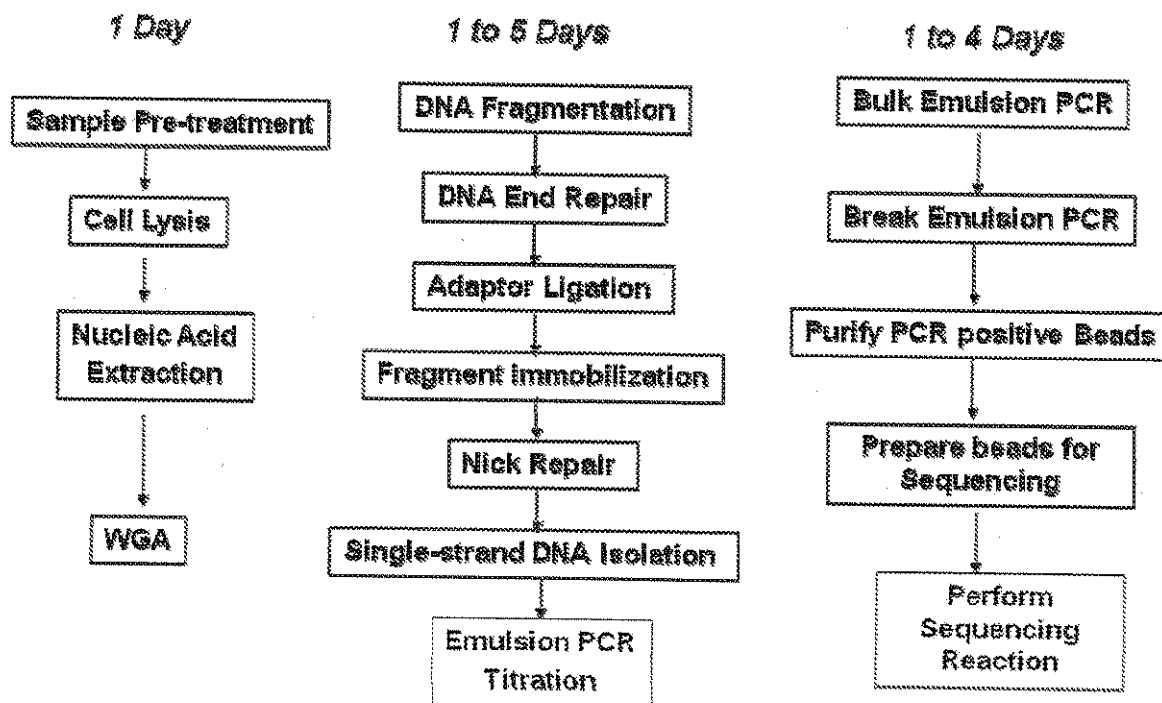


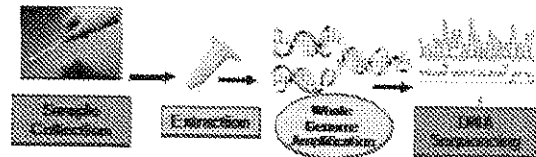
FIGURE 2

Disposable Microfluidic Sample Prep Development Cards

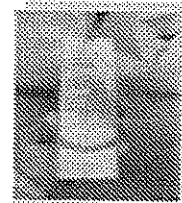
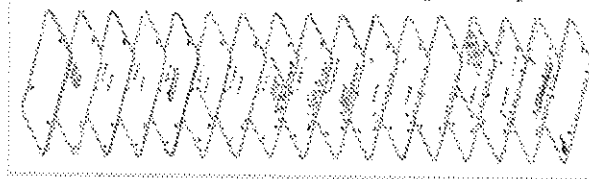
Design test and microfluidic sub-circuits:

- Cell lysis
- DNA Extraction
- Whole Genome Amplification

•All reagents stabilized and contained on the cards



From prototype subcircuits to integrated injection molded devices



- Each component of an assay reduced to a subcircuit
- Sub-circuit results confirmed against gold standard assay
- Sub-circuits integrated for total assay performance
- Integrated prototype design converted to commercial design

FIGURE 3

Integrated Sample Prep. Process

Key subcircuits of microfluidic cards.

1. Lysis
2. DNA Extraction
3. Whole Genome Amplification

All reagents stabilized and contained on the cards

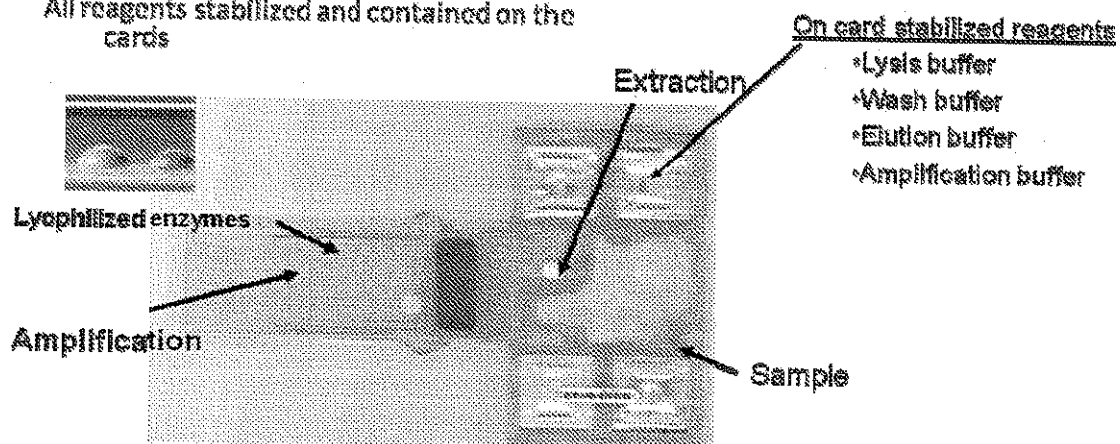
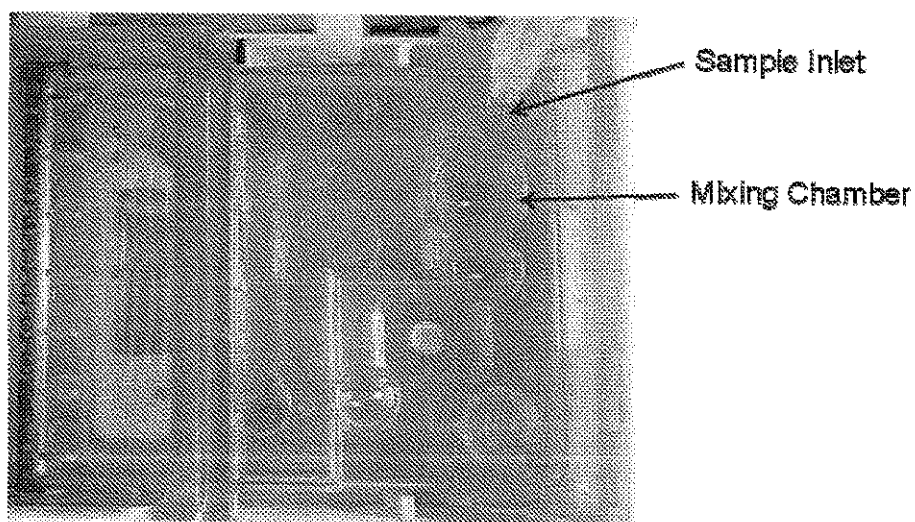


FIGURE 4

Sample Purification: Cartridge Fluidic Architecture

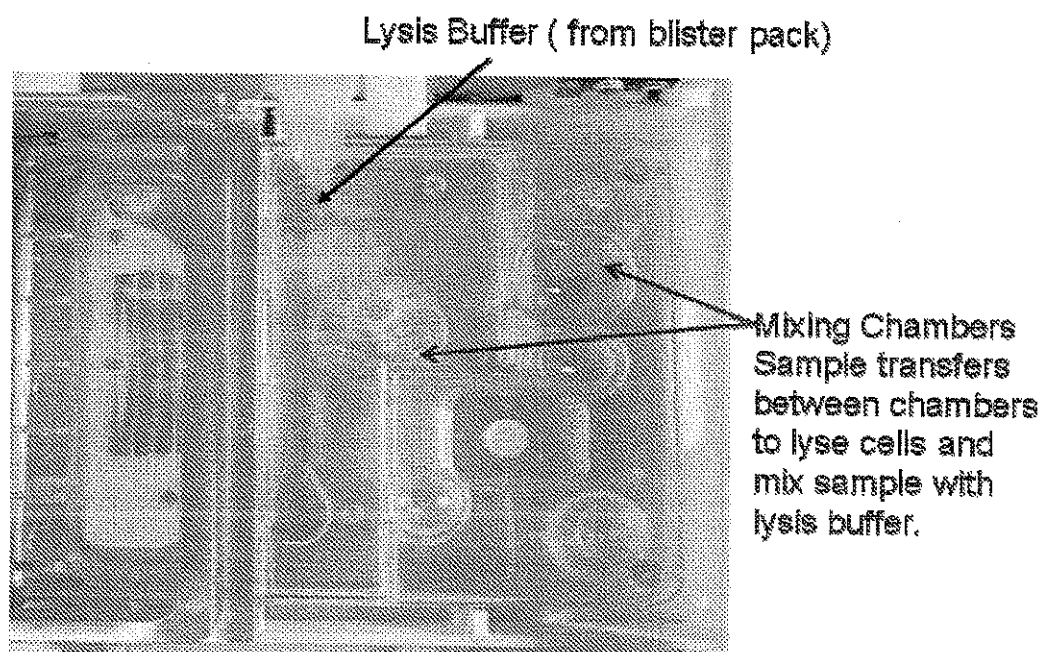
Note: buffer packs, covers and pneumatics removed to enable visualization



Step 1: Sample added to cartridge.

FIGURE 5

Sample Purification: Cartridge Fluidic Architecture

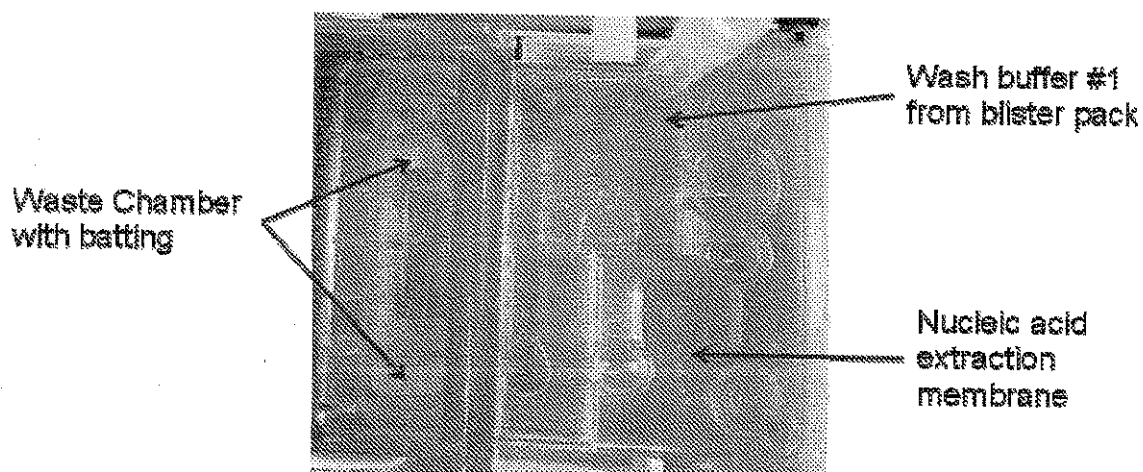


Step 2: Lysis buffer mix with sample.

FIGURE 6

Sample Purification: Cartridge Fluidic Architecture

Note: buffer packs, covers and pneumatics removed to enable visualization

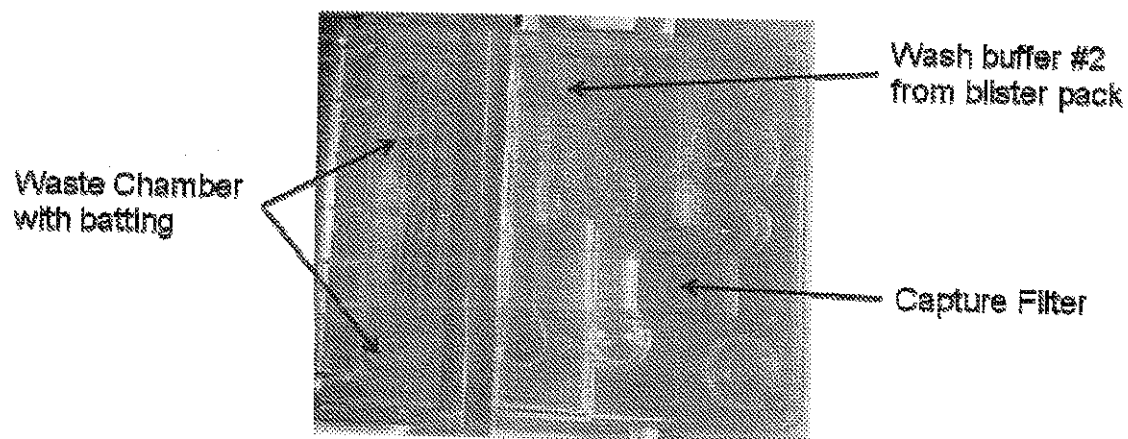


Step 3: Lysed mixture passed over capture filter and to waste chamber. Wash#1 passed over filter and to waste chamber.

FIGURE 7

Sample Purification: Cartridge Fluidic Architecture

Note: buffer packs, covers and pneumatics removed to enable visualization

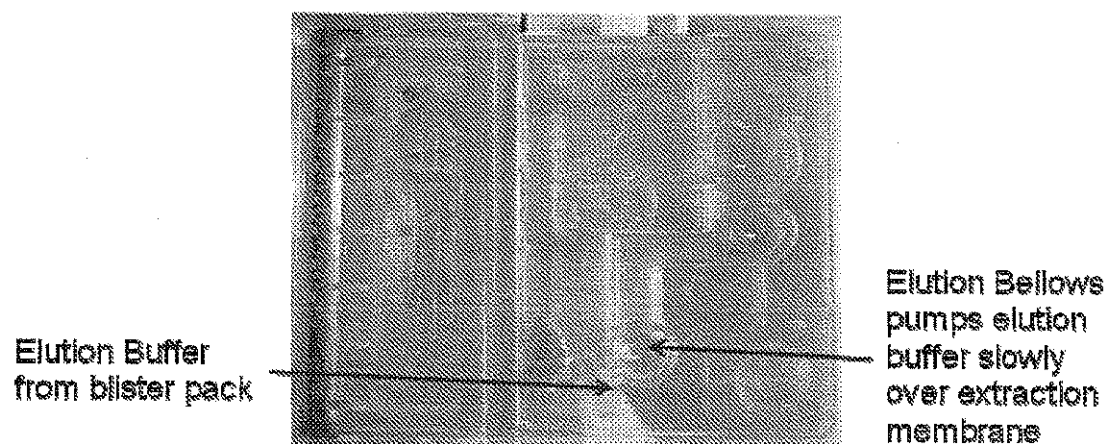


Step 4: Wash #2 passed through capture filter and to waste chamber. Filter air dried to remove wash #2.

FIGURE 8

Sample Purification: Cartridge Fluidic Architecture

Note: buffer packs, covers and pneumatics removed to enable visualization



Step 5: Elution buffer removes purified target from filter using elution bellows.

FIGURE 9

Reference Reagents and Reporter Assays to Determine lysis/Extraction Efficiency.

- Reference material needed to evaluate lysis and extraction.
- Three surrogate organisms references samples. :
 - *Bacillus anthracis* Sterne strain spores
 - QPCR: *recA* locus
 - *Staphylococcus aureus*
 - QPCR: *tufB* locus
 - *Klebsiella pneumoniae*
 - QPCR: *16s* locus
 - Frozen Stocks
 - Used QPCR to measure lysis and Extraction efficiencies

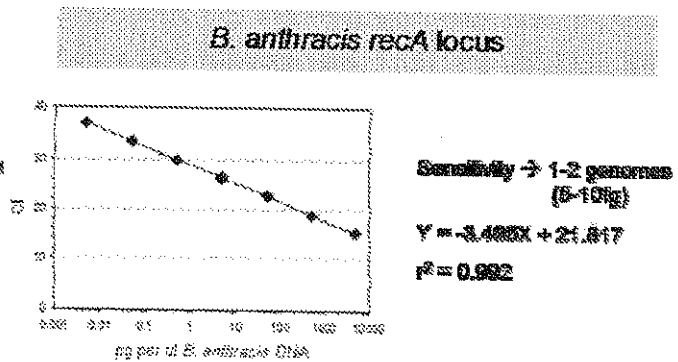
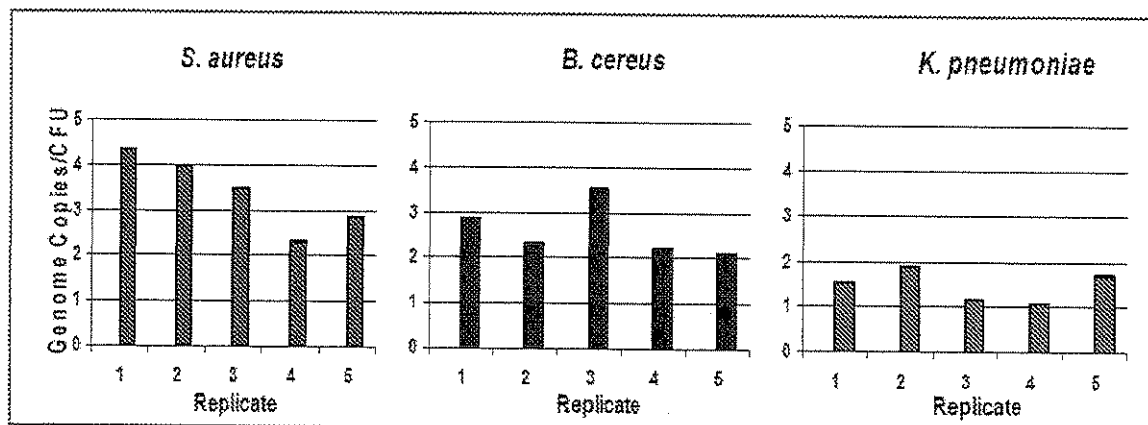


FIGURE 10

Results of final validation of lysis/extraction subcircuit. Inputs of 10,000 CFUs for *B. cereus* and *K. pneumoniae*, input of freshly grown culture for *S. aureus*.

FIGURE 11

Production of Contamination Free WGA Reagents.

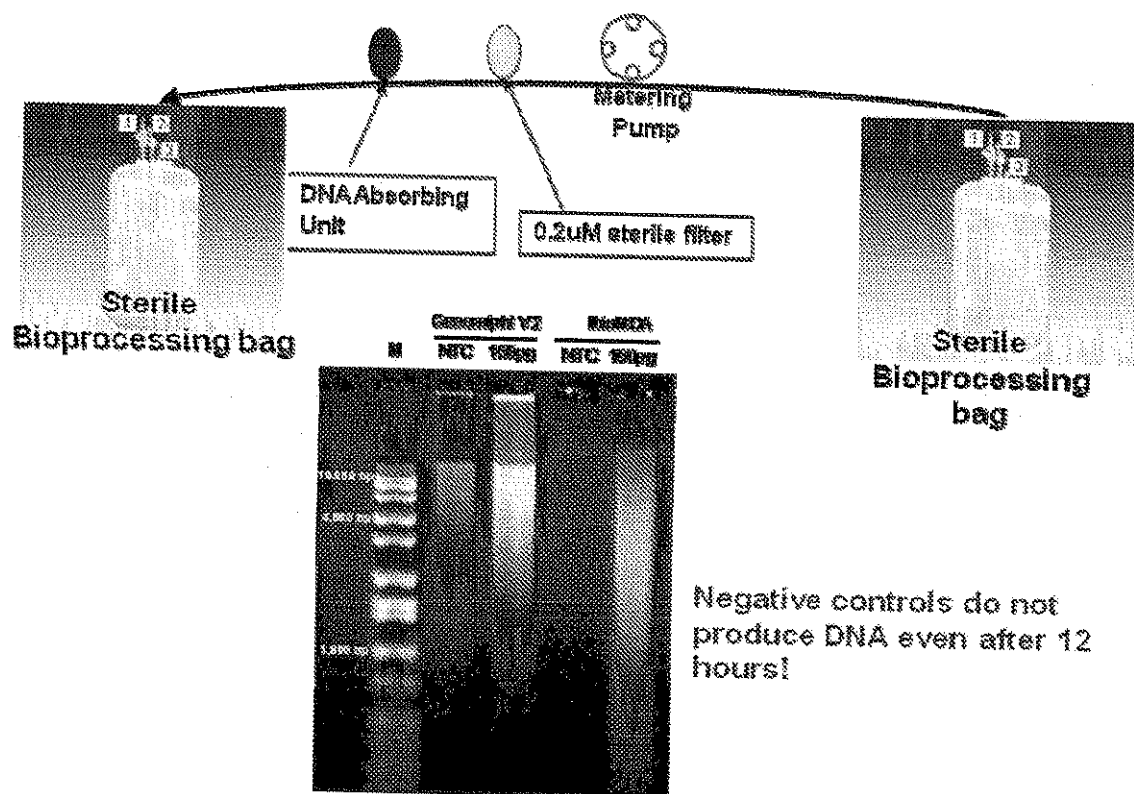


FIGURE 12

WGA: Cartridge Fluidic Architecture

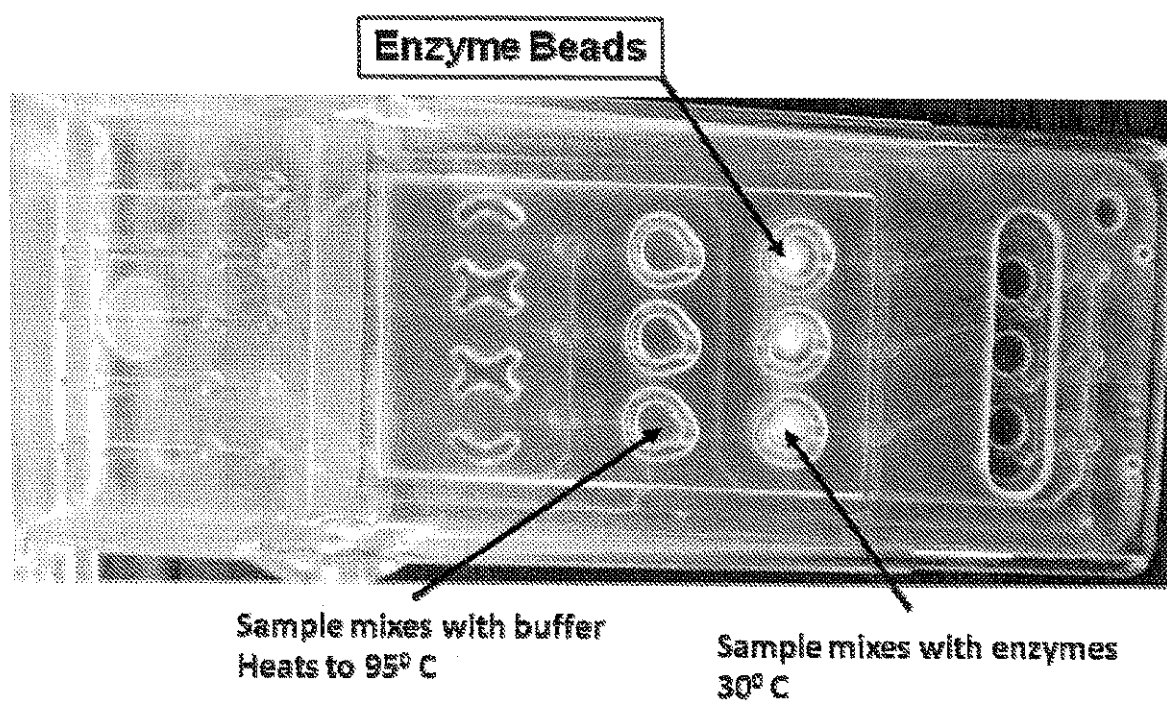


FIGURE 13

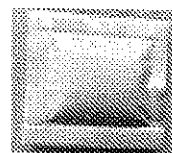
Reagent Stability

- **Baseline stability**
 - Extraction reagents
 - WGA buffer
 - WGA enzyme
- **Accelerated aging**
- **Buffer stabilization**
 - Storage in blister packs
- **Enzyme stabilization**
 - Lyophilization

Dried inside microfluidic
amplification card



Buffer Packs



Lyophilized Beads

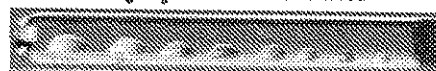


FIGURE 14

Reagent Stabilization: WGA Enzyme Mix

- **WGA enzyme mix is fragile**
 - A mixture of three mesophilic enzymes
 - Stable for only 5 days at room temperature
 - No commercial vendor of stabilized WGA enzymes
- **Lyophilization protects enzymes**
 - Removes water
 - Replaces with protectants and stabilizers (excipients)
 - Increases shelf life
 - Decreases sensitivity to heat

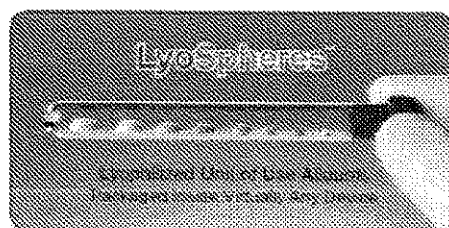


FIGURE 15

Excipient Selection

- 25 excipient formulations were tested for compatibility with the WGA enzyme mix
 - Dialysis of enzymes into excipients
 - Glycerol needs to be removed for lyophilization
 - WGA using dialyzed enzymes to determine enzyme activity
 - Excipients 17-19 showed increased activity relative to fresh enzyme
 - Excipients 17 & 19 carried forward for small scale lyophilization testing

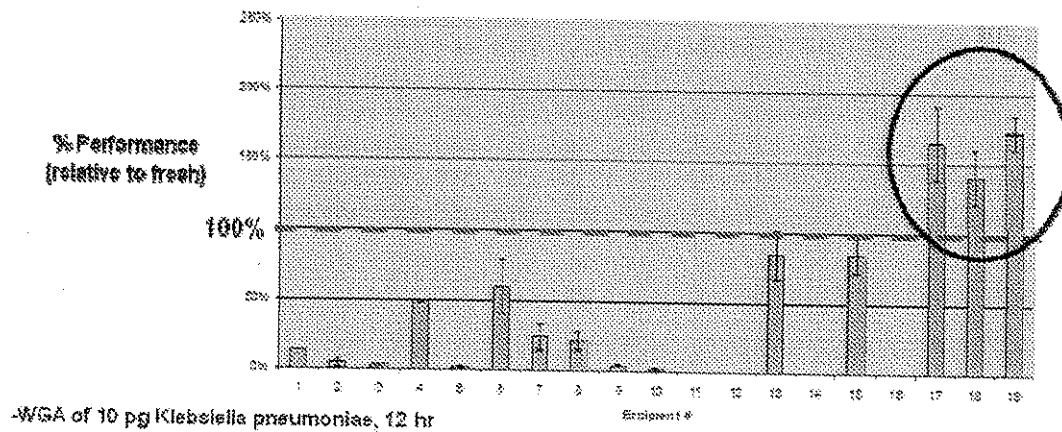


FIGURE 16

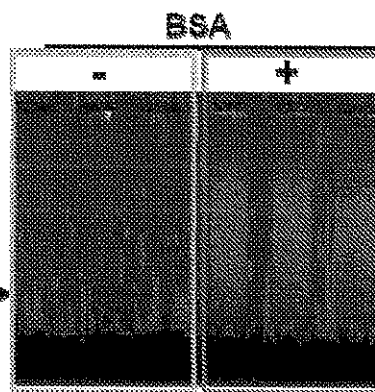
Small Scale Lyophilization Testing

- Combinations of enzyme mix were chosen to identify if specific enzymes were affected during the lyophilization process
- Lyophilized enzyme beads were
 - ~ mixed with the appropriate fresh enzymes (if applicable)
 - ~ used in WGA reactions with fresh WGA buffer

Enzyme Mixtures for Lyophilization

Mix #	Enz 1	Enz 2	Enz 3	BSA
1	●	-	-	-
2	-	●	-	-
3	-	-	●	-
4	●	●	-	-
5	●	-	●	-
6	●	●	●	-
7	●	-	-	●
8	●	●	●	●

No
Amplification

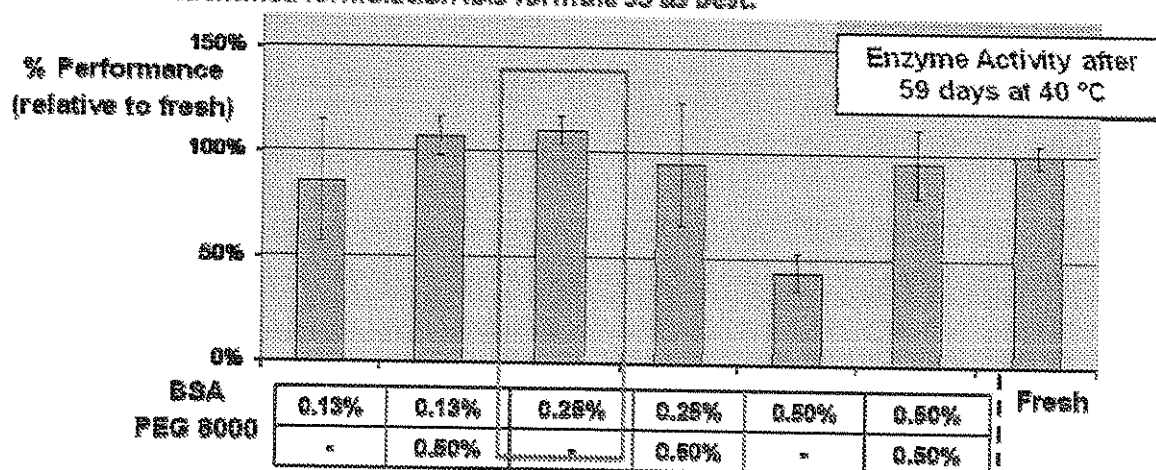


Amplification

FIGURE 17

Excipient Optimization (Fine Tuning)

- Lead excipient formulation optimization
 - BSA is important for stability
 - Test varying concentration of BSA
 - Evaluated effect of PEG-8000
 - Identified formulation Ibis formula 33 as best.

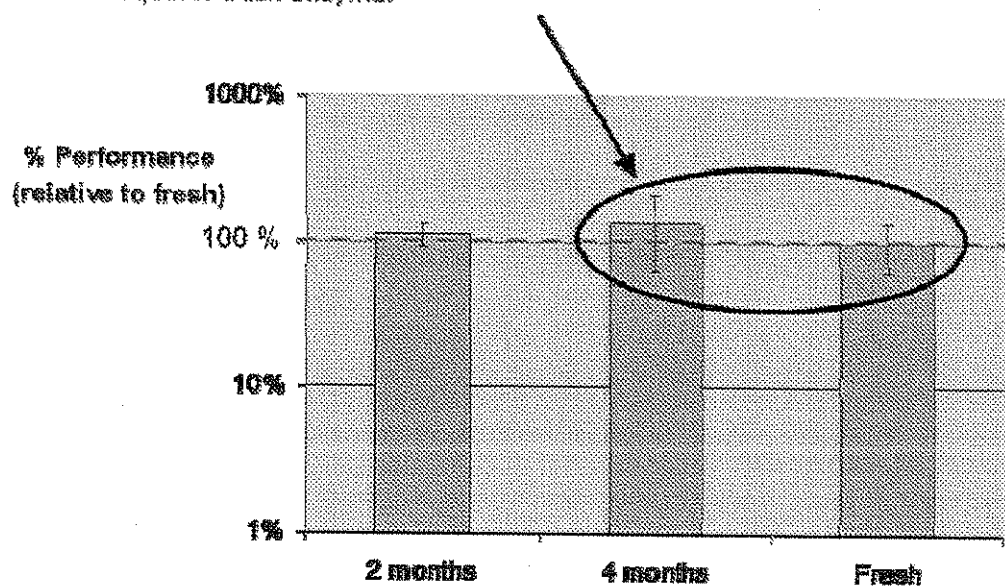


-WGA of 10 pg *Klebsiella pneumoniae*, 6 hr

FIGURE 18

Long Term Stability: Room Temperature

After 4 months at room temperature this formula 33 lyophilized enzymes performed equal to fresh enzyme.

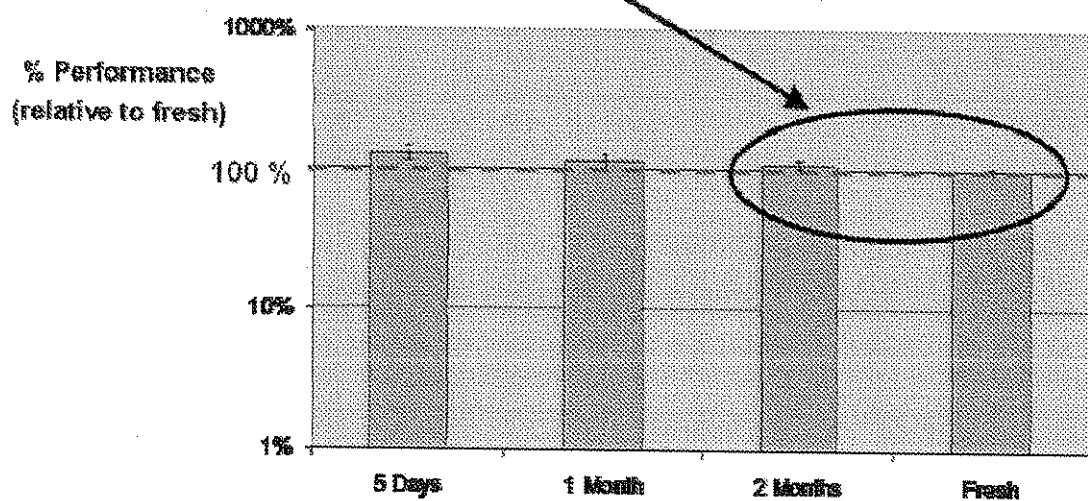


-WGA of 10 pg *Klebsiella pneumoniae*, 3 hr

FIGURE 19

Long Term Stability: Accelerated Aging (40 °C)

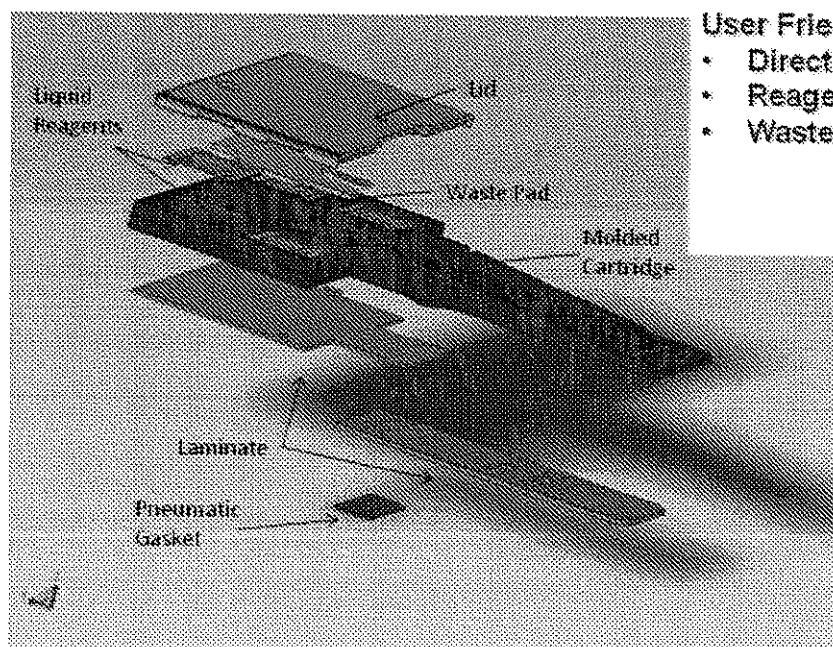
After 2 months at 40 °C (equivalent to ~ 5 months room temperature) (his formula 33 lyophilized enzymes performed equal to fresh enzyme



-WGA of 10 pg *Klebsiella pneumoniae*, 3 hr

FIGURE 20

Integrated Sample Prep. Process



User Friendly Design:

- Direct sample processing
- Reagents on card
- Waste containment

FIGURE 21

On Card Liquid Reagents

- Advanced on-cartridge buffer pack development
- Materials selected for chemical compatibility with assay Wash and Lysis buffers
- Molded sharps designed to puncture packet and release stored reagent
- Extraction reagents stable for over 8 months based upon 4 months accelerated testing parameters

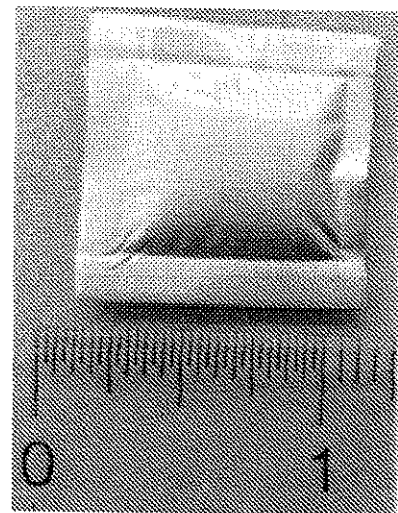


FIGURE 22

Processing Instrument

- Self contained unit with integrated processor, pneumatics, and card interfaces
 - Processor runs the instrument
 - IO may include USB, Ethernet
 - LCD provides status to user
 - Touch screen incorporates user controls
- Pneumatics system contains
 - Air pumps
 - Regulators
 - Manifold
 - Pressure/vacuum reservoirs
 - Valves
- Cartridge interfaces
 - Clamp
 - Pneumatic manifold
 - Heaters

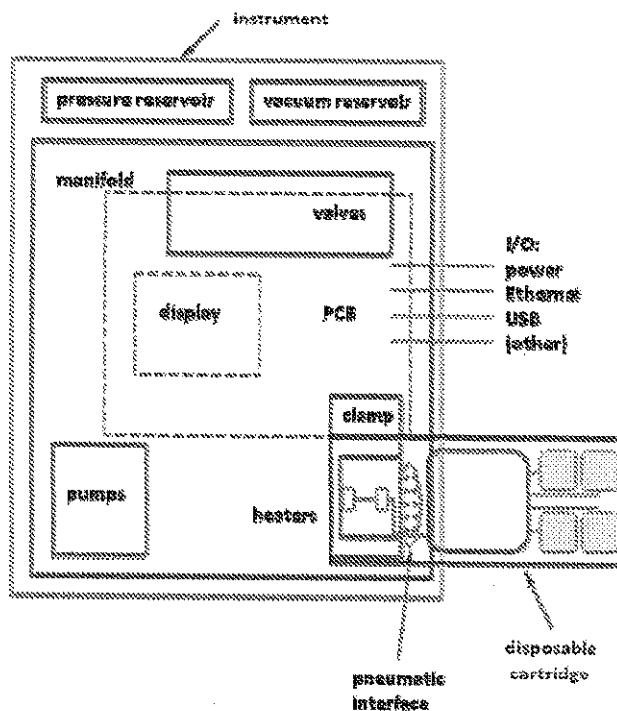


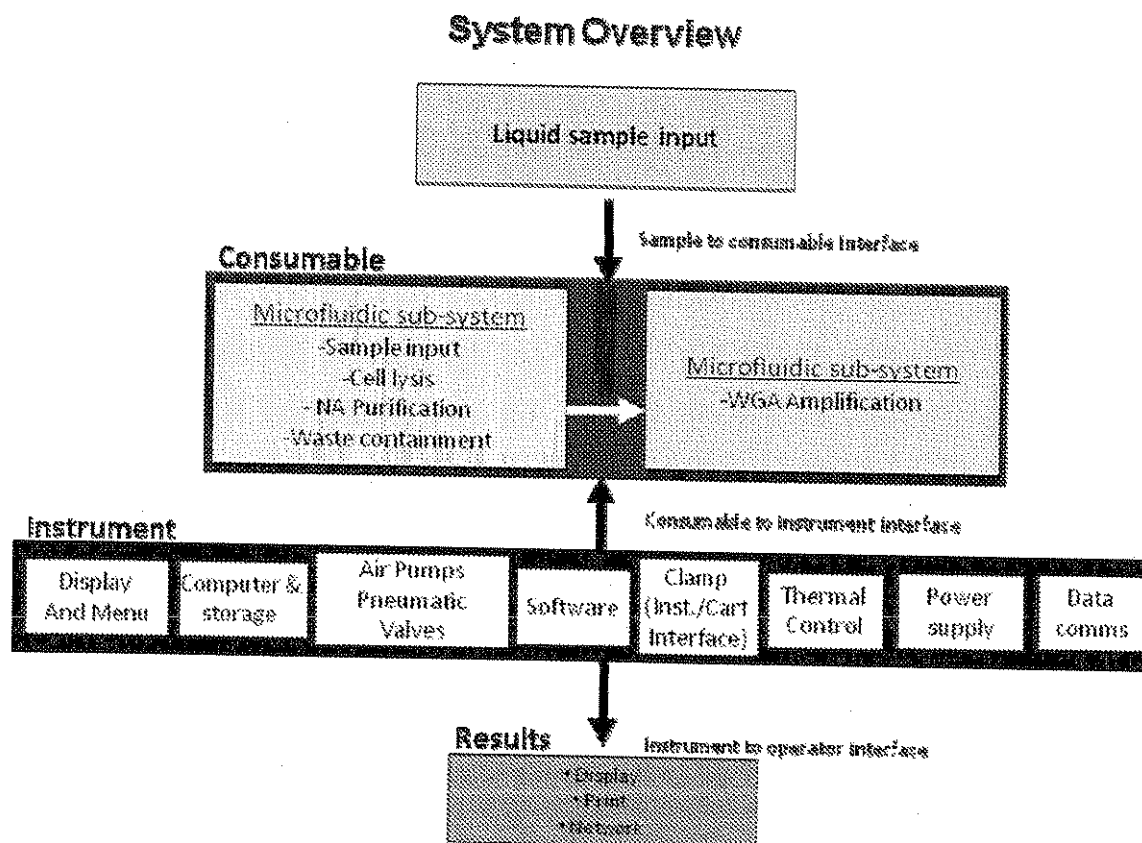
FIGURE 23

FIGURE 24

Fragmentation of DNA

First step in library prep is fragmentation of the HWM DNA

