



Optimizing MALDI-TOF mass spectrometry sequencing

NJ Governor's School of Engineering and Technology

Ami Greene, Mark Martinez, and Justine Langman



Introduction

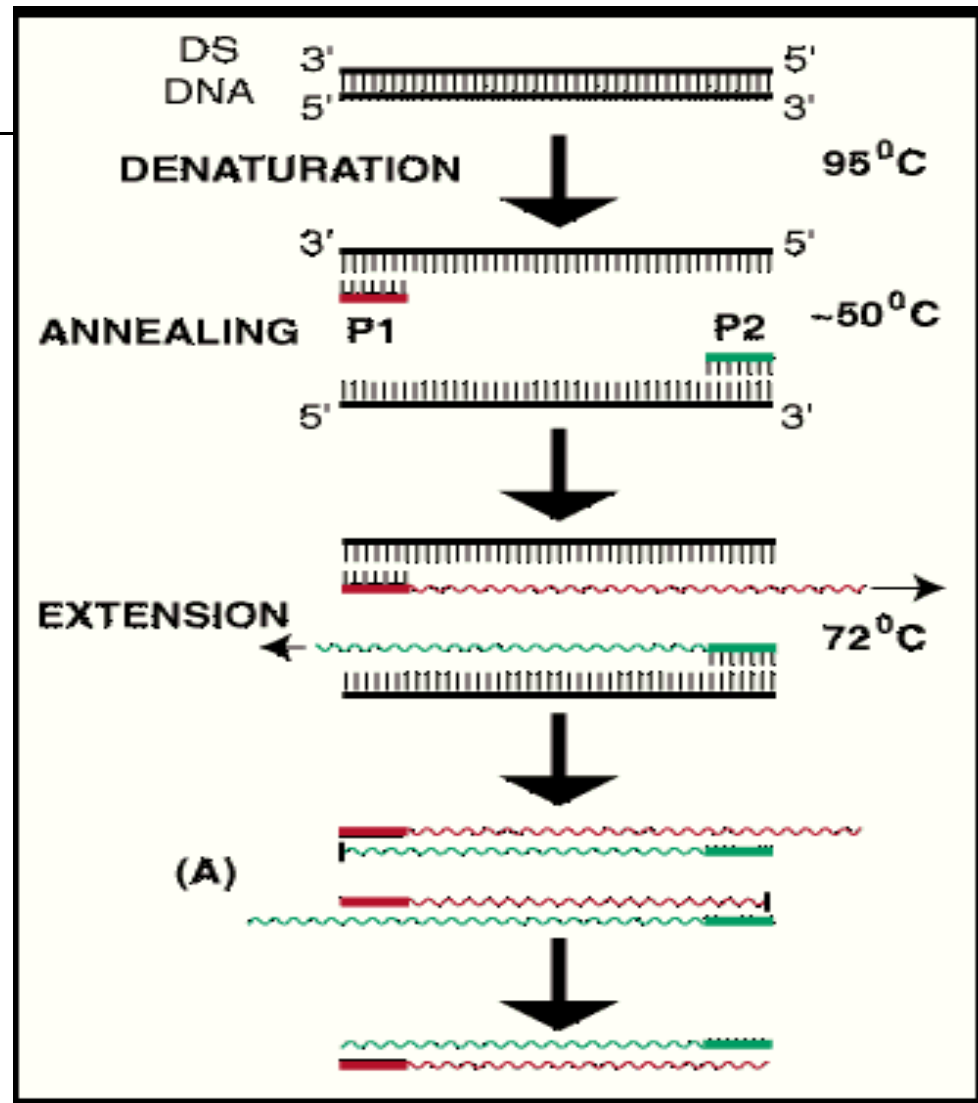
- Genetics is a growing field
- Efficient and Effective Sequencing



Outline

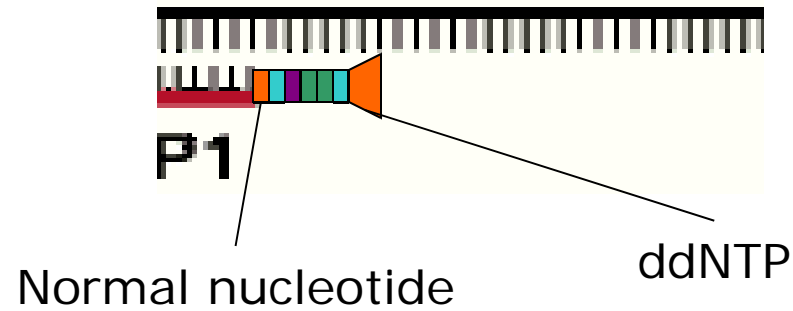
- Background
- Trial results
- Gene sequencing program
- Acknowledgments

PCR



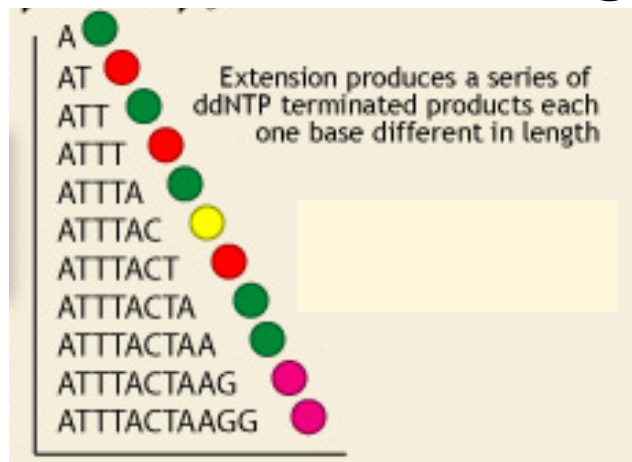
ddNTPs

- Terminate growing strand of DNA



ddNTPs

o 'Ladder' of DNA fragments formed



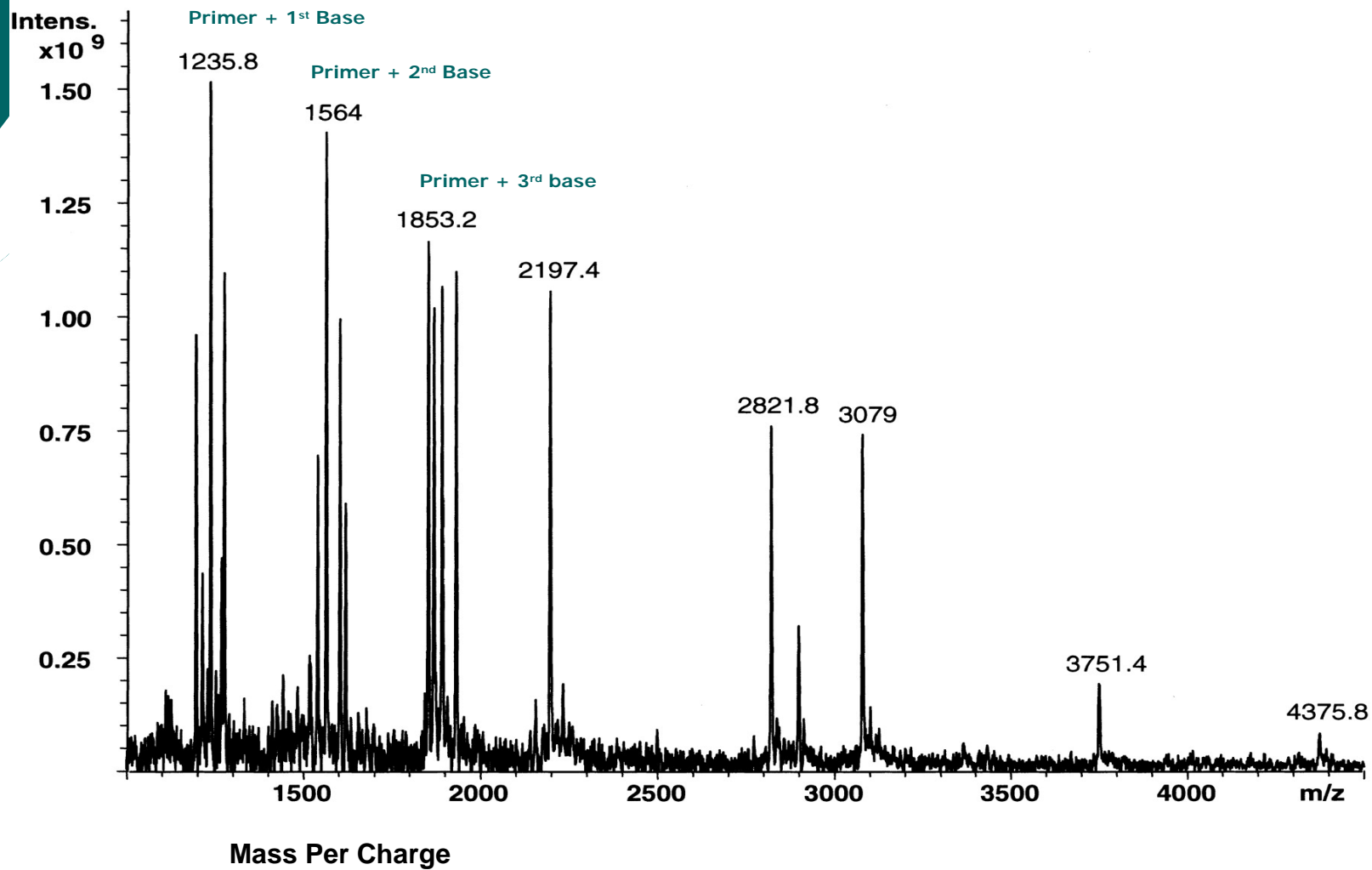


MALDI-TOF MS

- New method of gene sequencing
- Mass per Charge

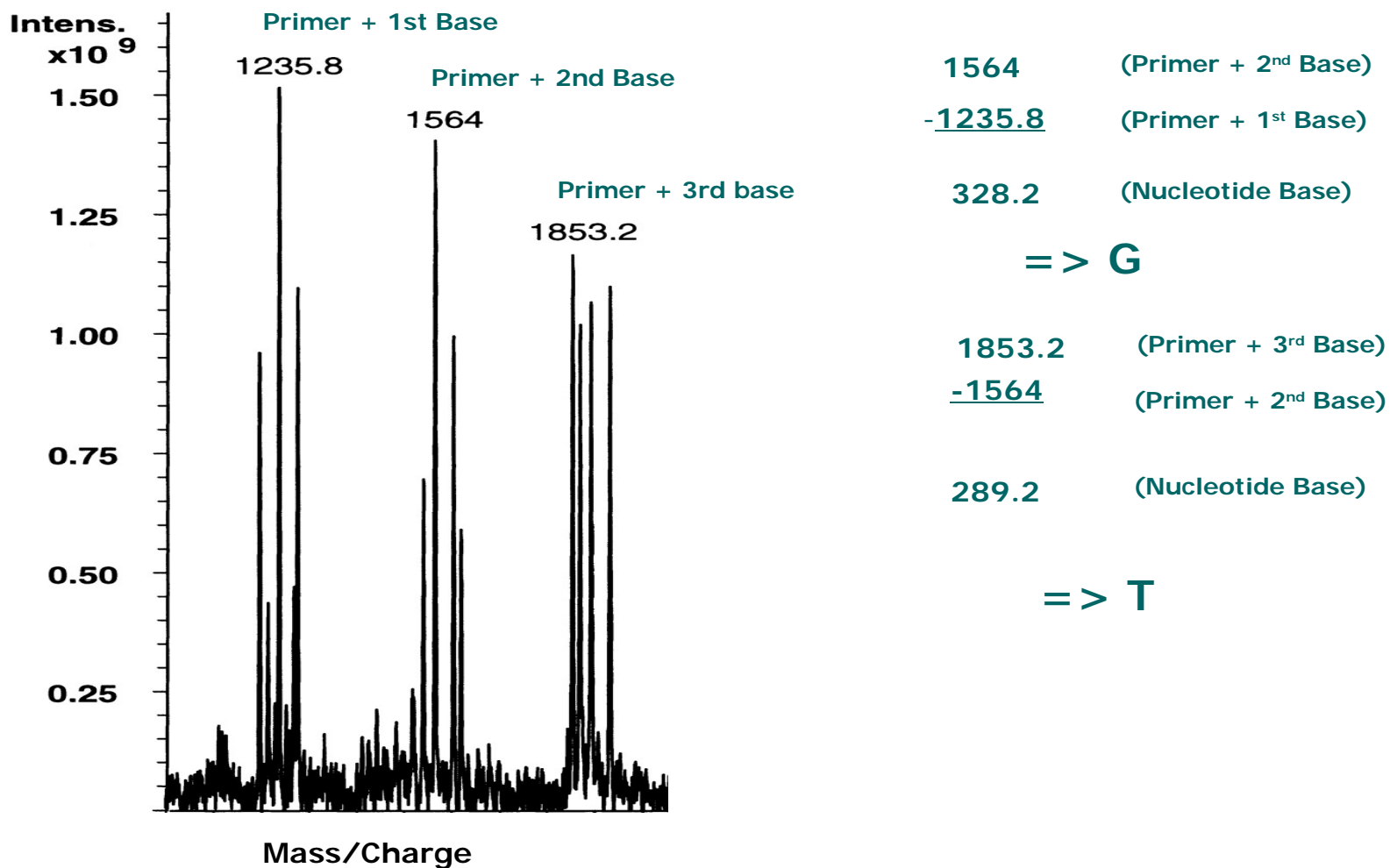
Reading MS data

B



Reading MS data

B





Objectives

- Increase peak size/intensity
- Decrease noise

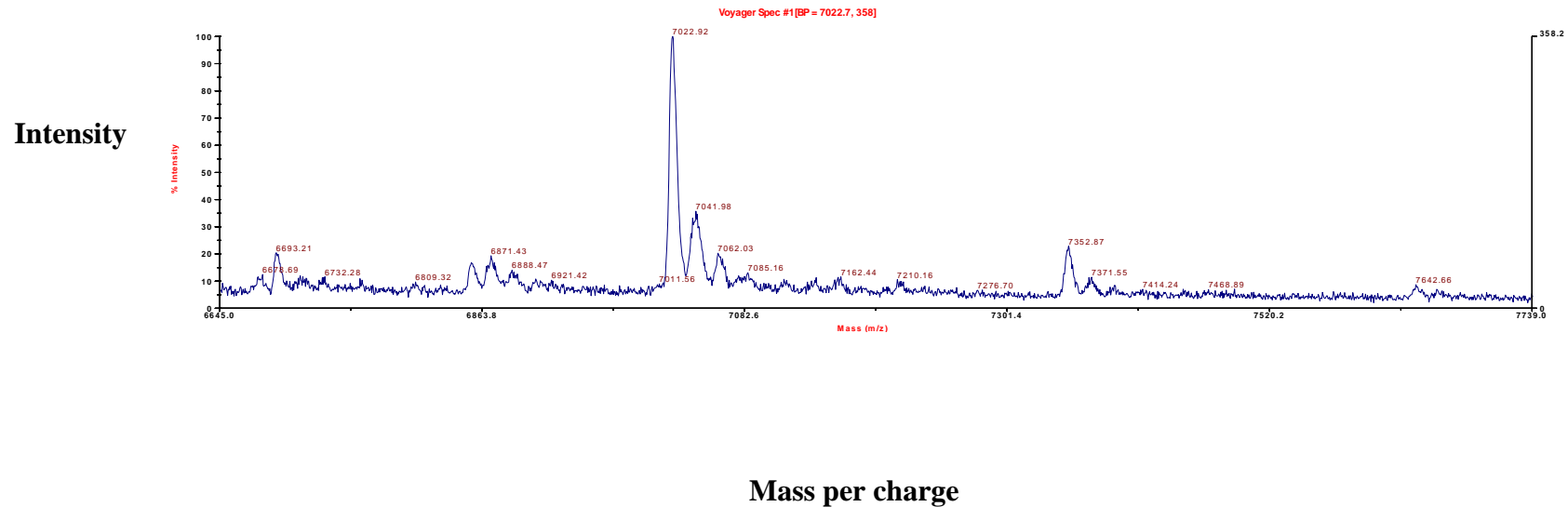


Outline

- **Background**
- Trial results
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Condition #1

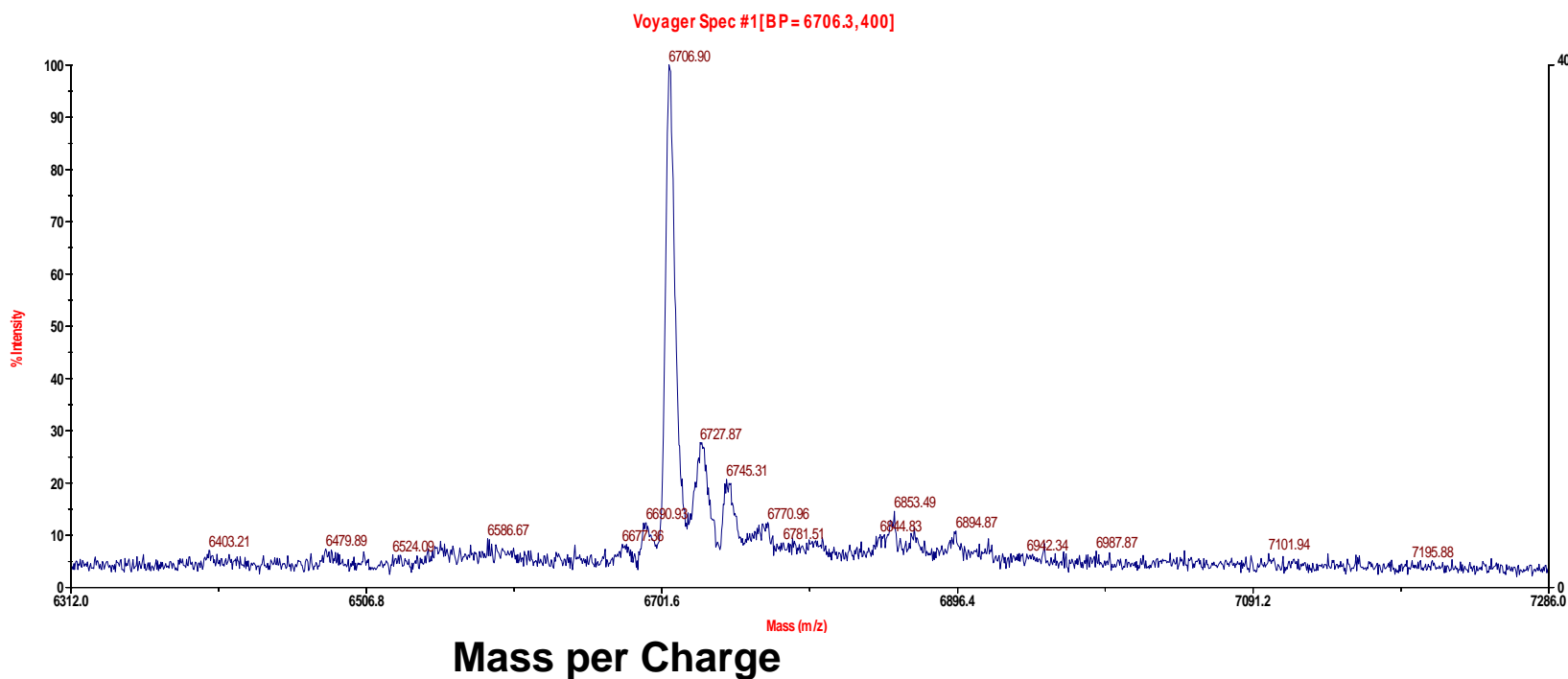
- Standard solution
- Annealing temperature – 57° Celsius
- Annealing time – 30 seconds; extension time – 30 seconds



No Improvement

Increase Annealing Time:

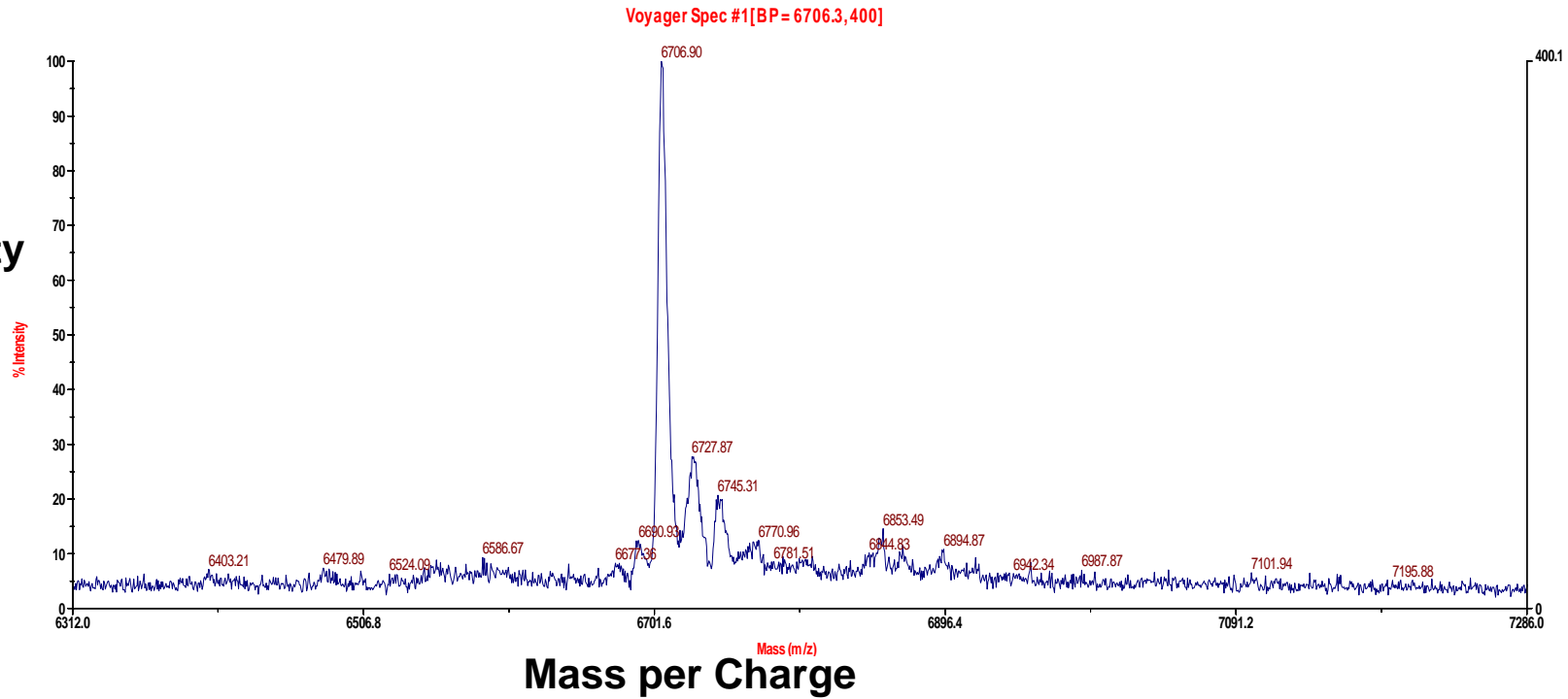
Intensity



No Improvement

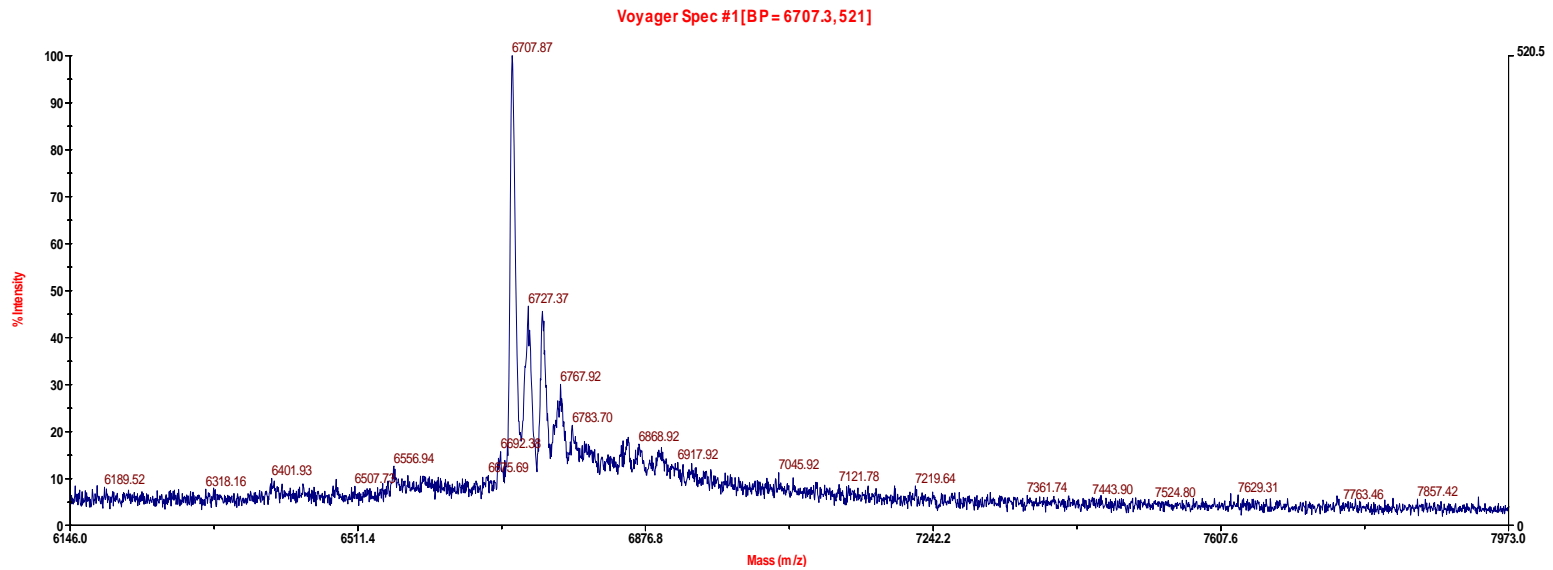
Increase Extension Time:

Intensity



No Improvement

Increase Annealing and Extension Times:

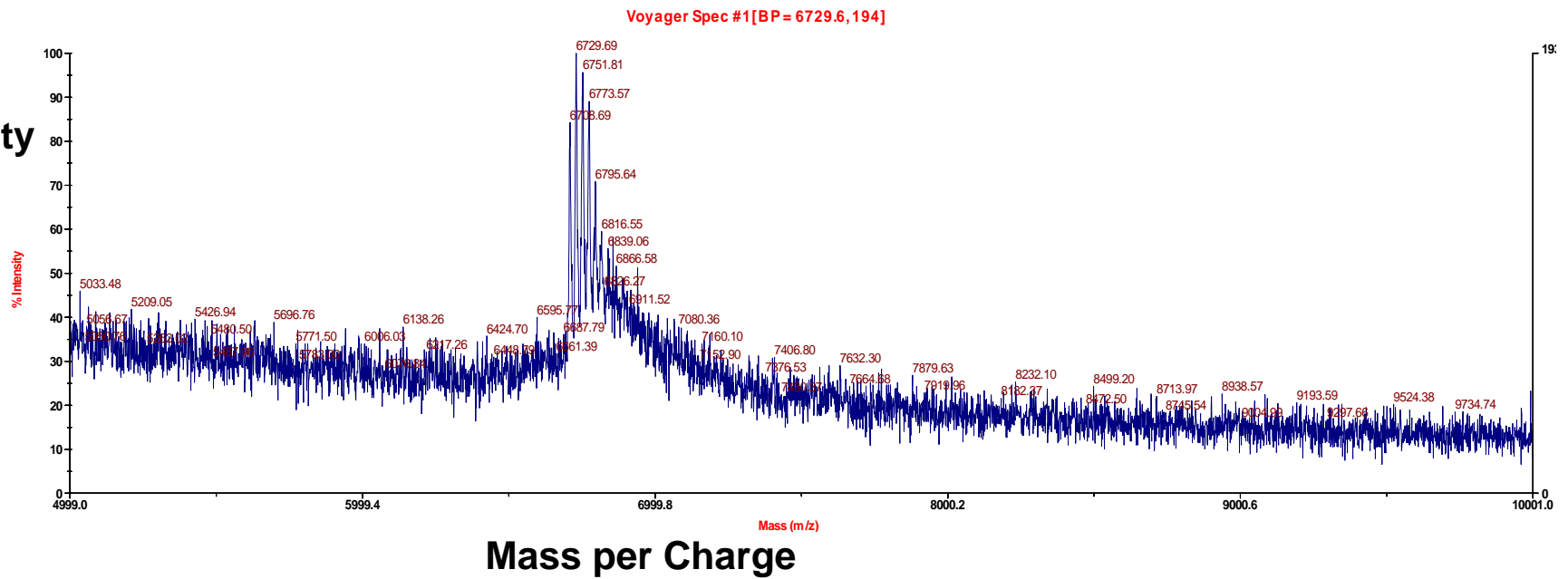


Mass per Charge

No Improvement

Match ddNTP ratios to those in Sequence

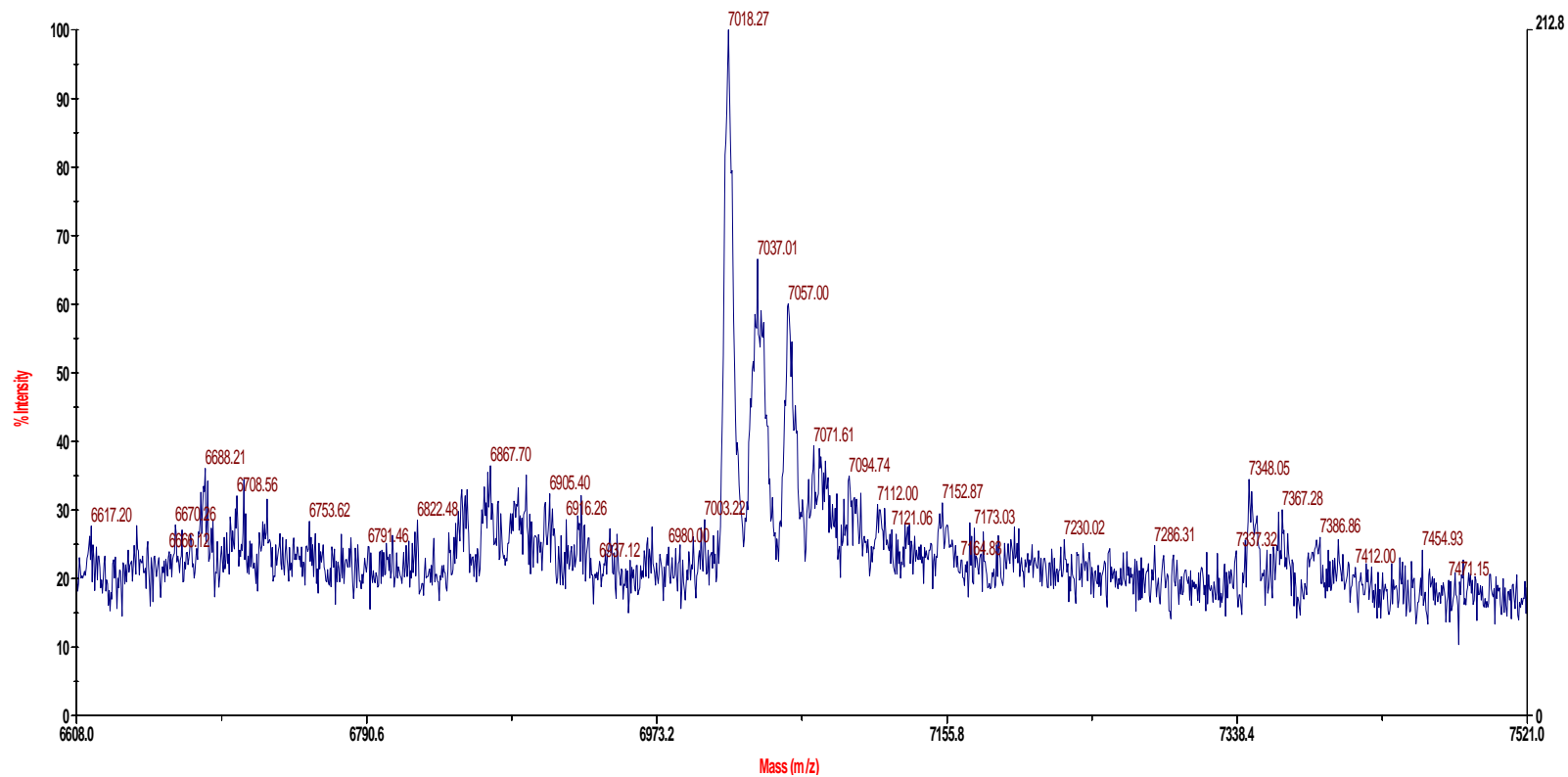
Intensity



Improvements

Decrease Buffer Volume

Voyager Spec #1 [BP=7018.2,213]

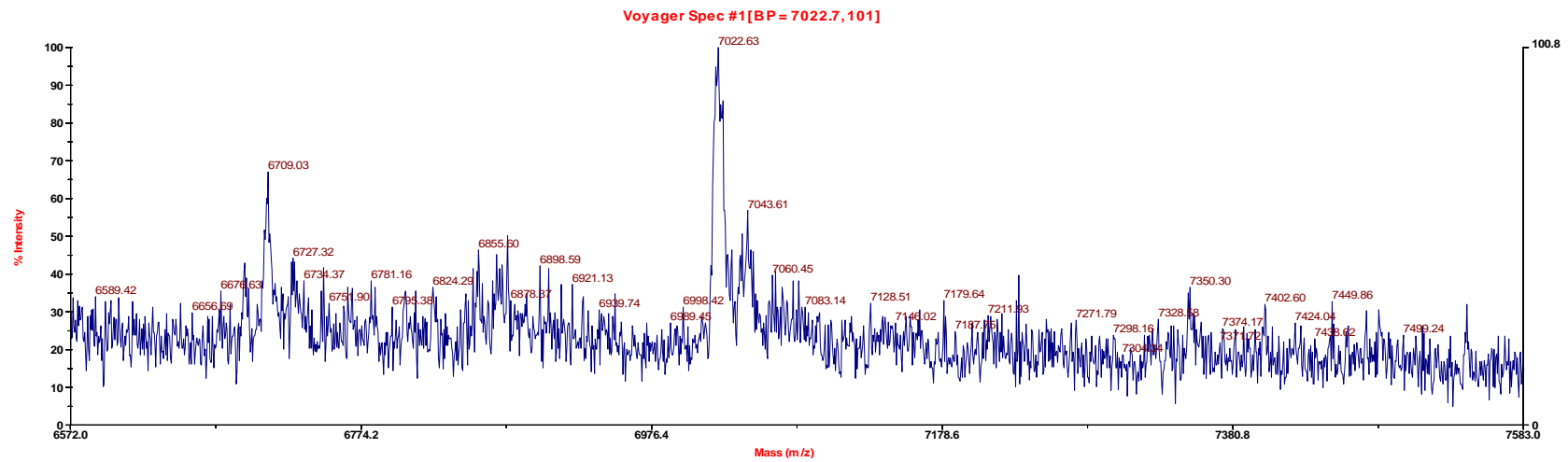


Mass per charge

Intensity

Improvements

Increase Annealing Temperature



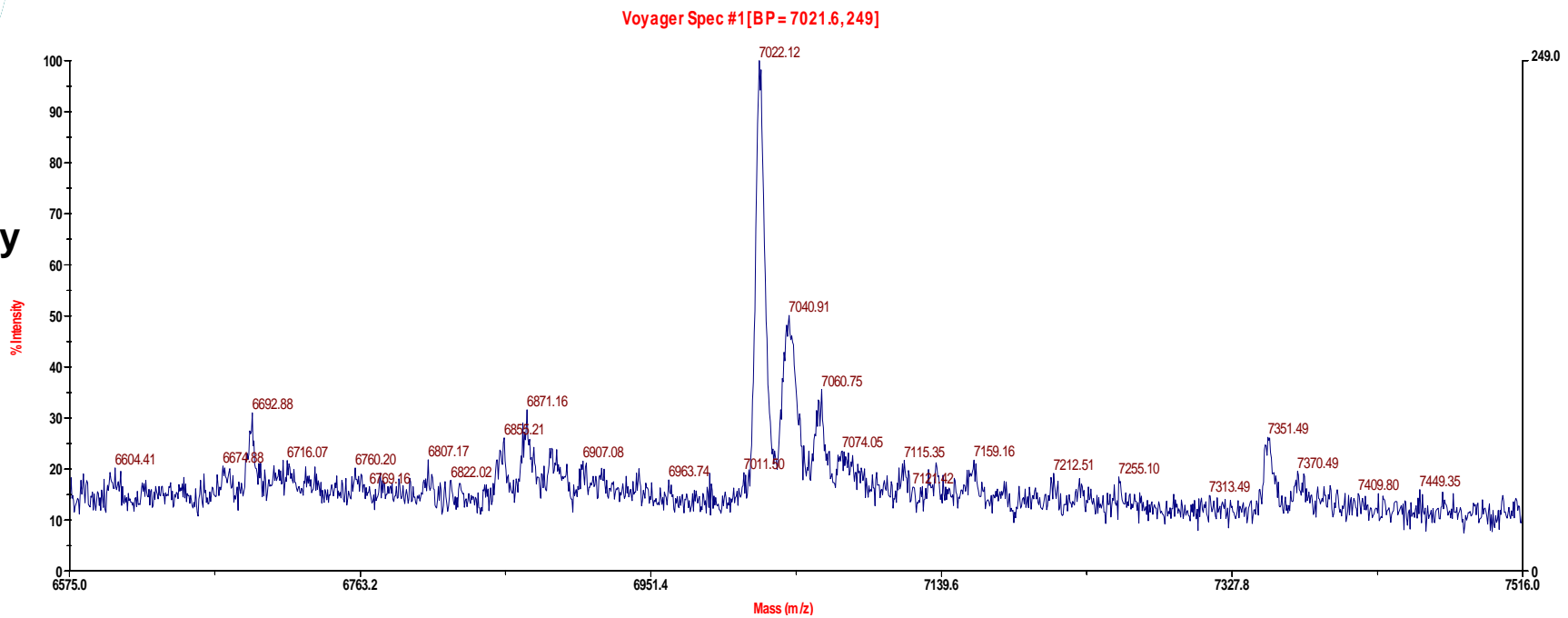
Mass per charge

Intensity

Improvements

Increase dNTP Volume

Intensity



Mass per charge



Further Research

- Increase the number of test trials
- Range the variables
- Test on different genes



Outline

- **Genomic technologies**
- **Trial results**
- Gene sequencing program
- Acknowledgments

Gene sequencing program

- Developed to facilitate sequencing process
 - Input: numerical MALDI-TOF peaks
 - Output: sequence of nucleotide bases

```
Editor - E:\GENEBasesPROGRAM\basic.m
File Edit Text Go Cell Tools Debug Desktop Window Help
[Icons] Stack
- 1.0 + ÷ 1.1 x [Icons]
16 -     peak(1,i)=input('Peak Mass (Including that of Primer:');
17 - end
18
19 -     delta=0;
20
21
22 -     if biotin~=1
23
24 -     for j=1:length
25
26 -         x=peak(1,j+1)-(peak(1,j)+delta);
27
28 -         if x <280.7 && x>265
29 -             base(1,j)='C';
30 -         elseif x>280.7 && x<293.7
31 -             base(1,j)='T';
32 -         elseif x>293.7 && x<305.2
33 -             base(1,j)='A';
34 -         elseif x>305.2 && x<320
35 -             base(1,j)='G';
36 -         else
37 -             base(1,j)='error';
38 -         end
39
40
41 -         if base(1,j)=='A'
42 -             delta=15;
43 -         elseif base(1,j)=='C'
44 -             delta=16;
45 -         elseif base(1,j)=='T'
46 -             delta=16;
47 -         elseif base(1,j)=='G'
48 -             delta=15;
49 -         end
50 -         base=char(base)
51
52 -     end
53 - end
```




Outline

- **Background**
- **Trial results**
- **Summary**
- **Gene sequencing program**
- **Acknowledgments**

Thank You

- Professor Sobin Kim, Dan Duffield, Diana Arellano
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Questions?

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