Utilizing 2DE gels for carcinogen screening

MaryEd Pratts

Overview

- Current method used for mutagen screening – The Ames Salmonella test

1. How it works – (S9)
2. Why a compliment would be helpful

- Our Approach - 2DE gels

1. How and why we use them
2. Current work and accomplishments
3. Future work

The Ames *Salmonella* assay

no mutagen present

mutagen present

•The Ames test consists in the detection of mutations by a bacteria that is histidine dependent: Salmonella typhimurium.

•Several strains of this bacteria, all carriers of a mutation in the his operon, can revert spontaneously to *His*+, and thus grow in a histidine-free medium.

•This very weak spontaneous reversion can be increased by mutagens, which allows the qualification of the mutagenic potential of these substances.

picture by Mary Pratts

S9 Rat Liver Enzymes

- Bacteria lack the oxidative enzyme systems for metabolizing some compounds to electrophilic metabolites capable of reacting with DNA.
- The metabolic activation system usually consists of a 9000x*g* supernatant fraction of rat liver homogenate in the presence of NADP and NADPH cofactors.

Limitations of Ames

- False positives
- False negatives
- Only compliments are extensive and usually require live animals
- Only cellular growth level, not any protein expression change

Use of 2DE gels

- Most commonly used technique in proteomics
- Proteins are separated by two different chemical properties – pH dependent isoelectric point (pI) and molecular weight
- Can easily be coupled with mass spec to identify the isolated proteins

Slide taken from Kelly Fowlkes

How 2DE gels work

<u>The first dimension:</u>

- Isoelectric Focusing (IEF)
- Protiens are applied to strip containing an immobilized pH gradient (IPG strip)
- Migrate to their pI point – net charge is zero

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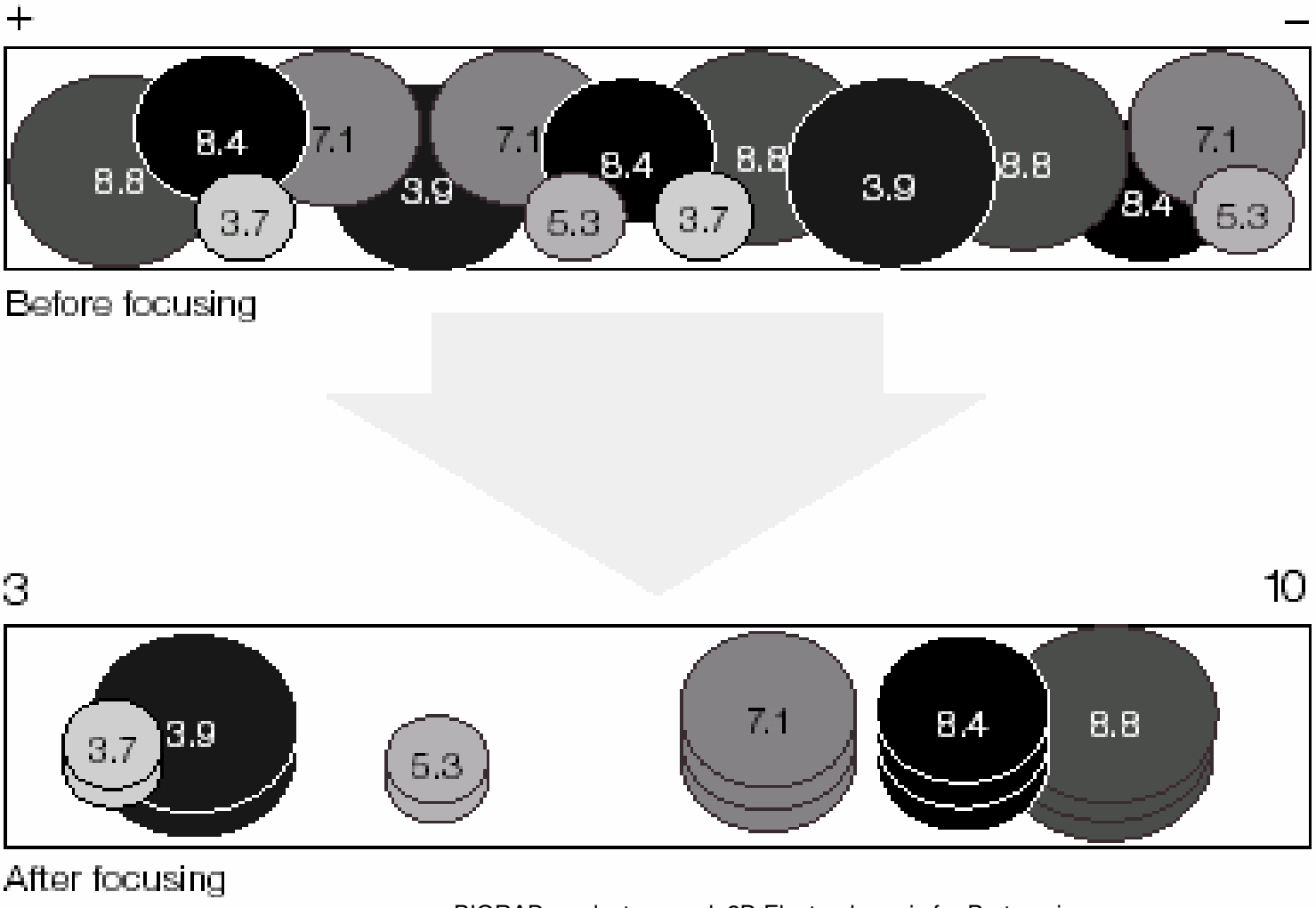
IEF



BIORAD product manual: 2D Electrophoresis for Proteomics

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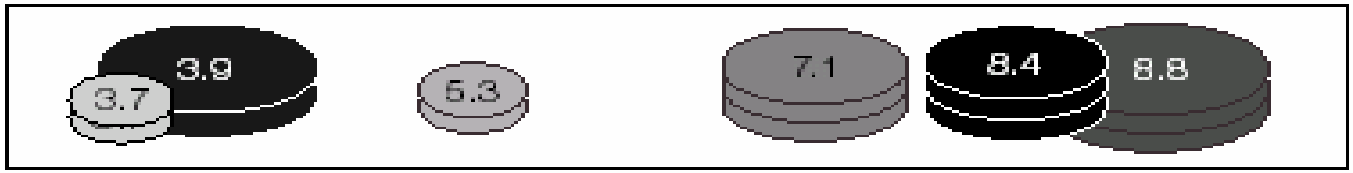
How 2DE gels work

The second dimension:

- IEF-focused proteins are equilibrated in SDS and reducing agents
- SDS coats the proteins in proportion to their mass
- Electric current is applied through the polyacrylamide gel and the proteins will migrate.

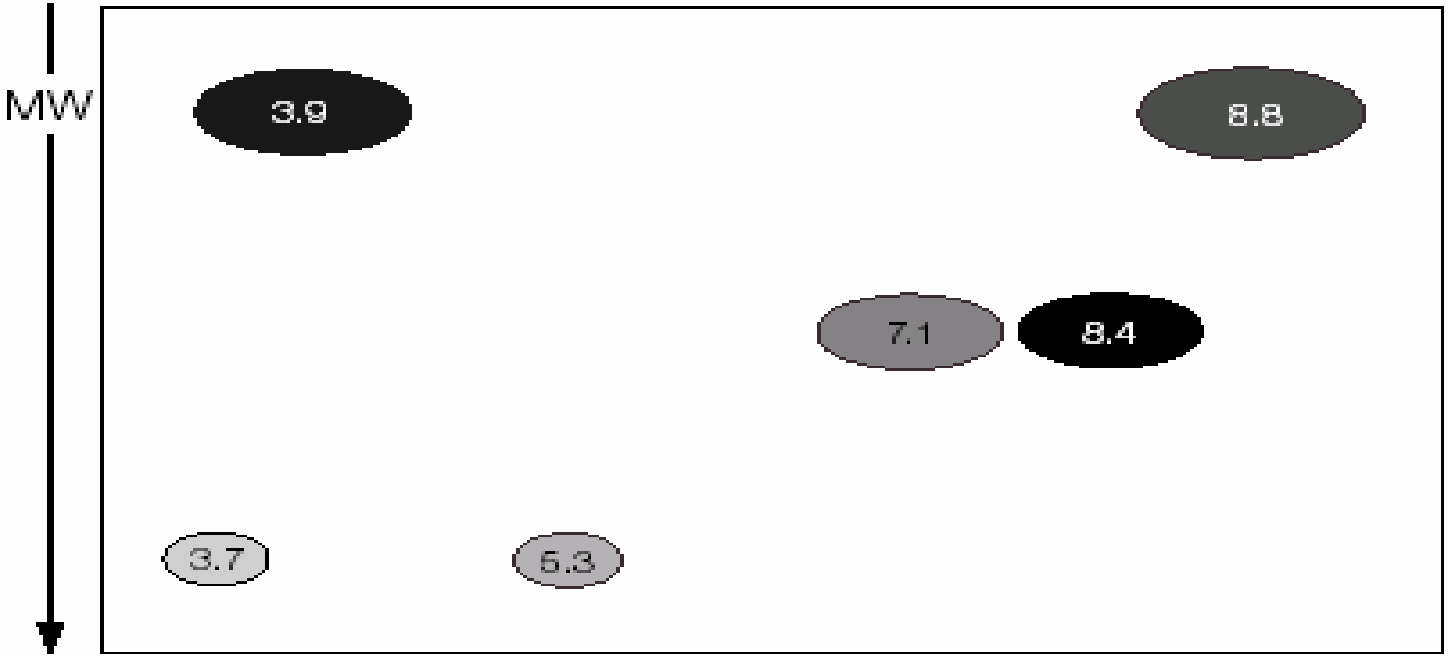
Slide taken from Kelly Fowlkes

SDS-PAGE



SDS-charged proteins in IPG strip

Apply to SDS-PAGE gel



SDS-charged proteins resolved according to size in SDS-PAGE gel

BIORAD product manual: 2D Electrophoresis for Proteomics

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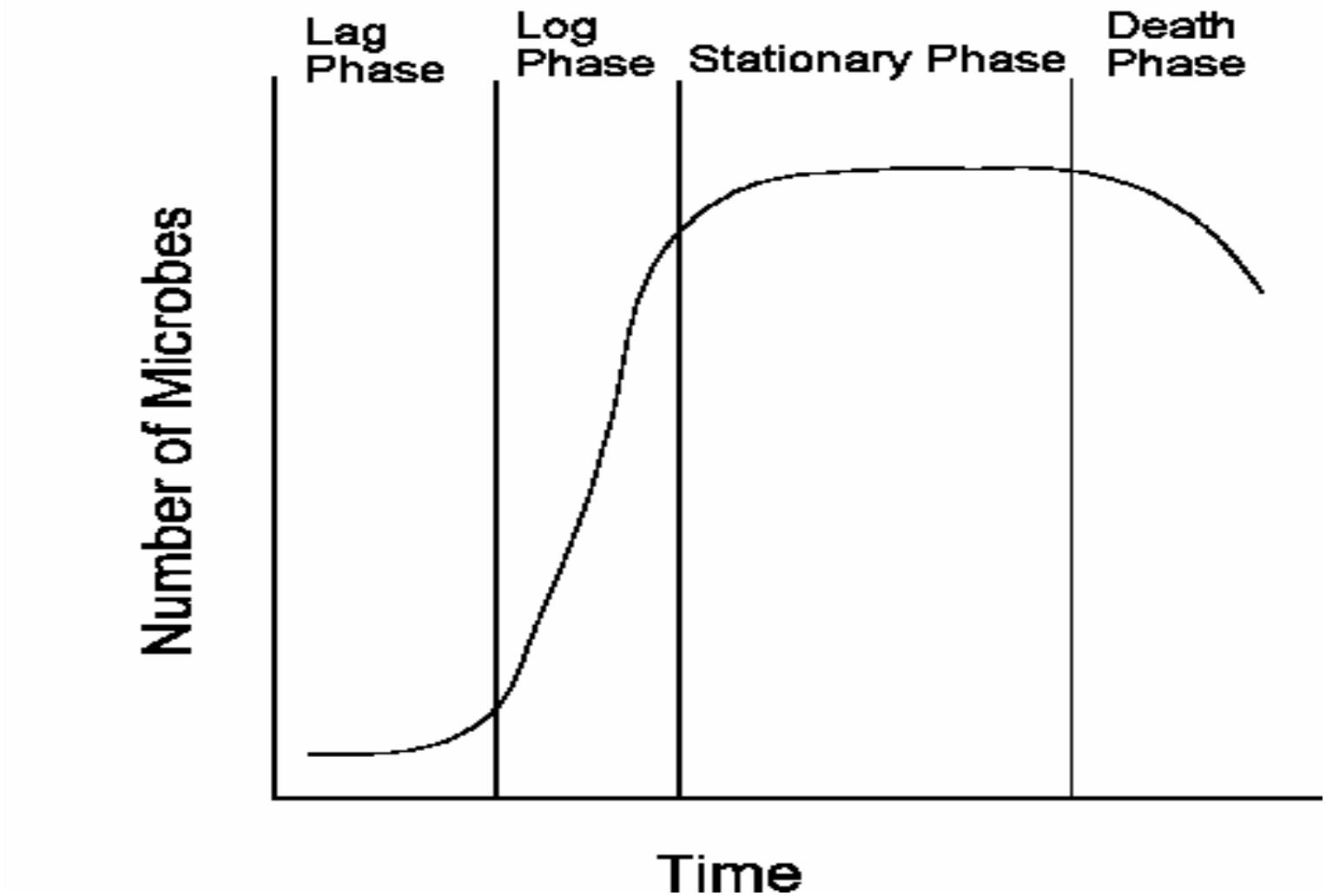
How we use 2DE

1. Grow the bacteria Pseudomonas putida on standard carbon sources in the presence of different carcinogens
2. Examine the proteins by 2DE
3. Identify the expressed proteins
4. Eventually replace the Ames Test

Sample Prep

- A bio-safe soil bacteria: *Pseudomonas putida* strains P.p.F1and P.p.KT2440 are used
- To determine when to extract the protein from cells (mid-log), a growth curve for a specific set of conditions is conducted

Bacterial Growth Curve



Sample Prep

- The preparation to extract the protein from the cells consists of: Centrifugation, Sonication, Microfugation, RNAase treatment, protein assay, and rehydration
- 2DE gels are then run on the protein sample, and the gels are then stained
- The gels are then scanned and dried
- Progenesis software is then used to analyze and compare gels

Gel Results

- We have the complete proteomic signature for that set of conditions
- Once the gels have been analyzed, we can identify biomarkers under specific growth conditions
- Mass spectrometry or other methods can then be used to find the identity of the biomarker proteins.

2DE gels - why

- In determining the biomarkers, we can also determine the differences in protein expression when a carcinogen is present in the growth conditions
- This method can be used as a test for carcinogens if common proteins (such as DNA repair) are expressed in the presence of all carcinogenic compounds
- This would be a more specific test than Ames which only tests on the cellular growth level

Group Accomplishments

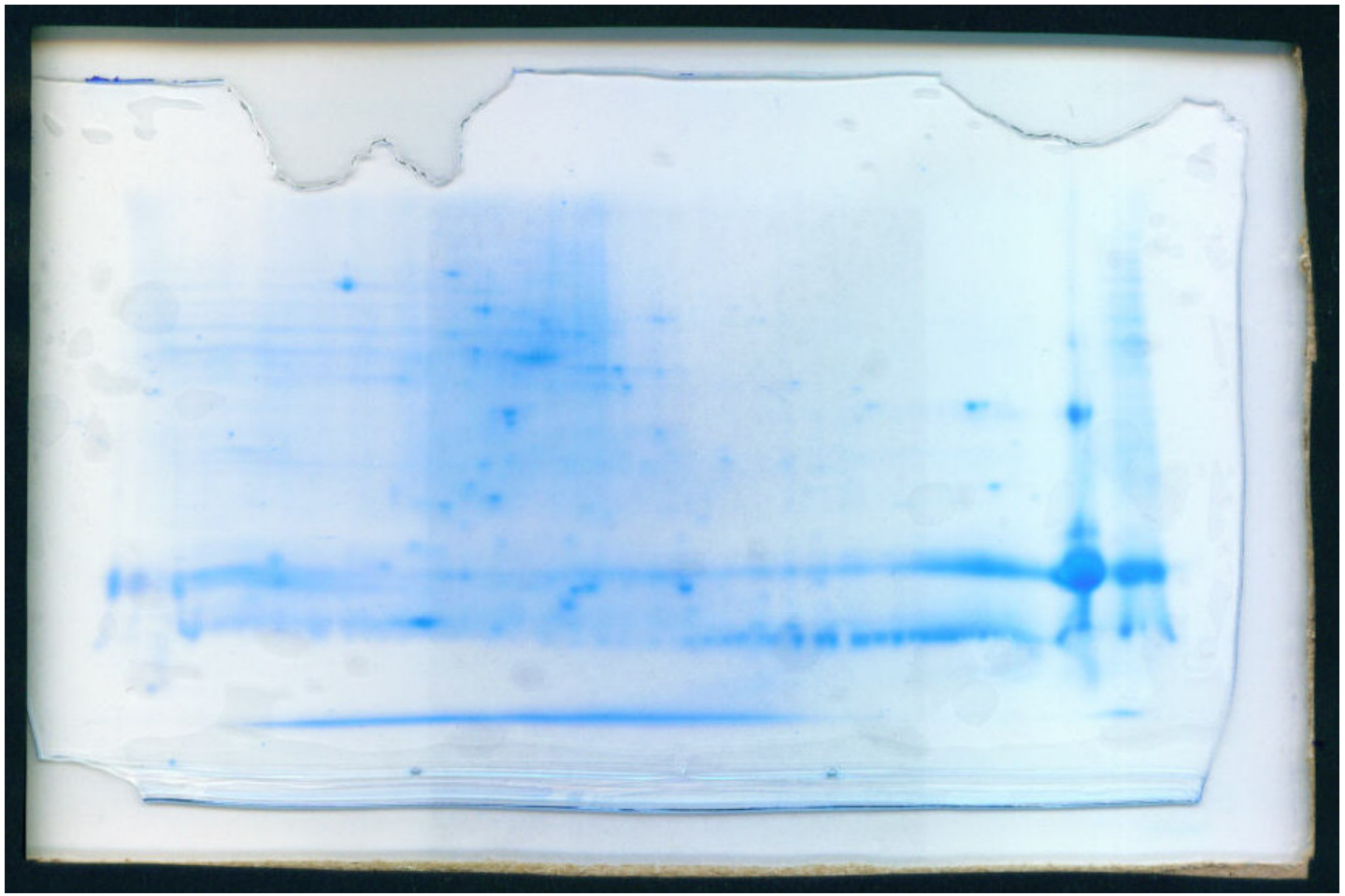
- Growth curves for P.p.F1 have been conducted using: Succinic Acid, Phenylethylamine, Benzoic acid, and Succinic Acid in the presence of 9-Aminoacridine.
- Growth curves for P.p.KT2440 have been conducted using: Succinic Acid, Phenylethylamine.

Group Accomplishments

- Gels have been run under all of these growth conditions
- Some gels have been analyzed and compared using the Progenisis software at the U of R proteomics lab
- Similarities and differences have been found in some of the gel comparisons

Gels

PH 3
High MW



PH 10

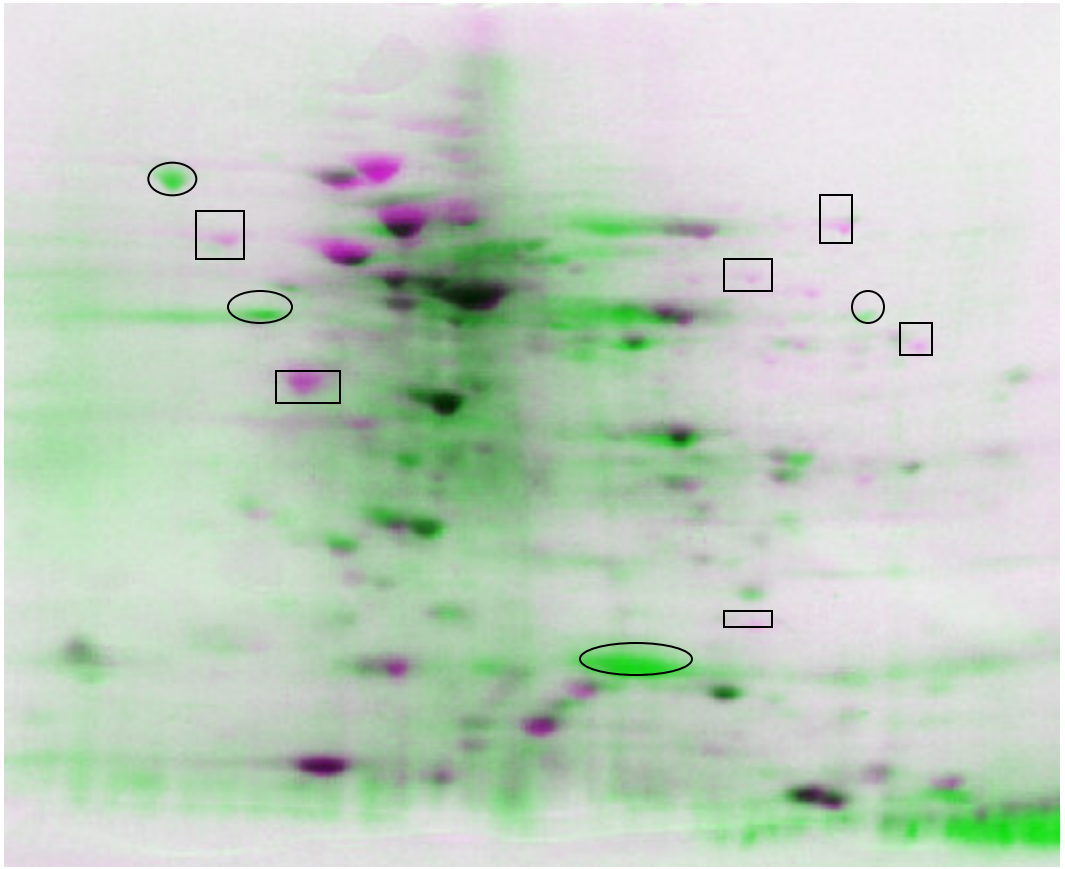
Low MW

Carbon Source: Succinic Acid Date:07-29-2004 Mary Pratts

2DE Gels – Compare – SA vs. PEA

PH 3

High MW



PH 10

Legend:
Circle: PS of PEA
Rectangle: PS of SA

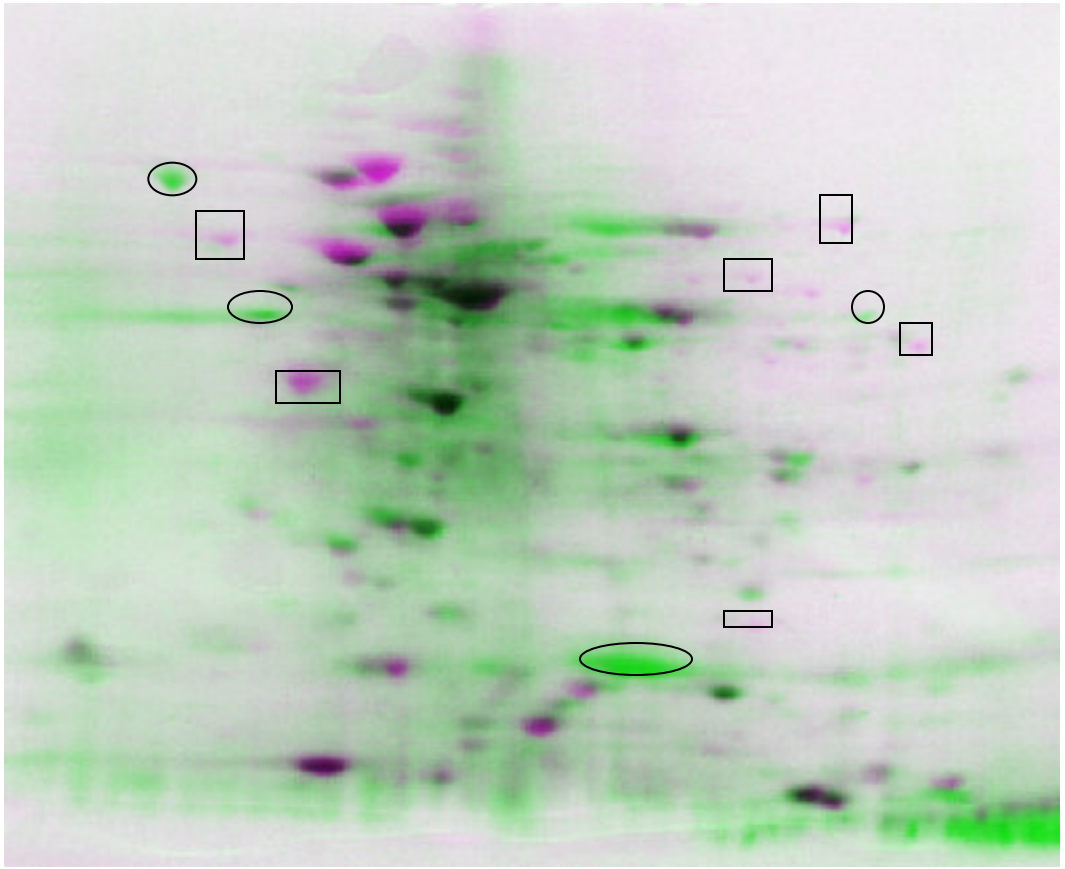
Low MW

Slide taken from Han Hoang

Overlaid SA reference gel (magenta) and PEA reference gel (green)

2DE Gels – Compare – SA vs. PEA

PH 3
High MW



PH 10

Legend:
Circle: PS of PEA
Rectangle: PS of SA

Low MW

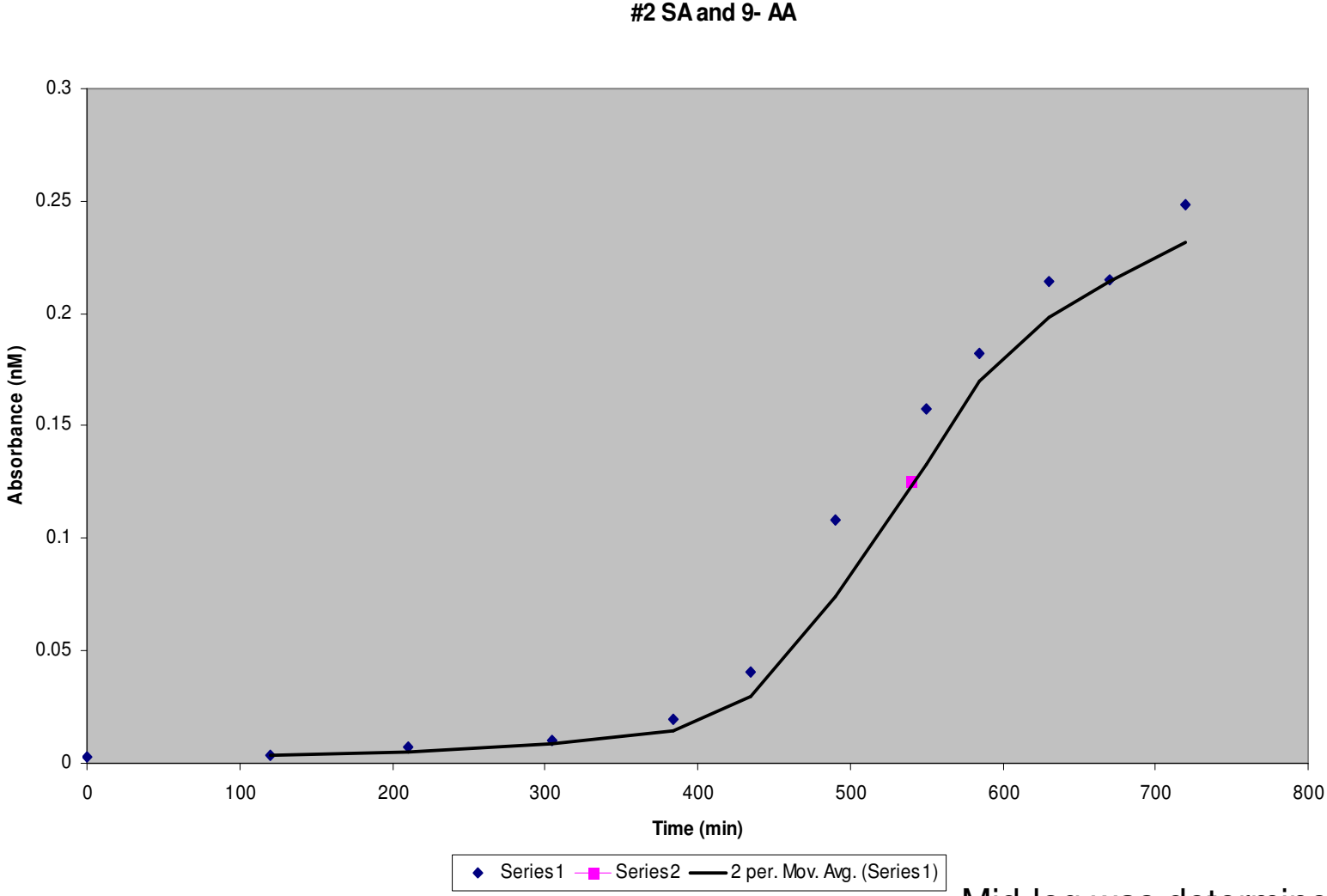
Slide taken from Han Hoang

Overlaid SA reference gel (magenta) and PEA reference gel (green)

Summer Research

- Learn the SOP specific to our research lab and study goals
- Practice these techniques and become proficient in running gels
- Conduct growth curve for P.p.F1on a Succinic Acid carbon source in the Presence of the mutagen 9-Aminoacridine
- Run initial gels for this set of conditions

Growth Curve: SA and 9-AA



Mid-log was determined to be approx. 9hrs.

Future Plans

- Run and analyze gels with Succinic acid and Benzoic acid carbon sources in the presence of: 9-Aminoacridine, Sodium azide, and Benzo(a)pyrene
- Consider the addition of S9 for metabolic activation when using Benzo(a)pyrene

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References

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