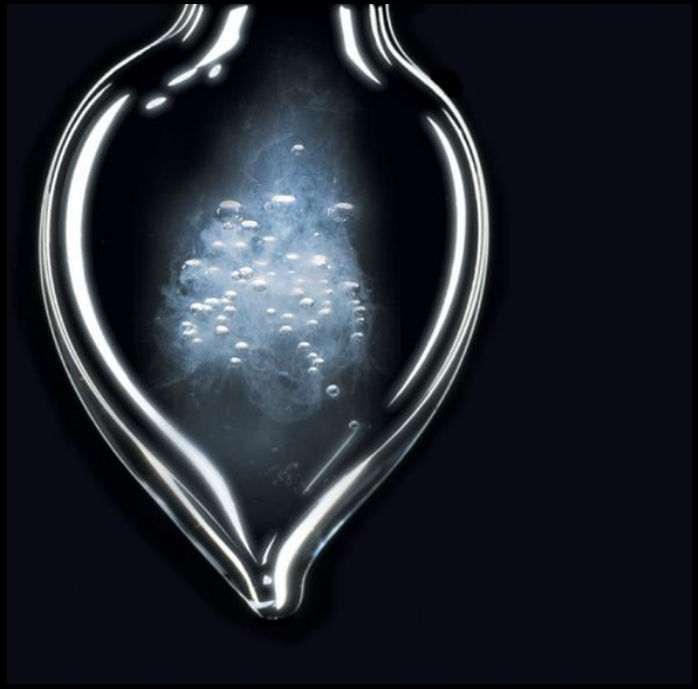

Biotechnology
CAPTIVATING SCIENCE EDUCATION
Explorer™
BIO-RAD
Professional Development

Biotechnology Explorer™ CAPTIVATING SCIENCE EDUCATION

BIO-RAD

Genes in a Bottle

Capture Your Unique Essence!



Biotechnology Explorer™ CAPTIVATING SCIENCE EDUCATION

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Genes in a Bottle Kit

Capture Your Unique Essence!

Instructors

Stan Hitomi
Coordinator – Math & Science
San Ramon Valley Unified School District
Danville, CA

Kirk Brown
Lead Instructor, Edward Teller Education Center
Science Chair, Tracy High School
and Delta College, Tracy, CA

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Curriculum and Training Specialist
Bio-Rad Laboratories

Essy Levy, M.Sc.
Curriculum and Training Specialist
Bio-Rad Laboratories

Why Teach DNA Extraction?

- **Links classroom to real-world science**
- **Tangible results**
- **Laboratory extensions**
  - cheek cell staining with Fast Blast DNA Stain (an excellent microscope activity)
  - staining precipitated DNA with Fast Blast DNA Stain
  - DNA electrophoresis/fingerprinting
- **Aligned with standards**
  - Reinforce the structures of the cell
  - DNA structure and function
  - Enzyme function

Scientific Inquiry

- Conduct human genomic DNA extraction procedure
- Extract DNA from cheek cells
- Precipitate and preserve DNA

Chemistry of Life

- Chemical and physical properties of DNA
- Properties of enzymes
- Solubility of biological molecules

Genetics

- DNA location, structure, and function
- Genes, chromosomes, and genomes
- Chromosomal inheritance
- Human genome structure

Evolution

- DNA and genetic variation among individuals
- Genetic inheritance

Cell and Molecular Biology

- Eukaryotic cell structure and organization
- Organelles and cell membranes
- Cell nucleus and DNA staining

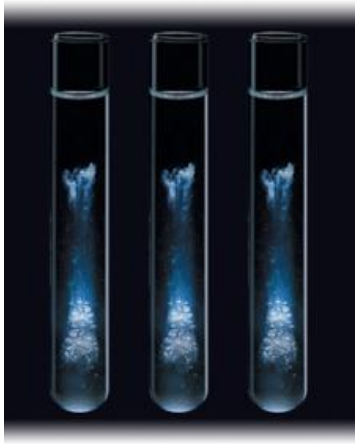
Environmental and Health Science

- Genetic testing
- DNA profiling
- Forensic DNA science

Genes in a Bottle Kit Advantages

- **Provides enough reagents for 36 student DNA extractions**
- **The activity fits into a single 45-minute period**
- **Curriculum Manual is set up in two sections:**
  - Basic level instruction (for grades 5-8)
  - Advanced level instruction (for grades 9-14)
- **This activity does not require any specialized equipment**
- **The product is accompanied by Bio-Rad's world-class technical support**
- **Cost effective**
- **Raise $$ for science programs**

Genes in a Bottle Workshop Timeline



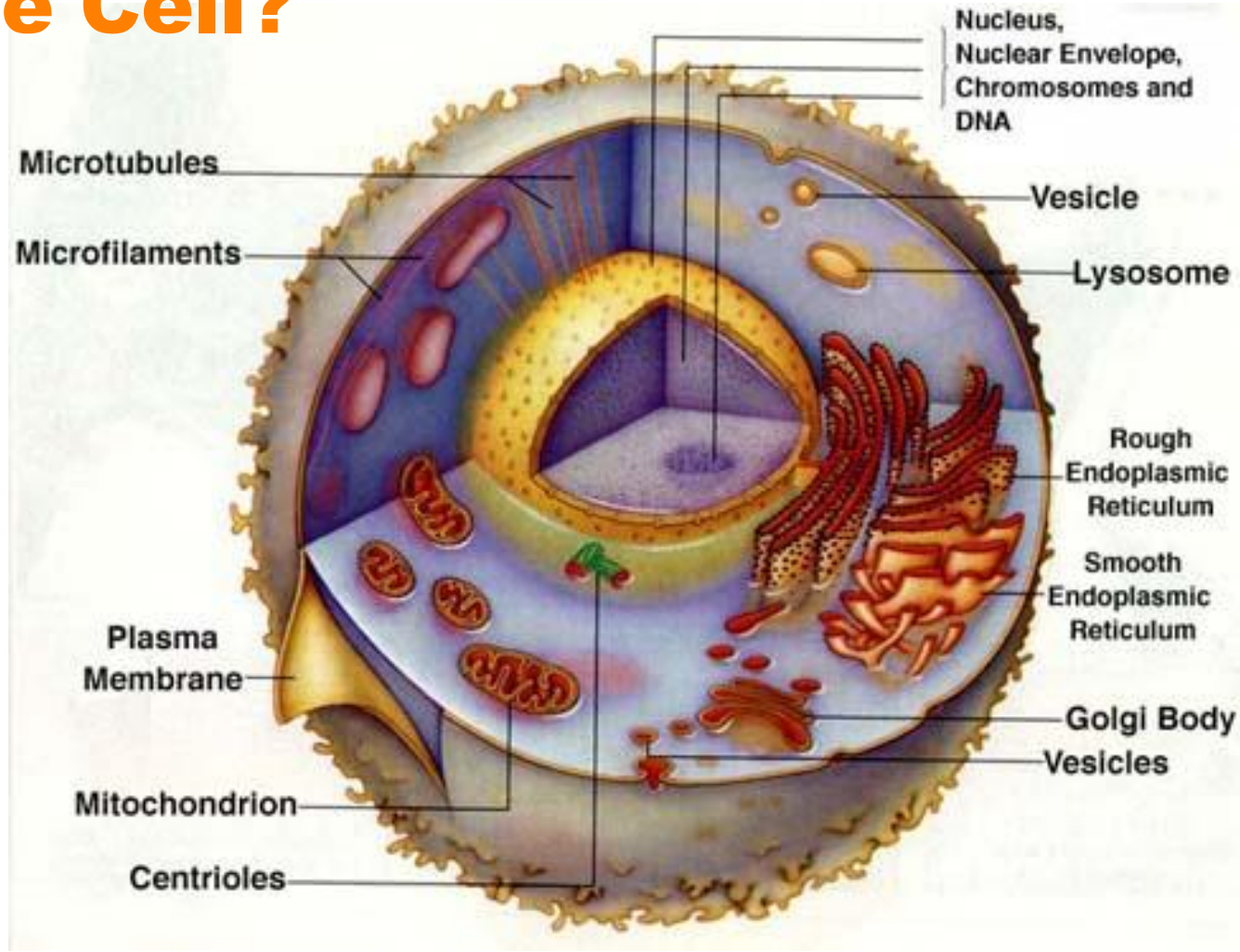
- Introduction
- Background on DNA Extraction
- Extract Genomic DNA from Cheek Cells
- Prepare DNA Necklaces

Relevance of DNA Isolation

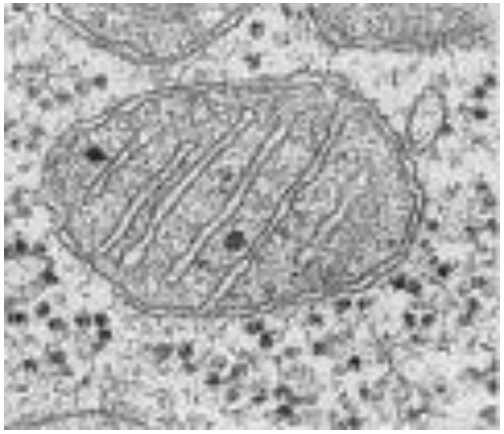
Isolation of DNA is often the first step before further analysis

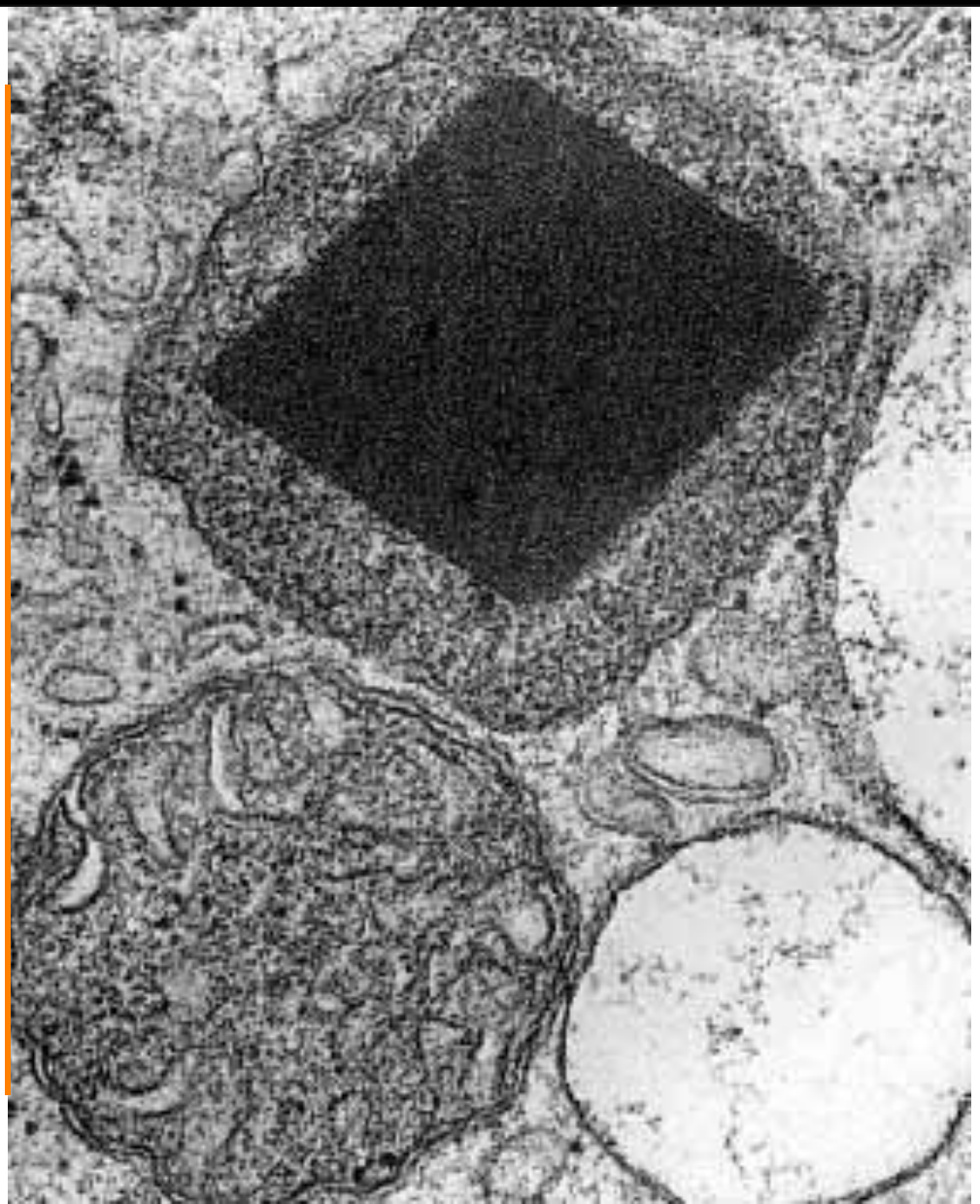
- **DNA profiling**
- **Cloning**
- **Disease diagnosis**
- **DNA sequencing**
- **Genetically modified organisms (GMO) -** agriculture, pharmaceutical
- **Environmental testing, biodefense**

Cell Bio 101: What are the Structures of the Cell?



Cell Structures





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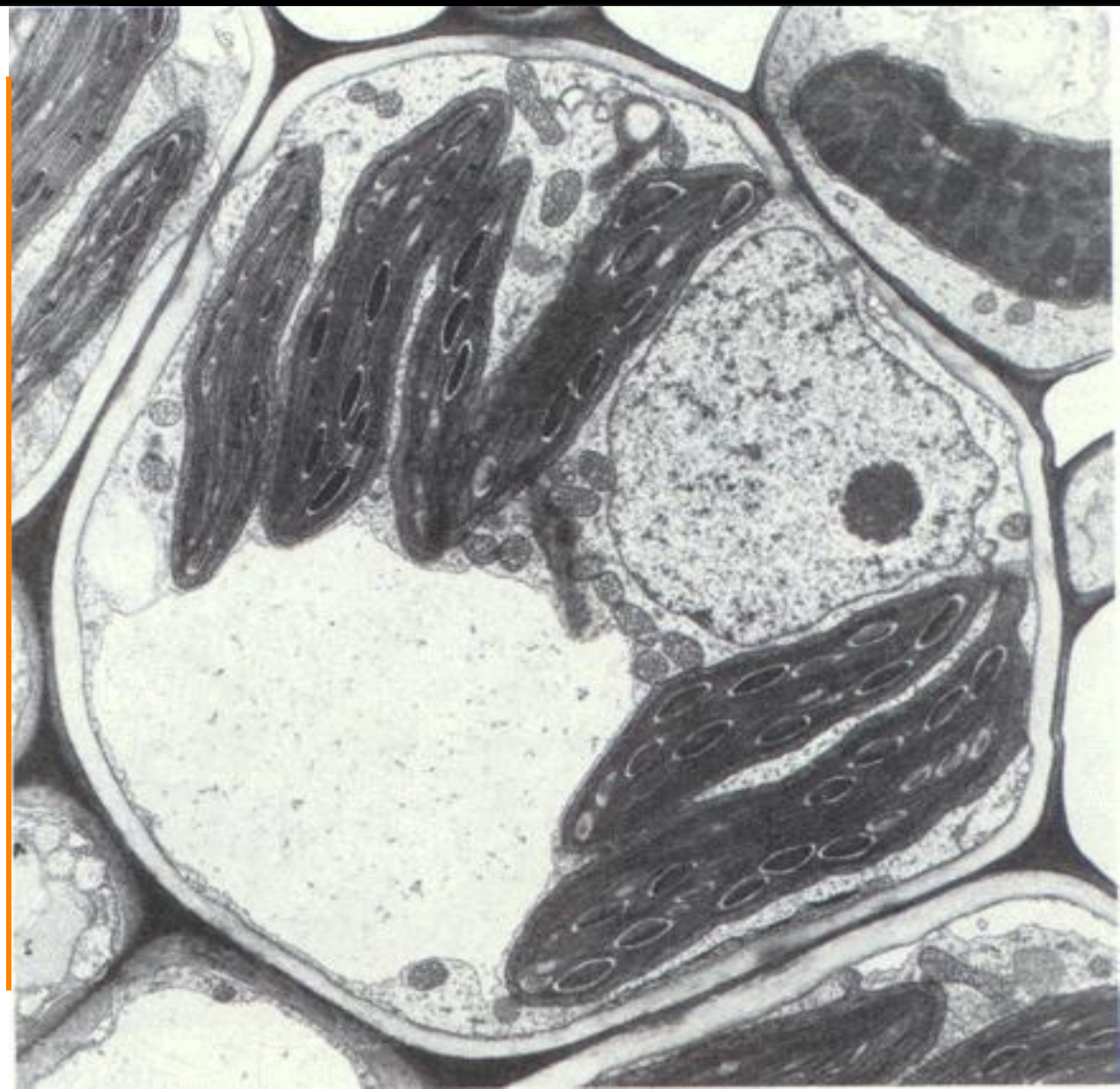
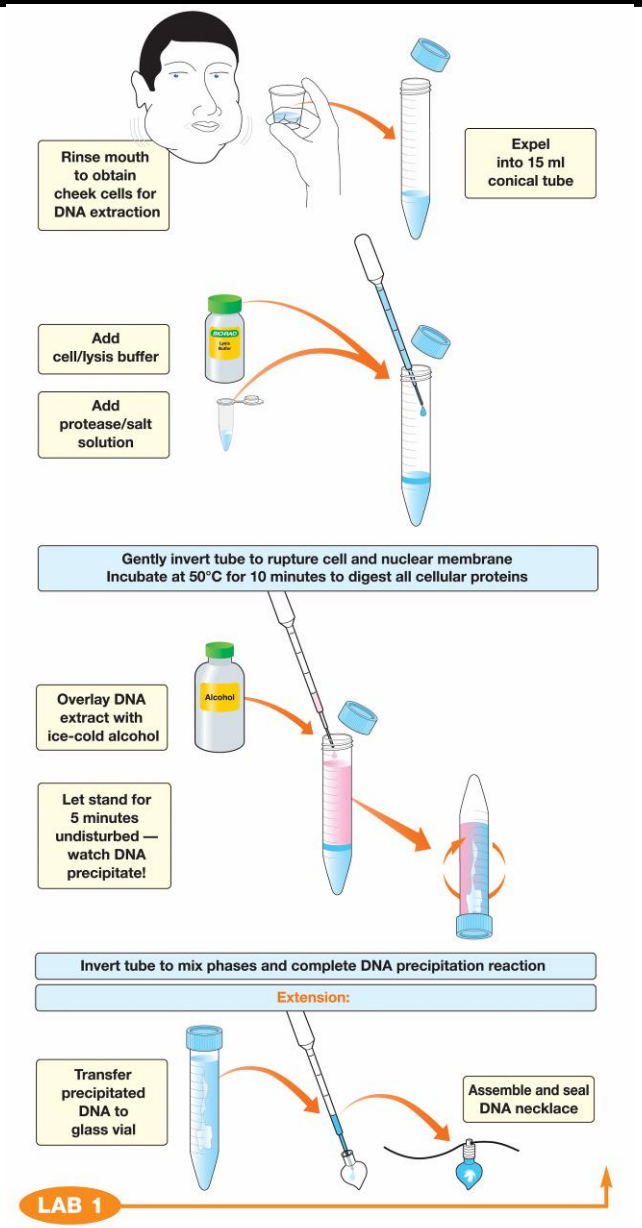
Cell Structures



Protocol Highlights: Genomic DNA Extraction

- Use a simple water mouthwash to collect cheek cells
- Add Lysis buffer to cells to break open cell and nuclear membranes and release nuclear contents
- Digest sample with protease to degrade proteins
- Precipitate DNA with cold alcohol in high salt

Genes in a Bottle: Procedure Overview



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DNA Extraction and Precipitation Workstation Inventory

4 Students/Workstation for a total of 36 Students

Teacher's (Common) Station

Water bath at 50°C

Ice-cold bottle of 91% isopropanol or 95% ethanol on ice

Students' Workstation (4 students per station)	**No. Required**
15 ml tubes each containing 3 ml water	4
Pink micro test tube labeled "prot", containing 1.25 ml of rehydrated protease + salt	1
15 ml tube labeled 'lysis' containing 10 ml Lysis buffer	1
Disposable plastic transfer pipets	6
Foam micro test tube holder	1
Permanent marker	1
Disposable paper cup or beaker for holding 15 ml tubes and subsequent waste collection	1
Necklace components or 1.5 ml flip-top tubes for DNA storage	1
Quick Guide	1

Ample Cell Collection is Critical for Success

For best results, make sure students spend the recommended amount of time collecting mouth cells.

Some users may find collecting mouthwash in a 15 ml tube difficult. As an alternative, instructors may wish to use a small drinking cup to dispense and collect mouth wash.

Laboratory Quick Guide

Quick Guide for DNA Extraction and Precipitation

1. Obtain 15 ml tube containing 3 ml water from your instructor. Label the tube with your initials.

2. Gently chew the insides of your cheeks for 30 seconds. It is NOT helpful to draw blood!

3. Take the water from the 15 ml tube into your mouth, and swish the water around vigorously for 30 seconds.

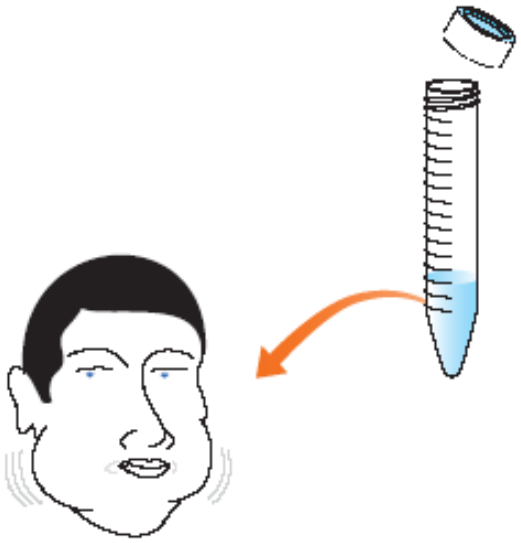
4. Carefully expel the liquid back into the 15 ml tube.

5. Obtain the tube of lysis buffer from your workstation, and add 2 ml of lysis buffer to your tube.

6. Place the cap on the tube, and gently invert the tube 5 times (don't shake your tube!). Observe your tube — do you notice any changes? If you do, write them down.

7. Obtain the tube of protease (prot) at your workstation. Add 5 drops of protease to your tube.

Laboratory Protocol



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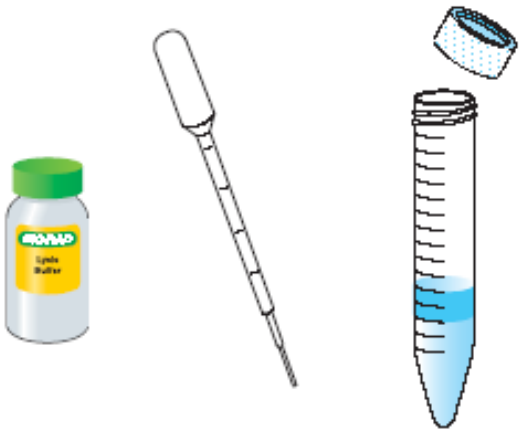

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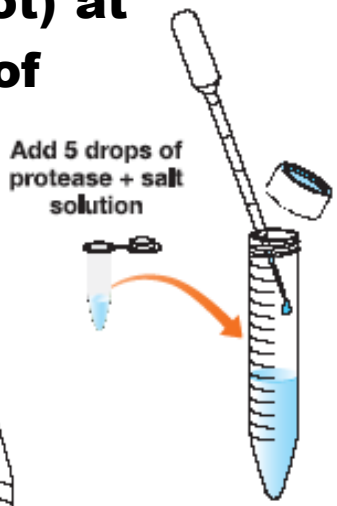
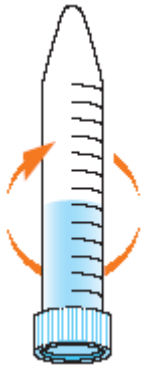


8. Place the cap on your tube, and gently invert it a few times.



9. Place your tube in a test tube rack or beaker in the water bath and incubate at 50°C for 10 minutes.

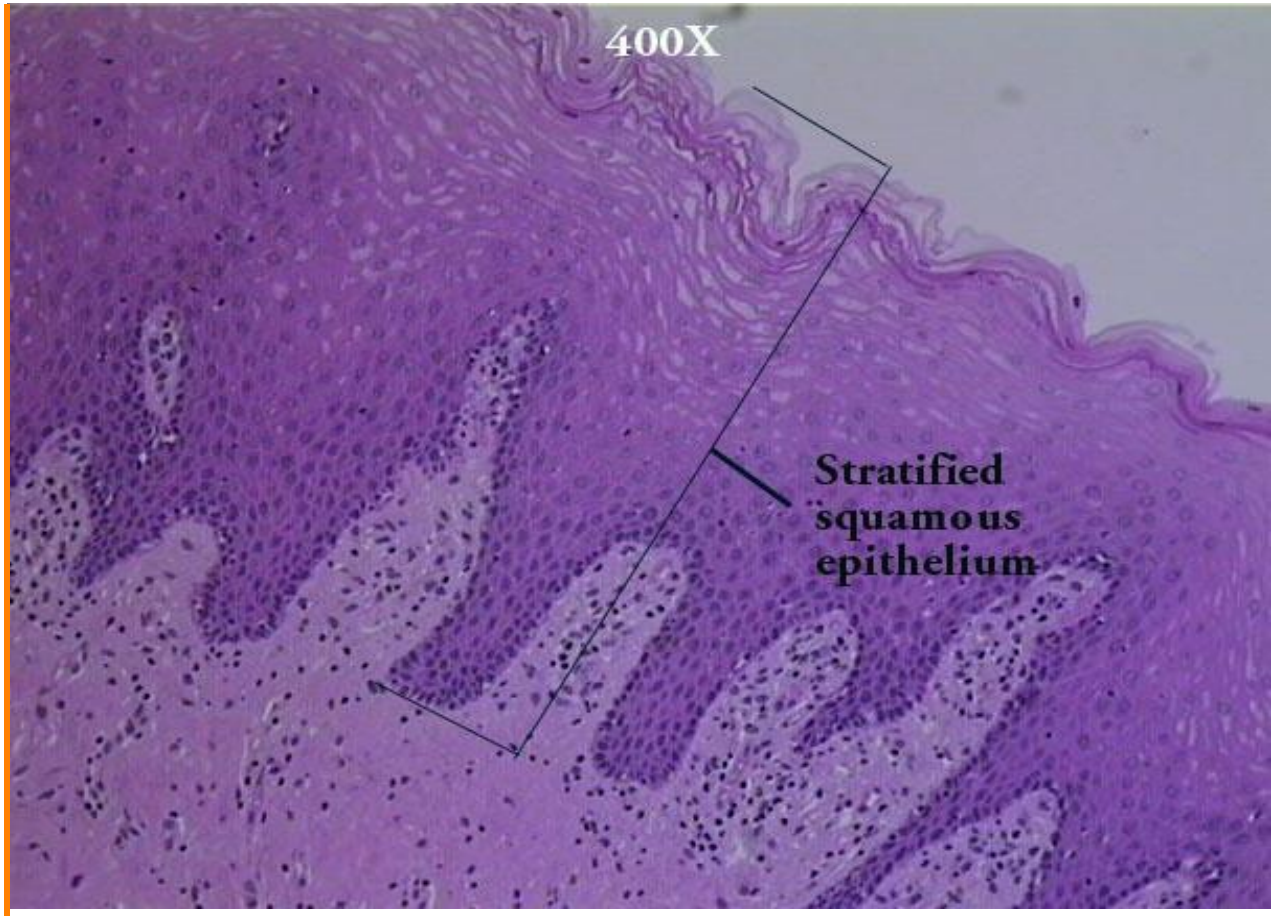
Genes in a Bottle Kit

Why Perform Each Step?

1. Cell Collection

Gently chewing the inside of the mouth combined with a water mouth wash is used to dislodge epithelial cells lining the mouth

Ample cell collection is **critical** for success.



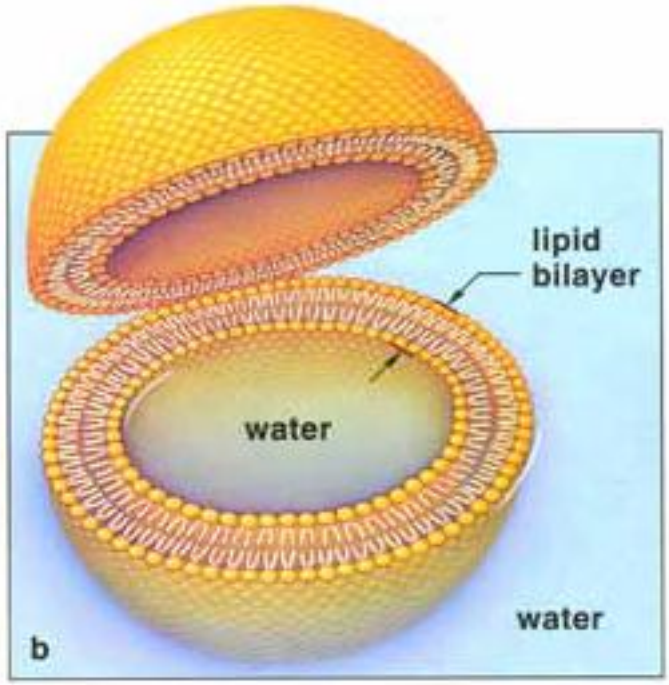
2. Lysis Buffer

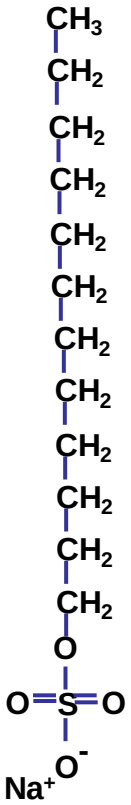
What is Lysis Buffer?

- 50 mM Tris-HCl, pH 8.0
- 1% SDS

Tris buffer to maintain the pH of the solution at a level where DNA is stable

1% SDS to break open the cell and nuclear membranes, allowing the DNA to be released into the solution (SDS also denatures and unfolds proteins, making them more susceptible to protease cleavage).





SDS

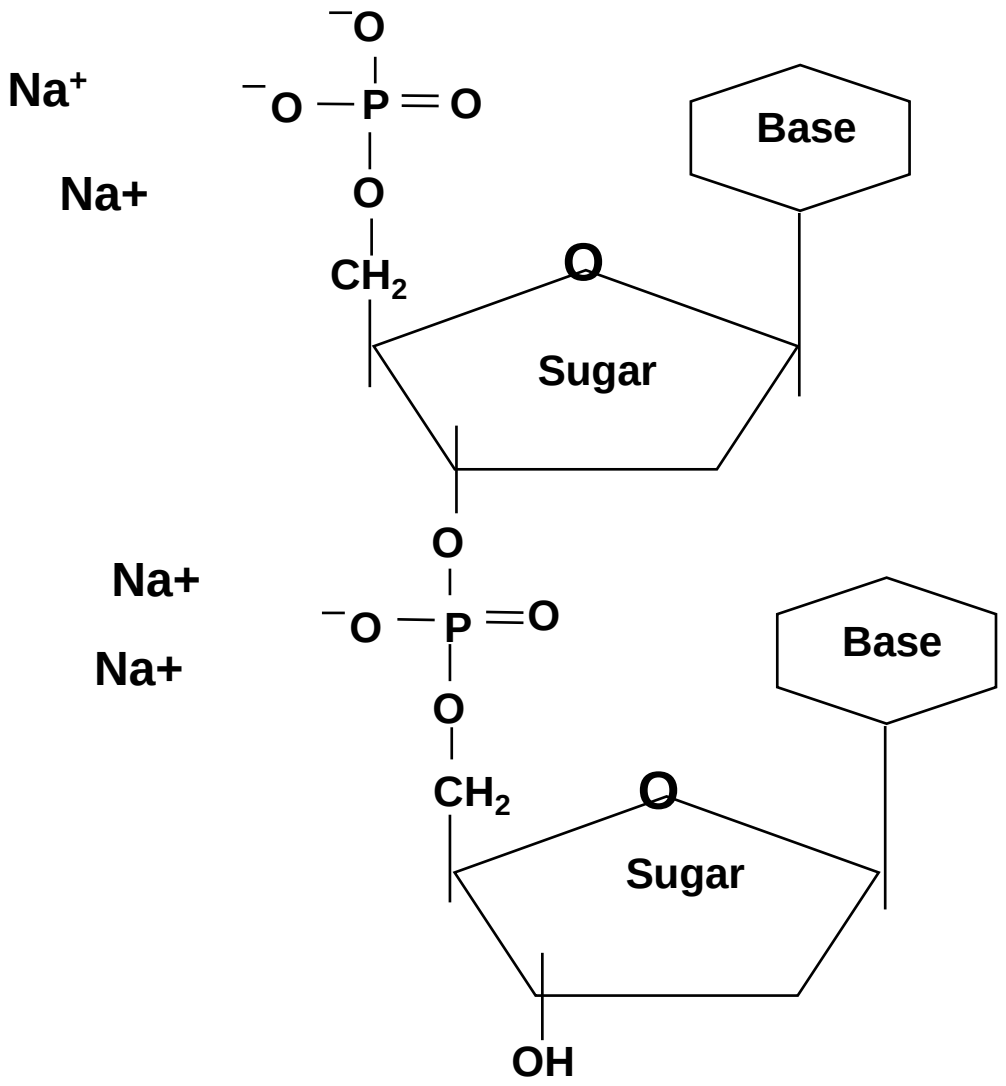
3. Why Add Protease?

- **Protease is added to destroy nuclear proteins that bind DNA and cytoplasmic enzymes that breakdown and destroy DNA.**
- **Protease treatment increases the amount of intact DNA that is extracted.**

4. Adding Salt

- The protease solution already contains salt
- Na^+ ions of NaCl bind to the phosphate groups of DNA molecules, neutralizing the electric charge of the DNA molecules.
- The addition of NaCl allows the DNA molecules to come together instead of repelling each other, thus making it easier for DNA to precipitate out of solution when alcohol is added.

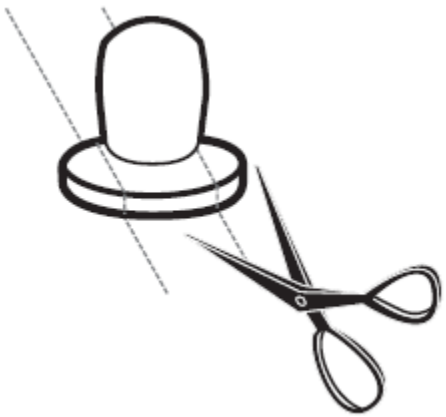
DNA Structure



5. Adding Ice Cold Alcohol?

- DNA does not dissolve in alcohol.
- The addition of cold alcohol makes the DNA clump together and precipitate out of solution.
- Precipitated DNA molecules appear as long pieces of fluffy, stringy, web-like strands.
- Microscopic oxygen bubbles “aggregate” , or “fuse” together, as the DNA precipitates.
- The larger, visible air bubbles “lift” the DNA out of solution, from the aqueous into the organic phase.

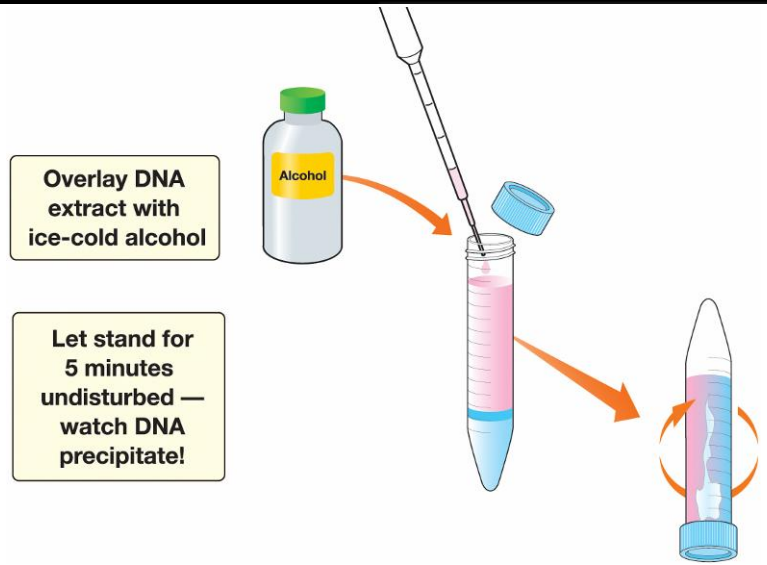
Prepare stoppers for necklaces



1. Remove stopper from vial
2. Trim “ears” off stopper on 2 sides
3. Replace in vial for later necklace creation

WHY? So the stopper doesn’t create an air pocket preventing the cap from seating fully on the vial

Laboratory Protocol

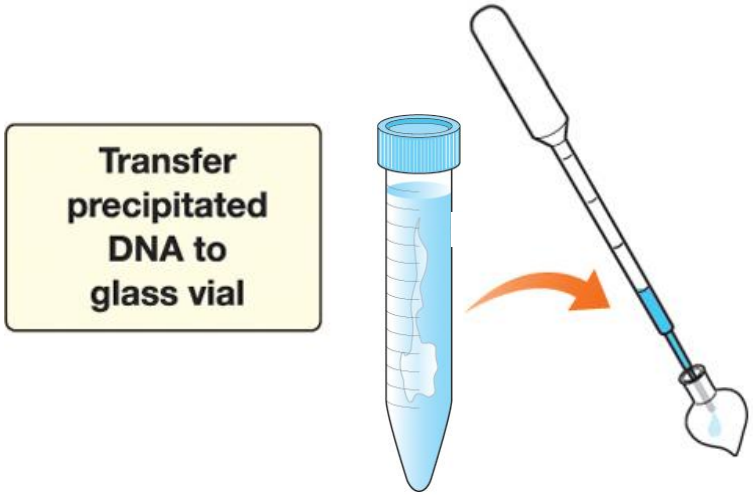


15. **Slowly add 10 ml of cold alcohol, holding the tube at a 45° angle. This will take repeated additions using the disposable transfer pipet.**

16. **Let stand undisturbed for 5 minutes at room temperature. *What do you see?***

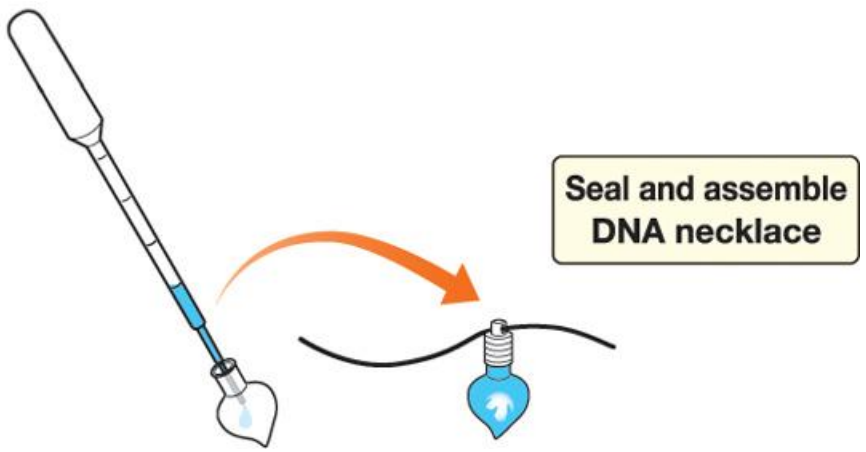
17. **Cap your tube, and very gently tilt tube on its side then turn upright about 10 times until both the water and alcohol phases have mixed and the DNA comes out of solution.**

Preserving DNA Sample: DNA Necklace Preparation



1. **Using a disposable plastic transfer pipet, carefully transfer the fluffy DNA strands you extracted into the small glass vial.**
   - **Transfer as much of your DNA and as little alcohol as possible.**
   - **The vial should be filled no higher than 2 mm from the top of the neck of the vial.**

Preserving DNA Sample: DNA Necklace Preparation



2. Firmly push the trimmed plastic stopper cap into the neck of the vial to seal the glass vial.

3. Slip the waxed cord through the silver cap.

4. Apply a small drop of super glue to the inside of the silver cap.

Preserving DNA Sample: DNA Necklace Preparation

5. Place the silver cap onto the top of the glass vial and press down firmly for 30 seconds. Allow the glue to dry for an additional 10–15 seconds, and then check for a complete seal.

6. After the glue has dried, tie the waxed cord.

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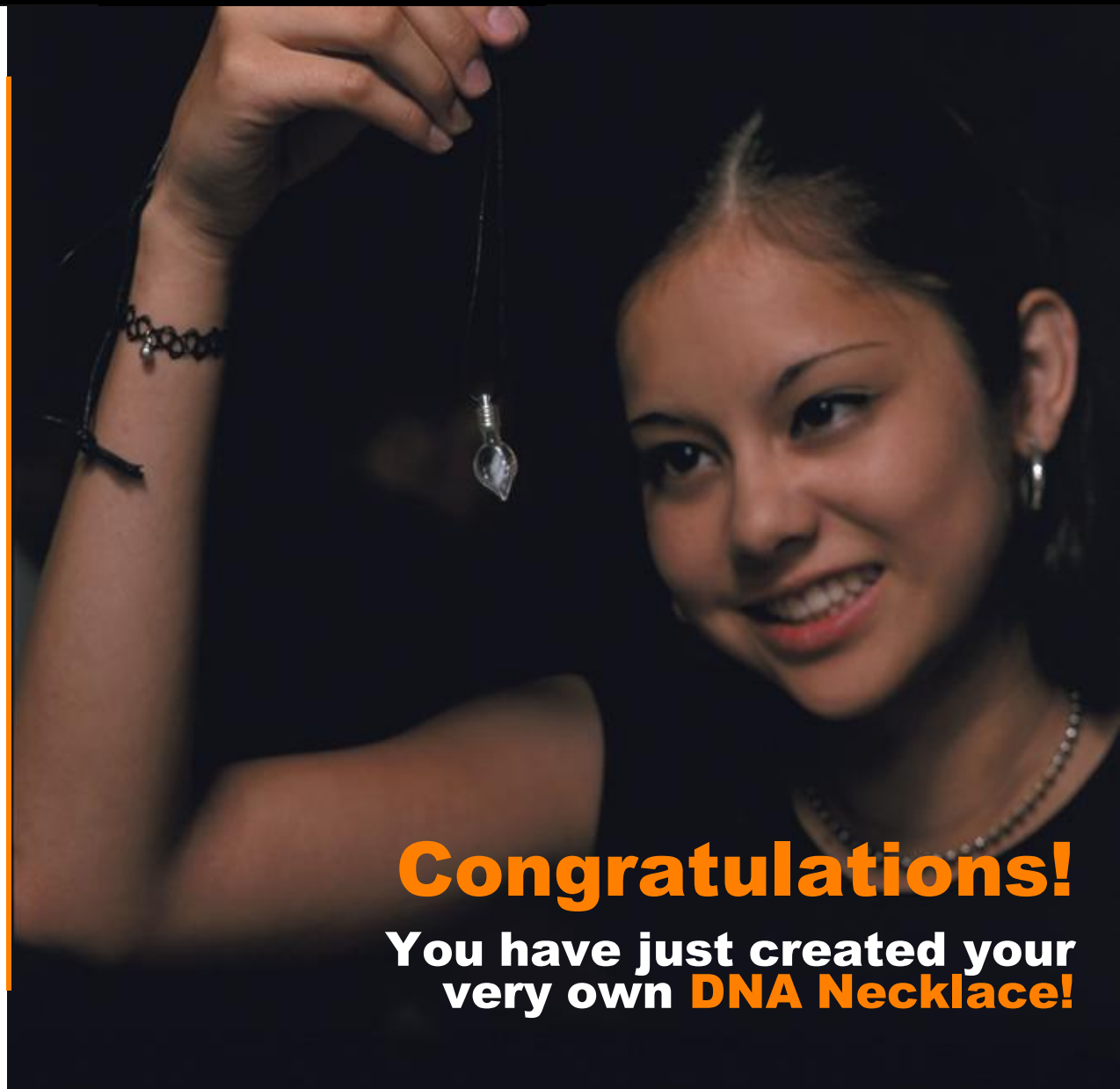

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How Long Does the DNA in the Necklace Last?

The DNA in the glass vial can last for years. Add more alcohol into the vial if some evaporation occurs.

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Genes in a Bottle Kit Components

(166-2300EDU)

Kit provides enough materials for 36 Students

Above Kit Contains:

(1) DNA Extraction Module (166-2000EDU)

(2) DNA Necklace Modules (166-2200EDU)

DNA Extraction Module (166-2000EDU):

(contains enough material for 36 students)

- Lysis buffer, 150 ml
- Powdered protease + salt, 1.5 g
- 15 ml tubes, 50
- Clear, flip-top microtubes, 60
- Multicolor, flip-top microtubes, 60
- Disposable plastic transfer pipets, 60
- Foam microtube holders, 10
- Curriculum, including a teacher's guide, a graphic quick guide, and separate student instructions for basic and advanced-level instruction

DNA Necklace Module (166-2200EDU):

(contains enough material for 18 DNA necklaces; order 2 modules for a classroom of 36 students)

- Glass vials, 18
- Silver caps, 18
- Plastic plugs, 18
- Waxed string, 18
- Super glue gel, 1 tube
- Instruction manual

Required Accessories Not Included in Kit:

- 91% isopropanol (available from drugstores) or 95% ethanol, 360 ml
- Container of ice, 1
- Permanent marker, 1–8

Recommended (Optional) Accessories:

Water bath with thermometer 166-0504EDU