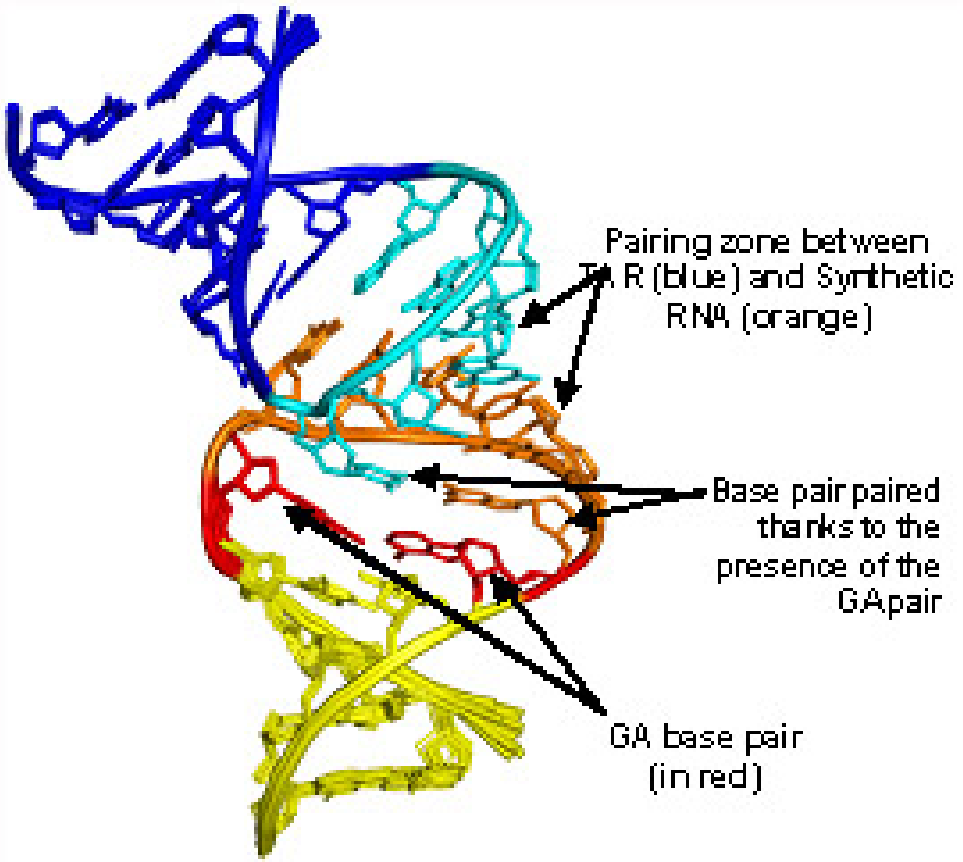
Imaging Nanoscale RNA

Jannine Tuttle

Mentor: Arkadiusz Chworos

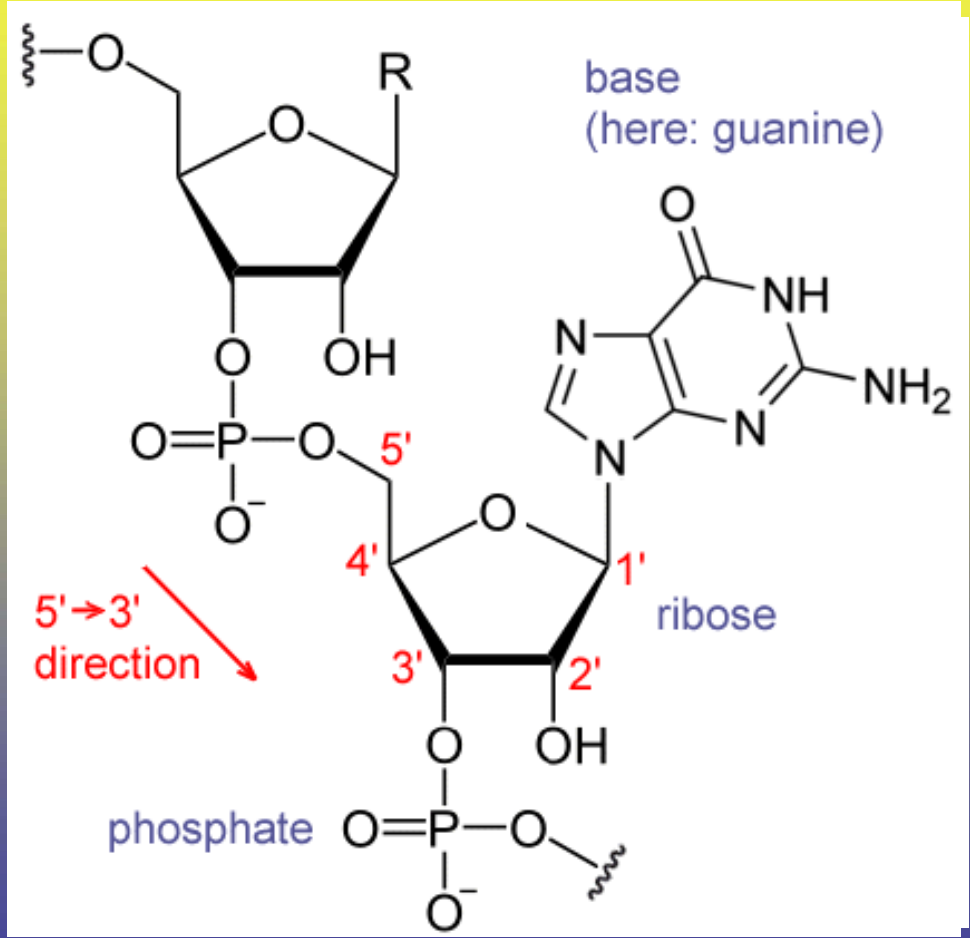
Engineering RNA Molecules



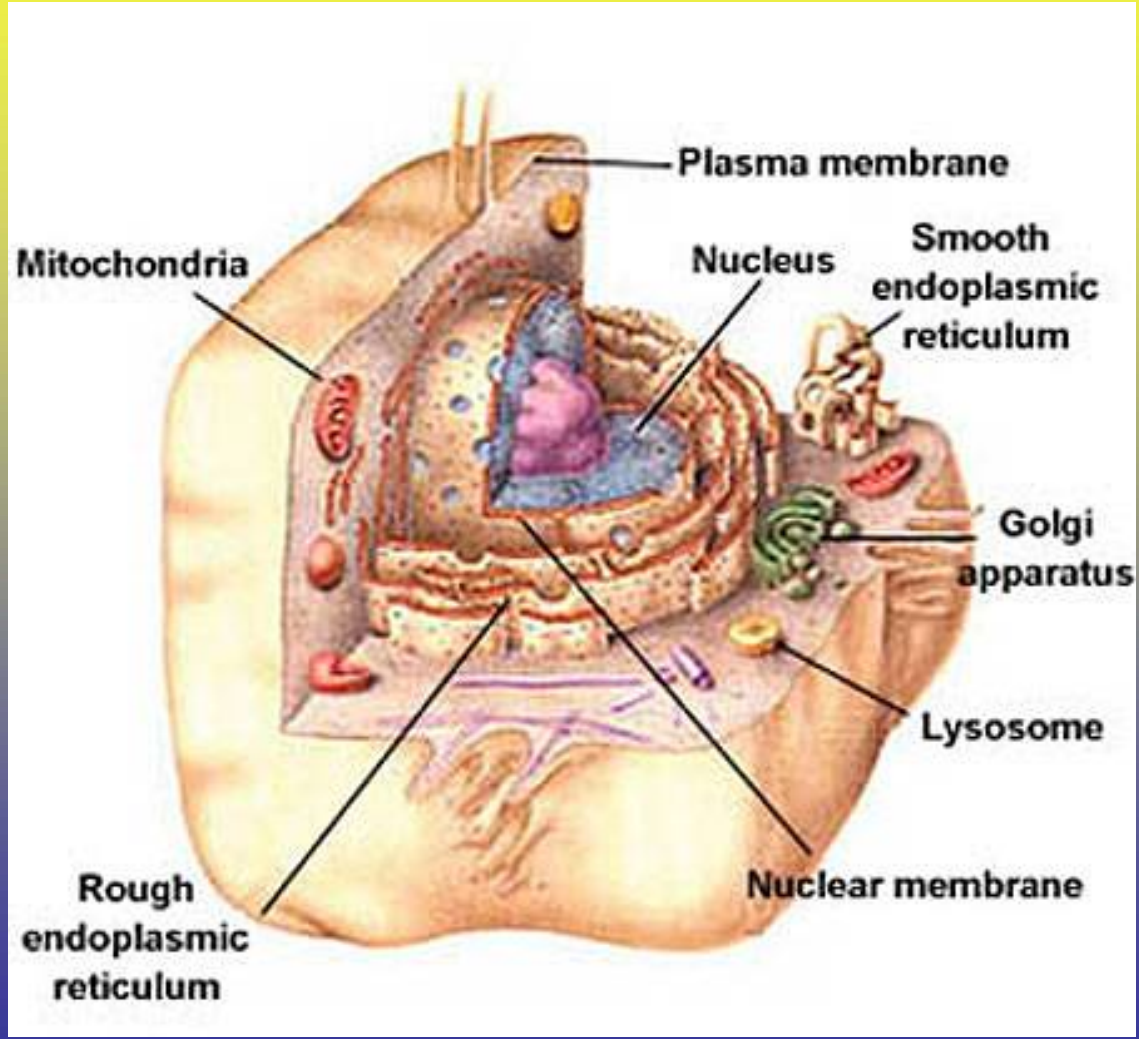
Kissing complex between HIV TAR sequence (dark blue and light blue) and synthetic RNA (yellow, orange and red)

What is RNA?

- Ribonucleic Acid
- Single Stranded chain of nucleotides (ribose, phosphate and nitrogen base A,G,U,C)



Where is RNA extracted from?



Why do we want to make synthetic molecules?

- Understand how they fold and interact with themselves or other molecules
- Therapeutic agent -iRNA gene silencer
- Target cell recognition and drug delivery

Not Using DNA
because it is so stable

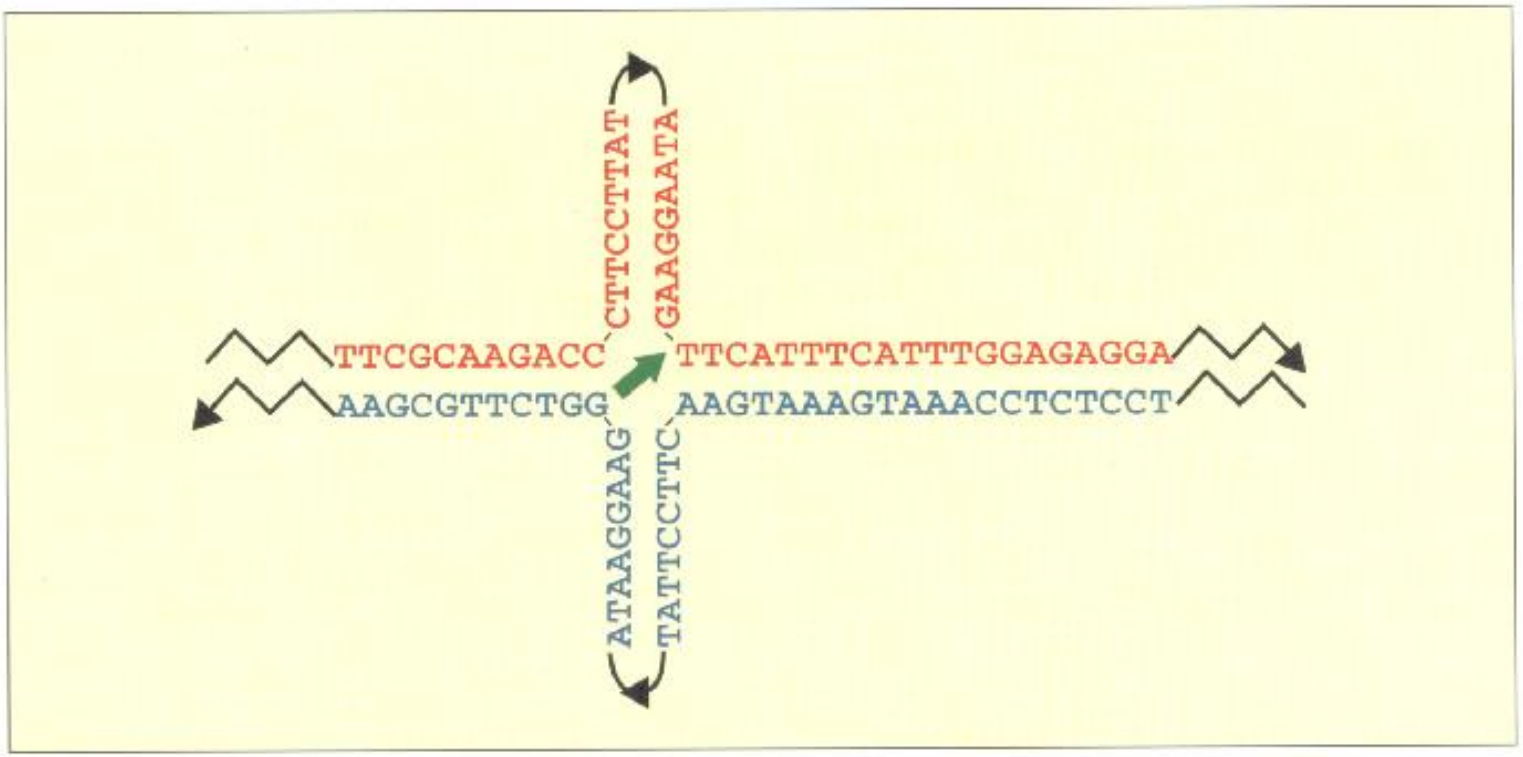
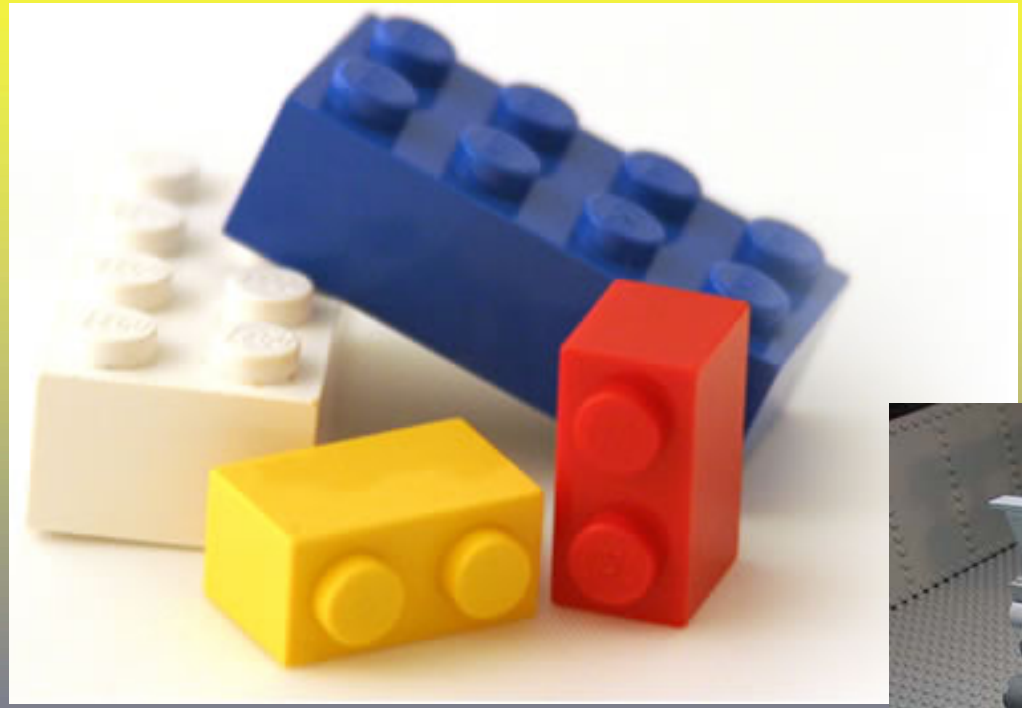


Fig. 17 *Transgene rearrangements often occur at regions rich in DNA secondary structure, such as the CAMV 35S promoter, which can form the cruciform structure shown above. This allows recombination to occur, as shown by the green arrow.*

DNA vs RNA



Why RNA Is Versatile

- Has additional OH in sugar so it can make multiple three dimensional bonds
- A-U and G-U pairing
- Forms A type double helix with base pairs buried deeper in helix

Funding

- National Institute of Health
- National Science Foundation
- MRL

UCSB Investigators

- Dr. Arkadiusz Chworos
- Wade Grabow
- Professor Luc Jaeger

Why Make a Hexagon?

- Try to understand folding of RNA
- Easier to make flat two dimensional object versus multidimensional objects

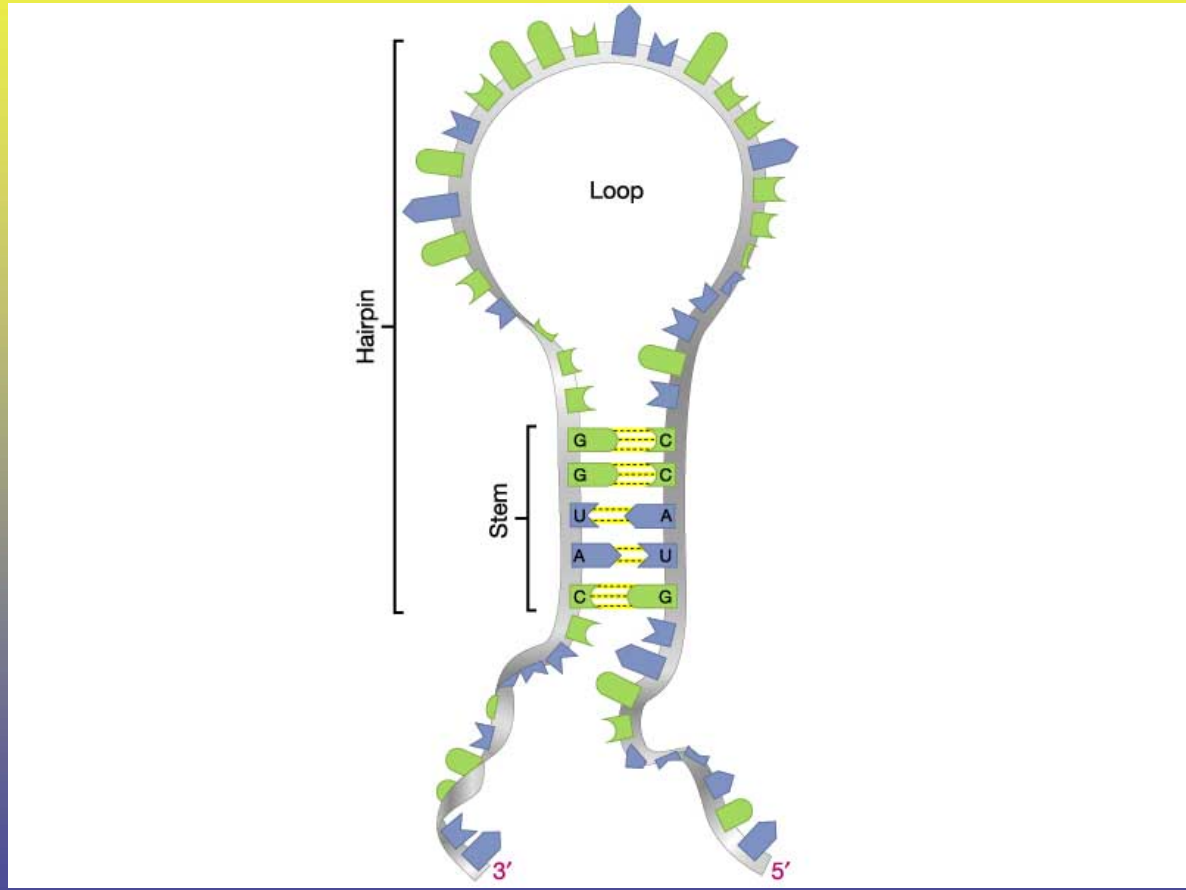
The Process

- Pick a shape
- Find a motif that fits that geometry
- Model to see if feasible
- Design Sequence
- Purchase DNA
- PCR and Convert to RNA
- Gel Electrophoresis
- Image with AFM
- Analysis

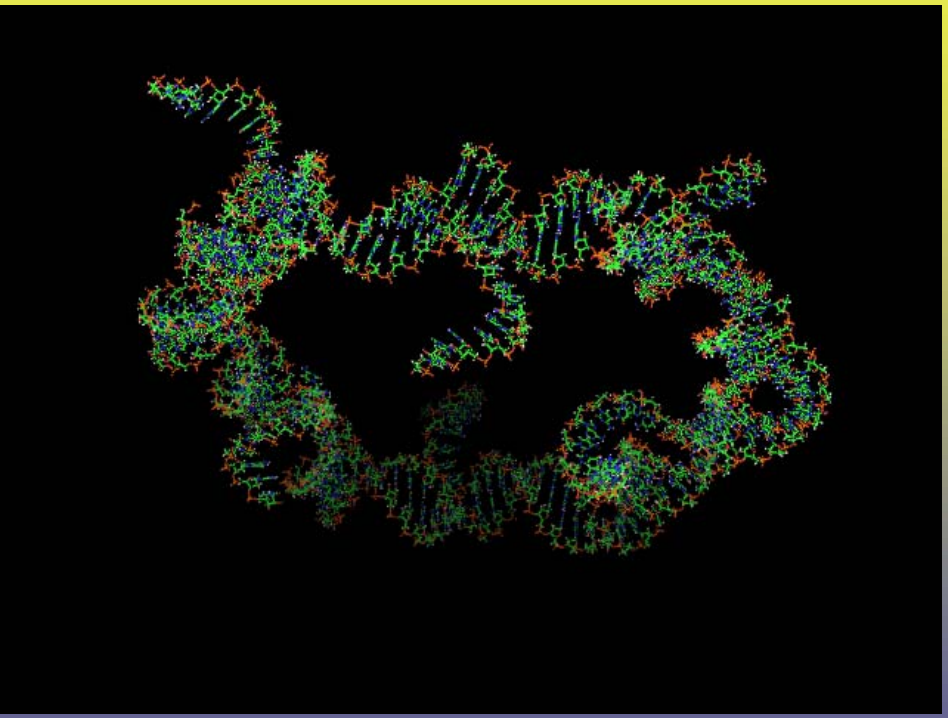
Goals and Questions

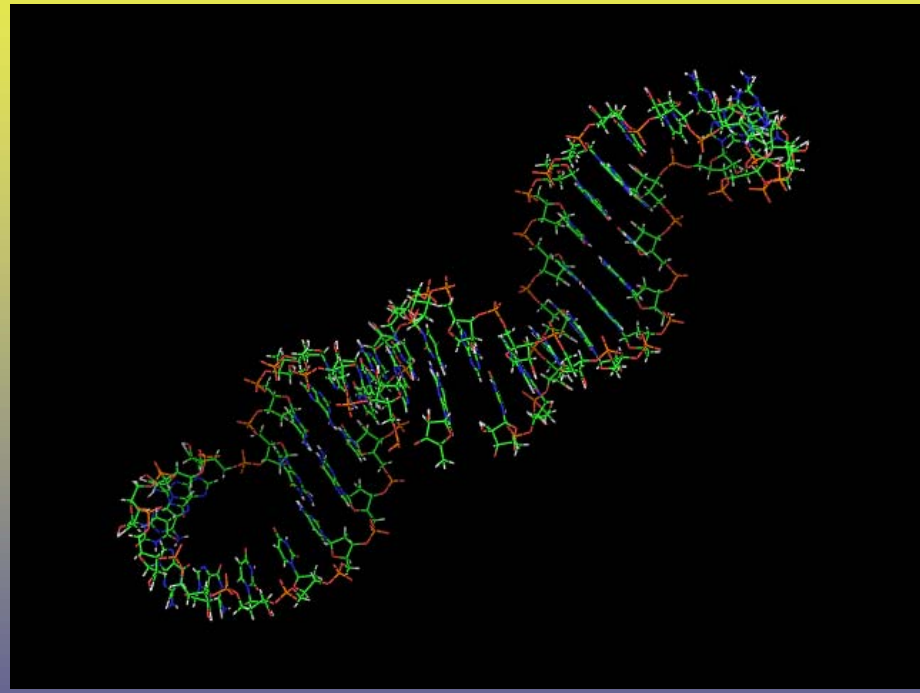
- Confirm what shapes you made
- Understand stability of structure temperature and longevity
- How do knicks impact assembly of RNA?
- If we alter environmental conditions, like temperature, how does that impact stability of structure?

Kissing Loop

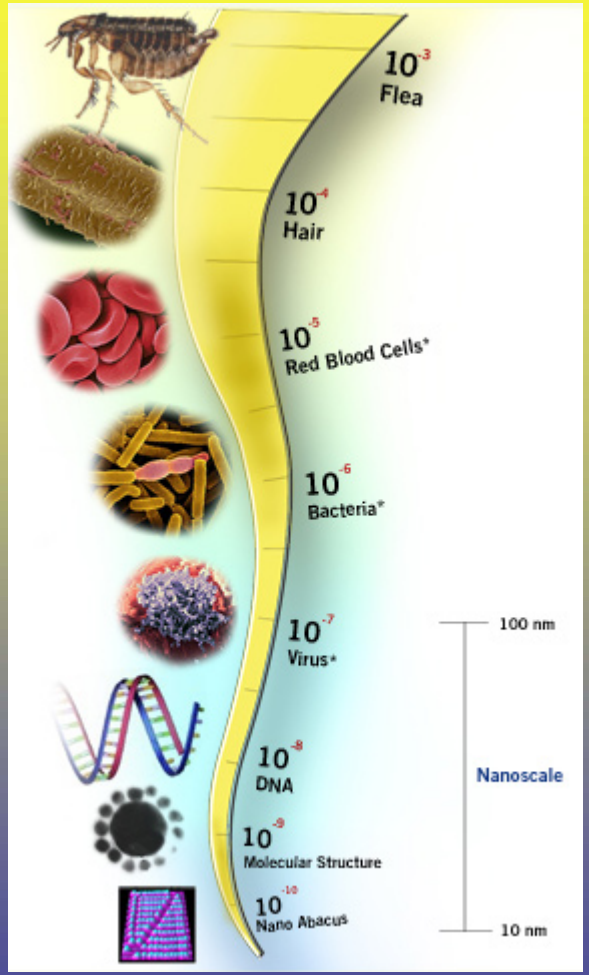


3-D Model





Purchase, PCR, and Convert



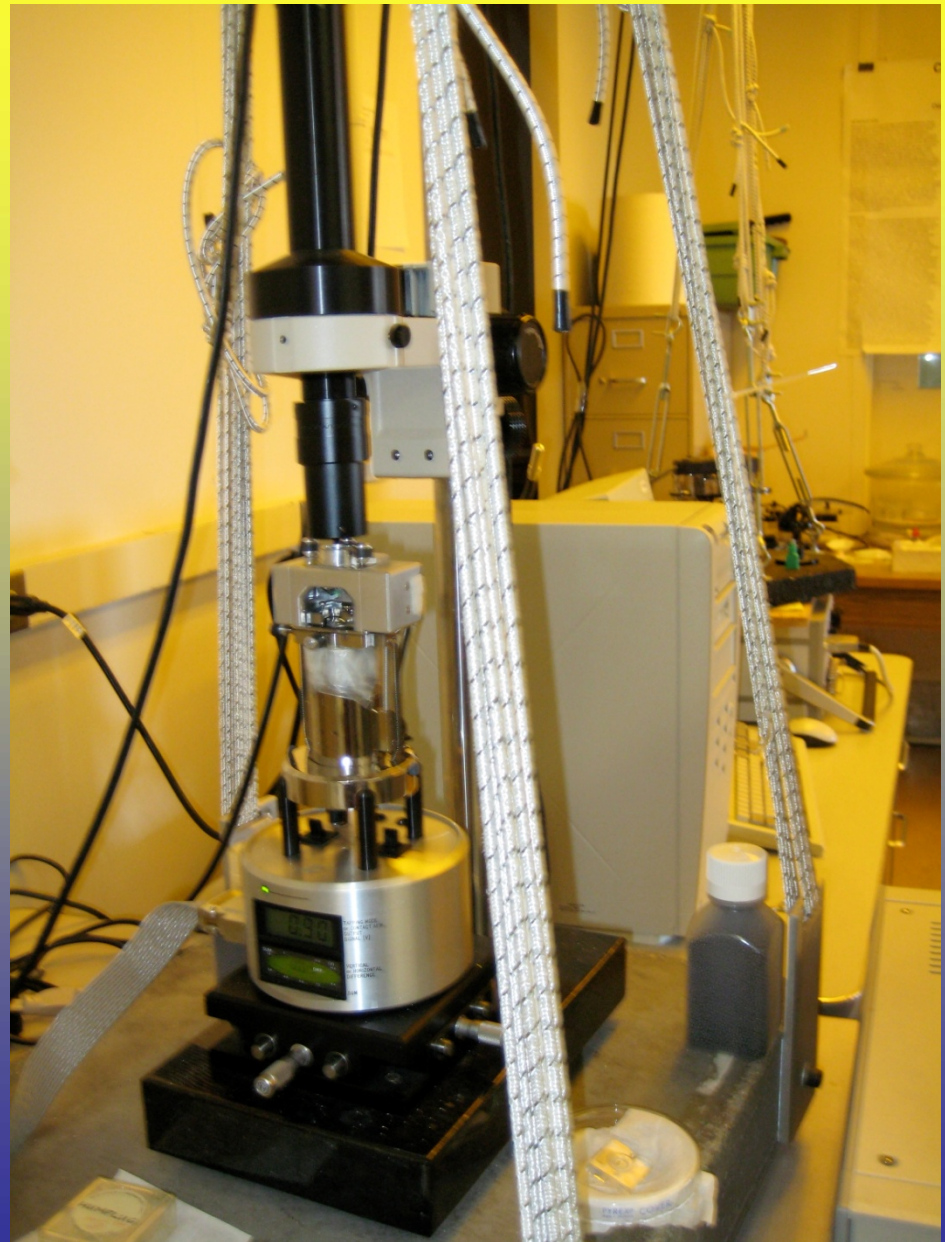
How do you know if you made it?

Benefits of Gel Electrophoresis

Run to confirm

- Abundance of Species
- Distribution of Species
- Relative Shapes

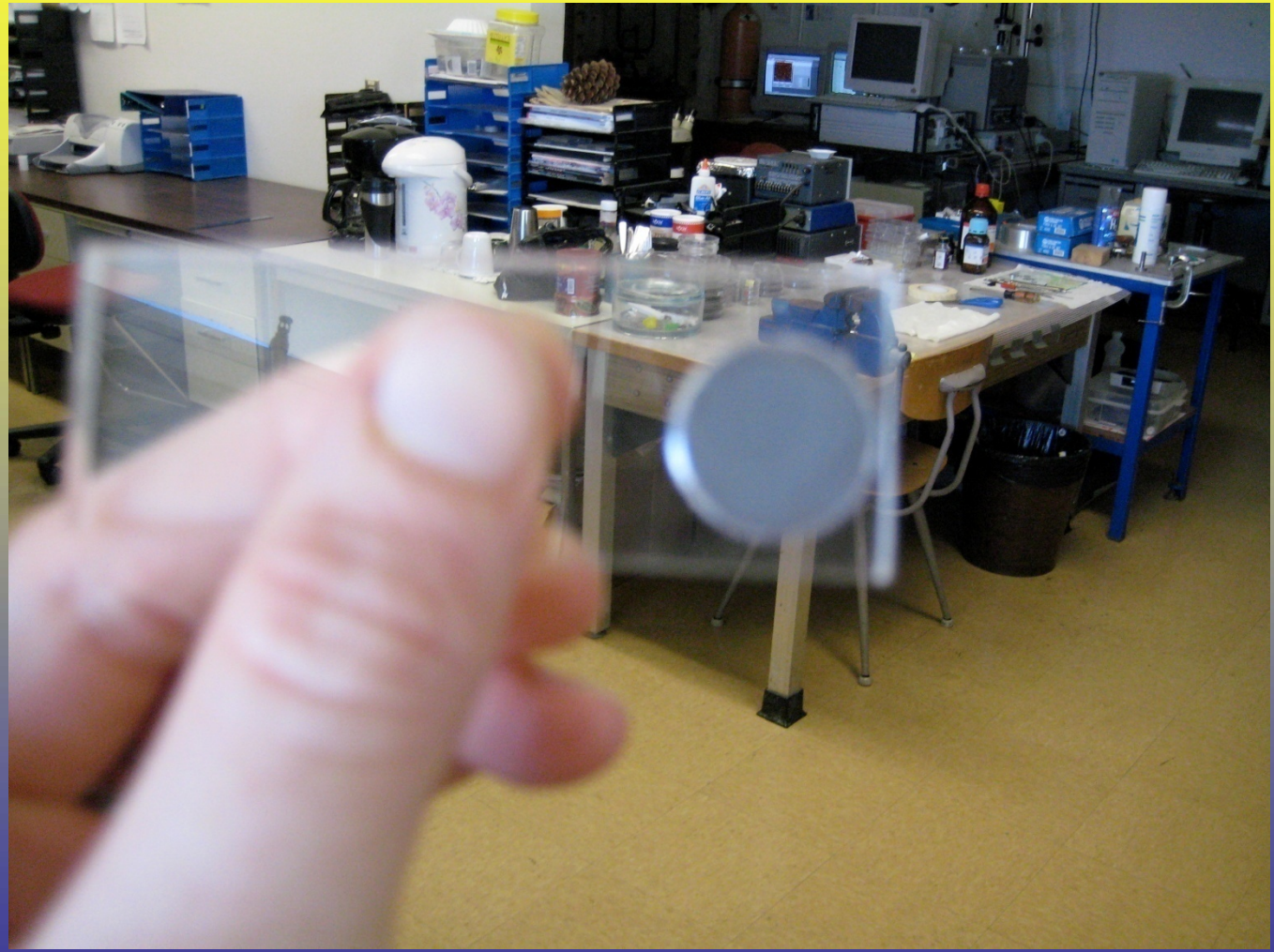
Atomic Force Microscope



Atomic Force Microscope

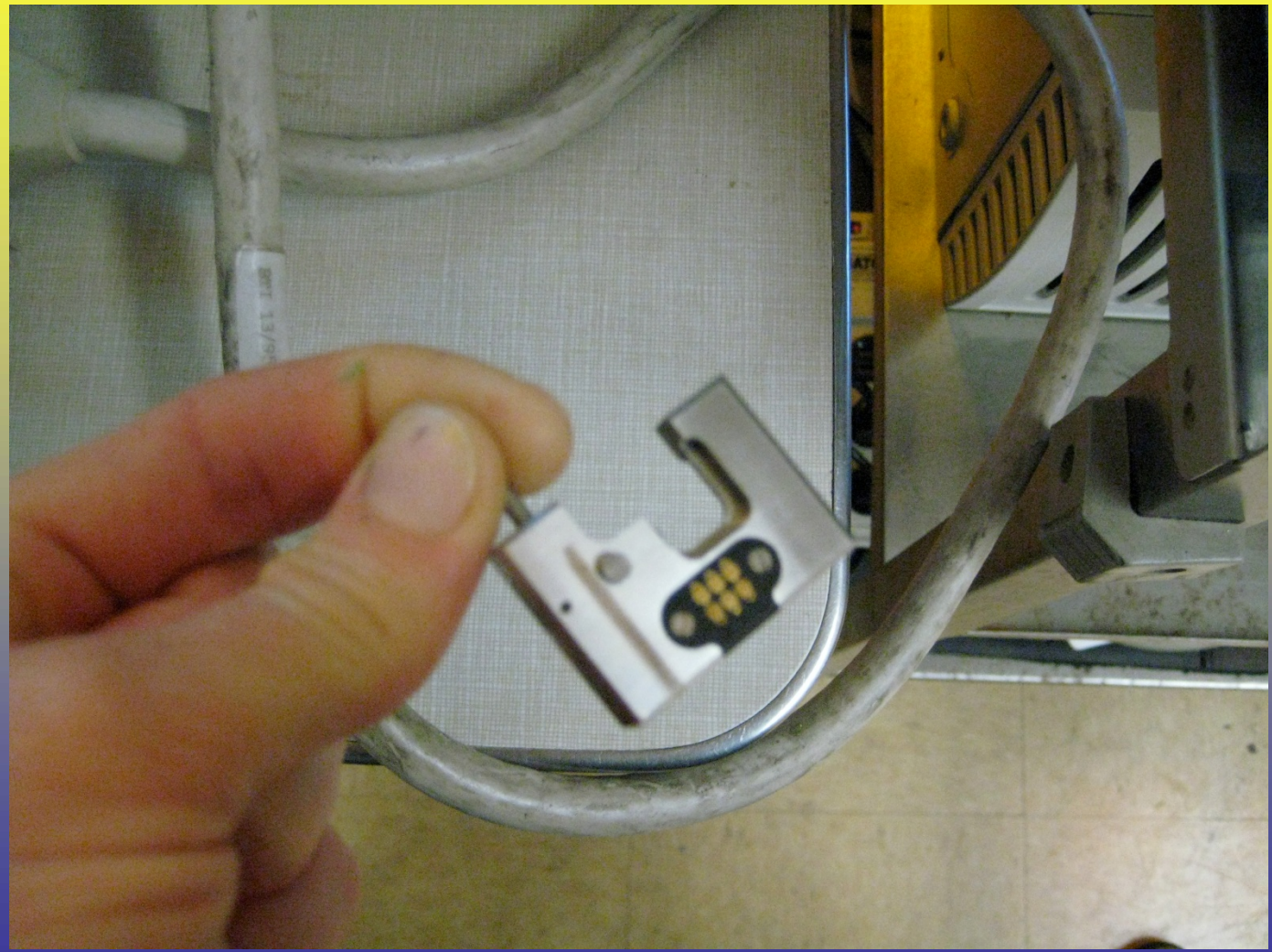
Preparing the Sample

Preparing the Sample



Preparing the Sample

Cantilever



Analysis

Quantitative analysis images

- Count abundance of different dimers (tetramer, hexamer and octomer)
- Measure size of each species

Future

- Analyze images
- Investigate whether altered RNA sequence improves stability of hexamer

THANKS!

- Arkadiusz Chworos Project Scientist
- Wade Grabow - PhD candidate
- Martina Michenfelder - MRL Education Coordinator