

**miniPCR™ Crime Lab: Missy Baker Gone Missing**

Missy Baker has gone missing. Two suspects are held by the police. Hair samples found in their cars must be analyzed by PCR to evaluate whether they match Missy Baker's DNA.

**The DNA Times** Free

**Missy Baker missing, police at a loss**

**The disappearance of a bakery shop owner raises serious concerns. The enigma befuddles police investigators. With two abduction suspects held in custody, local students volunteer to try to find the missing baker.**

**Boston, October 30<sup>th</sup>, 2025.** Husband Ned reported Missy "Sugar-Cup" Baker missing, fretful after not finding her at the bakery shop following his daily nap. The couple resides in the apartment above the pastry shop at 2 Middleborough Rd. a popular fixture in this usually quiet neighborhood. Anxiety takes hold in the community.



Quickly following the report of the missing baker (wheat-blond and thin as a breadstick) investigators identified two suspects, but both have remained silent after extensive interrogation. Following forensic police searches, investigators found blond hair strands in each of the suspects' cars.

DNA from these hair samples is now in the hands of science students, who will volunteer with DNA analysis equipment to help identify the alleged abductor.

Wooly mammoth finally cloned at  
 Brooklyn Zoo p.5



Transgenic kiwi solves  
 world hunger p.7



## Laboratory guide

### **A** PCR set up

- Label 4 PCR tubes** (200  $\mu$ L tubes) per lab group
  - 1 tube labeled "A": 'Hair DNA' extracted from Suspect A's car
  - 1 tube labeled "B": 'Hair DNA' extracted from Suspect B's car
  - 1 tube labeled "H": 'Control DNA' from a healthy individual
  - 1 tube labeled "D": 'Control DNA' from a CFTR deletion mutant



Also label each tube with your group's name on the side wall

- Add PCR reagents** to each 200  $\mu$ L PCR tube

	<b>Tube A</b>	<b>Tube B</b>	<b>Tube H</b>	<b>Tube D</b>
<b>Template DNA</b>	DNA found in Suspect A's car 5 $\mu$ L	DNA found in Suspect B's car 5 $\mu$ L	Control H 'Healthy' DNA 5 $\mu$ L	Control D 'Deletion' DNA 5 $\mu$ L
<b>3X Crime Lab Primer Mix</b>	10 $\mu$ L	10 $\mu$ L	10 $\mu$ L	10 $\mu$ L
<b>2X PCR Master Mix</b>	15 $\mu$ L	15 $\mu$ L	15 $\mu$ L	15 $\mu$ L
<b>TOTAL VOLUME</b>	<b>30 <math>\mu</math>L</b>	<b>30 <math>\mu</math>L</b>	<b>30 <math>\mu</math>L</b>	<b>30 <math>\mu</math>L</b>



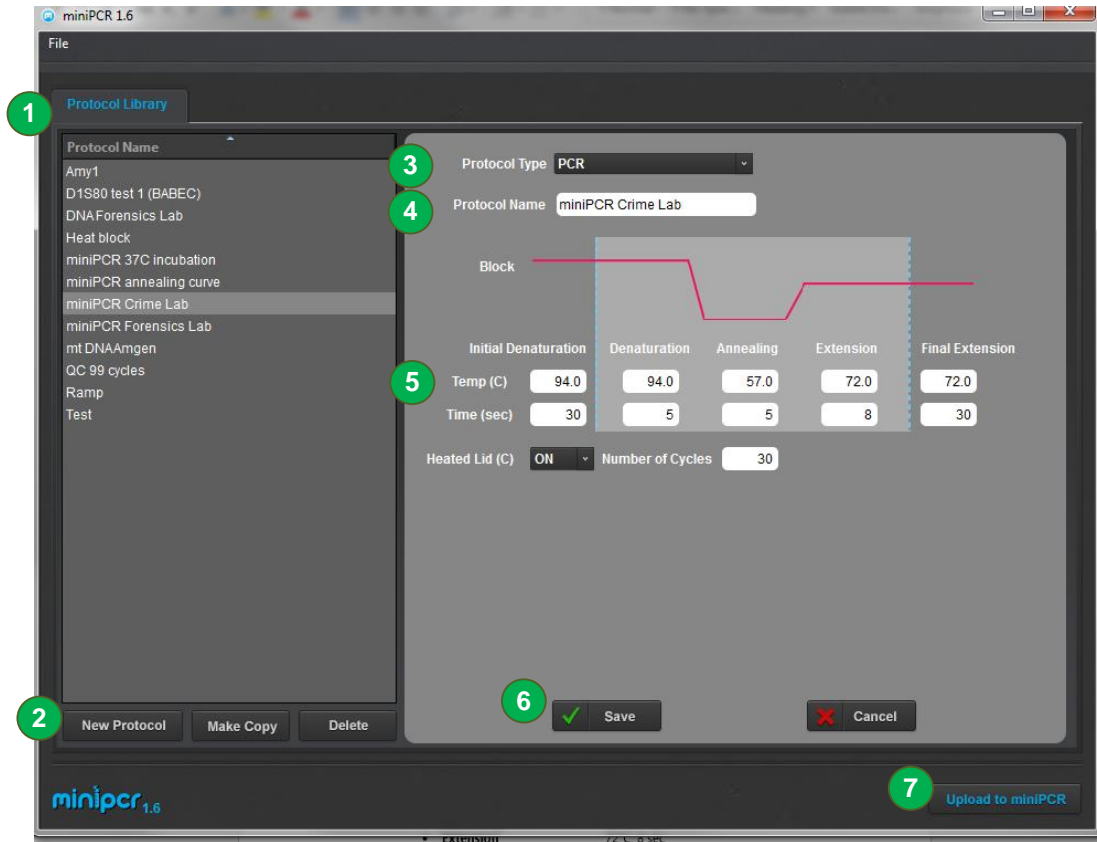
Use a micropipette to add each of the reagents.

Remember to change tips at each step!

- Gently mix the reagents by pipetting up and down 3-4 times, cap the tubes**
  - Make sure all the liquid volume collects at the bottom of the tube (if necessary, spin the tubes briefly using a microcentrifuge)
- Place the tubes inside the PCR machine**
  - Press firmly on the tube caps to ensure a tight fit
  - Close the PCR machine lid and tighten it gently

## B PCR programming and monitoring (illustrated using miniPCR™ software)

1. Open the miniPCR software app and remain on the "Protocol Library" tab



2. Click the "New Protocol" button on the lower left corner

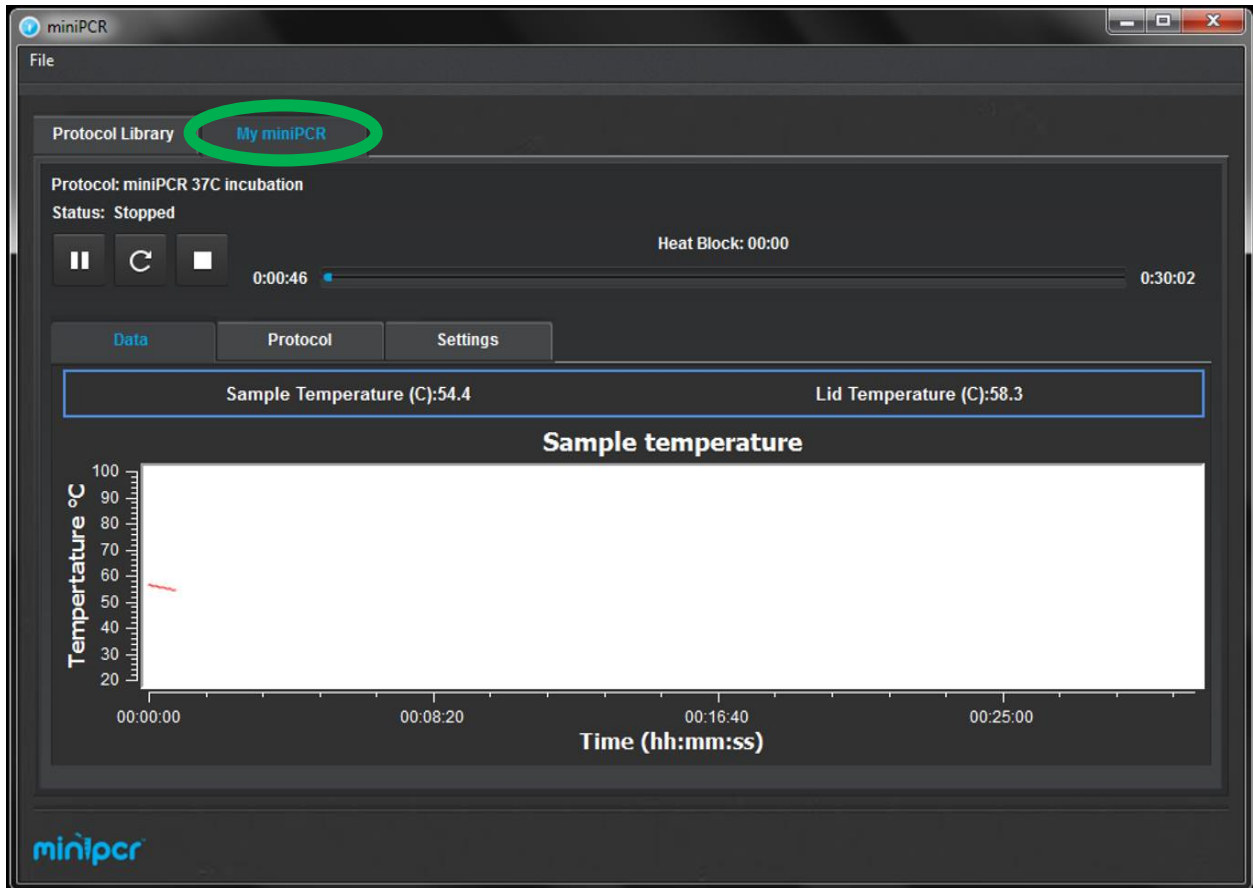
3. Select the PCR "Protocol Type" from the top drop-down menu

4. Enter a name for the Protocol; for example "Group 1 - Crime Lab"

5. Enter the PCR protocol parameters:

- **Initial Denaturation**      94°C, 30 sec
- **Denaturation**              94°C, 5 sec
- **Annealing**                  57°C, 5 sec
- **Extension**                  72°C, 8 sec
- **Number of Cycles**        30
- **Final Extension**         72°C, 30 sec
- **Heated Lid**                 ON

6. Click **"Save"** to store the protocol
7. Click **"Upload to miniPCR"** (and select the name of your miniPCR machine in the dialogue window) to finish programming the thermal cycler. Make sure that the power switch is in the ON position
8. Click on **"miniPCR [machine name]"** tab to begin monitoring the PCR reaction



*The miniPCR™ software allows each lab group to monitor the reaction parameters in real time, and to export the reaction data for analysis as a spreadsheet.*

Once the PCR run is completed (approximately 30-40 min), the screen will show **"Status: Completed"** and **all LEDs on your miniPCR machine will light up.**

You can now open the miniPCR lid and remove your PCR tubes.



**Be very careful not to touch the metal lid which may still be hot**



*The PCR product can now be stored up to 1 week in the fridge or 1 year in a freezer.*

## **Gel electrophoresis – Running the gel**

1. Make sure the gel is completely submerged in electrophoresis buffer
  - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).
  - Fill all reservoirs of the electrophoresis chamber and add just enough buffer to cover the gel and wells.
  
2. Load PCR samples onto the gel in the following sequence
  - **Lane 1:** 10µL DNA ladder
  - **Lane 2:** 15µL PCR from **Suspect A**
  - **Lane 3:** 15µL PCR from **Suspect B**
  - **Lane 4:** 15µL PCR from **Control H**
  - **Lane 5:** 15µL PCR from **Control D**

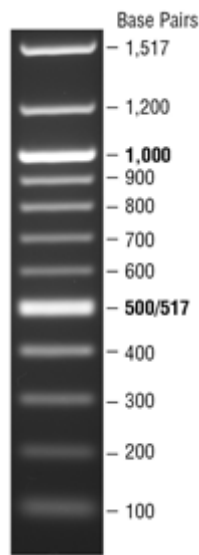


Note: there is no need to add gel loading dye to your samples.  
*miniPCR EZ PCR Master Mix* and *100 bp DNA Ladder* are Load-Ready™!

3. Place the cover on the gel electrophoresis box
  - Ensure the electrode terminals fit snugly into place
  
4. Insert the terminal leads into the power supply (not needed if using blueGel™)
  
5. If using blueGel™, simply press the “Run” button. Otherwise, set the voltage at 100-130V. Conduct electrophoresis for 15-20 minutes, or until the colored dye has progressed to about half the length of the gel
  - Check that small bubbles are forming near the terminals in the box
  - Longer electrophoresis times will result in better size resolution
  
6. Once electrophoresis is completed, turn the power off and remove the gel from the box (not needed if using blueGel™ which has a built-in illuminator)

## **D** *Size determination and interpretation*

1. Place the gel on the transilluminator (or turn on the blueGel™ illuminator)
  - *Wear UV-protective goggles if using UV light*
2. Verify the presence of PCR product
3. Ensure there is sufficient DNA band resolution in the 400-800 bp range of the 100bp DNA ladder



- Run the gel longer if needed to increase resolution
- DNA ladder should look approximately as shown

4. Document the size of the PCR amplified DNA fragments by comparing the PCR products to the molecular weight reference marker (100bp DNA ladder)
  - Capture an image with a smartphone camera
  - If available, use a Gel Documentation system