Primer design using Vector NTI

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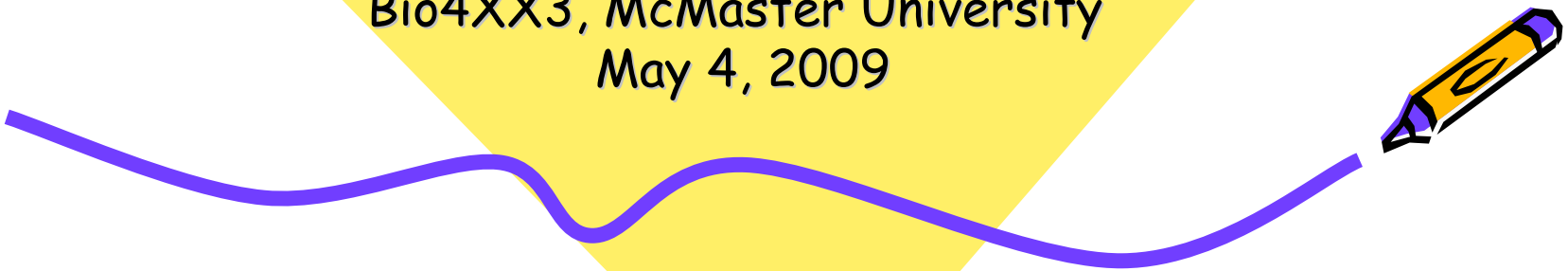

PCR

1. PCR: Amplification of target DNA sequences
2. Steps:
    1. Denaturation
    2. Annealing
    3. Extension

    } 30 cycles
3. Template + Primers + Taq Polymerase + dNTP + buffer ($MgCl_2$)

Primer design

1. Primer Length: 18-22 bases
2. Primer Melting Temperature: 52-58 °C
3. Primer annealing temperature
4. GC Content: 40%-60%
5. GC Clamp: GC at 3'
6. Primer Secondary Structures: try to avoid
7. Repeats: maximum 4 repeats: ATATATAT
8. Runs: maximum 4: AAAA
9. End Stability: less stability at 3', less false priming
10. Avoid Template secondary structure
11. Avoid Cross homology

PCR primer guidelines

- Tech note from Premierbiosoft
- http://www.premierbiosoft.com/tech_notes/PCR_Primer_Design.html

Assignment

- Design a pair of primers to amplify the *cm* gene of pCA24N.
- Send your candidate primers to bio4xx3@gmail.com (place "pACN24 primers" in the subject line

Vector NTI

- Data management program
- DNA and protein sequence storage and analysis
- Cloning and digestion map
- Sequence alignment
- Retrieve sequence directly from NCBI database
- Primer design