

Brooks Automation

Life Sciences Group

**Automated Nanoliter
Cycle Sequencing,
PCR
and more with the**

 **Parallab™₃₅₀**



Dr Harvey Dosanjh - Application Specialist



Outline

- Company Overview
- Technology Goals
- Product Information
- Parallax Data
- Summary



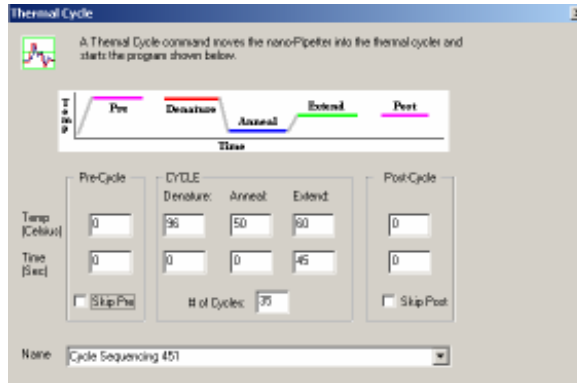
The Life Sciences Group

- Automation equipment developers for 15 years
- Developed a variety of technologies for the Human Genome Project
 - Thermal cyclers
 - Microarrayers
 - Manual and automated pipettors
 - DNA Synthesizers





Prepare Reactions



Thermal Cycle



Purify samples

All In One Automated Solution



Immediate Target Applications

- Cycle sequencing
- PCR
- SNP detection
- Genotyping
- Diagnostics



ABI Prism 3700



Amersham Biosciences
MegaBACE



ABI Prism 3730 XL



ABI 7900

Sample preparation for all these applications at a fraction of the cost

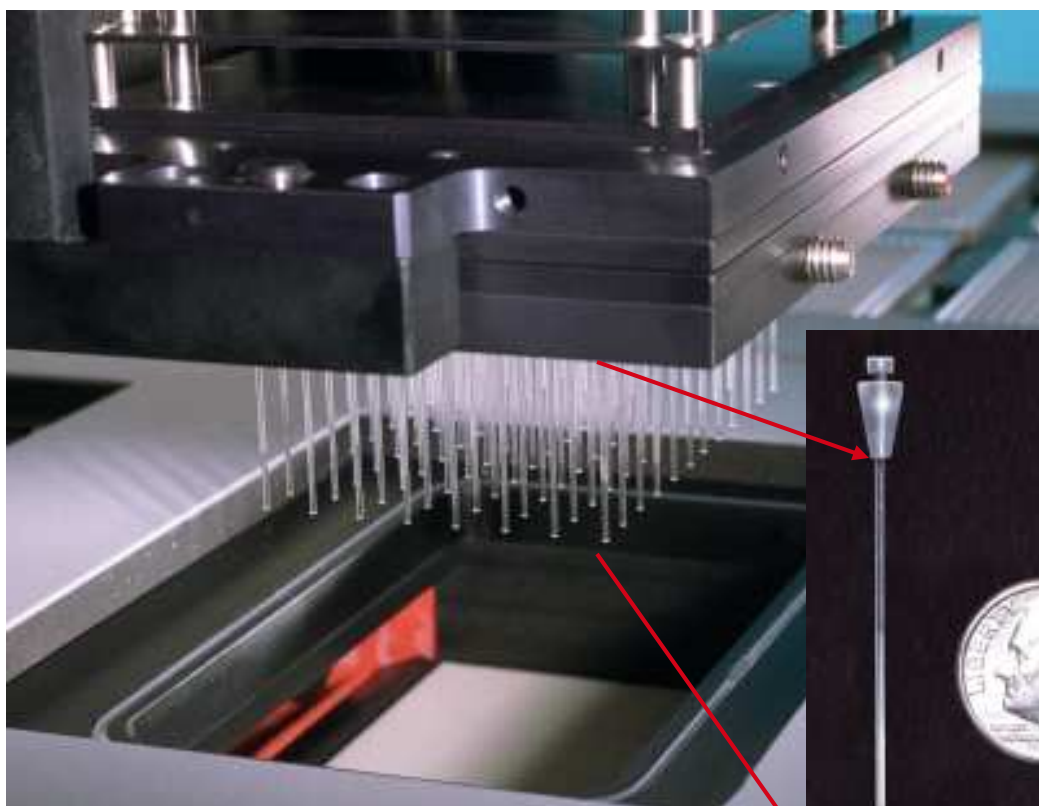


Cycle Sequencing Reaction Conditions

- 96 samples processed in parallel
- Under 1.5 hours to complete a reaction set
- 500nl total volume per sequencing sample
- On deck magnetic bead purification
- 190nl of Big Dye[®]
(1/42nd of a 1x Big Dye[®] reaction)



The Nano-Pipetter



Components

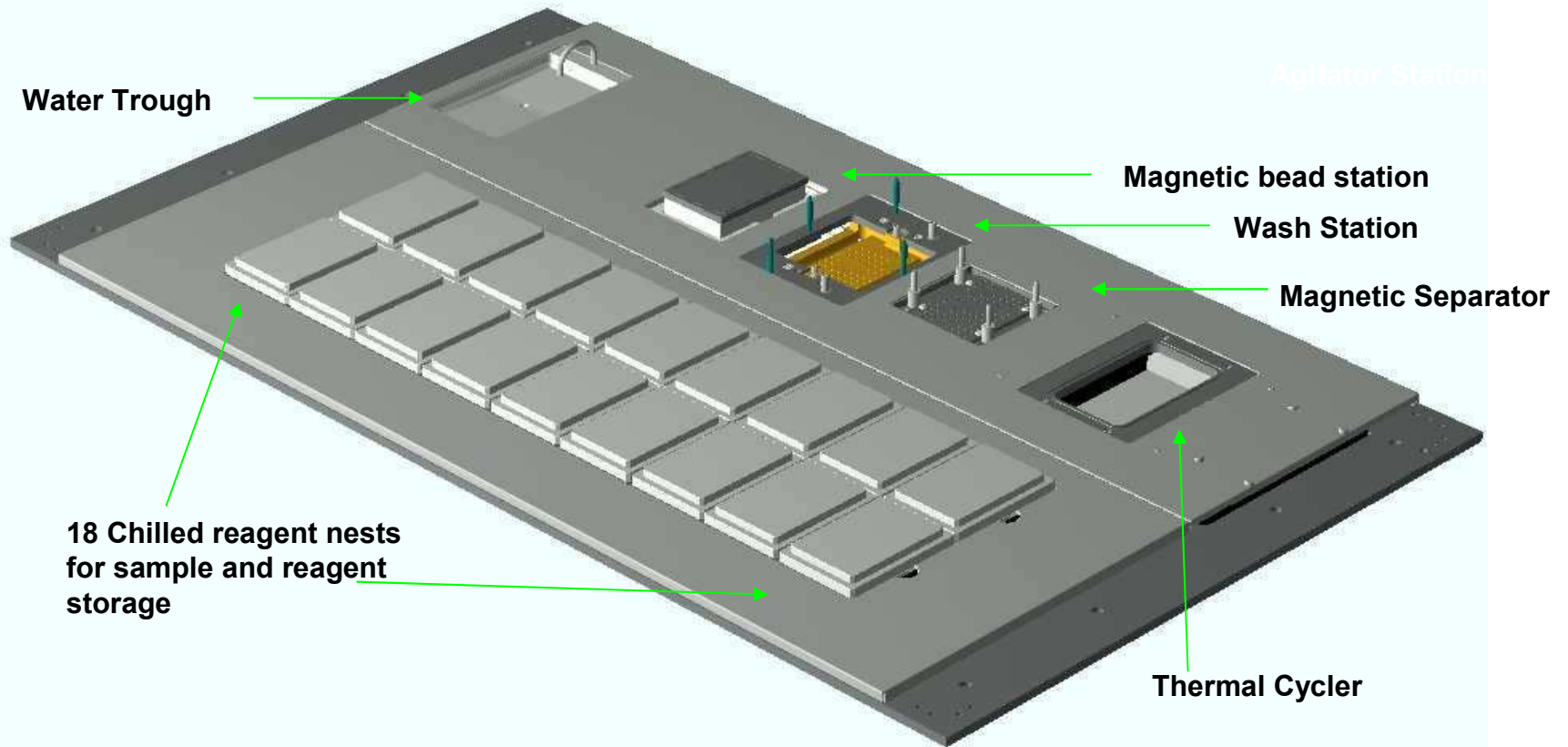
- Teflon tipped plungers
- Thin walled glass tubes

Performs all processing in the tubes

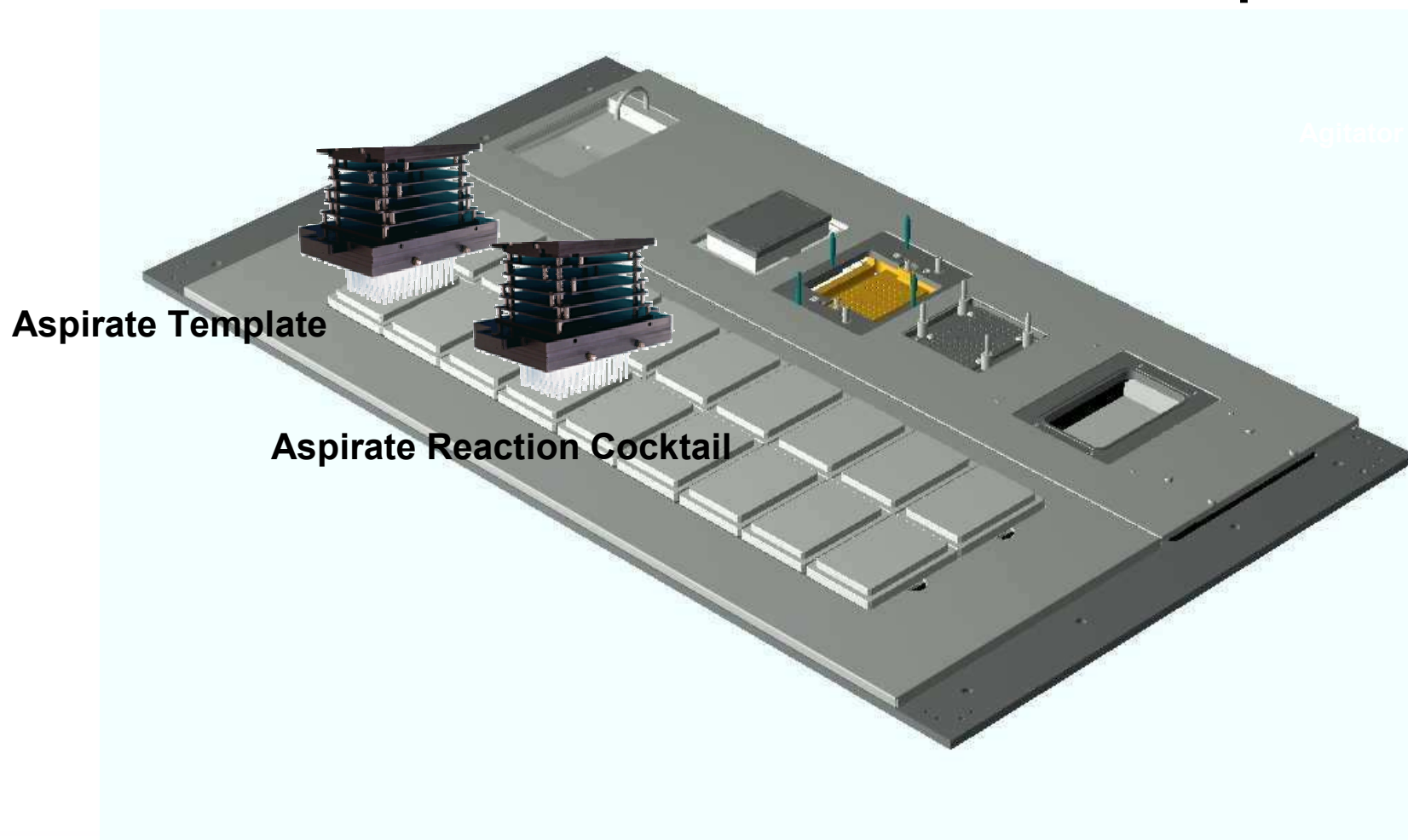
- Aspirating
- Mixing
- Thermal cycling
- Purifying
- Dispensing
- Cleaning for reuse

One of the 96 glass tubes

350 Workdeck Components



Parallab 350 Work Flow Reaction Setup



Mix Reagents in The Nano-Pipetter

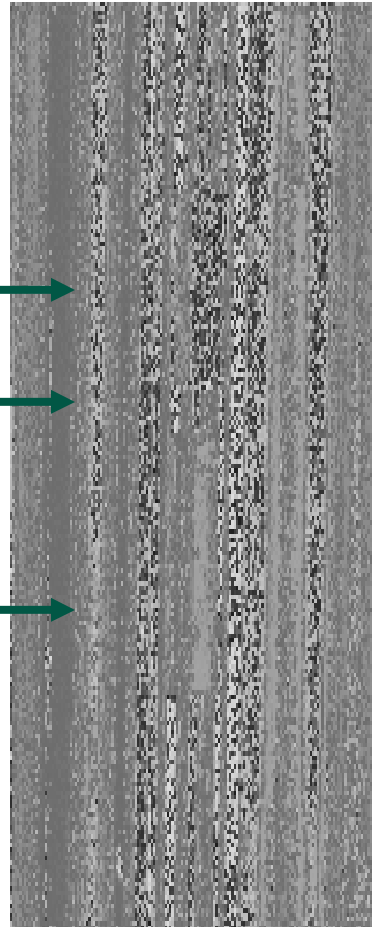
Dynamic Mixing

**High-speed
shuttling of
samples thoroughly
mixes**

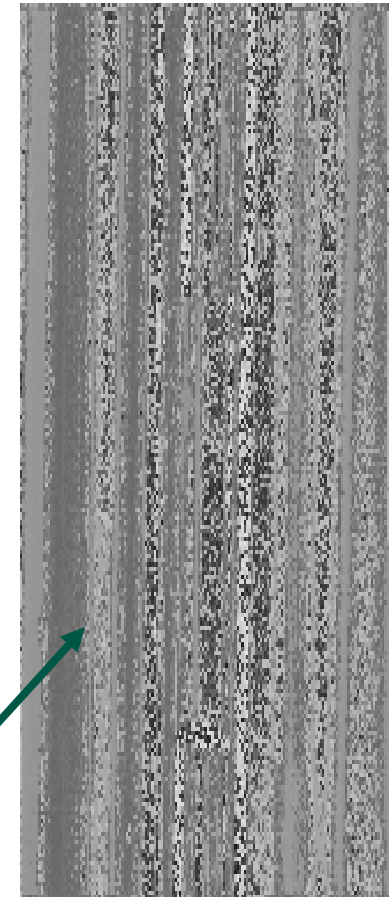
310 nanoliters
Big Dye Cocktail
and Primer

Air Gap

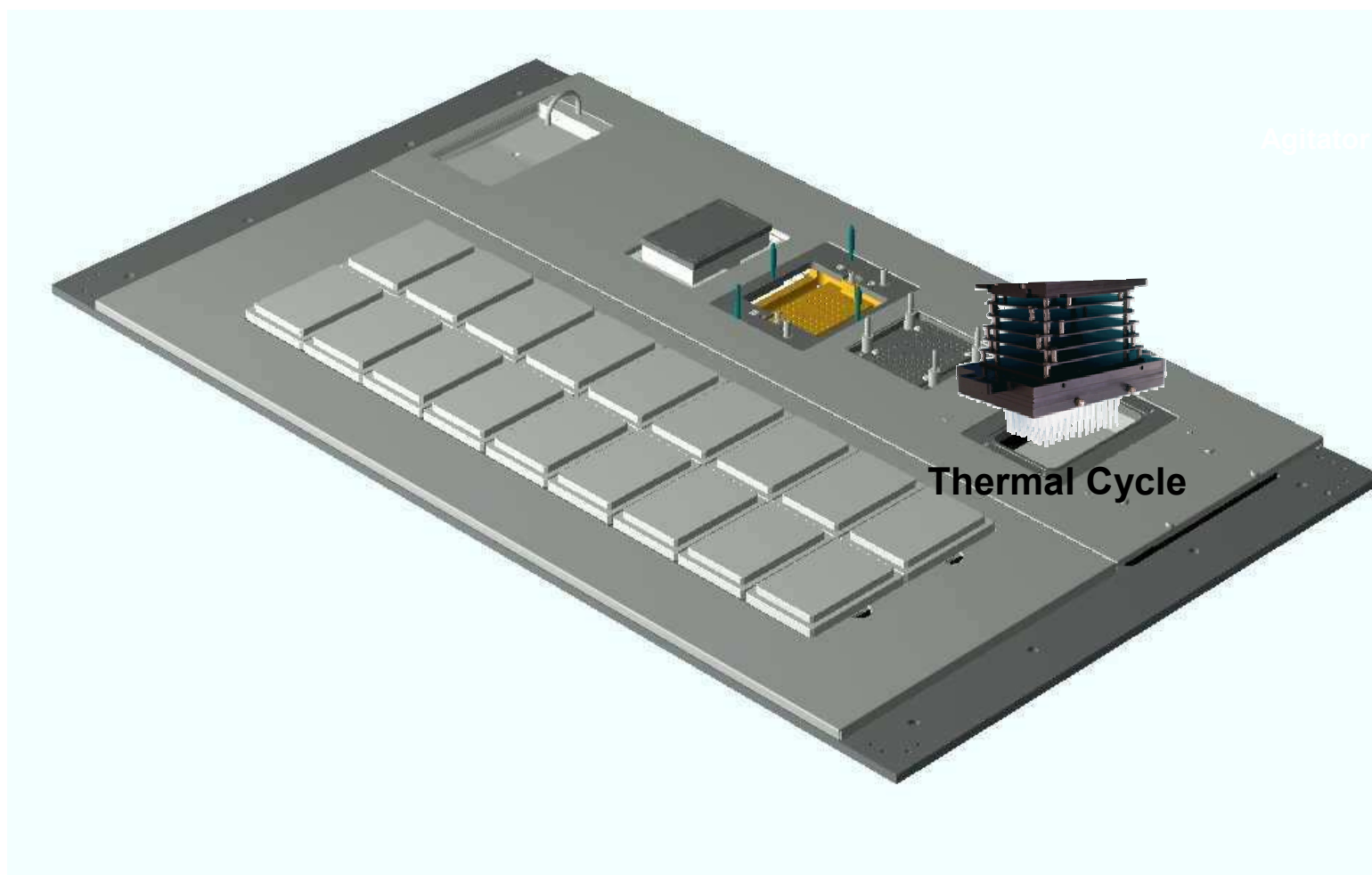
190 nanoliters
Template



Mixed solutions
=
500 nanoliters

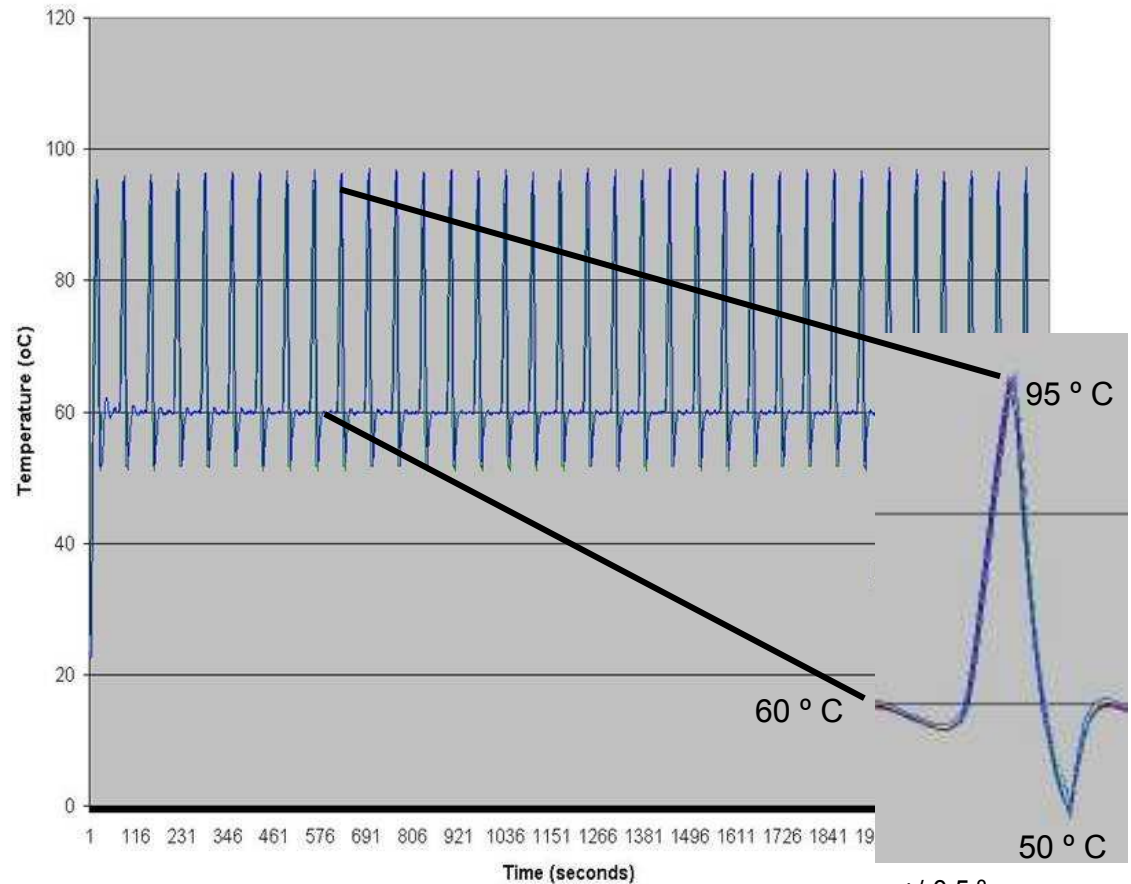


Parallab 350 Work Flow Thermal Cycle



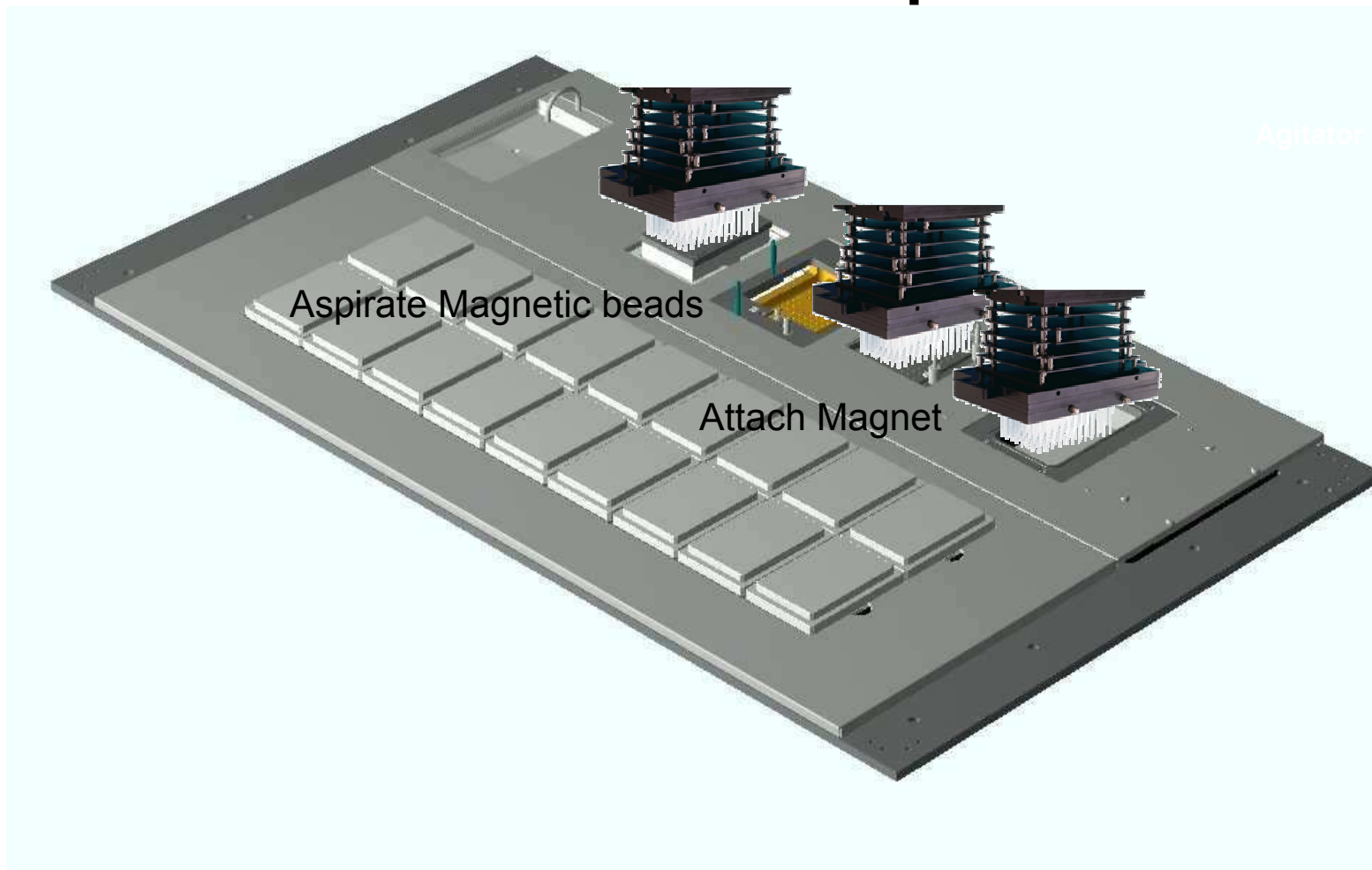
High Speed Air Based Thermal Cycler

- Exclusively designed for the Parallab™
- Program fast and accurate ramping speeds
 - (up to 15 °C/second)
- 35 cycle TC profile in 40 minutes
 - 95 °C for 0 seconds
 - 50 °C for 0 seconds
 - 60 °C for 45 seconds

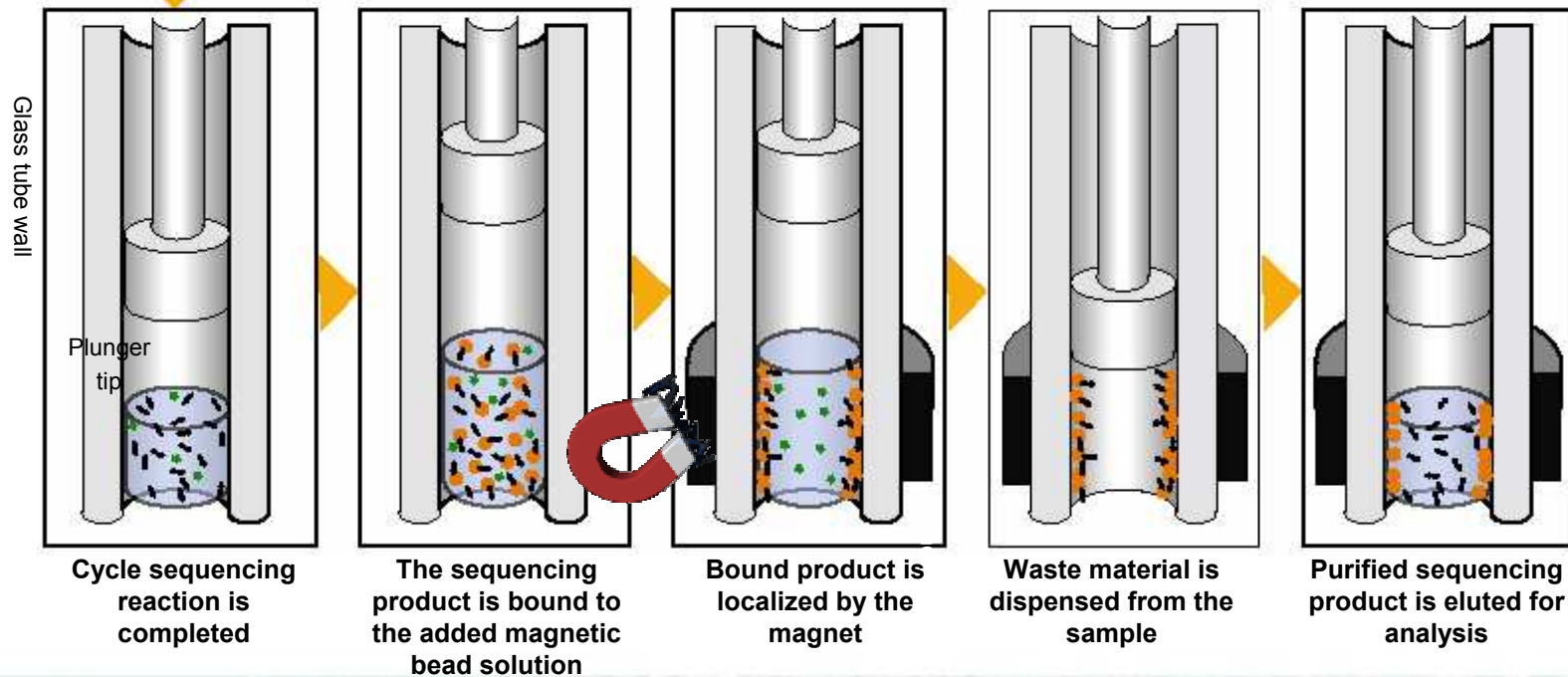


+/-0.5 ° accuracy
across chambers

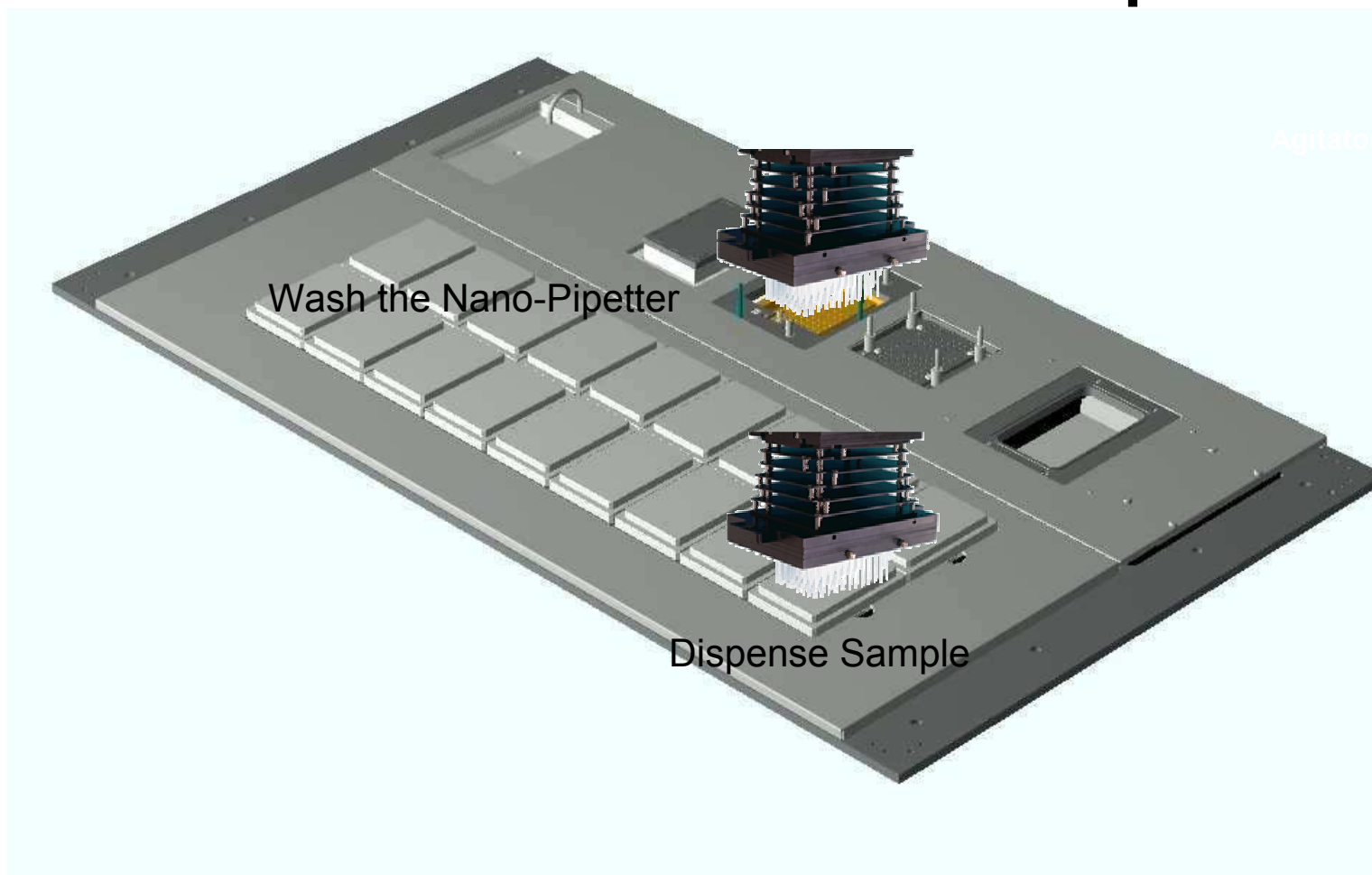
Parallab 350 Work Flow Sample Purification



Magnetic Bead Purification Done in the Glass Tubes



Parallab 350 Work Flow Run Completion



Repeated Use of Glass Tubes Yields Clean Product

Each round consists of:

- 1- A 35 cycle PCR amplification
- 2- Agarose gel analysis of sample (+)
- 3- A proprietary cleaning protocol
- 4- A 35 cycle amplification with no template to test for carry-over (Round 10 is 45 cycles)
- 5- Agarose gel analysis of sample (-)



213 base pair fragment shown

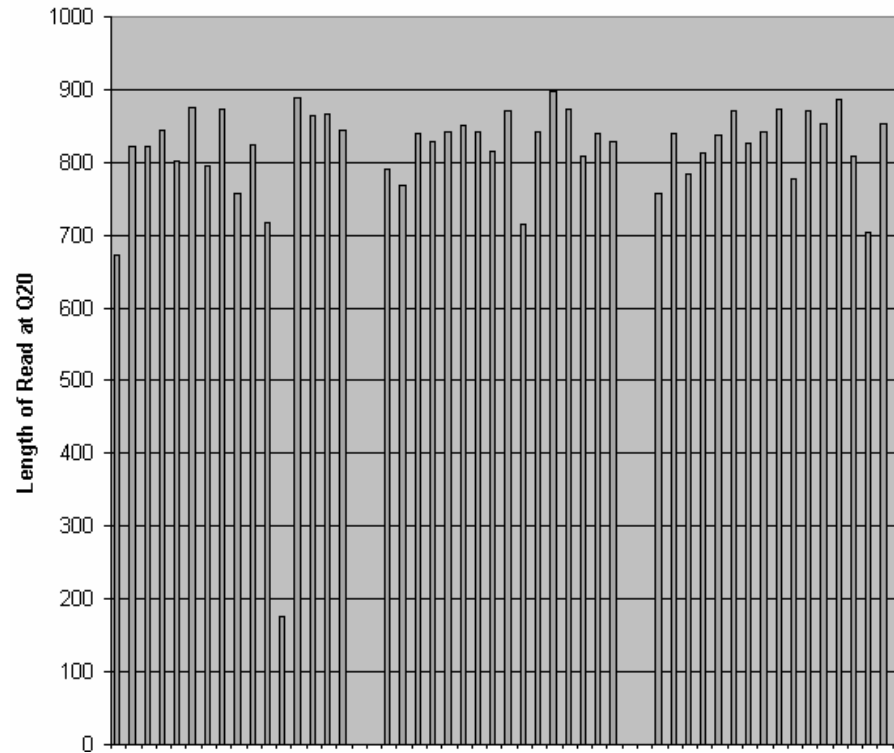
Rnd 1 Rnd 4 Rnd 7 Rnd 10
L (+ -) (+ -) (+ -) (+ -) L



No carry-over
even after 45
cycles of
amplification

Products from rounds 1, 4, 7 and 10, as described above, are shown from a single capillary
Zero carry-over and no inhibition on subsequent PCR** amplifications 5 μ l total reaction volume in glass tube

Big Dye Reduction

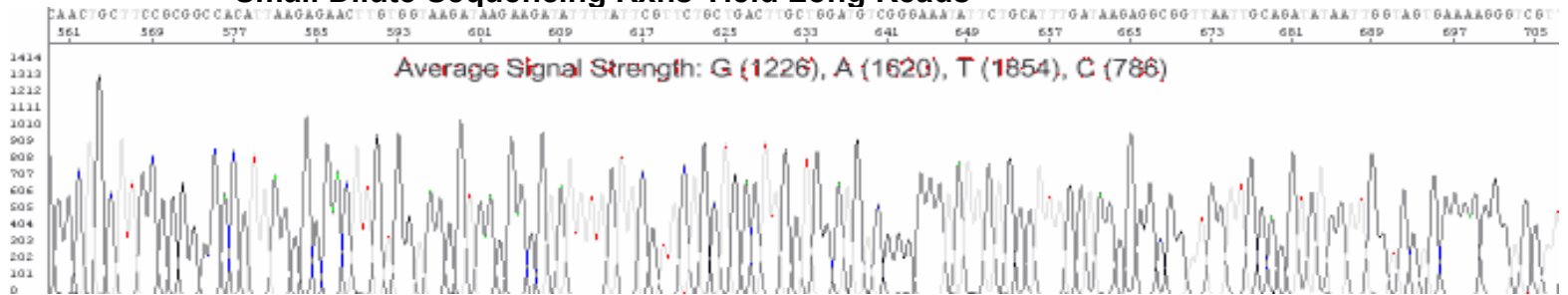


Big Dye per reaction:	190nl	95nl	47.5nl
Big Dye reduction	1 / 40 th	1 / 80 th	1 / 160 th

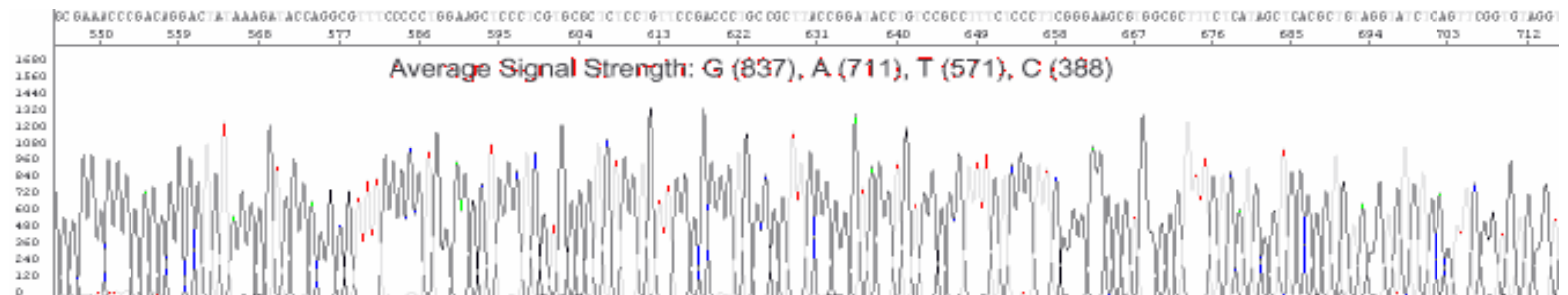
500nl sequencing reaction volume, thermal cycling conditions (96°C, 0 sec; 50°C, 0 sec; 60° C, 45 sec) X 35 cycles, SPRI purified; and sequenced on an ABI 3730xl

Small Dilute Sequencing Rxns Yield Long Reads

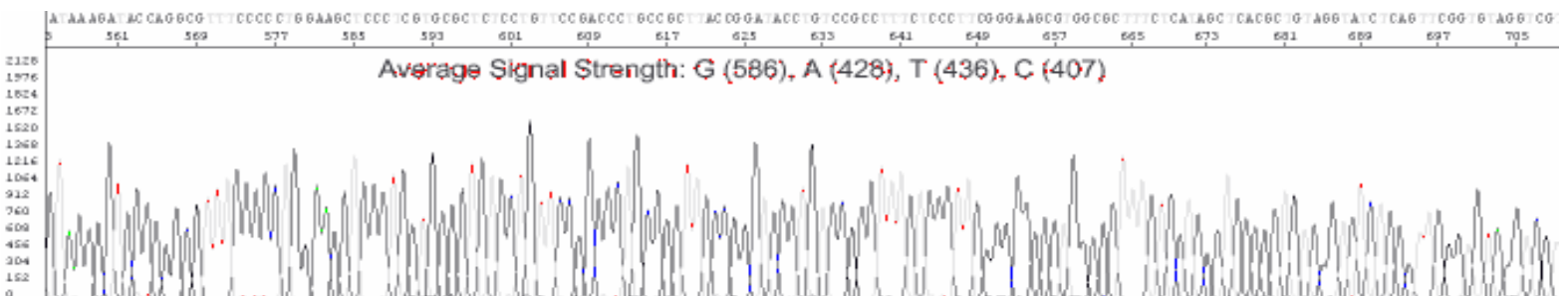
Control
841 q20
 Big Dye® Terminator
 Sequencing Std



1:42 reduction
905 q20
 500 nl reaction volume
 190 nl of Big Dye®
 SPRI purified



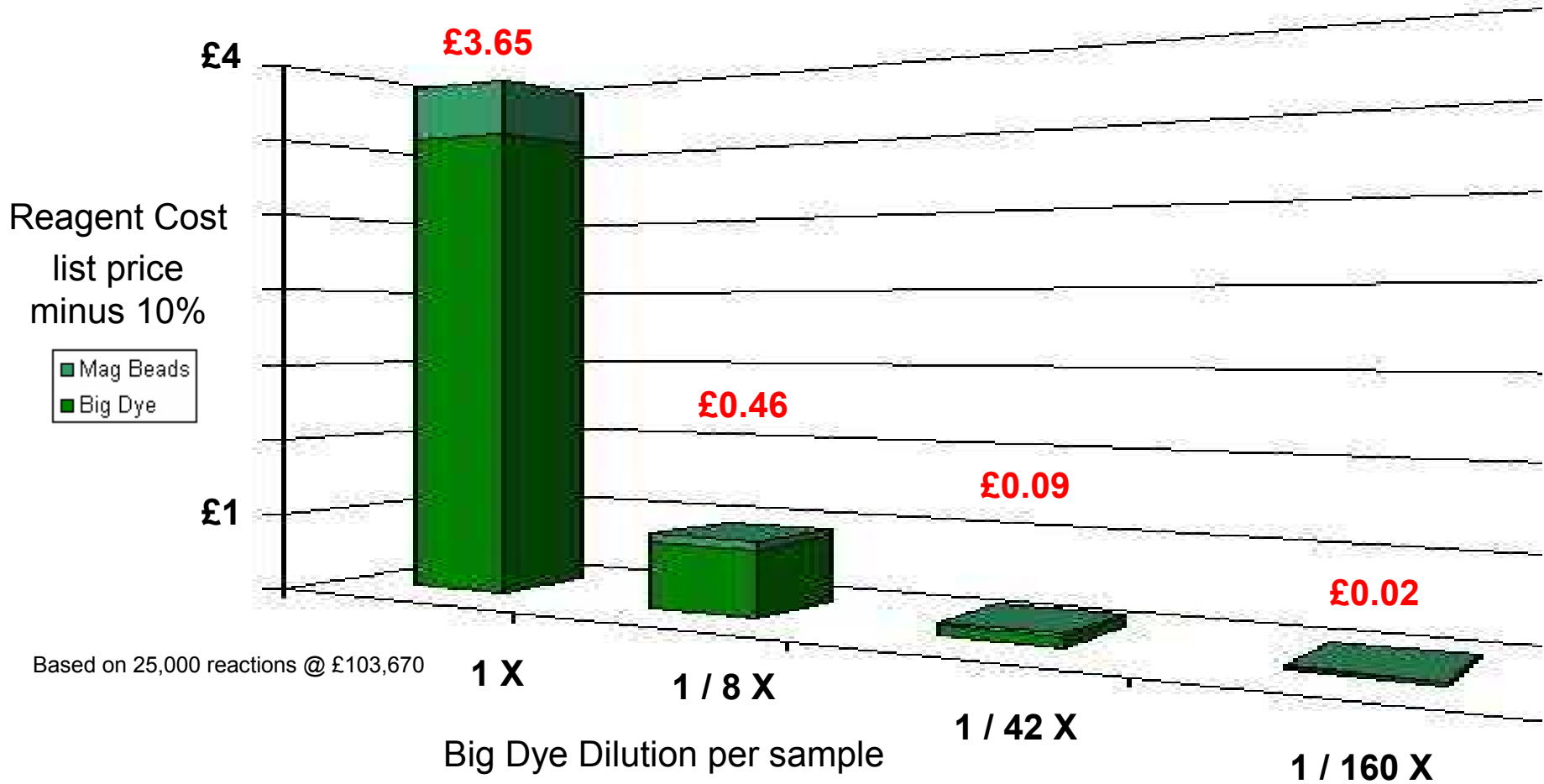
1:160 reduction
884 q20
 500 nl reaction volume
 50 nl of Big Dye®
 SPRI purified



Comparison of an ABI sequencing standard versus two dilute reactions performed in a Parallax 350 show some decrease in average signal strength but no loss of data quality or read length.

All samples sequenced on an ABI 3730 XL

Cost Savings with Reagent Use Reduction



Summary

Integrated Automation:

- Reaction setup
- Thermal cycling
- Sample purification
- 96 samples run in parallel
- Reusable glass reaction tubes

Advantages:

- Reduces reagent costs by using nanoliter volumes
- Completes 1,600 samples in 24 hours
- Lowers labour & error rates by unattended operation
- Saves valuable lab space
- Minimizes consumables

