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~Main Objective: To observe the effects of the NF-κB transcription factor on IKK knockouts~

First Step: Making Cultures

1) Plasmid Transformation
- Colonies of bacteria with transformed plasmid are grown and selected to grow more copies.

2) Plasmid DNA is purified through a mini-prep
- Selected colonies are placed into tubes and shaken overnight to promote further growth.

3) Run plasmids on a gel or cut with restrictive enzymes.
- Cultures are spun down in a centrifuge to isolate the bacterial pellet containing DNA.

Further Developments

~How do we determine IKK knockout?~

GENOTYPING

1) Animal sample’s DNA undergoes PCR
- DNA samples are loaded into PCR tubes and placed in a PCR machine for 2 ½ hours.

2) Analyze DNA samples through gel electrophoresis
- A current passes through the agarose gel and separates nucleic acids based on size.

3) Photograph gels and collect data.
- Various restrictive enzymes used to cut plasmids.
- Lanes #1-4 tested for IKK1
- Lanes #5-8 tested for IKK2
- Lanes #1, 2, 5, 6 are for sample 4887-2
- Lanes #3, 4, 7, 8 are for sample 5076-3
- Lanes #9-10 tested for IKK1 in Wild Type DNA
- Lanes #10-11 tested for IKK2 in Wild Type DNA

Homozygous indicates an IKK knockout

~How do IKK knockouts respond to stimuli?~

WESTERN BLOT

1) Neutralize samples with Bradford reagent
- The media is transferred to smaller tubes and centrifuged.

2) Load samples into SDS-PAGE gel and run it with blotting paper
- A current runs through the agarose gel and separates nucleic acids based on size. The dye from the samples will bleed onto the blotting paper.

3) Place blotting paper in buffer and on rotator
- The blotting paper goes through several buffer changes before imaging.

Western Blot Data

Samples: IKK2 -/- (knockouts) 0 ce, 10 ce, 30 ce, 60 ce, 90 ce, 120 ce
- Double bands indicate that the proteins correspond to IKK2 knockouts.
- 4887-2 tested positively for IKK1 knockout while 5076-3 tested positively for IKK2 knockout. The Wild Type DNA tested negative for IKK1 and IKK2 knockouts.

NF-κB Basics

NF-κB is a transcription factor that regulates several important genes crucial to intercellular and intracellular signaling.

NF-κB acts as an inhibitor for NF-κB. This protein inhibits NF-κB by masking nuclear localization signals, which keeps NF-κB in a state of inactivity. These transcription factors are thus confined to the cytoplasm and unable to express themselves.

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