Christopher Man, Hoffmann Lab

Andrew Caldwell, Mentor

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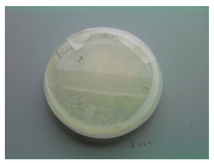
~Main Objective: To observe the effects of the NF-κB transcription factor on IKK knockouts~

First Step: Making Cultures

1) Plasmid Transformation

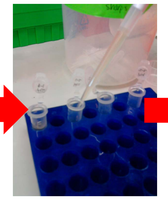


Colonies of bacteria with transformed plasmid are grown and selected to grow more copies.

Selected colonies are placed into tubes and shaken overnight to promote further growth.

2) Plasmid DNA is purified through a mini-prep

Cell cultures are removed from the shaker and ready to be centrifuged.

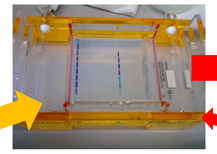


The media is transferred to smaller tubes and centrifuged.

Cultures are spun down in a centrifuge to isolate the bacterial pellet containing DNA.

Bacterial pellets are purified with various buffers in a mini-prep kit and resuspended in media.

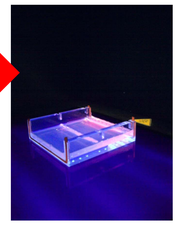
3) Run plasmids on a gel or cut with restrictive enzymes.



Samples loaded into the gel.

Various restrictive enzymes used to cut plasmids.

Gel imaging machine.



Gel inside the imaging machine. UV light helps highlight the bands of DNA.

Further Developments

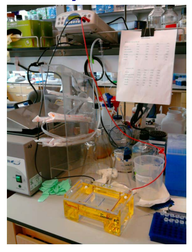
~How do we determine IKK knockout?~

GENOTYPING

1) Animal sample's DNA undergoes PCR

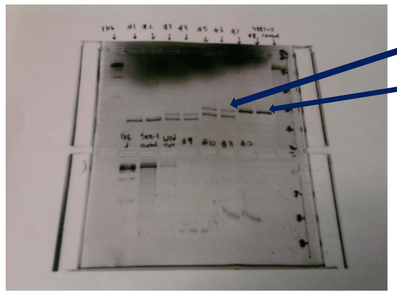
DNA samples are loaded into PCR tubes and placed in a PCR machine for 2 ½ hours.

2) Analyze DNA samples through gel electrophoresis



A current passes through the agarose gel and seperates nucleic acids based on size.

3) Photograph gels and collect data.



2 Bands = Heterozygous

1 Band = Homozygous

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.

Genotype Data

Genotyping the DNA for Animal Samples 4887-2, 5076-3, and Wild Type DNA

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

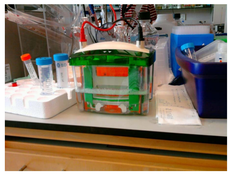
~How do IKK knockouts respond to stimuli?~

WESTERN BLOT

1) Neutralize samples with Bradford reagent

CBT buffer is mixed with Bradford reagent and mixed into small tubes along with sample protein.

2) Load samples into SDS-PAGE gel and run it with blotting paper



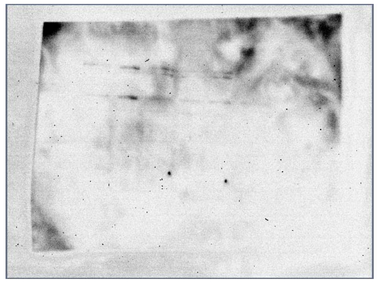
A current runs through the gel cassette and separates the proteins based on size. The dye from the samples will bleed onto the blotting paper.

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.**

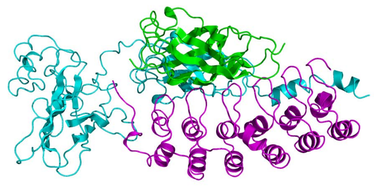
NF-κB Basics

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

NF-κB is a protein complex and its main function is to control the transcription of DNA. This complex is found in many types of organisms and is known to be involved in cellular responses towards stimuli, both exterior and interior. NF-κB has been known to be key in the regulation of cellular response towards infections. It is this regulation that scientists suspect may be the cause of cancer, autoimmune diseases, and septic shock.

IκBα is the inhibitor of NF-κB

IκBα 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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