Introduction to Molecular Diagnostics

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Overview

- Briefly consider central tenet of molecular biology
  - DNA, RNA, Protein
- Fundamental methods in analysis of nuclei acids
  - Polymerase chain reaction (PCR)
  - Modification of PCR for wide range of clinical molecular tests
- Goal
  - Start thinking about molecular techniques
  - Introduce simple molecular assays for clinical applications

Central Tenet of Molecular Biology

- Molecular Biology: study of fundamental building blocks of life
- DNA → RNA → Protein … → Phenotype
  - Change in DNA causes change in protein sequence, structure and function
- Molecular Diagnostics
  - Application of molecular biology (study of nucleic acids) in patient care
- Useful to study DNA/RNA sequence alterations when:
  - Phenotype is nonspecific
  - Underlying mechanism guides therapy

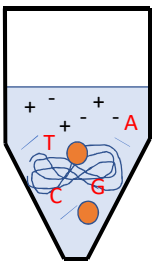
Analysis of DNA

- Molecular biology toolkit for manipulating DNA
- Mimic physiological processes *in vitro*

Biology	Purpose	*in vitro*
Helicase	DNA denaturation	Heat
Primase	Initiate synthesis	Primers
DNA polymerase	DNA extension	DNA polymerase

Target Amplification

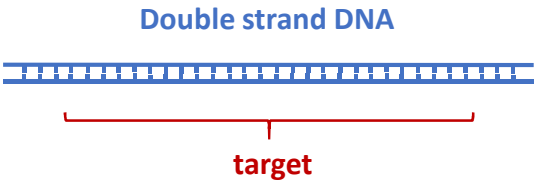
- Polymerase chain reaction
- Components:



- Water
- Buffer
- Template DNA
- Primers
- Nucleotides (dNTP)
- Polymerase
- Magnesium

- Cycles of denaturation, annealing, extension to amplify DNA product

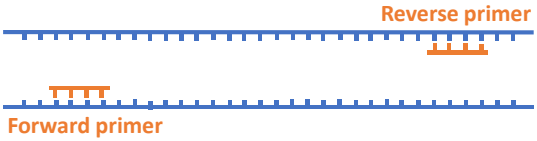
Target Amplification



Target Amplification

Step 1: Denaturation
94-98°C

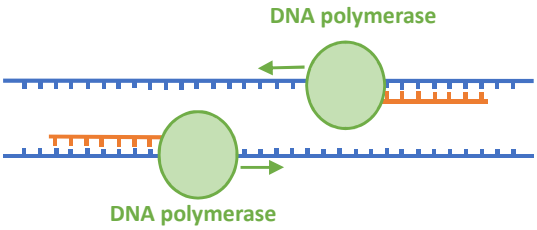
Target Amplification



Step 1: Denaturation
94-98°C

Step 2: Annealing
48-72°C

Target Amplification



Step 1: Denaturation
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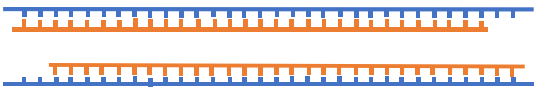
Step 2: Annealing
48-72°C

Step 3: Extension
68-72°C

Target Amplification



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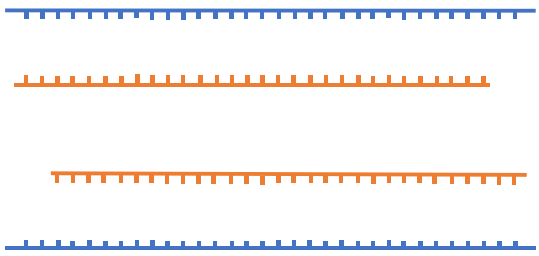

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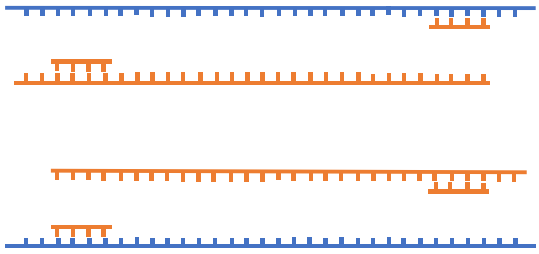

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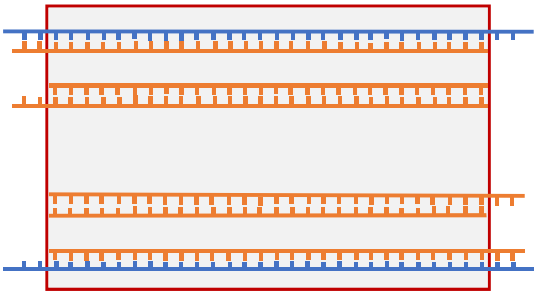

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Step 2: Annealing
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Step 3: Extension
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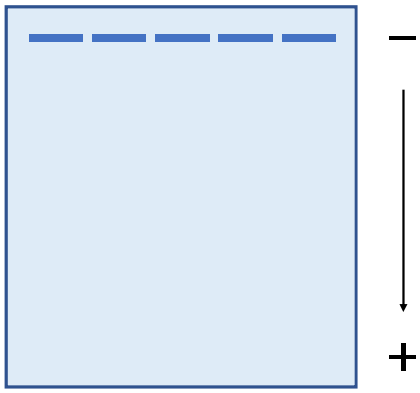
- Achieve amplification of a targeted region of DNA

Detect PCR Products

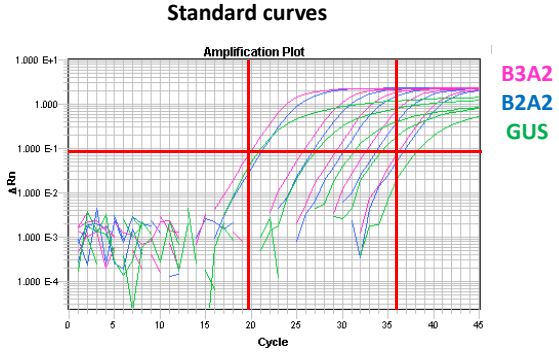
- Separation
  - Gel electrophoresis
  - Capillary electrophoresis
- Detection
  - DNA intercalating dyes
  - Radioisotopes
  - Fluorescent dyes for labeling nucleotides



Clinical tests that use PCR

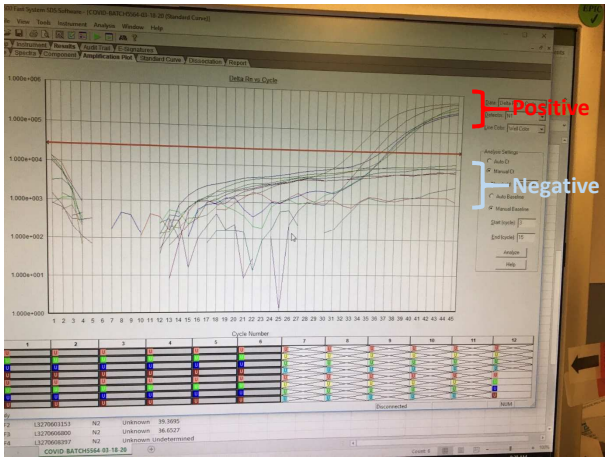
Quantitative PCR

- PCR
  - *BCR-ABL1* RNA transcript
  - Patients with CML on imatinib
  - Monitor for molecular evidence of recurrence/drug resistance
- Higher template quantity amplify at an earlier cycle



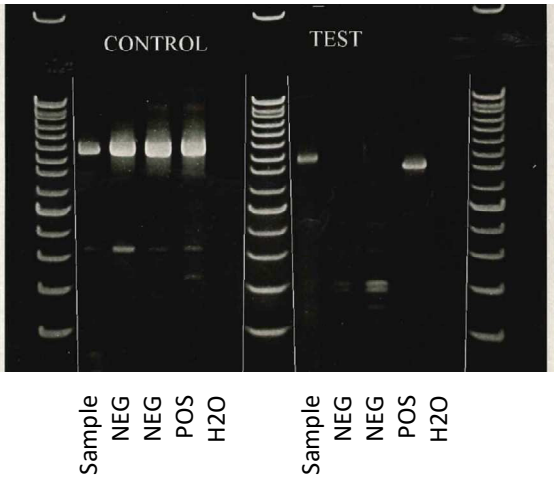
Quantitative PCR

- SARS-CoV2 testing



HPV Genotyping

- PCR
  - HPV
  - Control human DNA (*HBB*)



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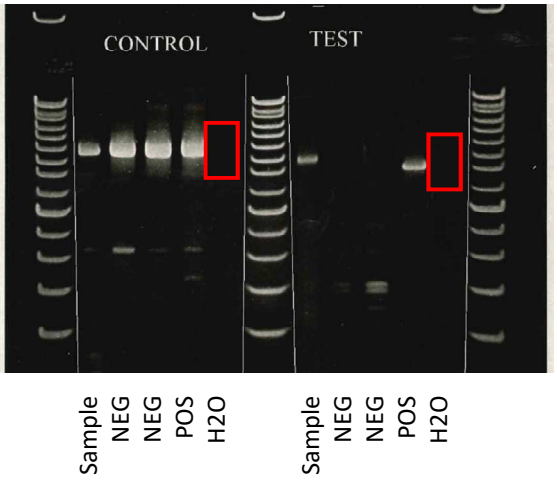

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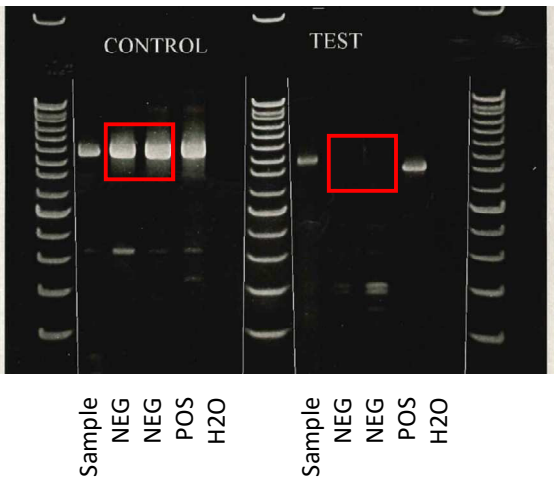

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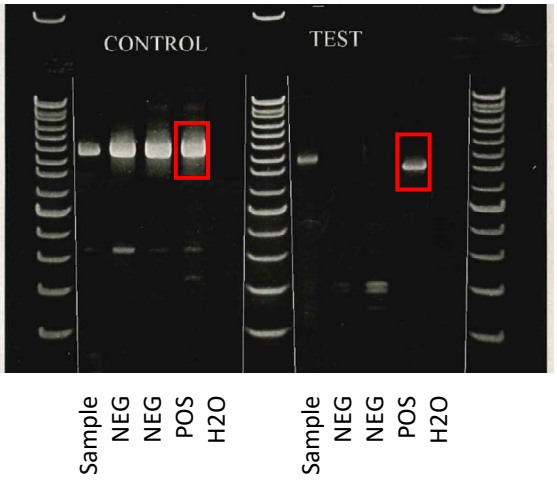

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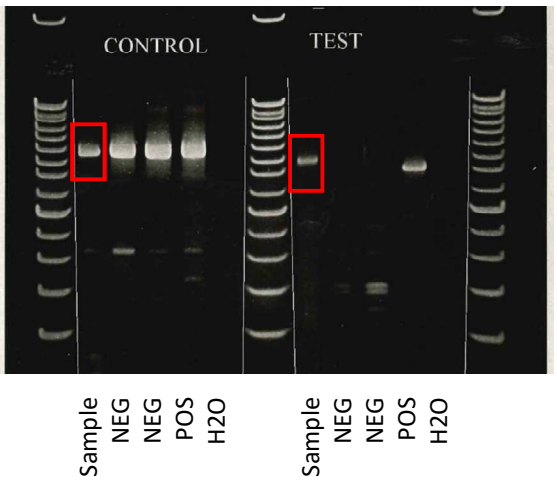
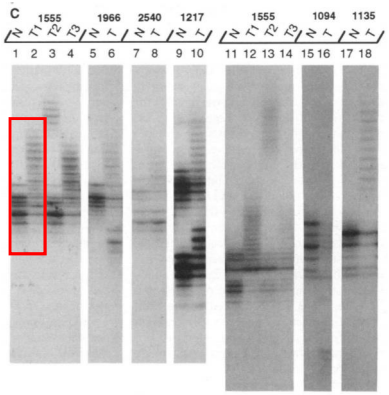


Microsatellite Instability Analysis

- PCR
  - Region of DNA repeat
  - Tumor-normal pair
  - Repeat length is altered by somatic mutation if mismatch repair machinery is deficient



Thibodeau SN, et al. Microsatellite instability in cancer of the proximal colon. Science. 1993 May 7;260(5109):816-9.

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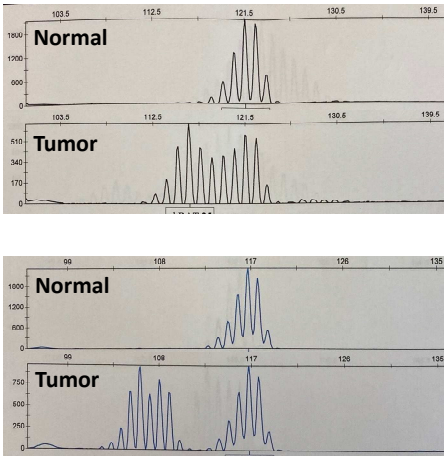
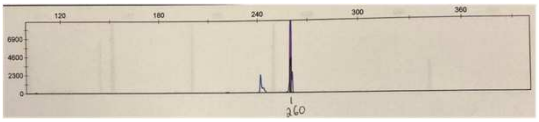


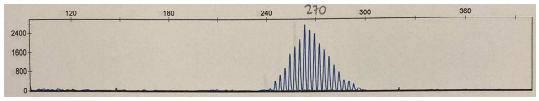
B Cell Clonality Testing

- IgH locus undergoes VDJ rearrangement
- PCR
  - Across region of rearrangement
  - If monoclonal B cells, all cells in population have same rearrangement



Monoclonal

  - If polyclonal B cells, mix of rearrangement sizes

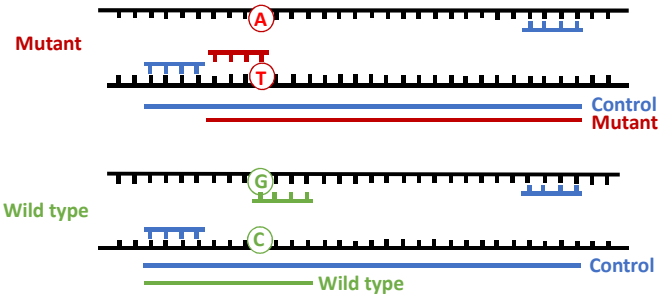


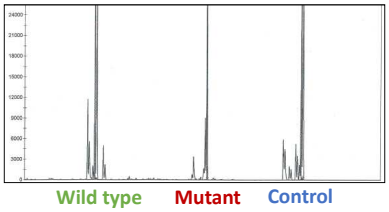
Polyclonal

Modified PCR to detect point mutations

Allele-Specific PCR

- Modification of PCR to detect mutation

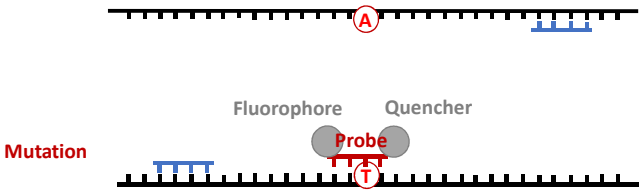




KIT D816V mutation in systemic mastocytosis

TaqMan probe PCR

- Probe provides specificity to wild type or mutant sequence



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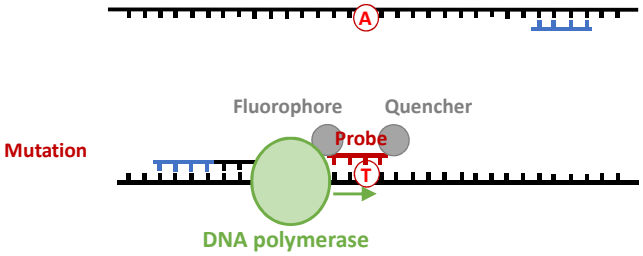

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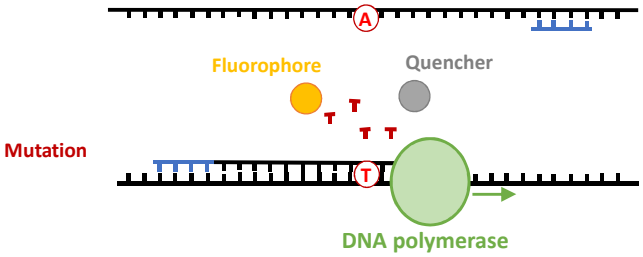
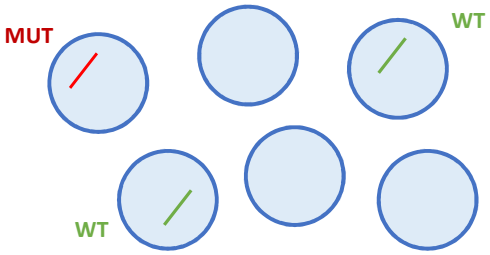
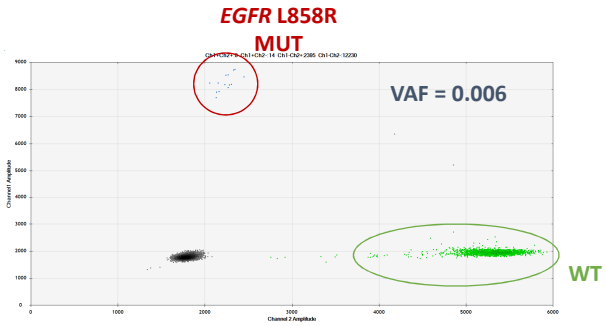


Droplet Digital PCR

- PCR from single DNA templates in microfluidic droplets





Modified PCR to detect mutations across a targeted region

Sanger Sequencing

- Ingredients
  - Water
  - Buffer
  - Template DNA
  - Primers
  - Nucleotides (dNTP)
  - Polymerase
  - Magnesium

Sanger Sequencing

- Ingredients
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  - Buffer
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  - Primers
  - Nucleotides (dNTP + labeled **ddNTP**)
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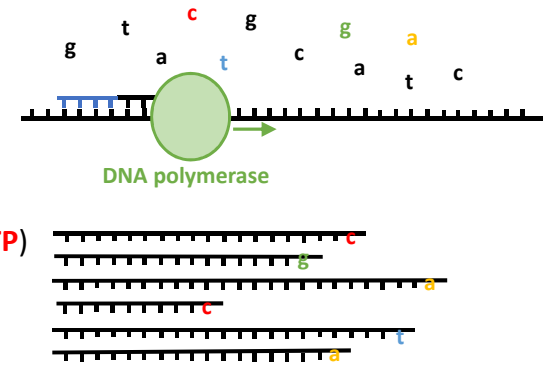
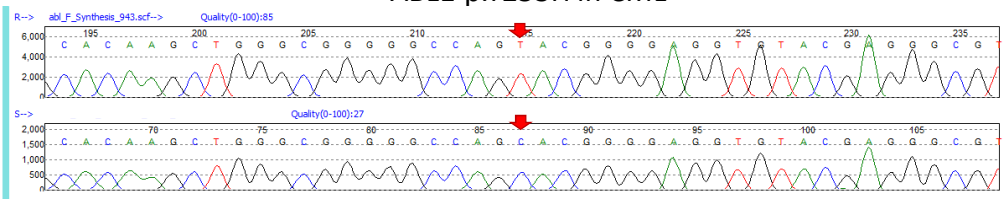


Sanger Sequencing

ABL1 p.Y253H in CML

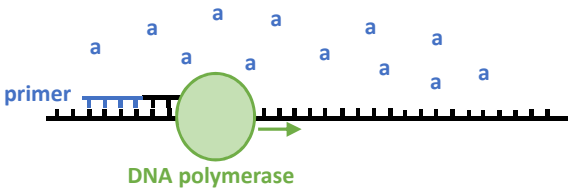
reference

sequence



Pyrosequencing

- Ingredients
  - Water
  - Buffer
  - Template DNA
  - Primers
  - Nucleotides (dNTP)*
  - Polymerase
  - Magnesium
- Method
  - Add one nucleotide at a time
  - Look for evidence of incorporation



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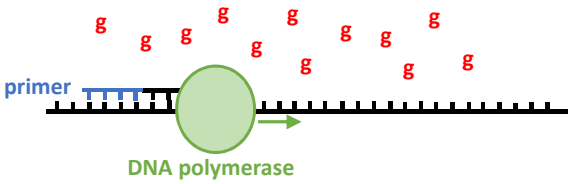

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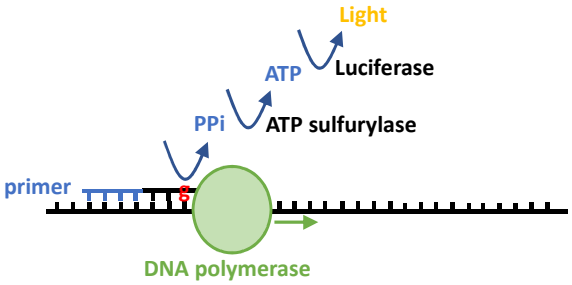
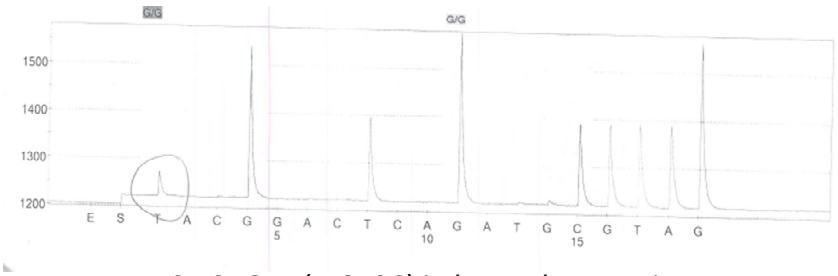


DNA chain extension creates *pyrophosphate*, which is detected in a subsequent reaction

Pyrosequencing



KRAS c.34G>T (p.G12C) in lung adenocarcinoma

Clinical Applications of Molecular Testing

- Cancer
  - Genetic disorder of mutations in oncogenes and tumor suppressor genes
  - Classification, prognostication, treatment
- Heritable Disorders
  - Pathogenic germline variants in diseases of Mendelian inheritance
  - Implication for patient and family members
- Microbiology
  - Detection and quantification of organisms
  - Detection of genetic mechanisms of drug resistance
- Identity testing
  - Test of polymorphisms within human population
  - Used for forensic testing, paternity testing, bone marrow engraftment, tissue identity

Summary

- Basic tenant of molecular biology
  - DNA sequence and disease phenotype
- Molecular laboratory techniques
  - Targeted amplification as a basis of advanced molecular techniques
- I hope you will enjoy the course today

@feidng