

***Optimizing the Small Core
Laboratory
“Tips and Tweaks”***

***Michelle Detwiler
Roswell Park Cancer Institute
Buffalo, N.Y. USA***



What is “optimizing”?

Every lab will have different criteria for what is considered “optimal”.

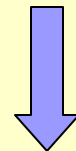
Some general criteria to consider:

Economizing and keeping costs down

Providing highest quality data possible

Quickest turnaround time

Creating the smoothest workflow in the laboratory



Satisfied customers that keep coming back

Customer requests and receiving samples



Preparing sequencing reactions



Thermocycling samples



Purification of sequencing reactions



Electrophoresis of samples



Distribution of results


Customer
Requests
and
Receiving
Samples

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Roswell Park Cancer Institute DNA Sequencing website

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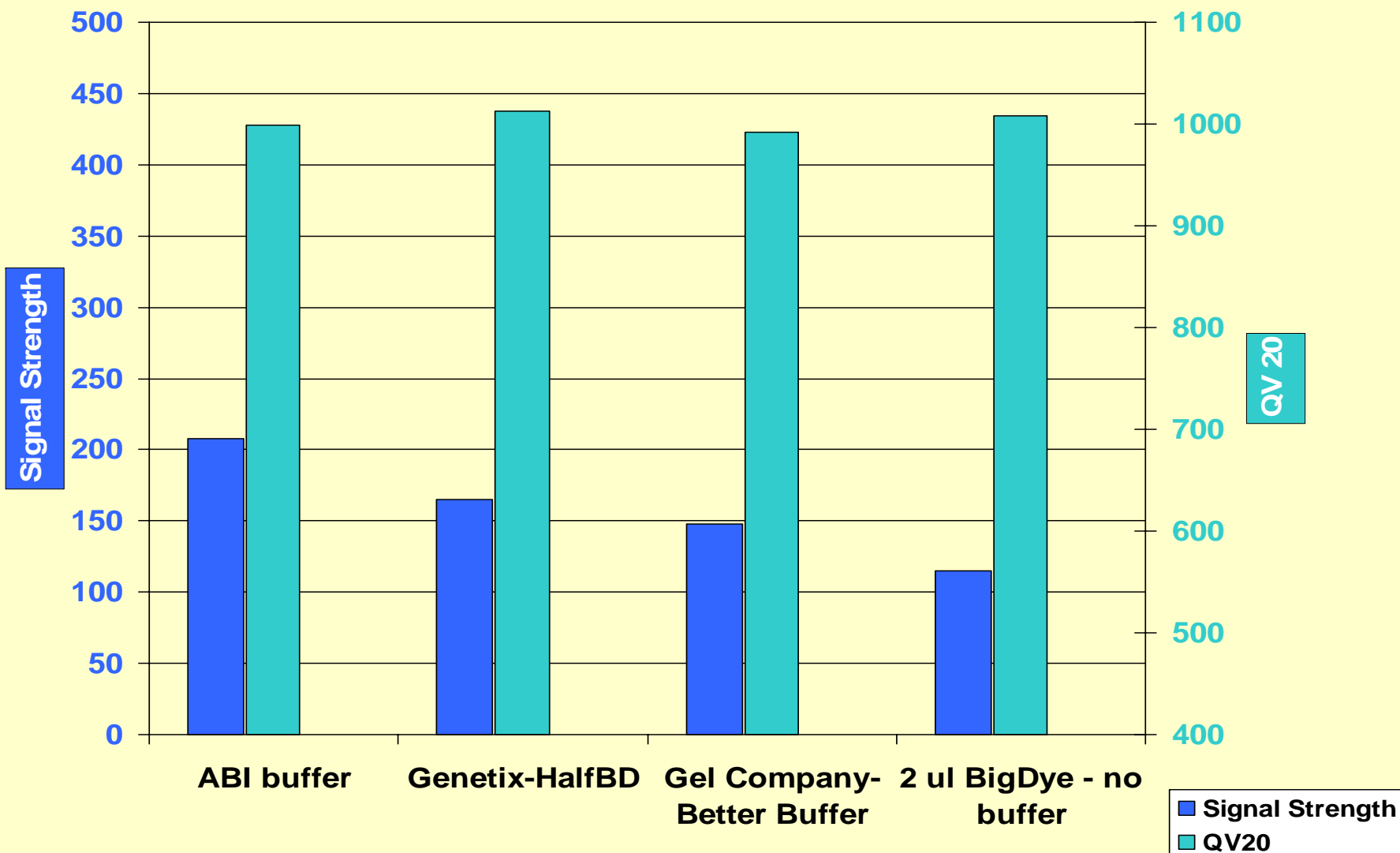
Preparing Sequencing Reactions

Dilution buffer comparisons

Brands evaluated:

- **Applied Biosystems BigDye Terminator 5x Sequencing Buffer**
- **Gel Company - Better Buffer**
- **Genetix – HalfBD**
- **No dilution buffer at all**

Dilution Buffer Comparisons



Dilution Buffers **Cost Comparisons**

| Manufacturer | Price per ml |
|--|---|
| Applied Biosystems 5x Sequencing Buffer | -Free to a point; \$33-\$36/ml |
| Gel Company Better Buffer | \$98-\$113/ml |
| Genetix HalfBD | \$169-\$213/ml |
| No buffer | Free |

Thermal cycling samples

Fast Cycle Sequencing method

Developed for the AB9800 Fast Thermal Cycler

96C for 1 min – 1 cycle

96C for 10 seconds

50C for 5 seconds

60C for 1 minute, 15 secs

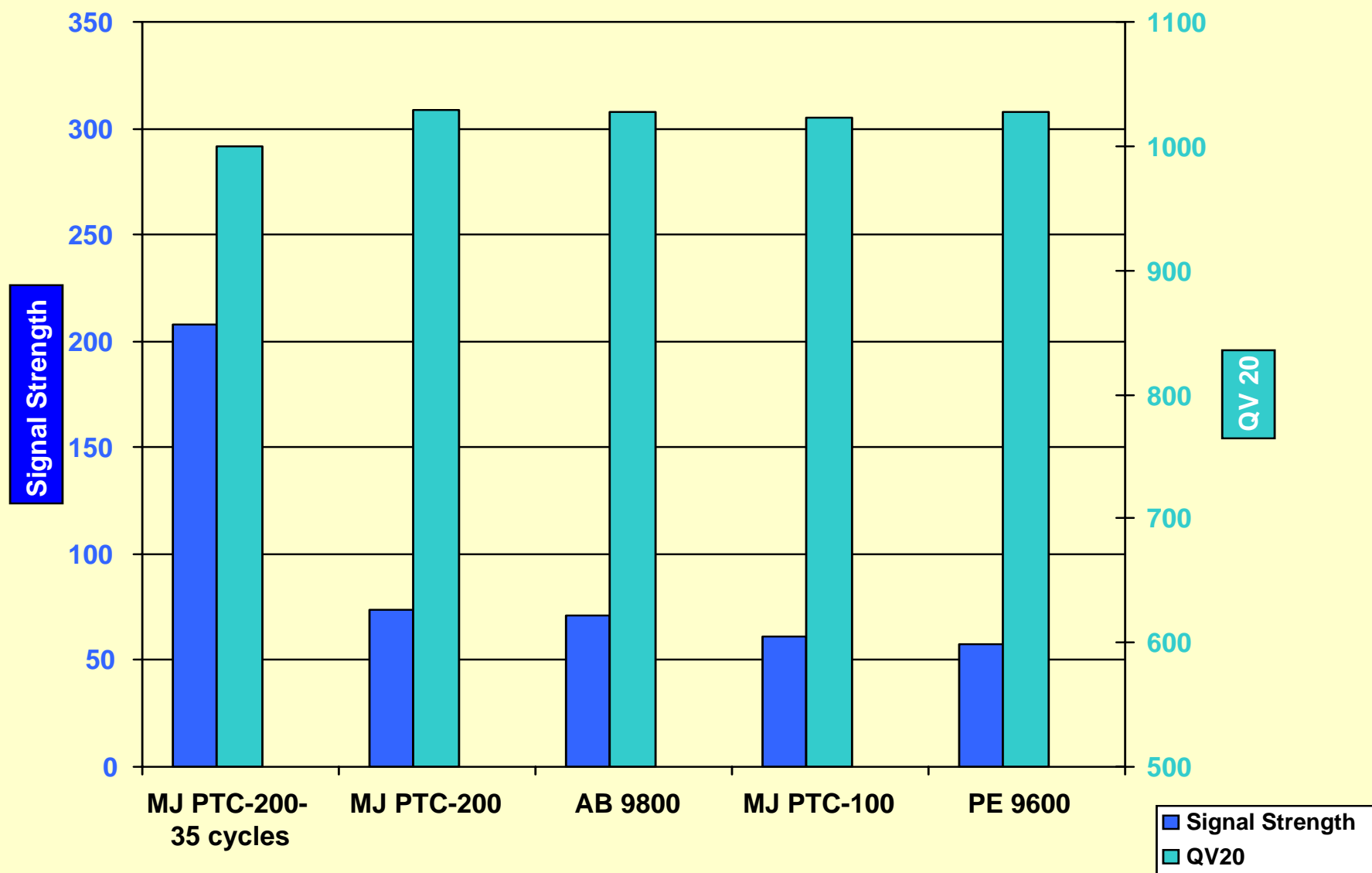
} **25 cycles**

**Projected time to complete full cycle sequencing run:
As little as 50 minutes**

Thermal Cycler Models Evaluated

- **Applied Biosystems 9800 Fast Thermal Cycler**
- **Perkins Elmer 9600 Thermal Cycler**
- **MJ Research PTC-100**
- **MJ Research PTC-200**

Thermal Cycler Comparisons



Thermal Cyclers Time per FastSeq run

| Manufacturer | Time per run |
|--|---------------------|
| Applied Biosystems 9800 Fast Thermal Cycler | 57 minutes |
| MJ Research PTC-200 | 72 minutes |
| PE 9600 | 74 minutes |
| MJ Research PTC-100 | 84 minutes |
| MJ Research PTC-200 – standard cycle seq protocol | 220 minutes |

Purification of reacted samples

Purification Methods Evaluated

Gel filtration:

- Edge Biosystems (deep-bed)
- Genetix genCLEAN (shallow-bed)
- Sephadex/Millipore home-packed plates

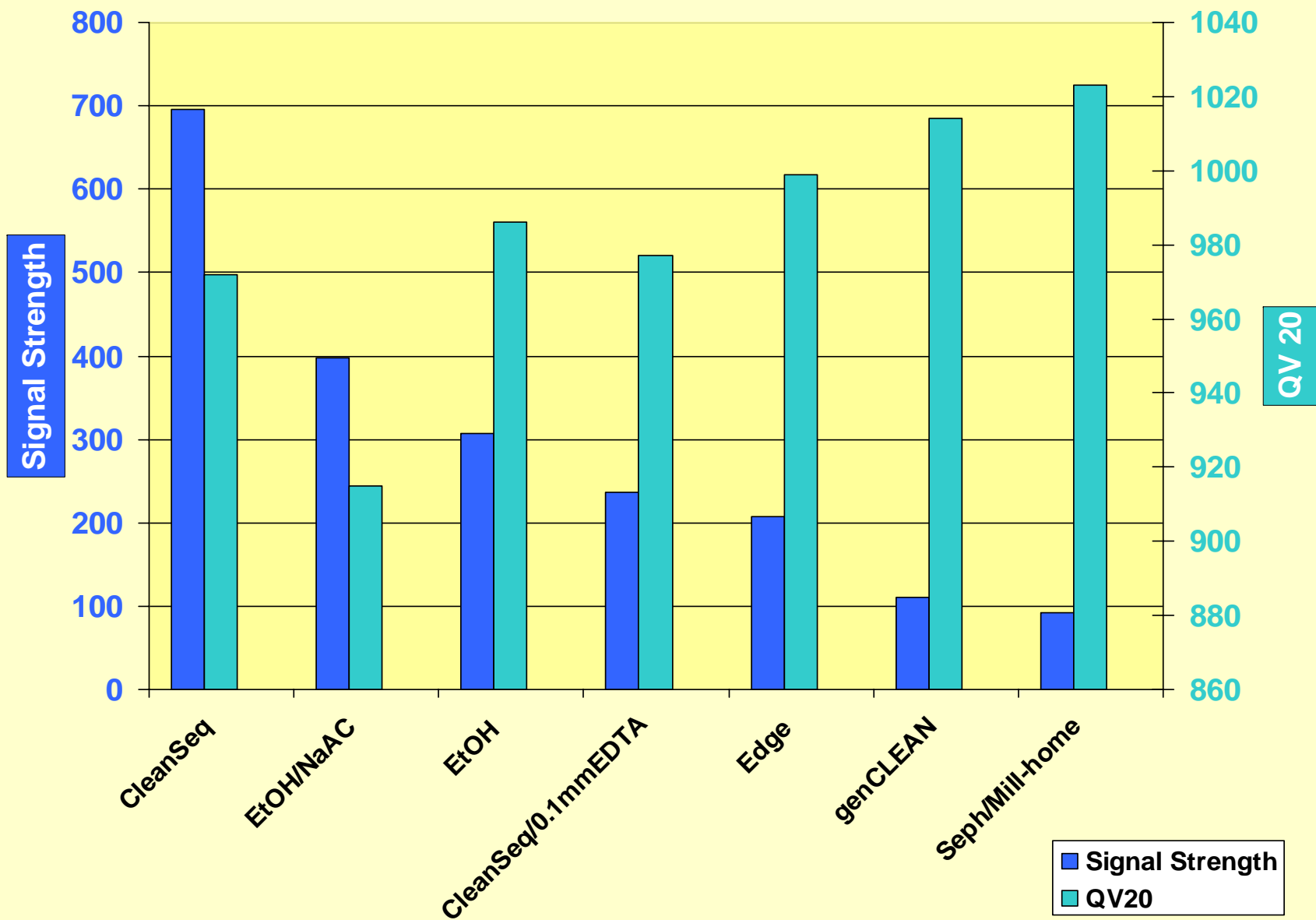
Ethanol precipitation:

- EtOH/EDTA
- EtOH/EDTA/NaAC

Magnetic beads:

- Agencourt CleanSeq

Purification Method Comparisons



Purification Methods **Cost Comparisons**

| Manufacturer | Cost per sample |
|--------------------------------------|------------------------|
| Edge Biosystems | 0.68 |
| Genetix genCLEAN | 0.44 |
| CleanSeq | 0.39 |
| Sephadex/Millpore home-packed | 0.15-0.23 |
| EtOH precipitation methods | 0.02 |

Additional tests performed

Test #1. Testing Agencourt's claim that, by overlaying samples with mineral oil, samples can sit for up to 6 days with no dye degradation and no adverse effect on electrophoresis.

Test #2. Vigorous remixing of gel filtration bed with 200 ul water after first centrifugation step, followed by a second spin, can improve signal strength of samples.

(thanks to Matt Schudt, Wadsworth Center, NYS Department of Health)

Test #1

Mineral oil overlay

| | Signal Strength | QV20 score |
|-----------------|-----------------|------------|
| CleanSeq | 695 | 972 |
| CleanSeq-6 days | 868 | 952 |
| | | |
| Sephadex | 92 | 1023 |
| Sephadex-6 days | 77 | 924 |

Test #2

2nd wash of Sephadex-packed plates

| | Signal Strength | QV20 Score |
|----------------------------------|-----------------|------------|
| Sephadex- One spin | 92 | 1023 |
| Sephadex- Two spins with wash | 231 | 1013 |

Electrophoresis of samples

What is a 3130?

A hybrid of the 3730 and the 3100

Primarily a hardware upgrade:

- Sapphire pump replaces the upper polymer block and syringes**
- Cell detector heater for more reproducible migration of fragments – most useful for fragment analysis**
- Runs with Data Collection v3.0 and Sequencing Analysis v5.2**

Disadvantages of the
3130

**Expensive
upgrade - \$30,000!**

**This is about one-
fourth the cost of
a new instrument.**

Advantages of the 3130

- **No more syringes and upper polymer block!**
- **Washing of blocks and pump is automated**
- **Bubble removal is automated – but uses lots of polymer to perform this task**
- **Can perform automated water washes of arrays –**
(Reduce by half the syringe injection speed and syringe plunger speed in .calib file)

Much less disassembly involved



Less manipulation of array



Increased array life!

**The use of POP-7 is now supported
on the 3130.**

**But do you need to upgrade to a 3130 to
enjoy the benefits of using POP-7?**

NO!!

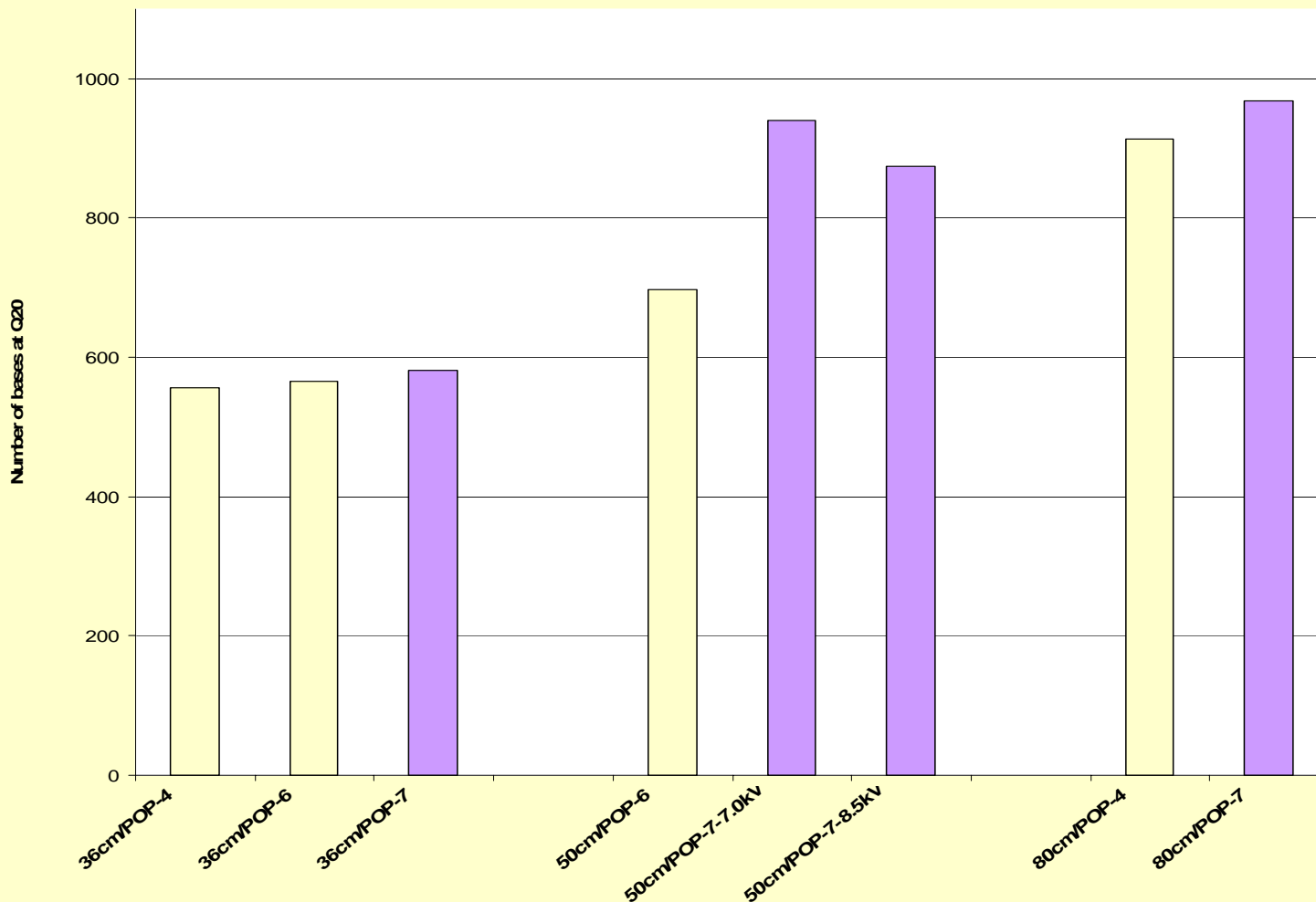
What are the advantages of using POP-7 on the 3100?

- Shorter run times with improvement in high-quality read length. When compared to sequencing with POP-6, read length is dramatically increased.**
- Can be significantly cheaper than “3100” polymers – IF you can purchase in greater volumes.**

Comparison of supported vs. modified modules

| | Standard ABI POP-6 module (50cm array) | Standard ABI POP-4 module (80cm array) | Modified module for POP-7 “standard read” (50cm array) | Modified module for POP-7 “long read” (50cm array) |
|---------------------------|--|---|--|--|
| Q20 bases | 650-700 | 900-950 | 850-900 | 900-950 |
| Total run time | 2 hours, 30 mins | 3 hours, 40 mins | 2 hours, 5 mins | 2 hours, 45 mins |

Comparison of supported protocols vs. modified POP-7 protocols - average number of bases with Phred Q20 score



Yellow = Supported Protocols

Purple = Modified POP-7 Protocols

Comparison of POP-7 prices for 3730 vs. 3130 users

| | Size of package | Price per package | Price per ml |
|--------------------|------------------|-------------------|----------------|
| <u>3730</u> | 25 ml bottle | \$990 | \$39.60 |
| | 10 pack (250 ml) | \$3,300 | \$13.20 |
| | 30 pack (750 ml) | \$6,600 | \$8.80 |
| | | | |
| <u>3130</u> | 3.5 ml bottle | \$150 | \$42.85 |
| | 7 ml bottle | \$365 | \$52.14 |

Distribution of results

What is the best way for your customers to view and analyze their data?

- Paper hard copy**
- LIMS built-in viewer**
- Commercially available programs**
- Freeware chromatogram viewers**

Applied Biosystems

Sequence Scanner

Acknowledgements

Timothy Hamp

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