

Techniques in Molecular Genetics

Polyacrylamide Gel
Electrophoresis
(PAGE) of Proteins

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PAGE Electrophoresis-Principle

PAGE can be used to separate proteins and nucleic acids.

Polyacrylamide is a crosslinked polymer of acrylamide and bis-acrylamide

Properties of the gel, especially limiting pore size, are determined by the total concentration of acrylamide-bisacrylamide (%T) and conc. of bis-acrylamide to total acrylamide (%C)

PAGE Electrophoresis-Polymerization

Two additional chemicals play important roles in the polymerization process.

Ammonium Persulfate---produces initiating free radicals when dissolved in water

TEMED- also produces free radicals

PAGE Electrophoresis-Other Chemicals



Sodium Dodecyl Sulfate

β -mercaptoethanol

PAGE Electrophoresis-Discontinuous

Most denaturing gels are composed of a *stacking gel* and a *separating gel*

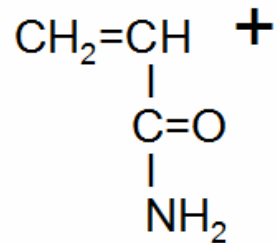
Stacking gel- low porosity, 2 pH units below running buffer

-allows proteins to form a compressed band after a few min.

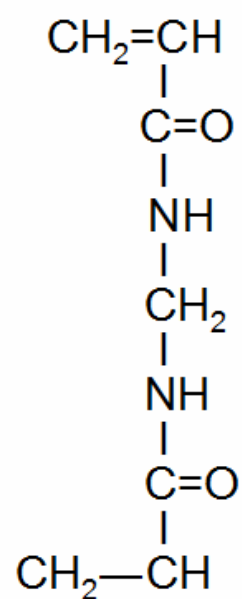
Separating gel- sieves protein according to size

PAGE Electrophoresis-Monomers

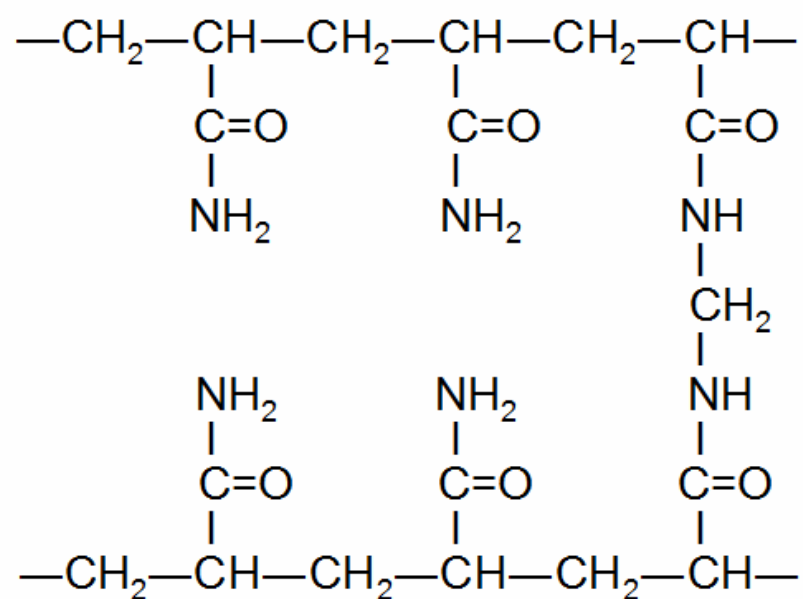
Acrylamide



Bis-acrylamide



Polyacrylamide



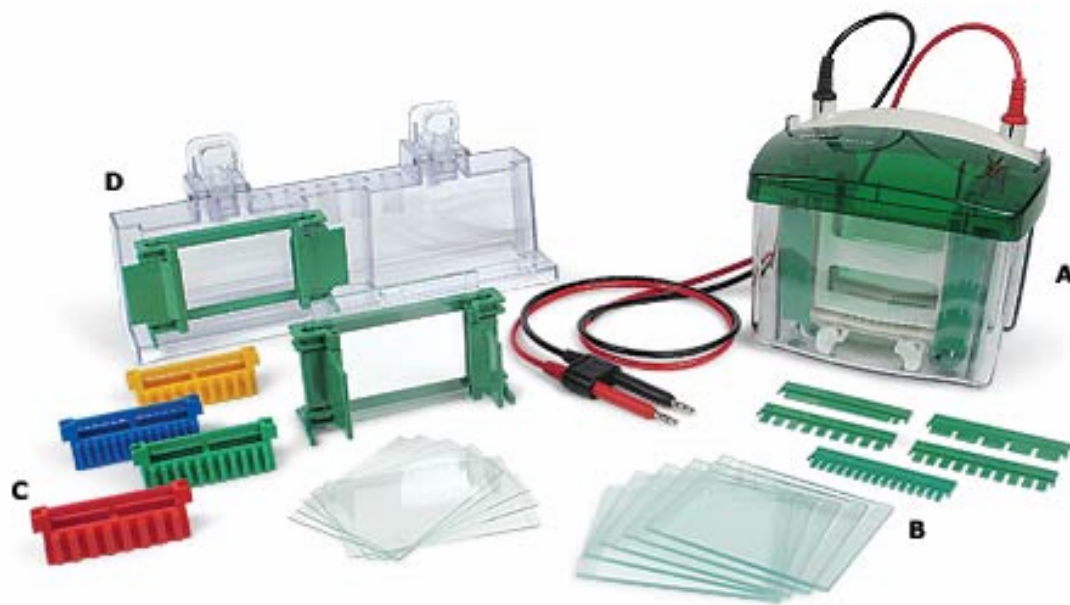
PAGE Electrophoresis-Types

- Native (non-denaturing)
- Two dimensional
- Gradient
- Isoelectric focussing

Common Problems in Using PAGE

Problem	Common Cause	Solution
Unequal lane width	Varying salt conc	Wash samples before
Vertical streaking	overload	Reduce sample
No bands	Not enough protein	Increase protein
Skewed bands	various	Use equal volumes, equal salt conc. in samples
Skewed bands at sides	Standard volume different from sample volume	Make standards up in sample buffer and use same volume as test samples

PAGE Electrophoresis Equipment



- A-Electrophoresis Cell
- B-Glass Plates
- C-Combs
- D-Casting Stand

Electrophoresis-Principle

Electrophoresis i

Fig. 2. Assembling the Mini-PROTEAN 3 cell.

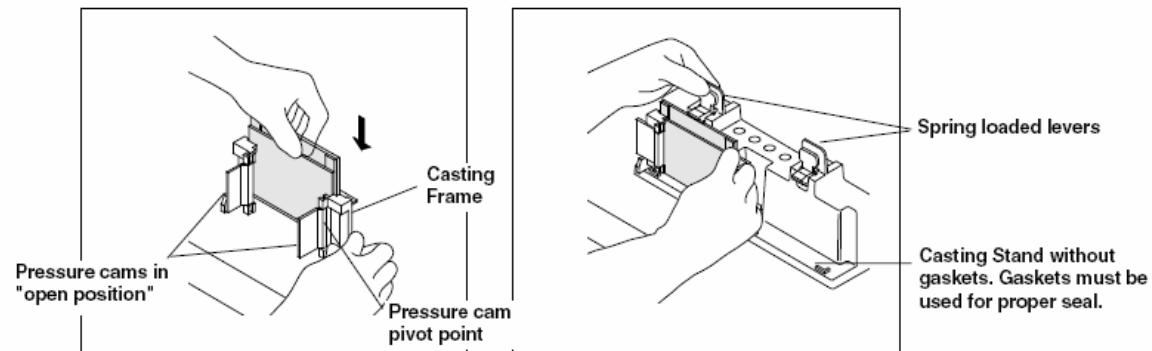
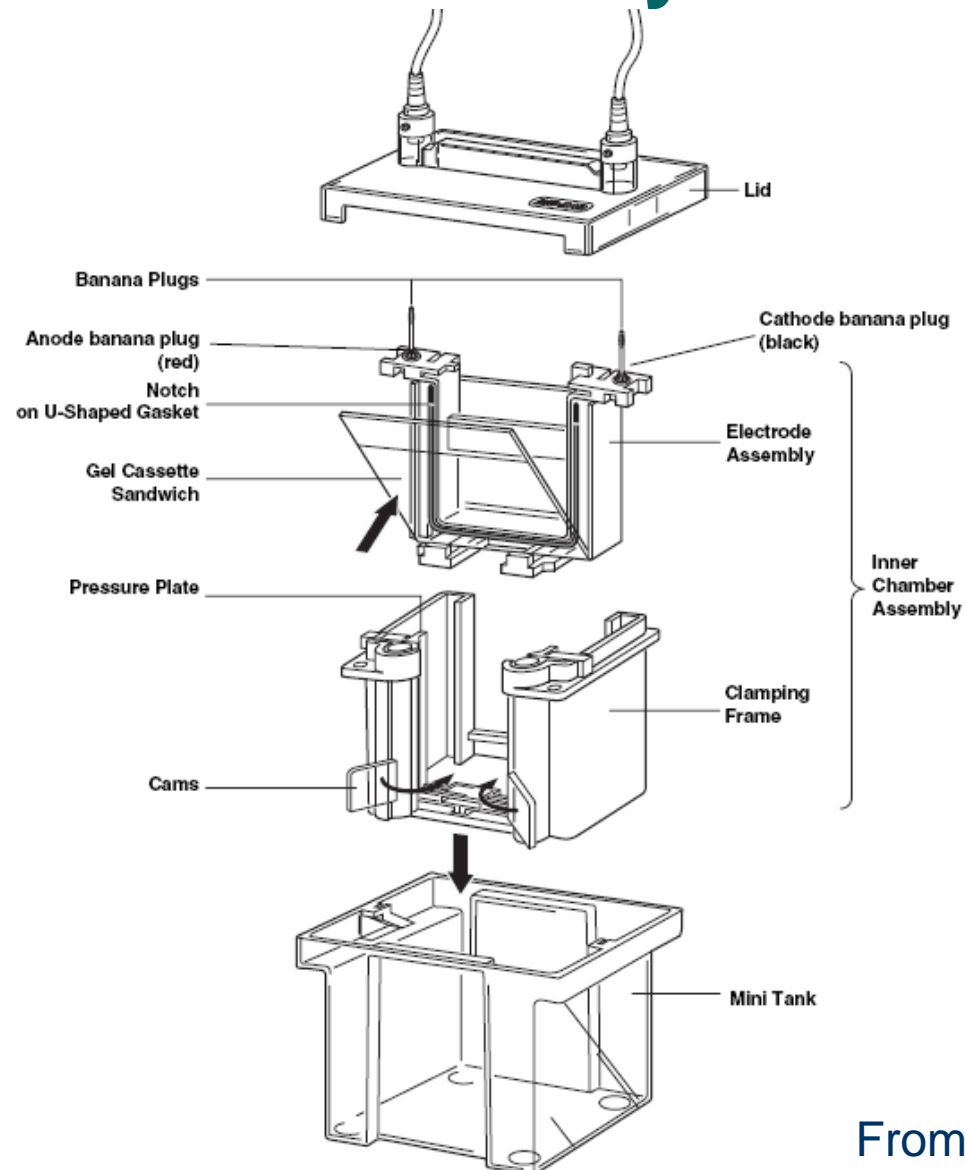


Fig. 3. Assembling the Mini-PROTEAN 3 Casting Frame and Casting Stand.

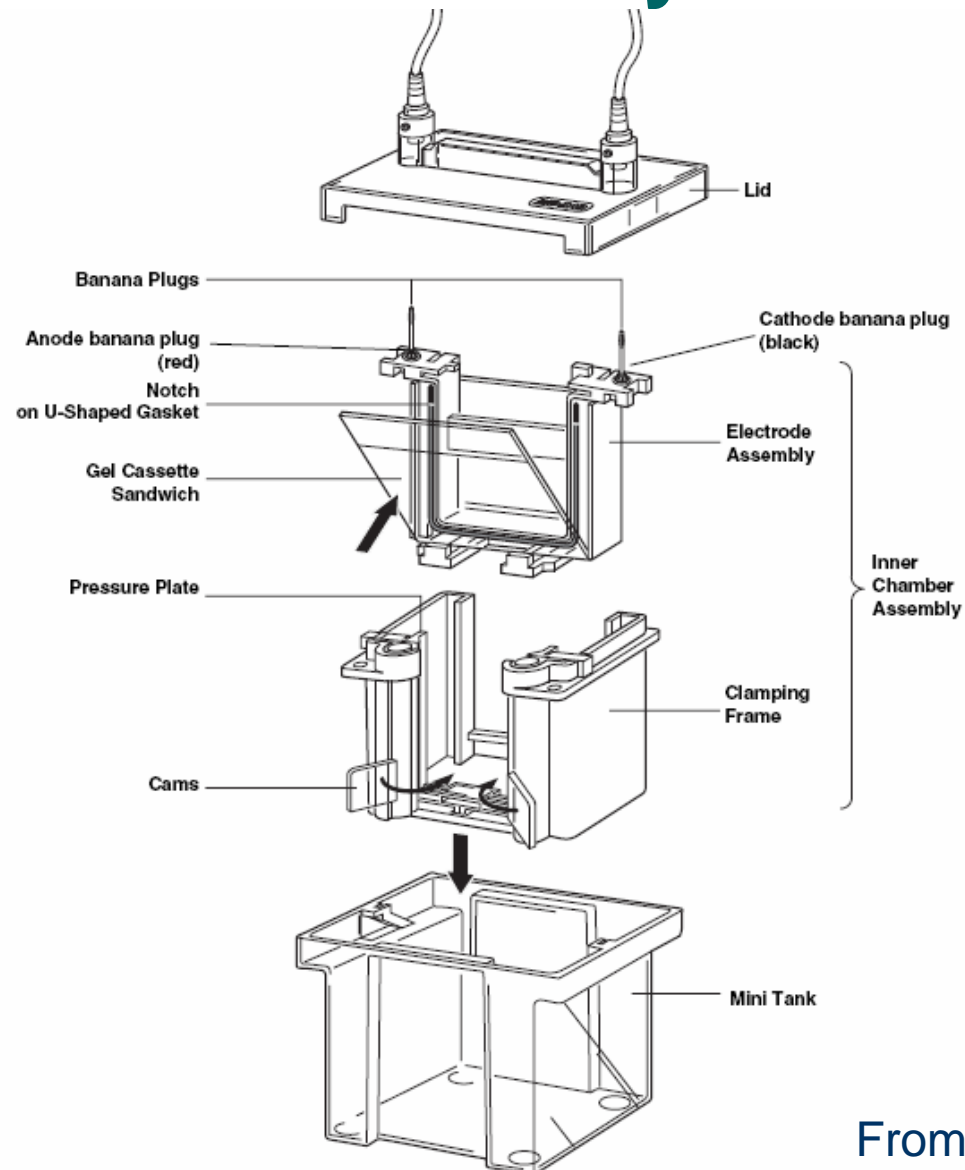
From Biorad Manual

Using the Biorad PAGE System...



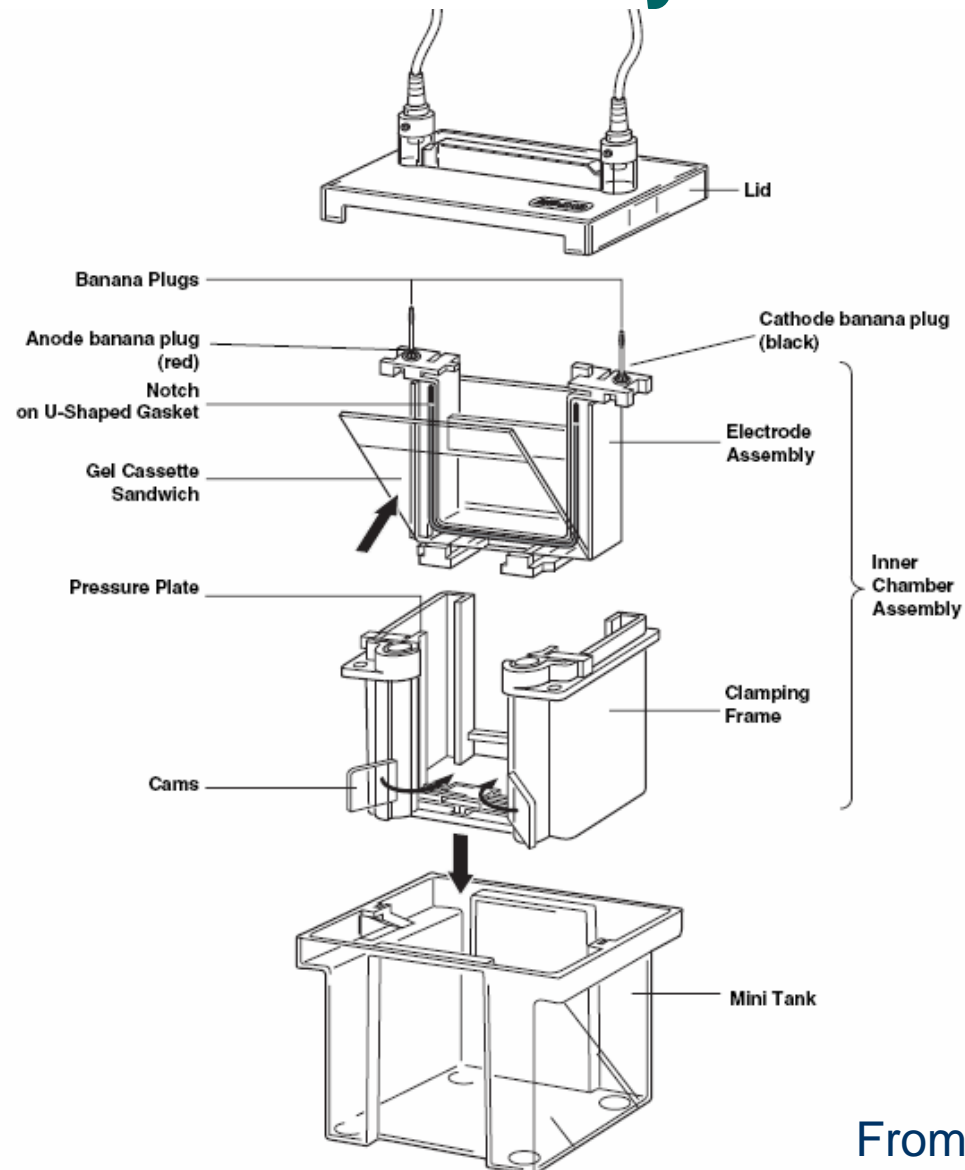
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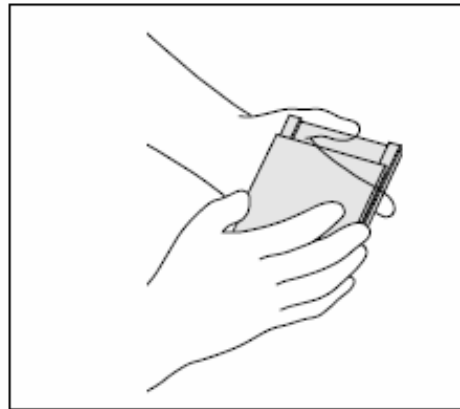
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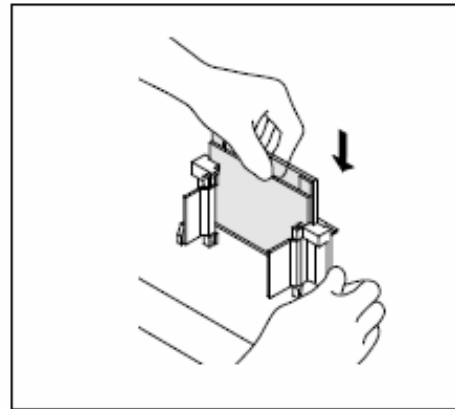


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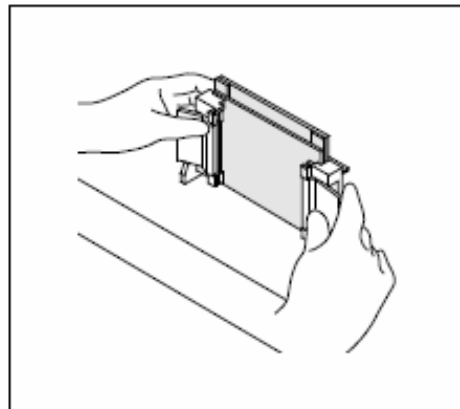
Using the Biorad PAGE System...



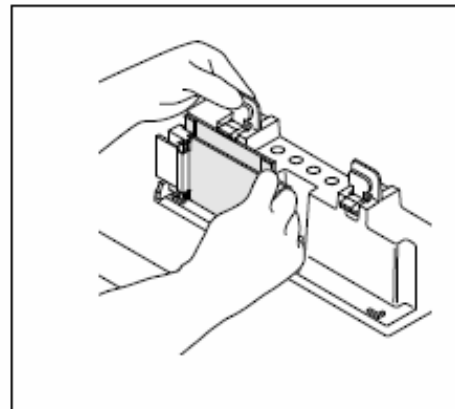
4a. Place a Short Plate on top of the Spacer Plate.



4b. Slide the two plates into the Casting Frame keeping the Short Plate facing front.



4c. Lock the pressure cams to secure the glass plates.

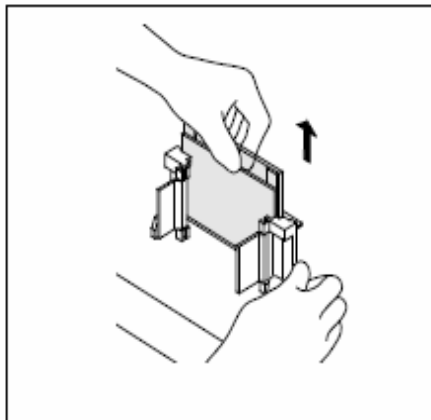


4d. Secure the Casting Frame in the Casting Stand by engaging the spring loaded lever.

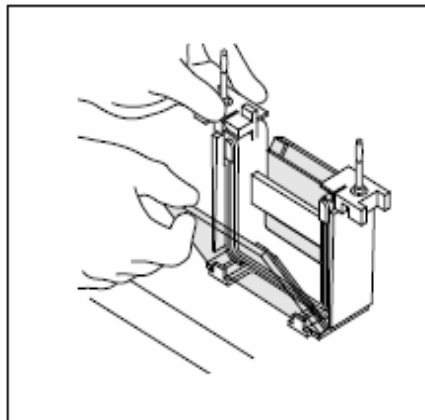
Fig. 4. Assembling the Mini-PROTEAN 3 casting stand and frame.

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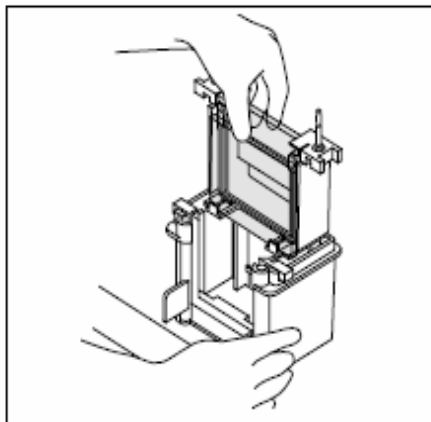
Using the Biorad PAGE System...



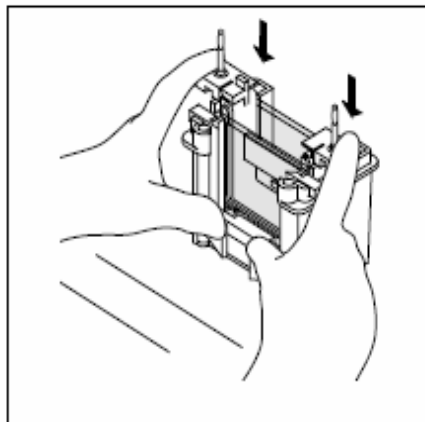
5a. Remove the Gel Cassette Sandwich from the Casting Frame.



5b. Place Gel Cassette Sandwich into the Electrode Assembly with the Short Plate facing inward.



5c. Slide Gel Cassette Sandwiches and Electrode Assembly into the clamping frame.



5d. Press down on the Electrode Assembly while closing the two cam levers of the Clamping Frame.

Using the Biorad PAGE System...

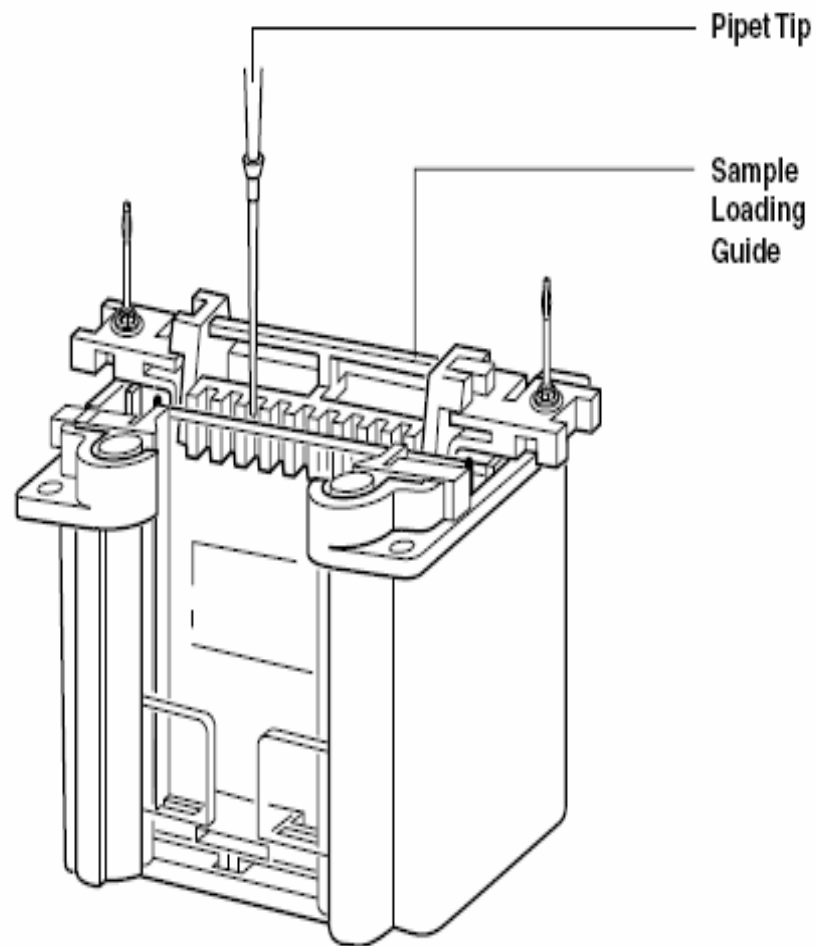


Fig. 6. Using the Sample Loading Guide (patent #5,656,145).

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