GENETIC MECHANISMS OF CARDIOMYOPATHY: FLNC KNOCKOUT MODELING AND MOUSE GENOTYPING

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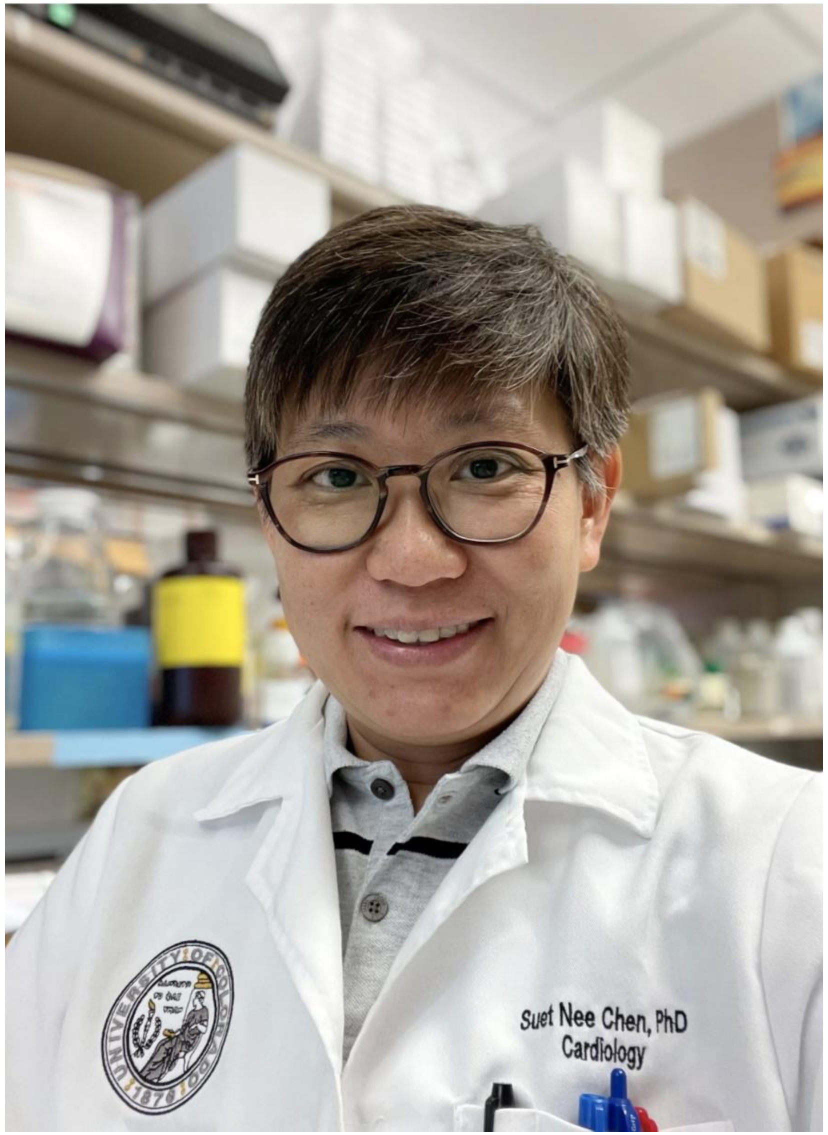
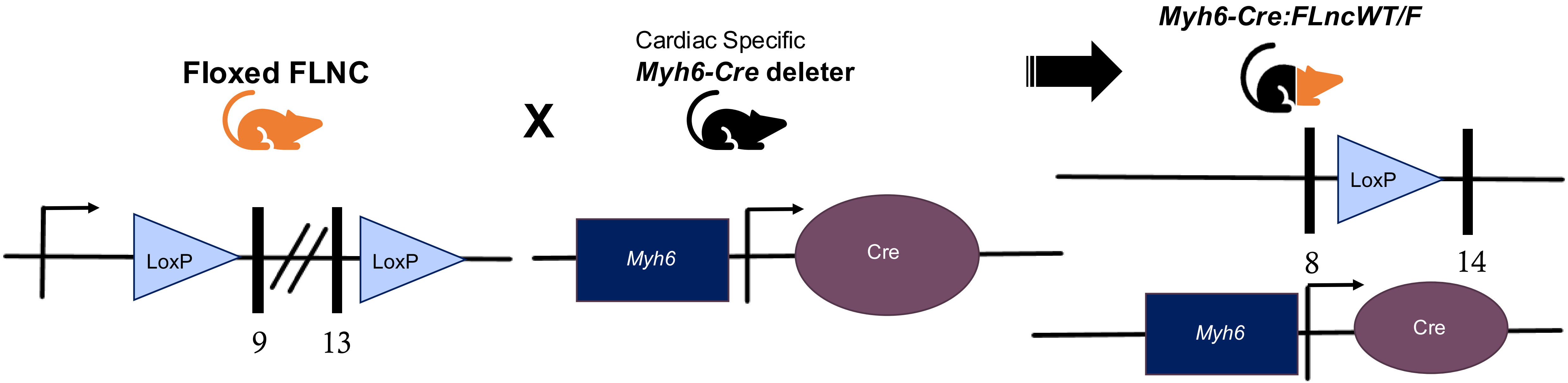
Mechanistic and Translational Genomics in Inherited Cardiomyopathy

- **Research Focus**
  - Genetic drivers of cardiomyopathy
  - Mechanistic pathways linking mutations to disease
  - Precision, mechanism-based therapeutic strategies
- ***FILAMIN C (FLNC)* as a Model Gene**
  - Key cytoskeletal protein at **Z-disc / costamere / Intercalated Disc**
  - Maintains cardiomyocyte structure and mechanical stability
  - *FLNC* mutations → **DCM/ACM**, arrhythmias, early remodeling
- **Approach**
  - Gene-edited & patient-derived hiPSC-CMs
  - Cardiac-specific mouse knockout models
  - Multi-omics + advanced imaging
  - Functional assays (contractility, electrophysiology)
- **Goal**
  - Identify actionable disease mechanisms
  - Develop targeted therapies for inherited cardiomyopathies



DISEASE MODEL: CARDIAC SPECIFIC FLNC KNOCKOUT MICE



MOUSE GENOTYPING PROCESS

1. TAIL TRANSECTION

Purpose: Performed to obtain a small tissue sample for DNA extraction and genotyping

Procedure: A tiny section (1-3mm) of a mouse (3 weeks or younger) tail tip is removed using sterile scissors or a scalpel

Anesthesia/Analgesia: local/general anesthesia or analgesia can be used to minimize pain and distress

The tail is disinfected, bleeding is stopped, and mouse is monitored for normal activity and healing

2. DNA EXTRACTION

DNA extraction isolates genomic DNA from tissue samples to enable downstream applications such as PCR genotyping. The process typically includes the following steps:

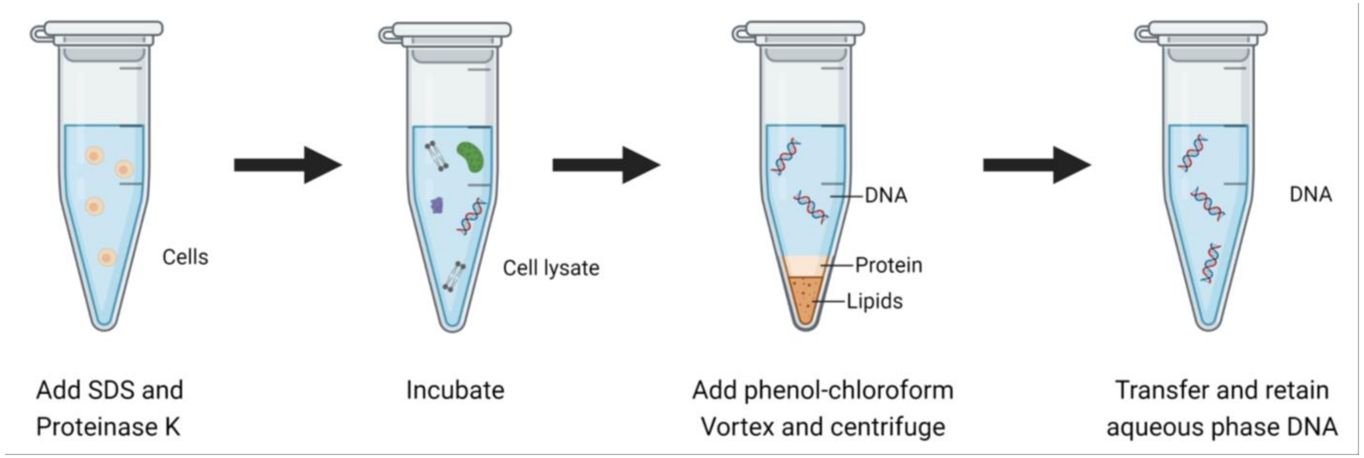
Sample preparation: Sample tail tip is placed into microcentrifuge tube for processing.

Cell Lysis: A cell lysis buffer (containing proteinase K) is added to break open cellular and nuclear membranes releasing DNA into solution.

Incubation: Samples are incubated overnight to allow complete digestion of proteins and tissue, ensuring efficient DNA release.

DNA purification: Protein and cellular debris are removed from sample through precipitation or spin-column purification, leaving purified DNA in the supernatant.

extracted DNA is transferred into a clean microcentrifuge tube and stored appropriately (typically at 4°C for short-term or −20°C for long-term storage) for

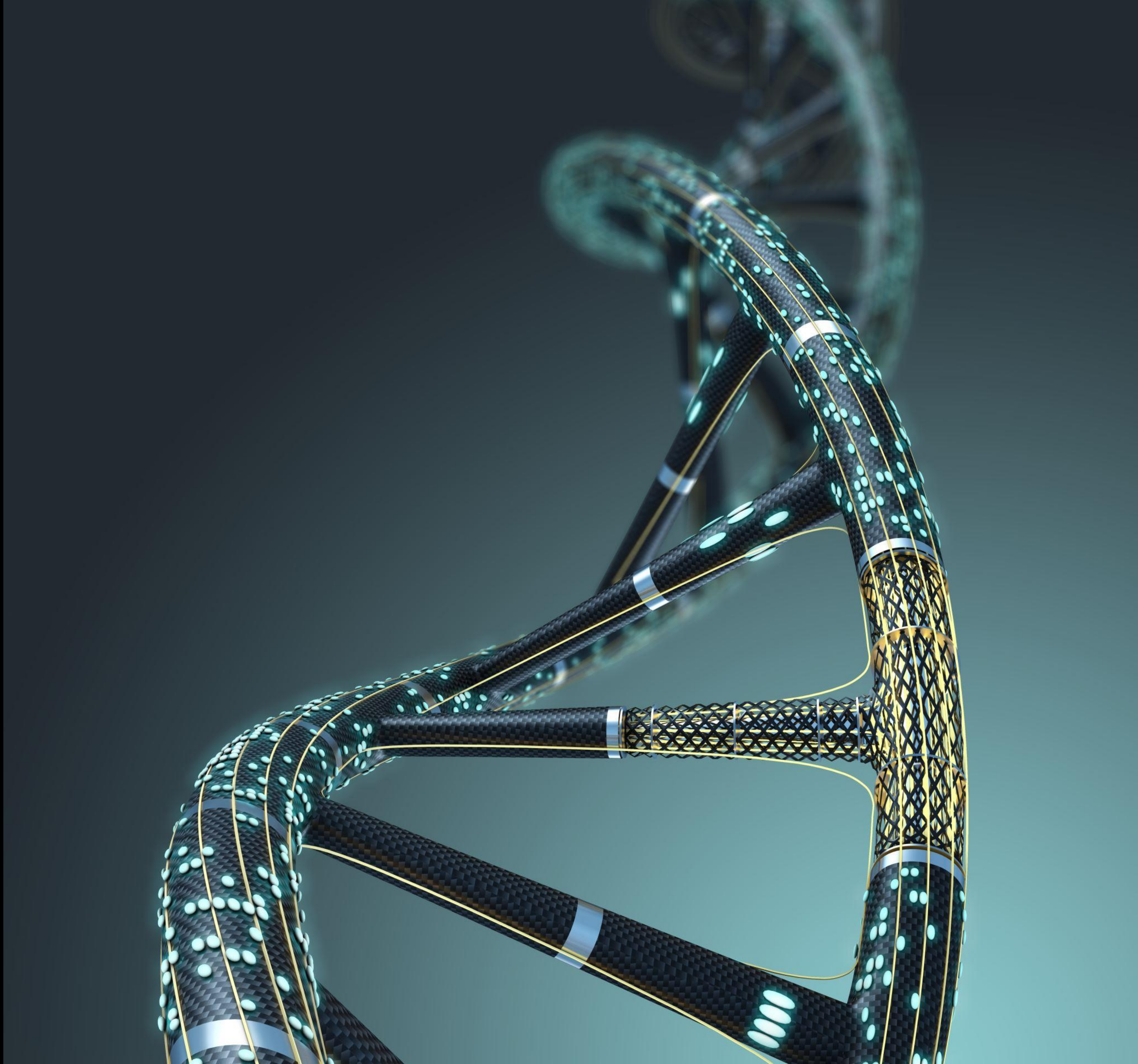


3. DNA MEASUREMENT

Accurate DNA quantification is critical to ensure that downstream applications (e.g., PCR, genotyping, library preparation) are not inhibited by suboptimal DNA concentration or contaminants. Measuring DNA also allows confirmation of sample purity.

NanoDrop Spectrophotometry

- Most common and rapid method for assessing DNA concentration.
- Measures nucleic acid abundance based on UV absorbance at 260 nm.
- Provides purity ratios (A260/A280, A260/A230) to detect protein, salt, or solvent contamination.
- Requires only 1–2 µL of sample, making it ideal for limited volumes.



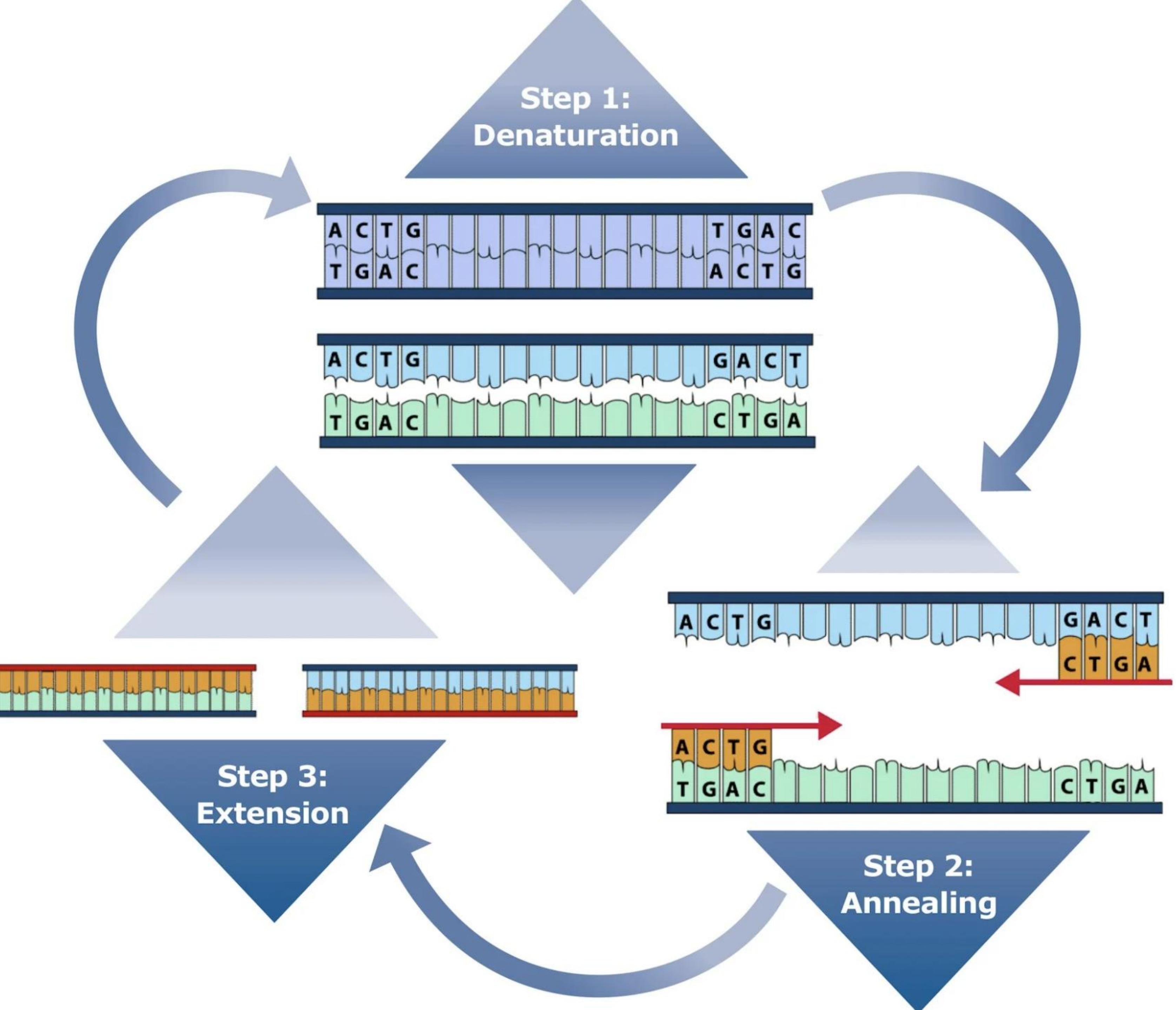
4. POLYMERASE CHAIN REACTION

- **Purpose**: Polymerase Chain Reaction (PCR) is a laboratory technique used to rapidly amplify specific DNA segments, generating millions of copies from a small starting amount of genomic DNA. This enables precise detection and analysis of genetic sequences.
- **Applications**
  - **Genotyping** and identification of alleles
  - **DNA cloning** for downstream molecular biology
  - **Medical diagnostics**, including detection of pathogens or mutations
  - **Forensic analysis** and genetic fingerprinting
  - **Research applications** requiring targeted DNA amplification

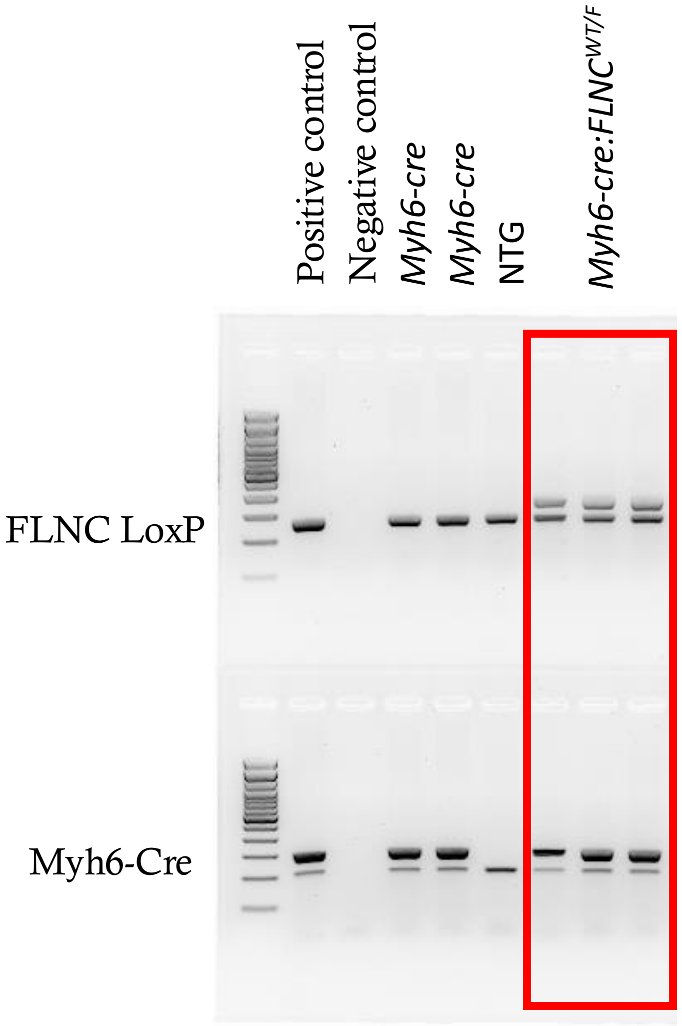
COMPONENTS OF A PCR REACTION

- A typical PCR master mix contains:
  - **Template DNA** – genomic DNA extracted from mouse tissue
  - Forward and Reverse Primers – short oligonucleotides that flank the target sequence
  - dNTPs – building blocks for DNA synthesis
  - PCR Buffer – maintains optimal pH and salt concentration$MgCl_2$ – cofactor required for polymerase activity
  - DNA Polymerase (e.g., Taq polymerase)
  - Nuclease-free water to bring reaction to final volume
- *Best practice: Prepare a master mix to reduce pipetting error.*

4. POLYMERASE CHAIN REACTION



- **The exact program varies by primer set, but a general program includes:**
- **Initial Denaturation:** 95°C for 2–3 minutes
- **PCR Cycling (30–35 cycles)**
  - **Denaturation:** 95°C for 30 seconds
  - **Annealing:** 55–65°C for 30 seconds (primer-specific)
  - **Extension:** 72°C for 30–60 seconds (depends on amplicon length)
  - **Final Extension:** 72°C for 5 minutes
  - **Hold:** 4°C indefinitely



5. AGAROSE GEL ELECTROPHORESIS

- **Purpose in Genotyping**
  - Agarose gels allow visualization of PCR products to determine the presence or absence of specific alleles.
- Genotyping Results Interpretation
  - Example: Wild-Type vs. Floxed Allele
    - **Wild-type allele**: Produces a PCR band at a specific size (e.g., smaller fragment).
    - **Floxed allele**: Produces a larger band due to the insertion of loxP sites sequence.
    - A mouse may show:
      - WT/WT: Only wild-type band
      - Flox/WT (heterozygous): Both bands present
      - Flox/Flox: Only floxed band
    - This size-based resolution enables accurate identification of mouse genotypes.

CONCLUSION

- Reliable genotyping requires high-quality DNA extraction, accurate measurement, and optimized PCR setup.
- Agarose gel electrophoresis allows clear visualization of alleles, enabling distinction between wild-type, heterozygous, and floxed/mutant genotypes.
- Consistent technique and proper documentation ensure reproducible, publication-quality results.
- These workflows support accurate mouse colony management and strengthen the mechanistic cardiomyopathy research conducted in Dr. Chen's laboratory.

Thank you!

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