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

- 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 9000xg 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
How 2DE gels work

The first dimension:

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

SDS-charged proteins in IPG strip

Apply to SDS-PAGE gel

MW

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

BIORAD product manual: 2D Electrophoresis for Proteomics

Slide taken from Kelly Fowlkes
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.F1 and 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

Number of Microbes

Time

Lag Phase | Log Phase | Stationary Phase | Death Phase
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

Carbon Source: Succinic Acid
Date: 07-29-2004
Mary Pratts
2DE Gels – Compare – SA vs. PEA

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

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

PH 3
High MW

PH 10

Low MW

Slide taken from Han Hoang
2DE Gels – Compare – SA vs. PEA

Legend:
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Legend:
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Low MW

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

Slide taken from Han Hoang
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.F1 on 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. 9 hrs.
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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