

ABRF

NARG 2000-2001 DNA Synthesis Study

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PART I: Survey Data

The current status of participating DNA synthesis core facilities is shown below compared to the NARG 1995 survey, illustrating a relatively stable environment over the last two years--only a few laboratories indicated future changes. Both studies indicated that many core facilities regularly practice quality control (QC) of outgoing oligonucleotides. Those that do not QC still regard high oligonucleotide quality as essential to their facility's success.

Figure 1: DNA Synthesis/Whole Facility Start Year

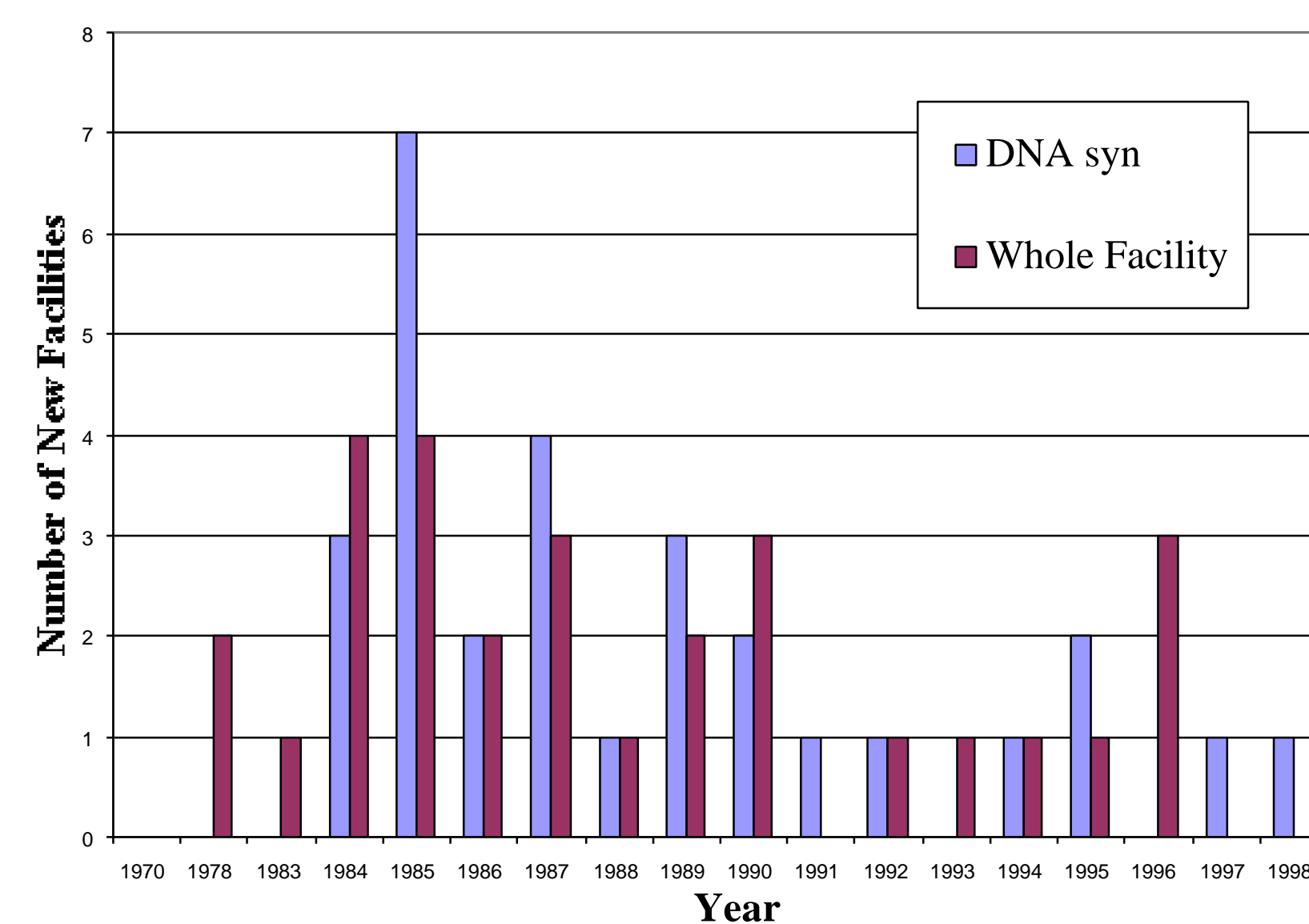


Table 2: Quality Control Issues

| Question | Non-profit (n=23) | Commercial (N=6) |
|--|-------------------|------------------|
| Percent of yearly oligonucleotides checked by QC | 48 | 74 |
| Percent of Labs that QC 100% of the oligos | 39 | 67 |
| Percent of Labs that QC 0% of the oligos | 17 | 0 |
| Preference to QC every oligonucleotide | 78 | 67 |
| Percentage of outgoing oligonucleotide failure considered acceptable | 0.8(n=22) | 2.4 (n=5) |
| Failure rate range | 0-5 | 0-6 |
| QC method of choice: MALDI | % labs 52 | 50 |
| Preference for a combination of QC methods | % labs 87 | 83 |
| Trityl monitoring as one of the preferred choices | % labs 43 | 0 |

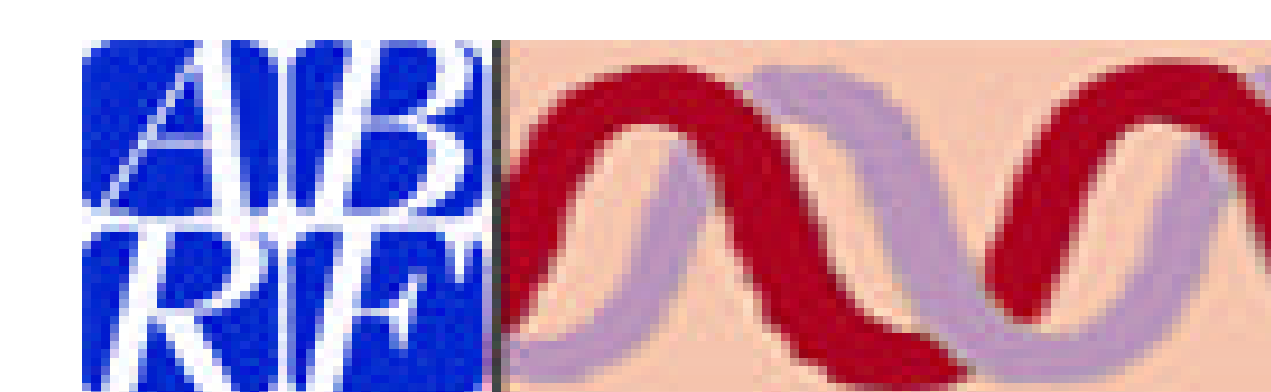
Table 1:
Participating DNA
synthesis core
facilities

| | 2000-2001 Survey | | 1995 Survey | |
|---|------------------|------------|------------------|--------------|
| | Non-profit | Commercial | Non-profit | Commercial |
| Total | 23 | 6 | 35 | 4 |
| ABRF member | 19 | 5 | ND(50% of staff) | ND |
| Average start year for the facility | 1988 | 1991 | 1987 | 1991 |
| Budget subsidized by institution | 20 | 4 | 17 (n=28) | ND |
| Provide other services | 23 | 5 | 32 | ND |
| Perform independent research | 13 | 5 | | |
| Average annual total of oligonucleotides | 4433 | 12,746 | 4940 | 77,667 |
| Range of annual total of oligonucleotides | 150-12,500 | 375-50,000 | 250-28,000 | 3,000-15,000 |
| Increase in demand for oligonucleotides | %labs 52 | 83 | 37(n=26) | 42 |
| No change in demand for oligonucleotides | %labs 22 | 16 | ND | ND |
| Decrease in demand for oligonucleotides | %labs 26 | 0 | 28 | 0 |
| Discontinue DNA synthesis | 3 | 0 | 4(n=32) | 0 |
| Typical synthesis scale | 40 nmol | 40 nmol | 40 nmol | 40 nmol |
| Percentage of purified oligonucleotides | 37 (n=21) | 36 | 44(n=33) | 67 |
| Most frequently used purification method | OPC | OPC | OPC | OPC |
| Percent of modified oligonucleotides | 10 | 35 | ND | ND |

Table 2: Quality Control Issues

PART I- Conclusion:

- ≈ Moderate participation (31 out of 111 facilities listed in ABRF directory participated)
- ≈ Majority of facilities started with DNA synthesis and include more than one service, predominantly peptide synthesis/protein sequencing, DNA sequencing, and mass spectrometry (data not shown)
- ≈ Stability of facility environment since only 3 facilities (non-profit) closed
- ≈ QC is not regularly performed on all outgoing oligonucleotides



Nucleic Acids Research Group

PART II: Quality Control Is Important To Detect Oligonucleotide Quality

DNA synthesis chemistry and automated DNA synthesizers can reliably deliver high purity oligonucleotides when they are properly maintained. However, this study finds that a CORE facility must practice thorough quality control to assure the products are always of the highest quality.

Research Participants:

20 DNA synthesis cores:

- 16 non-profit
- 4 commercial

30 DNA synthesizers tested:

- ABI 392
- ABI 394
- ABI 3948
- ABI Expedite
- Beckman Oligo 1000

Each core provided five samples:

- Homopolymer A 20 mer
- Homopolymer G 20 mer
- Homopolymer C 20 mer
- Homopolymer T 20 mer
- Mixed base 30mer

Rational:

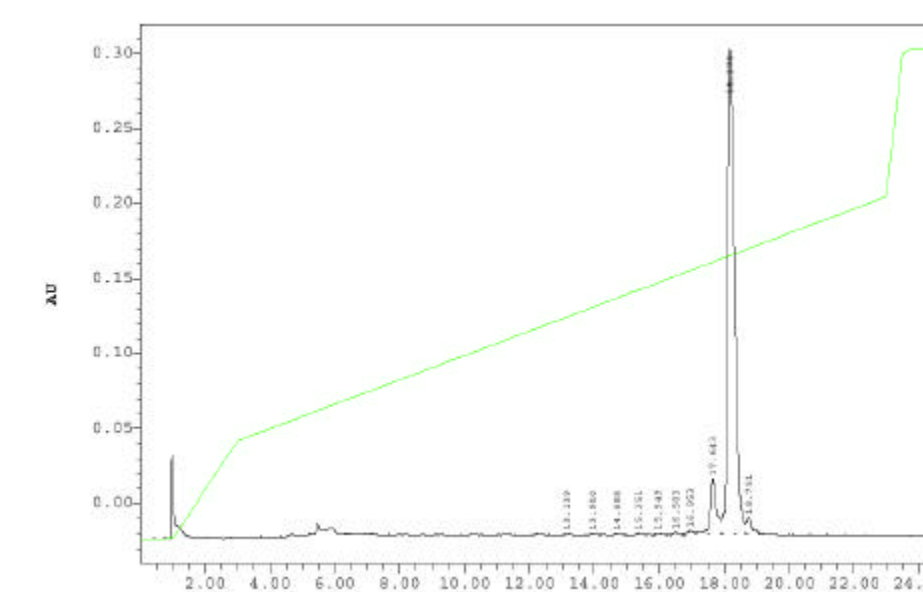
Problems in DNA synthesis may be nucleotide-specific and can be detected by using homopolymers.

Details:

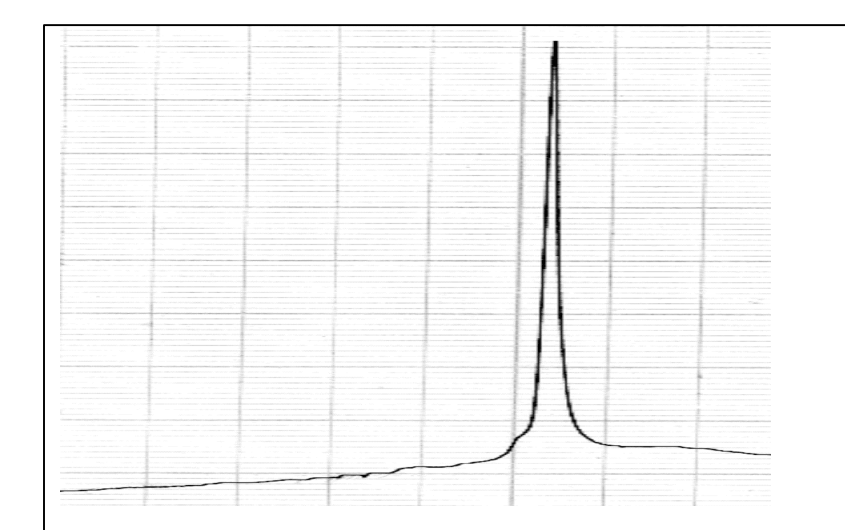
Yeung, A. T. and Miller, C. G. A general method of optimizing automated DNA synthesis to decrease chemical consumption to less than half. *Anal. Biochem.* **187**:66- 75, 1990.

Chromatograms of gel-purified oligonucleotides

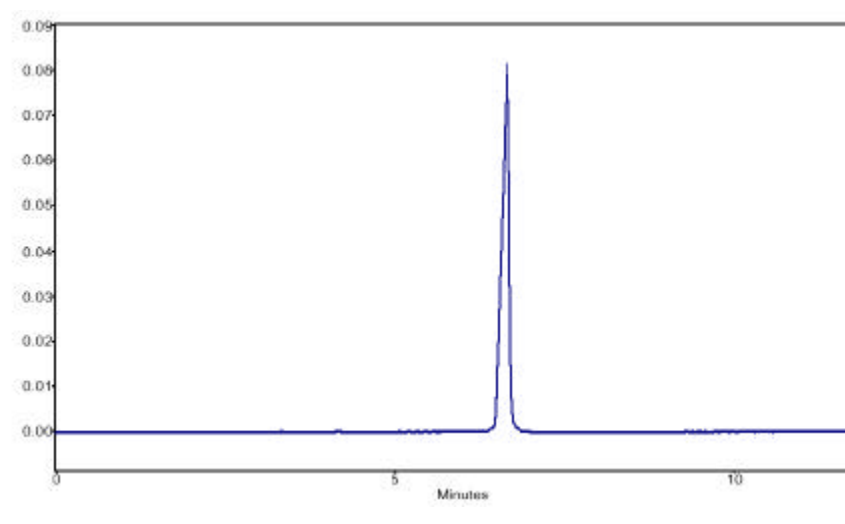
A20 mer, anion-exchange pH 7, 5M urea



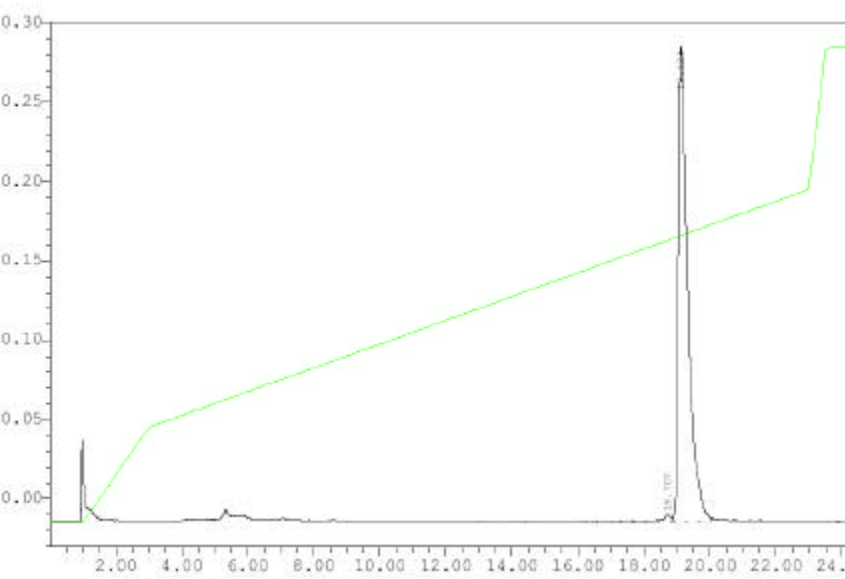
G20 mer, anion-exchange pH 12.5



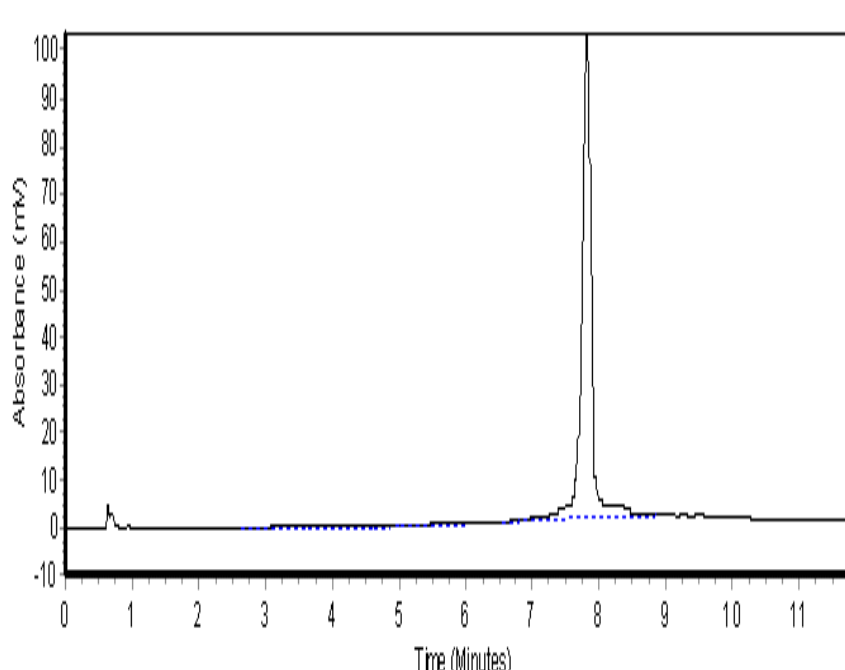
C20 mer, capillary electrophoresis



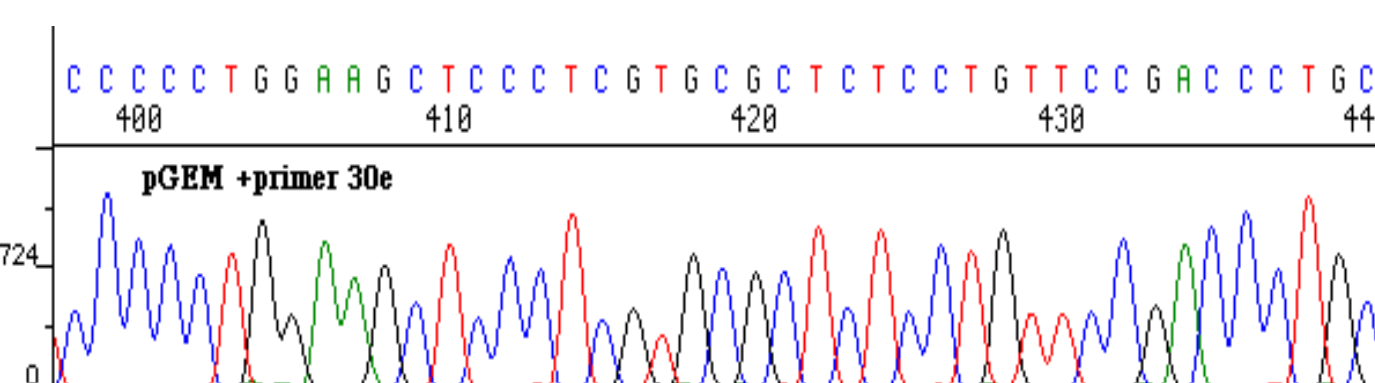
T20 mer, anion-exchange pH 7 5M urea



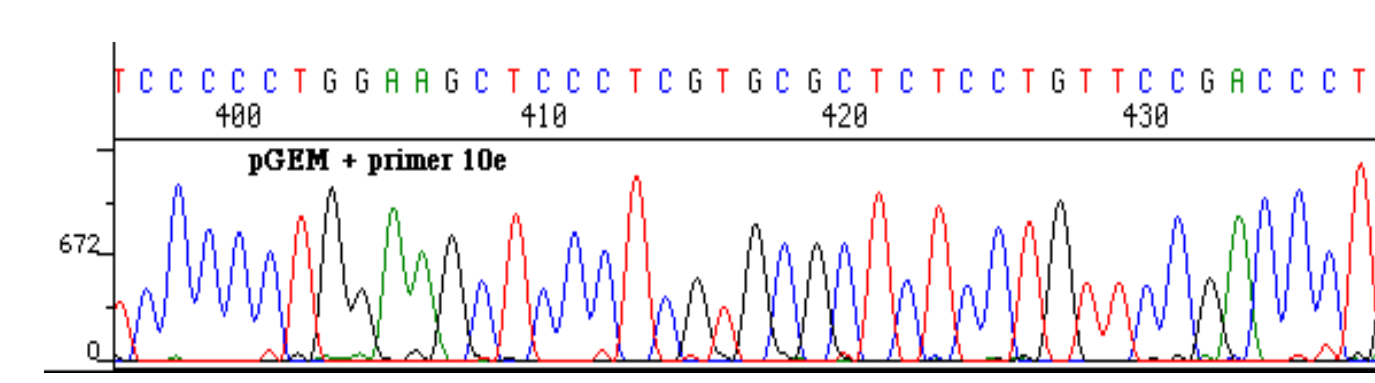
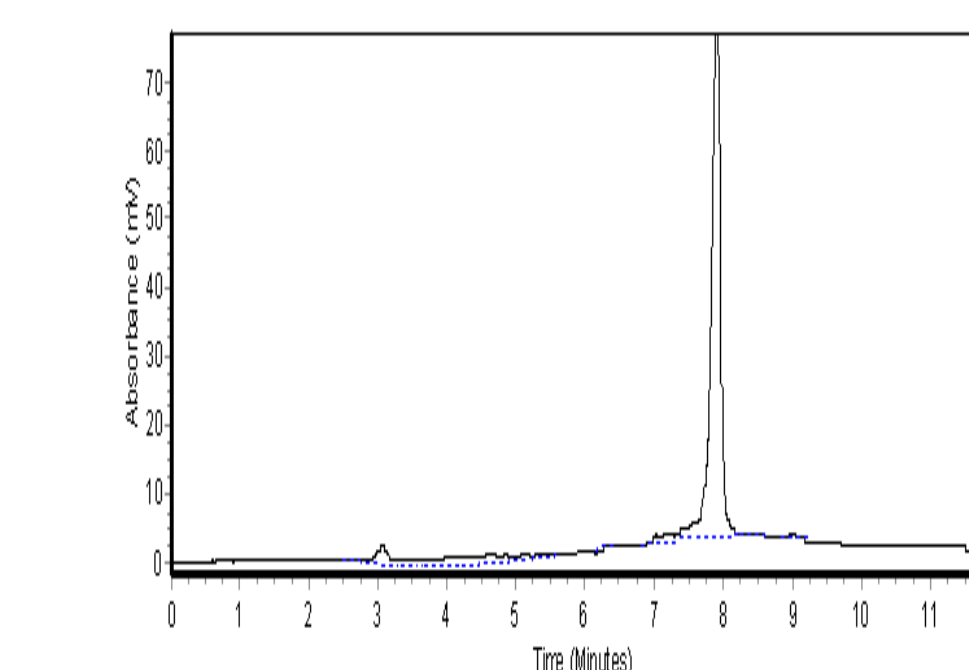
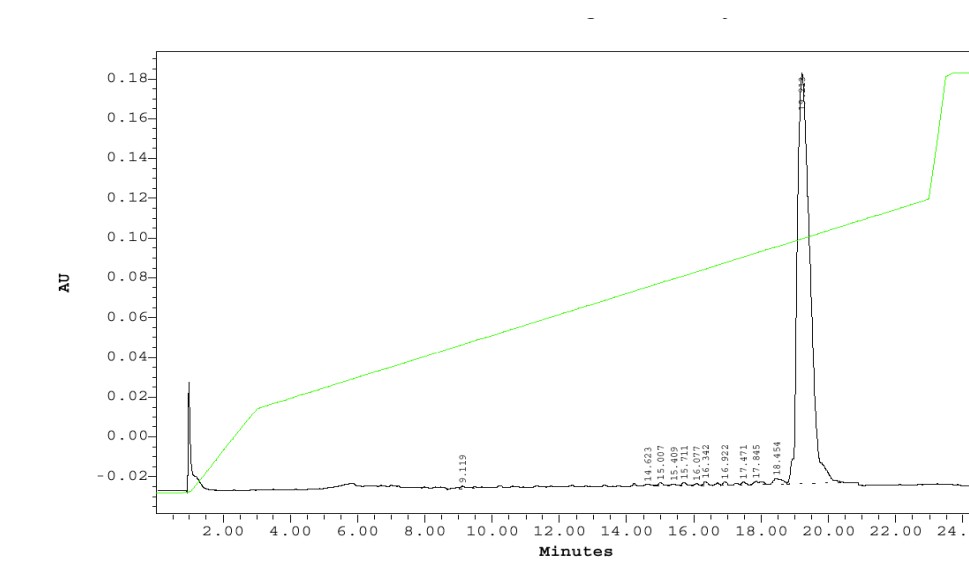
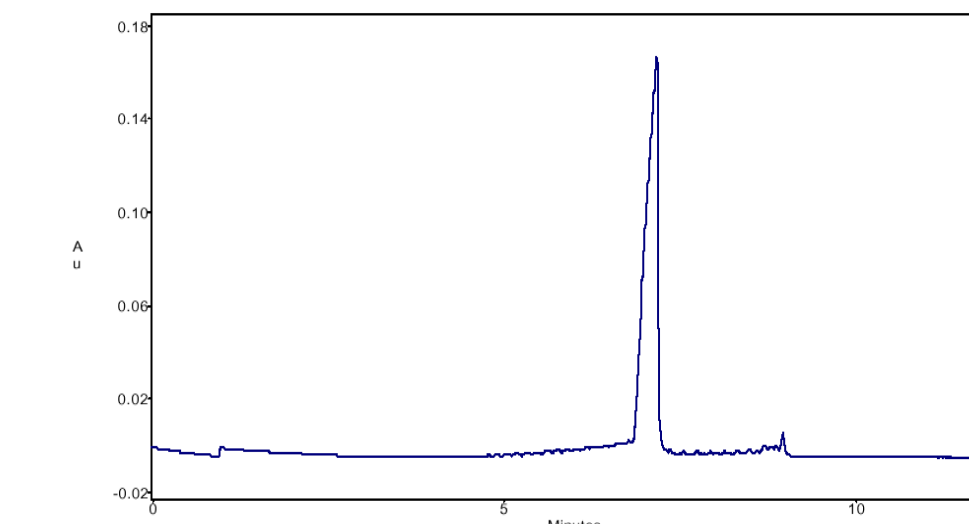
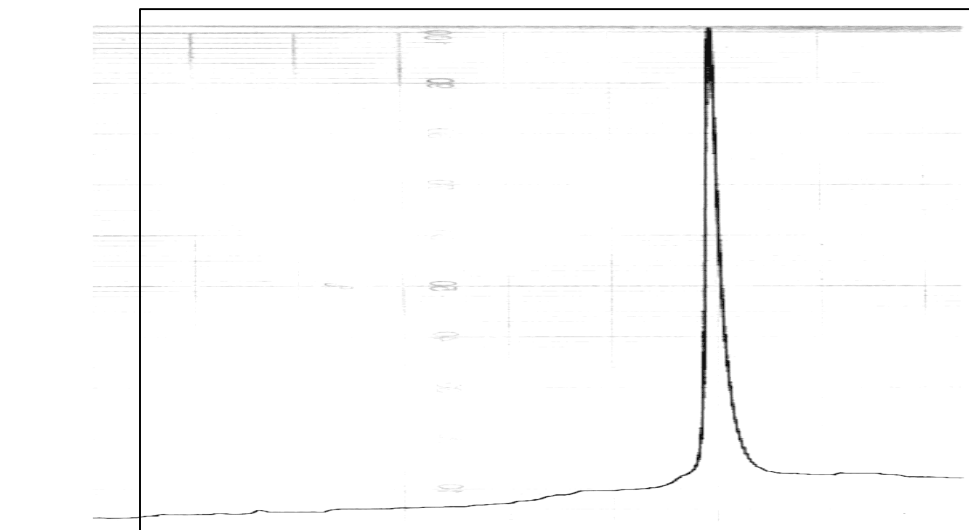
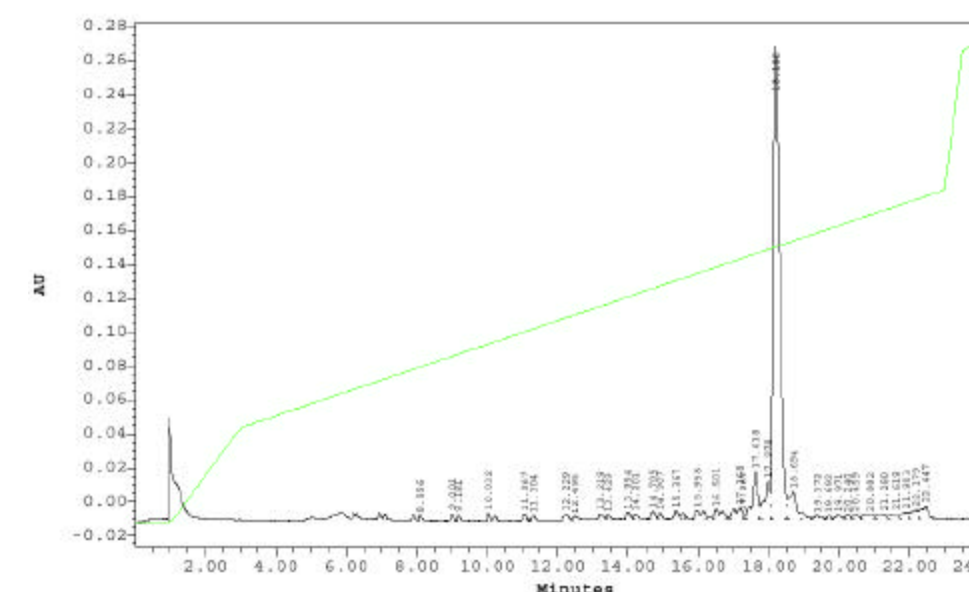
Mixed-base 30mer, DHPLC



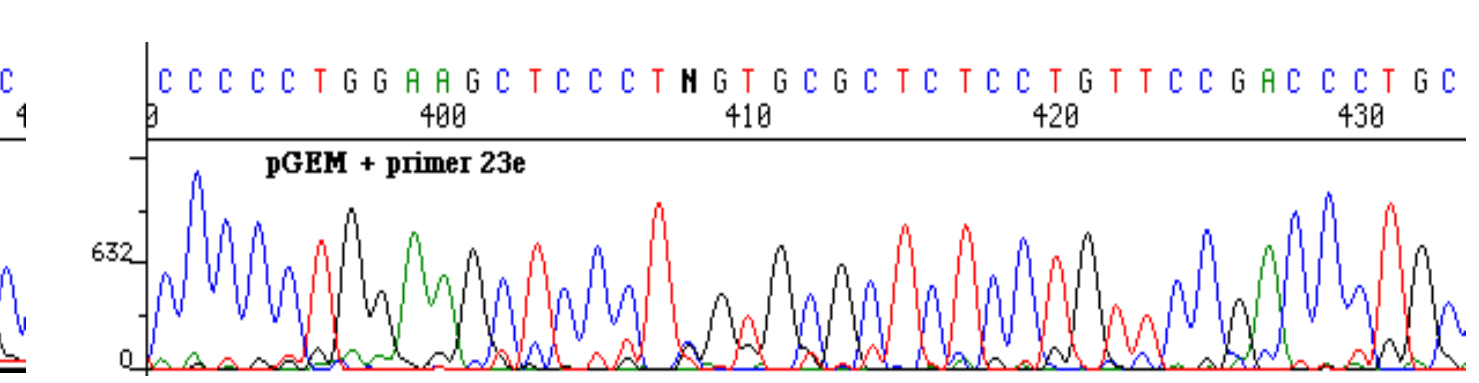
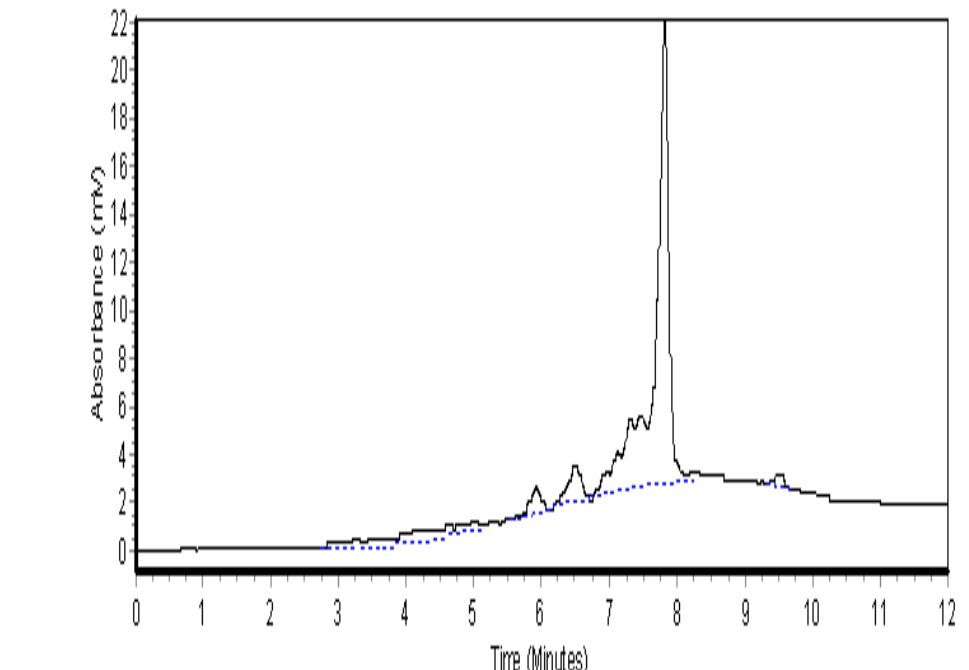
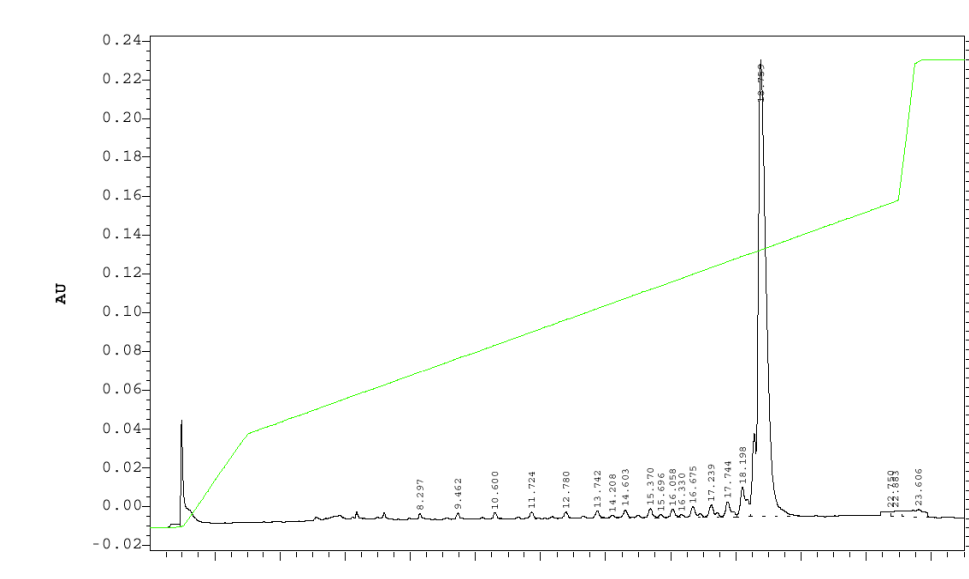
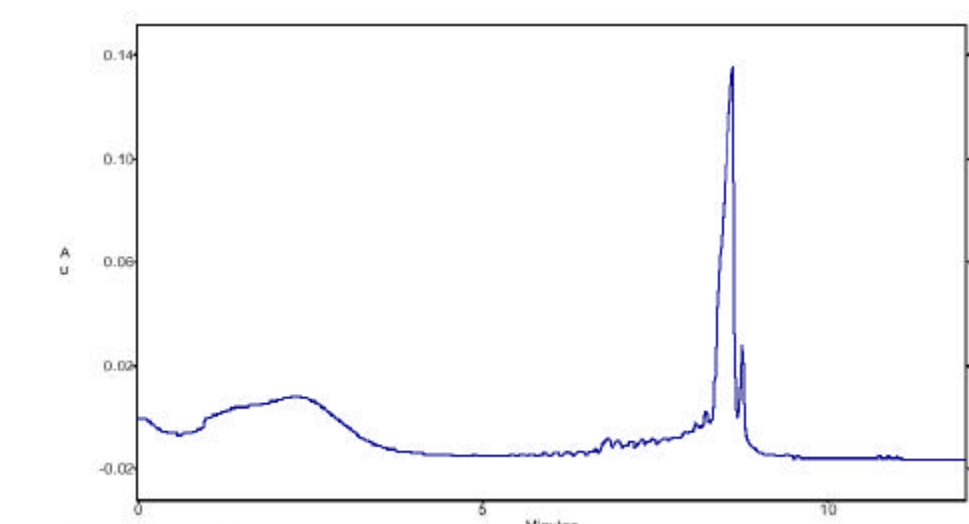
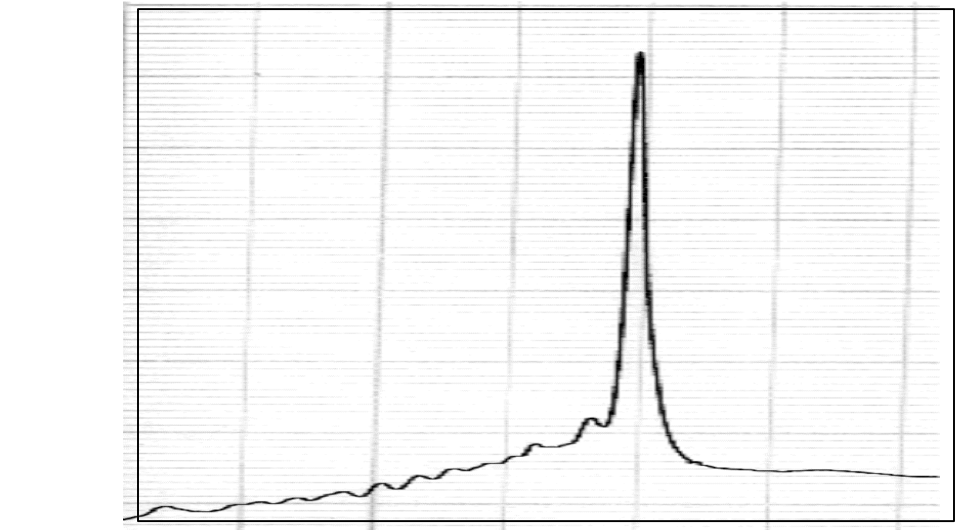
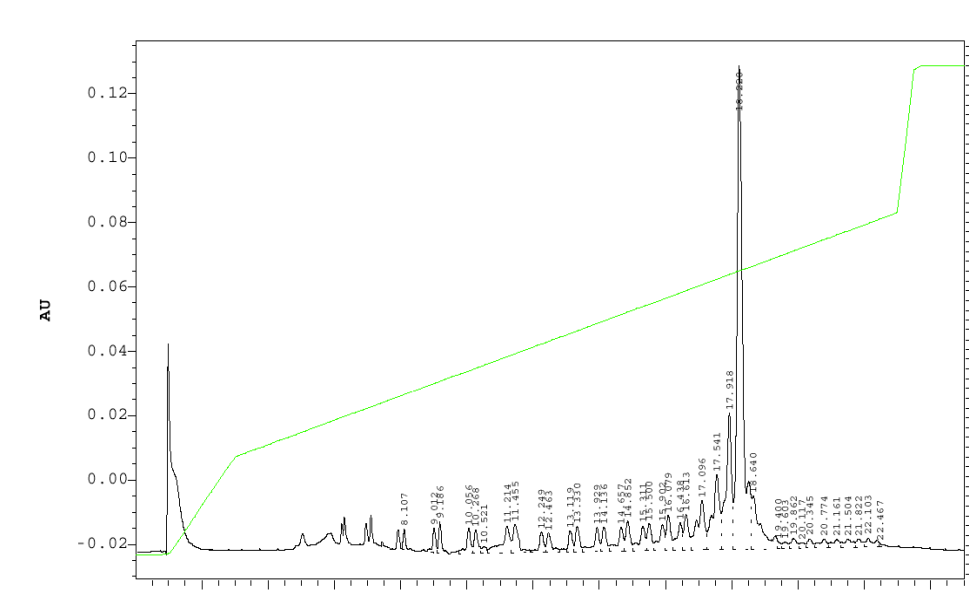
DNA sequencing Pgem with 30 mer primer



Oligonucleotides from instruments with good QC



Oligonucleotides from instruments with low QC



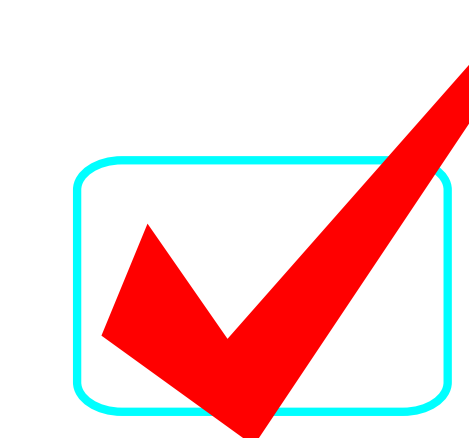
Even instruments that make good primers can have relatively non-optimal column positions

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 31 | 32 | 33 | | |
|------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|--|--|
| Code | 3 | 4 | | 1 | 1 | 8 | 8 | 8 | 8 | 3 | 3 | 3 | | | | | | | | | | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | |
| | 6 | 9 | 2 | 7 | 7 | 4 | 5 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| | 3 | 9 | 1 | # | # | 6 | # | # | # | # | # | # | # | 8 | 8 | 9 | 0 | 4 | M | 1 | 2 | 1 | 8 | # | # | # | 8 | 4 | 1 | | | |
| | 0 | 0 | 4 | 1 | 2 | 1 | 1 | 2 | 3 | 4 | 2 | 1 | 3 | 9 | 4 | 3 | 8 | 9 | C | 2 | 8 | 7 | 6 | 1 | 2 | s | 1 | 0 | 0 | 0 | | |
| A30 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| G30 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C30 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| T30 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

■ Non-optimal column positions

PART II-Conclusion:

- A core facility that QC 100% of the oligonucleotides will detect instrument and chemical failures immediately.
- Test of heteropolymer oligonucleotides will show if the amount of full-length species is sufficient .
- Use of homopolymers in QC can identify situations where the synthesis of some of the four nucleotides are compromised.



Announcements:

- The data files of all the 744 oligo QC analysis chromatograms can be found at:
<ftp://ftp.fccc.edu/yeung/outgoing/>
They files are organized according to lab entry number, or by method of analysis.
- If you have oligos that you like us to analyze for you, please send them to Tony Yeung as one A260/vial according to the format of this study:
<http://ABRF.org>
<http://128.218.106.86/NARG2001/intro2001.html>
- Please stay in touch with us. Thank you for your participation.