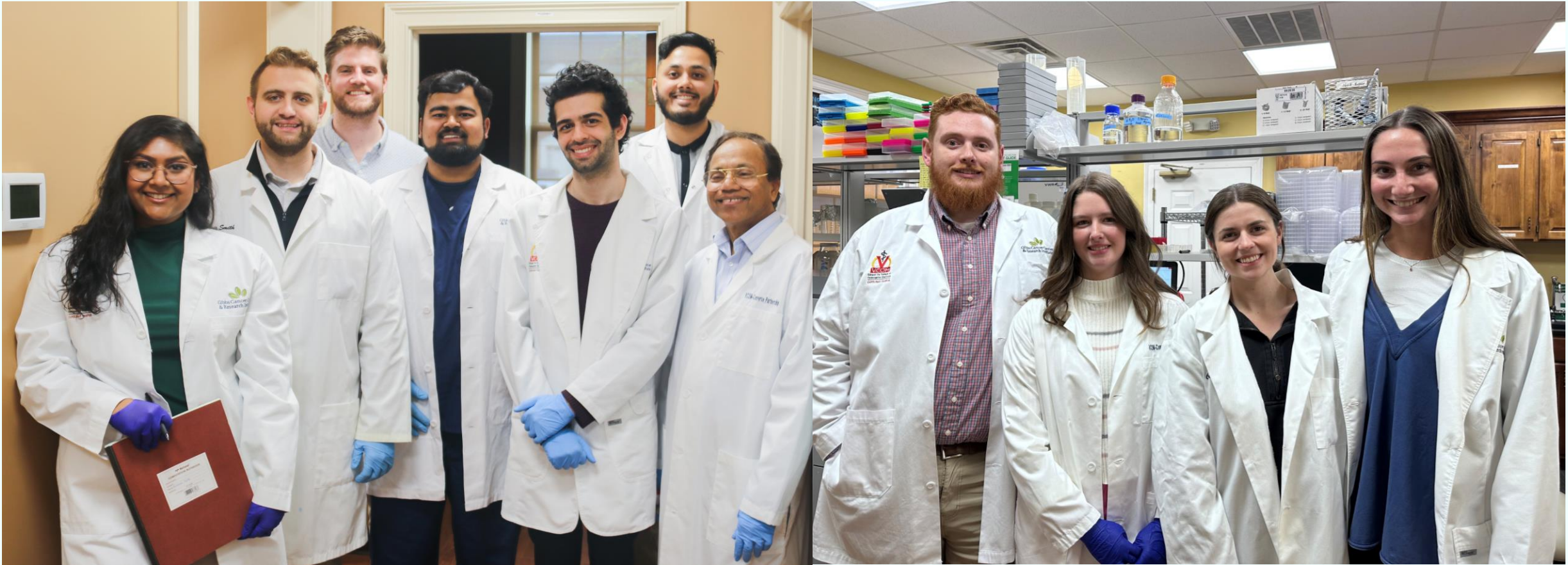


Noncanonical DNA structures and Diseases

Bidyut Mohanty, PhD

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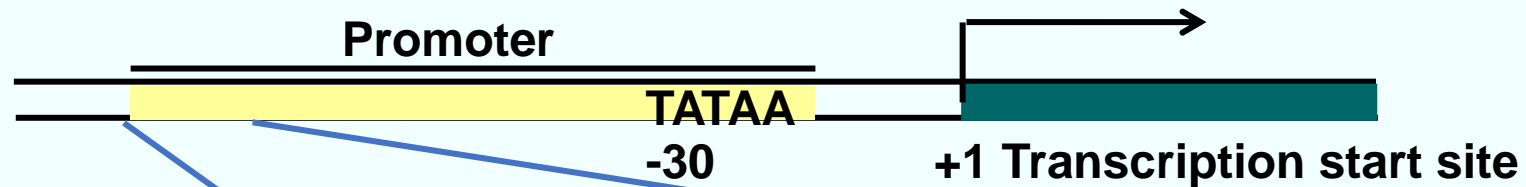
Researchers (from left to right):

Krishna Patel, **Christopher Smith**, **Levi Diggins**, **Venkata Kolluru**, **Christopher Syed**, **Chirag Lodha**, **Bidyut Mohanty**, **Daniel Ross**, **Olivia Lewis**, **Rachel Daley**, **Rebecca Corallo**.

DNA



PROMOTER-PROXIMAL REGIONS OF SEVERAL ONCOGENES CONTAIN G/C-RICH DNA SEQUENCES



c-MYC G strand:

5'-TGGGGAGGGTGGGGAGGGTGGGGAAGG-3'

c-MYC C strand:

3'-ACCCCTCCCACCCTCCCACCCTTCC-5

BCL-2 G strand:

5'-AGGGGCGGGCGCGGGAGGAAAGGGGGCGGGAGCGGGGCTG-3'

BCL2 C strand:

3'-TCCCGGCCGCGCCCTCCTTCCCGCCCTCGCCCCGAC-5'

G-rich and C-rich DNA form unusual structures

c-MYC G strand:

5'-TGGGGAGGGTGGGGAGGGTGGGGAAGG-3'

c-MYC C strand:

3'-A**CCCC**T**CCC**A**CCCC**T**CCC**A**CCCC**TTCC-5

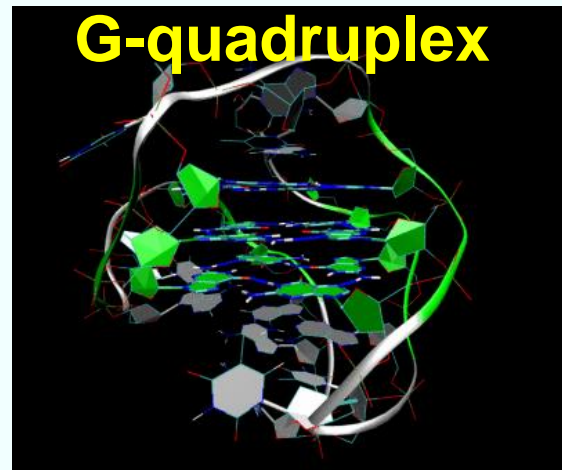
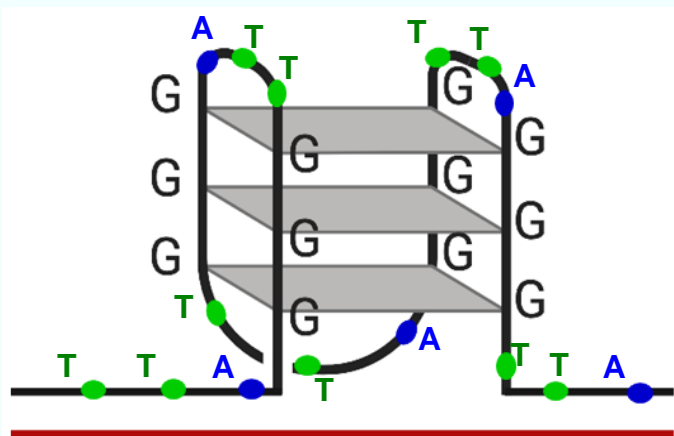
BCL-2 G strand:

5'-AGGGG**CGGG**CG**CGGG**AGGAAGGGGG**CGGG**AG**CGGG**GCTG-3'

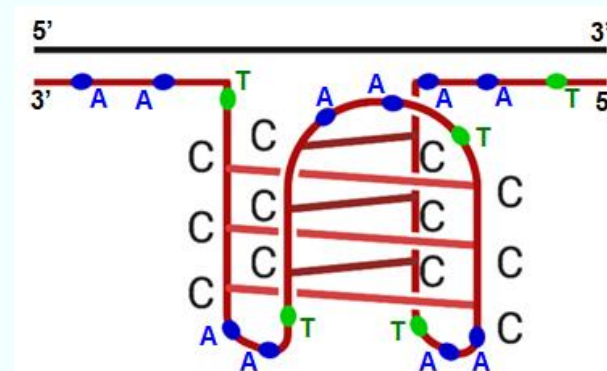
BCL2 C strand:

3'-T**CCCC**G**CCC**GCG**CCCTC**CTT**CCCC**G**CCCTC**G**CCCC**GAC-5'

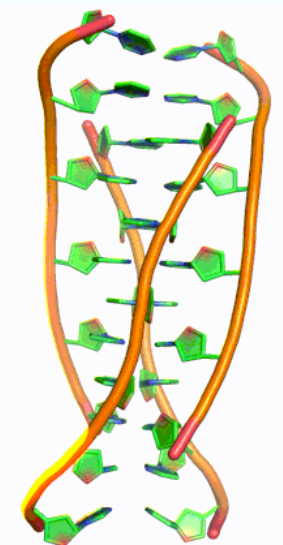
G-rich DNA forms G-quadruplex



C-rich DNA forms i-motif



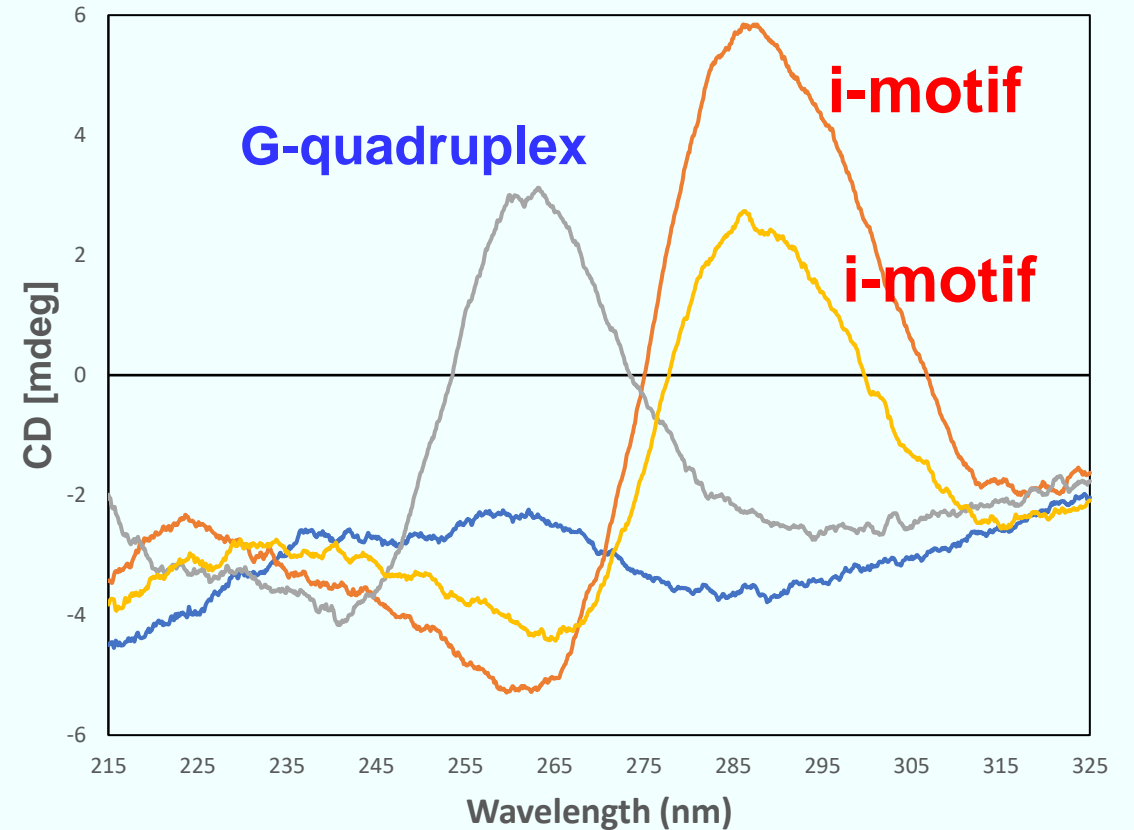
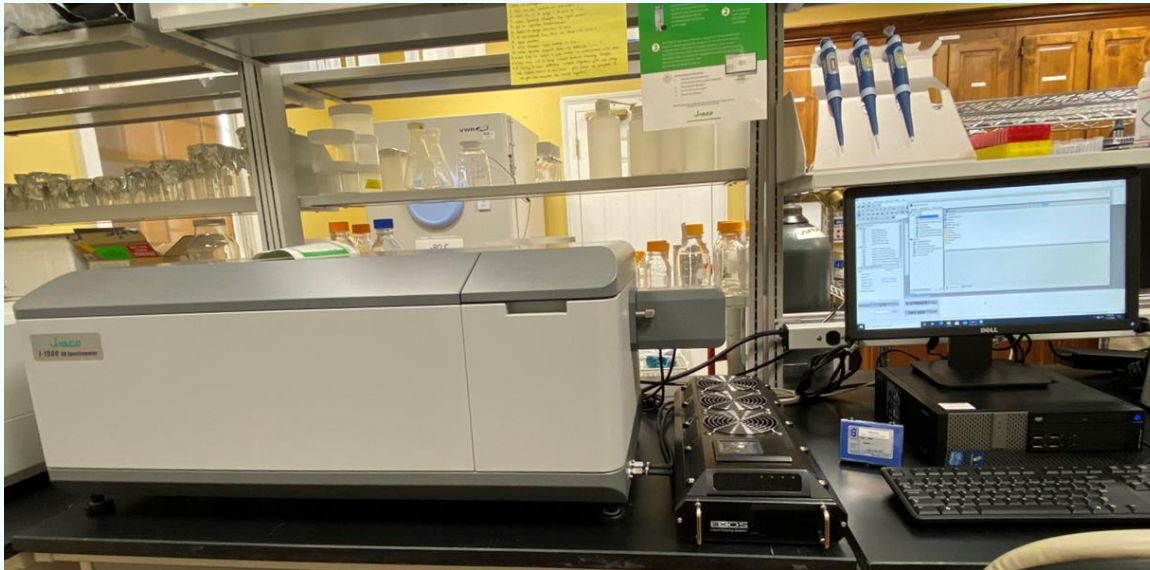
i-motif



How do you analyze noncanonical DNAs?

Circular Dichroism (CD)

CD spectroscope



Contact:

Daniel Ross

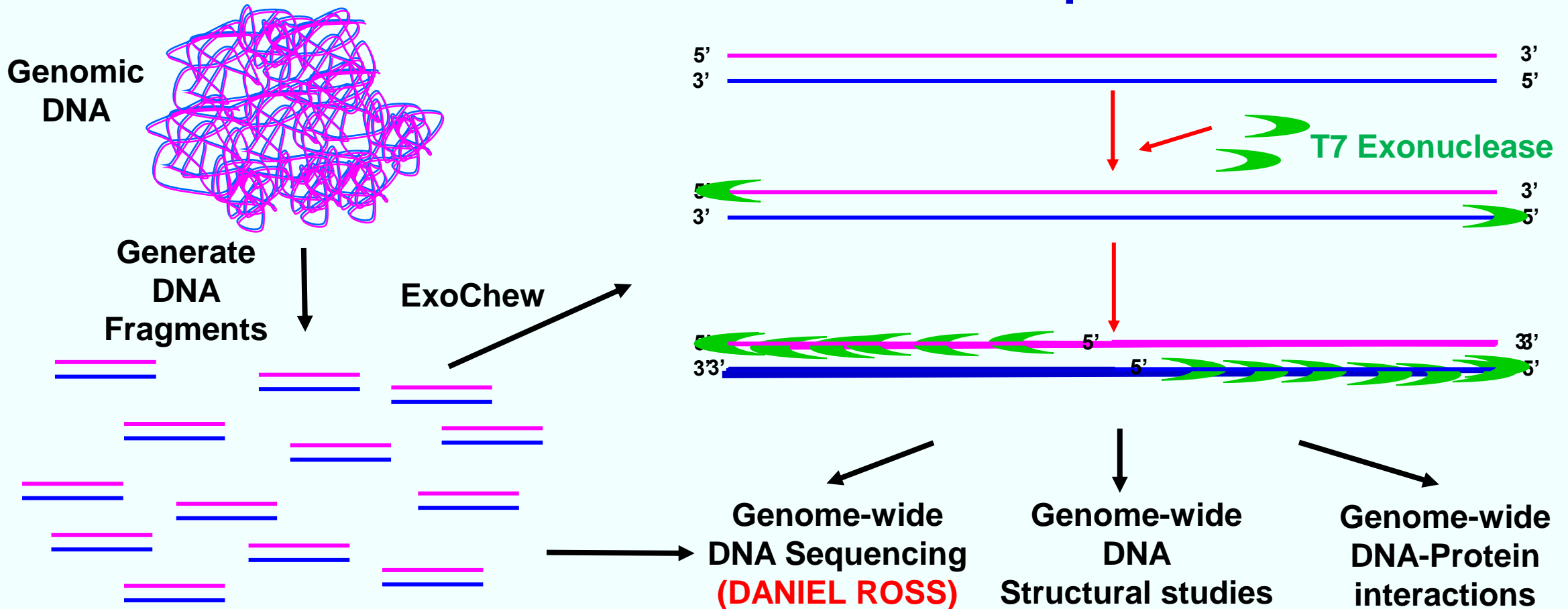
Magnolia Building
VCOM-Carolinas

— Buffer — Myc C — Myc G — BCL2

Levi Diggins, Rachel Daley, Rebecca Corallo
Daniel Ross, Olivia Lewis

How to study noncanonical DNA structures genome-wide?

Generate an ssDNA library from a dsDNA library by
Novel **ExoChew** technique:



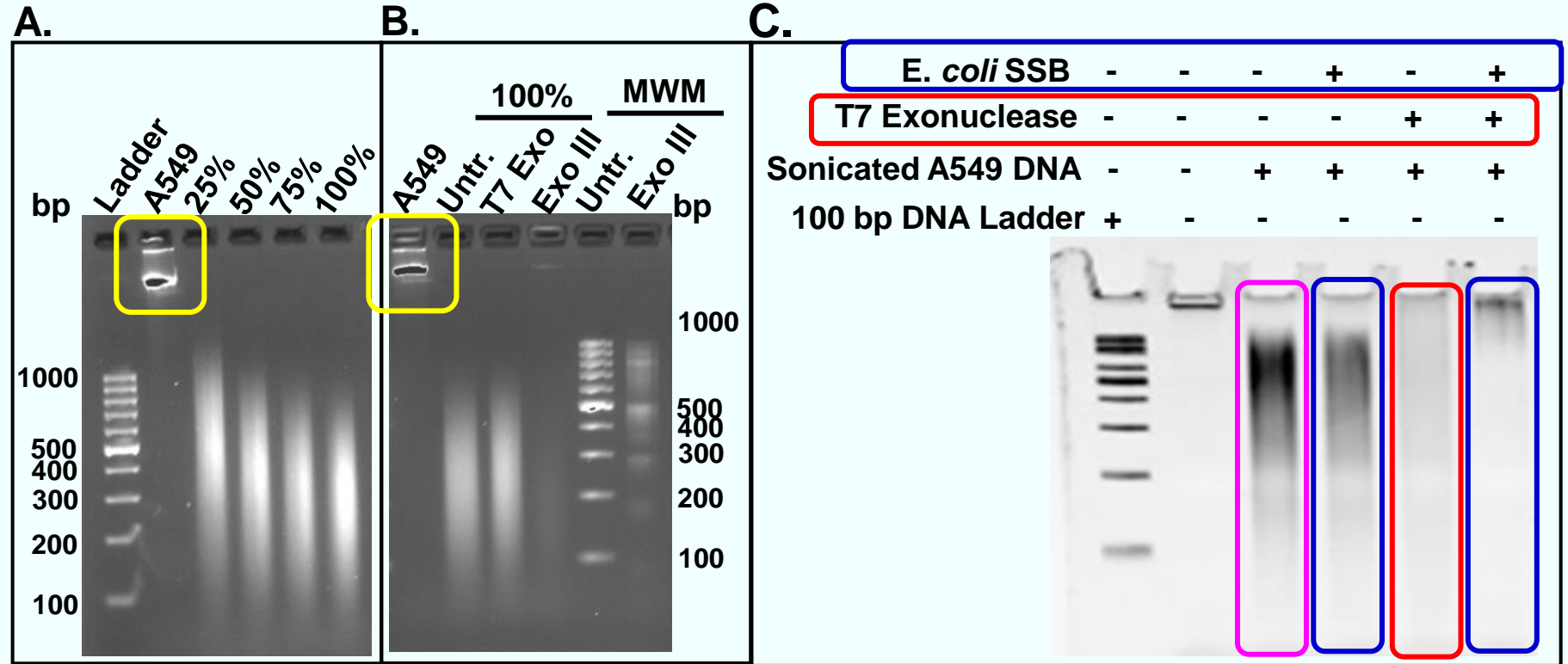
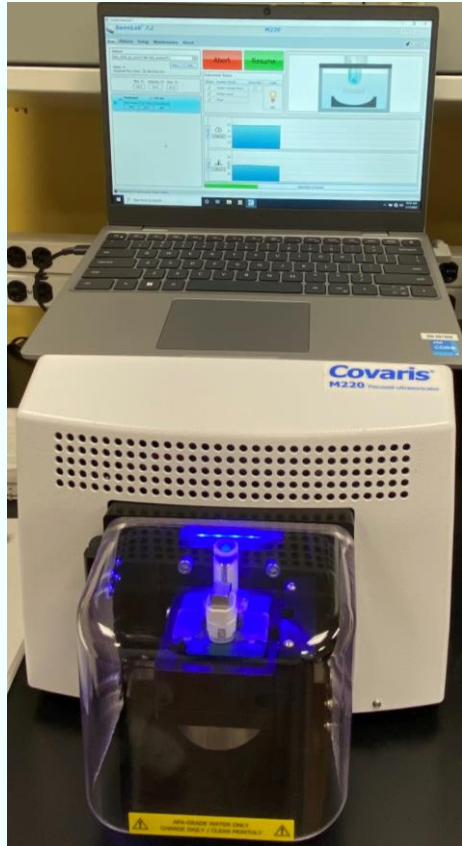
Patel, K., Lodha, C., Smith, C., Diggins, L., Kolluru, V., Ross, D., Syed, C., Lewis, O., Daley, R. and Mohanty, BK. (2023) ExoChew: An exonuclease technique to generate single-stranded DNA libraries. Biorxiv. doi: <https://doi.org/10.1101/2023.10.02.560524>. 2023.

Thank you!

Questions?



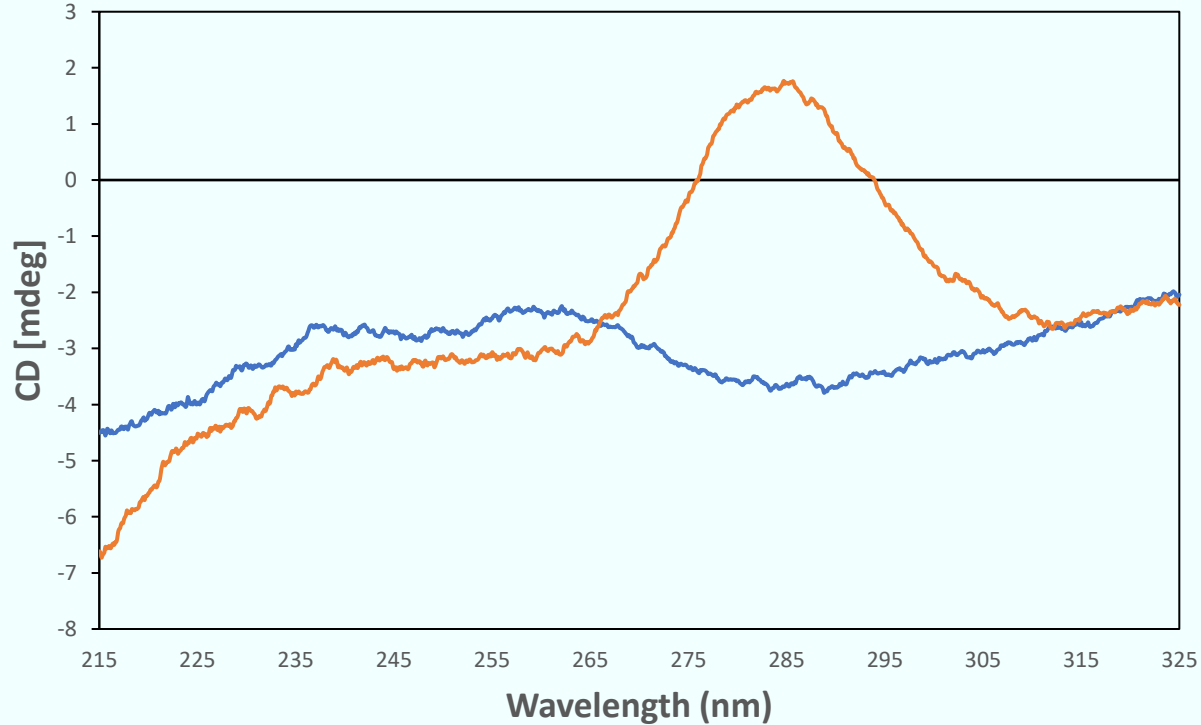
ExoChew with human genomic DNA



Both i-motif and G-quadruplex can form on same strand of DNA with (CCCCGG)₁₄ at high pH

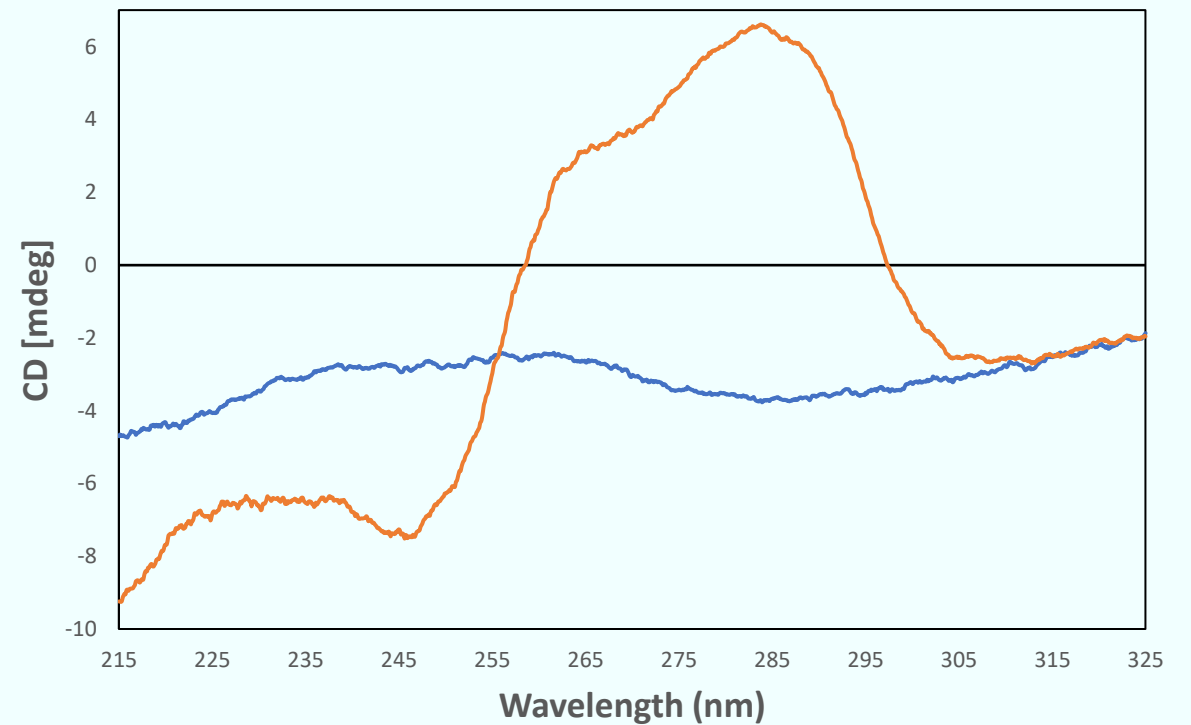
i-motif is formed at low pH
Peak at ~ 287 nm

NaCaco pH 5.5



G-quadruplex and i-motif are formed at high pH by (CCCCGG)₁₄

Tris KCl pH 7.5

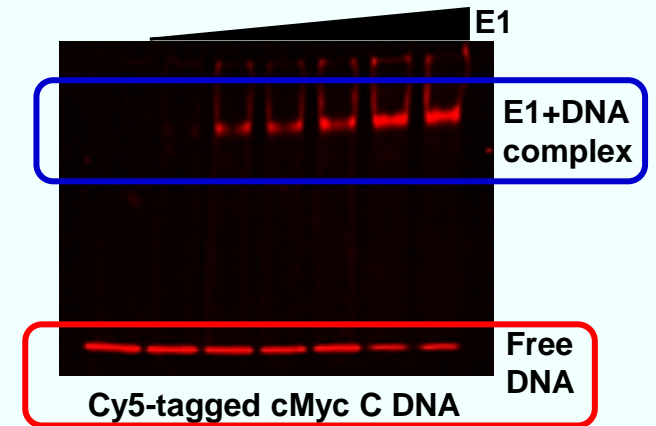
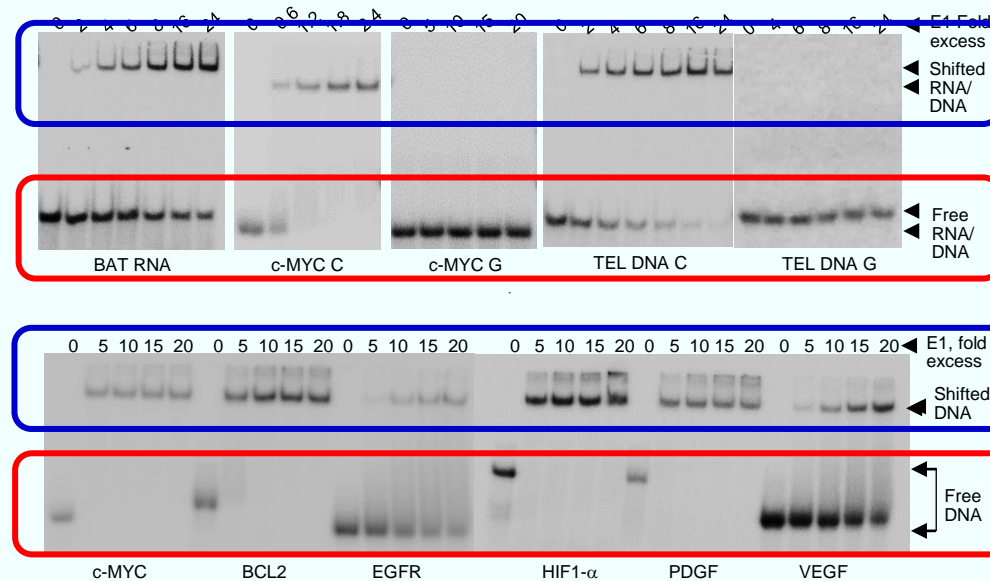
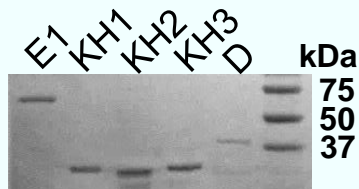
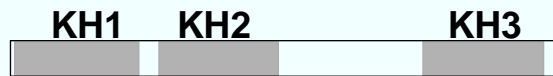


Electrophoretic mobility shift assay (EMSA)

c-MYC C: 5'-CCTTCCCCACCCTCCCCACCCTCCCCA-3'
 c-MYC G: 5'-TGGGGAGGGTGGGGAGGGTGGGGAAAGG-3'
 TEL DNA C: 5'-TAACCCTAACCCTAACCCTAACCCTAACCCTAA-3'
 TEL DNA G: 5'-TTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTA-3'
 BCL2 C: 5'CAGCCCCGCTCCCGCCCCTTCCTCCCGCGCCCGCCCCT-3'

hnRNP E1 protein:

1. Binds ssDNA and RNA.
2. Binds poly-C sequences.
3. Three domains:
---KH1, KH2 and KH3.



ExoChew can be used to generate libraries of chromosomal DNA and find all sequences that bind hnRNP E1

Genome-wide sequencing facility at VCOM-Carolinas

SEQUENCE –

- dsDNA libraries,
- ssDNA libraries and
- specific groups ssDNAs

E. coli genome – a paradigm – 4.5 X 10⁶ base pairs
Human- ultimate goal- 3.9 X 10⁹ base pairs

Oxford Nanopore DNA sequencing carried out by Daniel Ross at VCOM Carolinas.
Sequence analysis carried out by Christopher Syed at VCOM Carolinas.

	No Alignments	One Alignment	Two + Alignments
<i>E. coli</i>	3.48%	84.57%	11.95%
Strep. Pyogenes	99.73%	0.22%	0.05%
Mus musculus	99.24%	0.65%	0.11%
Homo sapien	99.75%	0.19%	0.06%