# Dideoxy Sequencing Reaction

Note: The below is the outline provided in the video: Below that is the outline of relavent ideas that I think you should know.

**Radiolabeled Primers**

**Add Buffer, Primer, Radiolabeled ATP**

**Add T4 polynucleotide Kinase**

**Thermal Cycle Dideoxy Sequencing Reaction**

**Template Primer Mix: Primers, DSDNA Template, Thermal Stable TAQ**

**Nucleotide Mixes- 4 for each sequence**

**Template Primer Mix: Nucleotide Mixes**

**Thermal Cycle 30 Times**

**Results**

This idea is a big advance, as earlier techniques require SSDNA (recall earlier video.)

*ATP is used to label primer as *P is transferred to 5'free end of primer (via polynucleatide kinase)

Primer to target DNA template is usually 100:1 (EX. 0.01 pM template to 1.0 pM of primer

Plasmid or lambda DNA is often used as a positive control.

PCR tubes are usually 0.5 ml and thin walled to allow for more rapid temperature flux.

Stop buffer role: Loading dye (bromophenol blue and sucrose.)
Formamide (to create SS DNA)
Chelating agents: EDTA to capture divalent cations.

Enzyme Evolution (Polymerases)
1. Klenow From bacteria… Has lots of exonuclease activity
2. T7 (high quality and fidelity, can make the longest runs
3. TAQ: Temperature cycle stable polymerase