CONCEPT: 2D-ELECTROPHORESIS

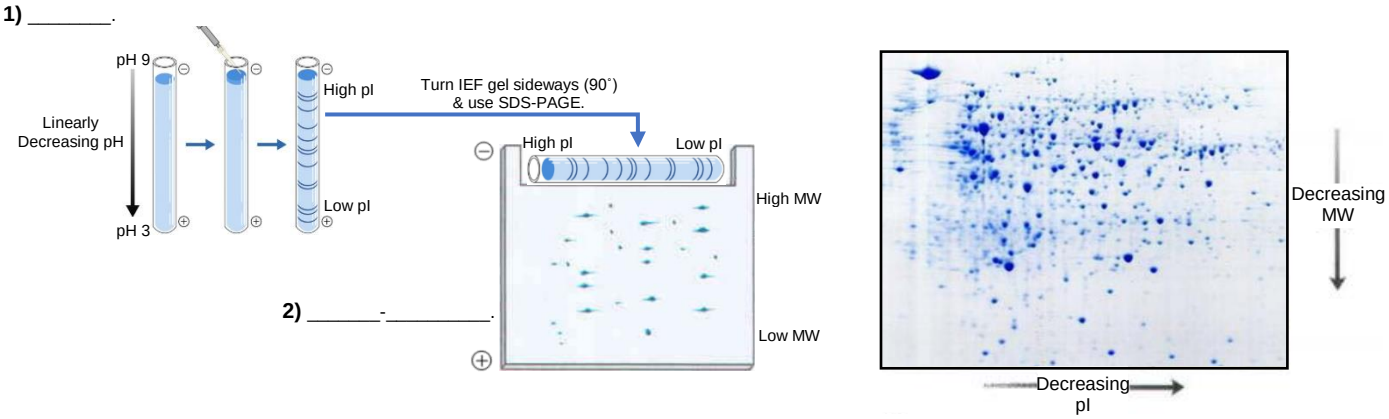
●2D-Electrophoresis: a *combination* of _____ focusing followed by _____-PAGE in the perpendicular direction.

□ 2D-Electrophoresis accomplishes _____ tasks that either technique *fails* to do on their own:

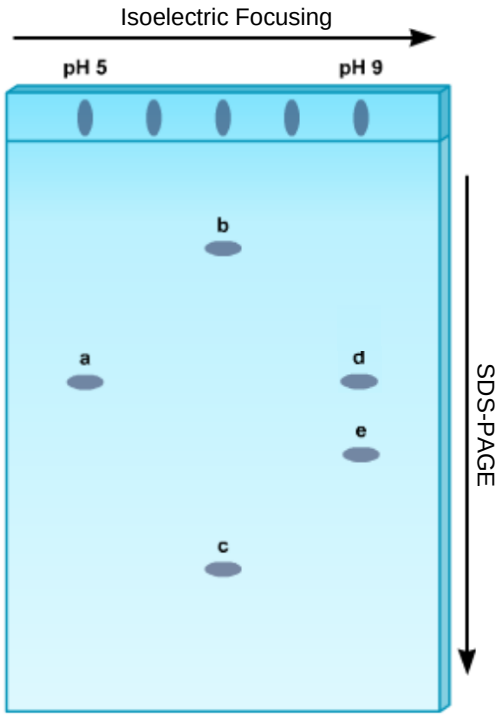
1) Separates proteins with *identical* ____, but *different* molecular weight.

2) Separates proteins with *identical* molecular _____, but *different* pI.

EXAMPLE: 2D-Electrophoresis.



PRACTICE: Use the results of the two-dimensional electrophoresis gel below to answer the following questions.



A) Which protein or proteins have the highest pI value?

a) Protein a.

b) Proteins b & c.

c) Proteins d & e.

B) Which protein or proteins have the highest molecular weight?

a) Protein a.

b) Protein b.

c) Protein c.

d) Proteins d & e.

C) Which protein or proteins have identical molecular weights?

a) Proteins a & d.

b) Proteins b & c.

c) Proteins d & e.

d) None. Each has a unique weight.

CONCEPT: 2D-ELECTROPHORESIS

PRACTICE: Which of the following is true in 2D-electrophoresis?

a) Spots on the gel corresponds to protein subunits.
b) The 1st step involves separating proteins by MW.
c) SDS is necessary to separate proteins by pI.
d) Proteins with identical pI but different MW separate.

PRACTICE: An average protein will not be denatured by:

a) A detergent such as sodium dodecyl sulfate (SDS).
b) Heating to 100˚C.
c) Iodoacetic acid.
d) A sudden change from pH 7 to pH 13.
e) Urea + β-mercaptoethanol.

PRACTICE: The first step in two-dimensional gel electrophoresis generates a series of protein bands by isoelectric focusing. In a second step, a strip of this gel is turned 90 degrees, placed on another gel containing SDS, and electric current is again applied. In this second step:

a) proteins with similar isoelectric points become further separated according to their molecular weights.
b) the individual bands become stained so that the isoelectric focus pattern can be visualized.
c) the individual bands become visualized by interacting with protein-specific antibodies in the second gel.
d) the individual bands undergo a second, more intense isoelectric focusing.
e) proteins in the bands separate further because the 2nd electric current has the opposite polarity of the 1st current.

PRACTICE: Sketch the result of 2D gel electrophoresis on the following four proteins (see chart) & label them clearly.

Protein	MW	pI	Charge at:		
			pH = 6	pH= 7	pH = 8
A	62,457	8.1	32.2	10.9	0.7
B	115,471	5.69	-7.3	-29.6	-42
C	17,183	7.7	7.4	2.1	-0.7
D	69,366	6.29	3.4	-3.4	-20.2