



## Lab Update

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- Review of Question
- RT PCR
- Analysis of PAGE Results
- Quantitative PCR
- MiniReport



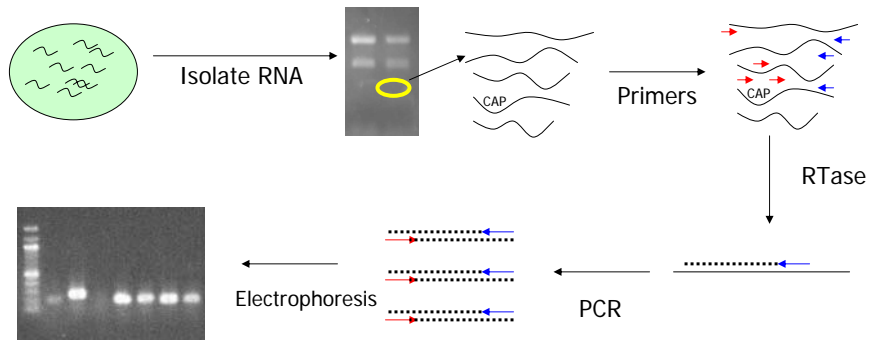
## Research Question

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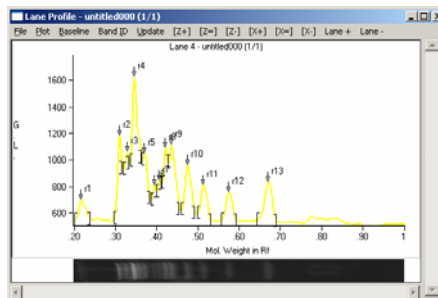
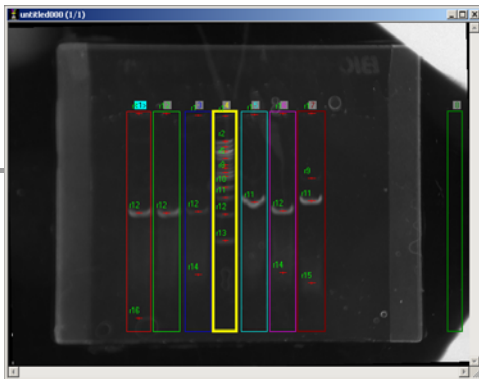
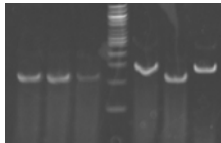
- What are the physiological roles of the two GRP170 genes in *Caenorhabditis elegans*?
- Reminder
  - GRP170 is a large ER chaperone.
  - Other ER chaperones that are important for ER Protein folding are induced by agents that inhibit protein folding in the ER (UPR)
- Experiment: Compare the levels of grp170a and grp170b in worms induced for UPR and control worms.
- Significance: Changes in grp170 during the UPR would suggest that 170 plays a role in protein folding in the ER.

## RT PCR

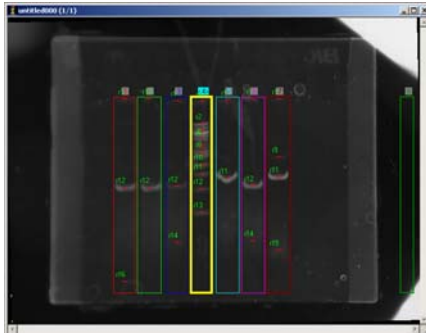
- Technique for detecting specific mRNA's in pool of total cellular RNA



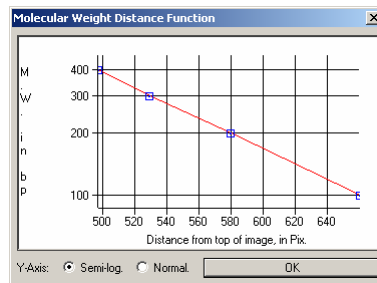
## PAGE Results



## Size (bp) and Amount (ng) of Bands



Computer Generated Std Curve



## Results

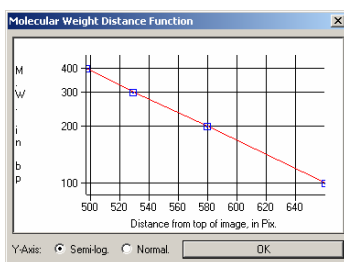
Microsoft Excel - Book1

Lanes:	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
Rows	(mol.w.)	(amount)	(mol.w.)	(amount)	(mol.w.)	(amount)	(amount)
r1	2917	4.3711	2917	2.4145	2789	3.7092	2739
r2							3.6258
r3							2839
r4							7.7516
r5							2917
r6							5.4065
r7							2943
r8							3.211
r9							
r10							
r11							
r12							
r13							
r14							
r15							
r16							
r17							
r18							
r19							
r20							
r21							
r22							
Sum							
In Lane							
Sheet1	Sheet2	Sheet3					

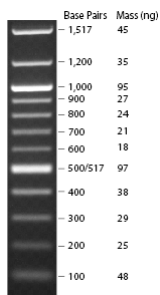
Size of band in lane 1

## Size of PCR products (bp)

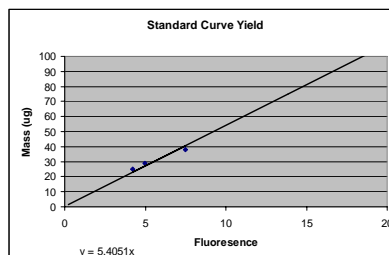
Gene	Predicted PCR products	Estimated DNA on Gel
CAP-1	207 bp	208/206 bp
GRP170a	212 bp	215/218 bp
GRP170b	266 bp	268/275 bp



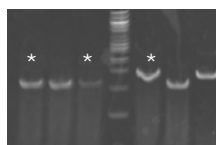
## Amount of DNA (ng)



Size (bp)	Fluor	Yield (ng)
400	7.5101	38
300	4.9715	29
200	4.2373	25
CAP1	26.9	145
170a	11.4	61.6
170b	31.7	171



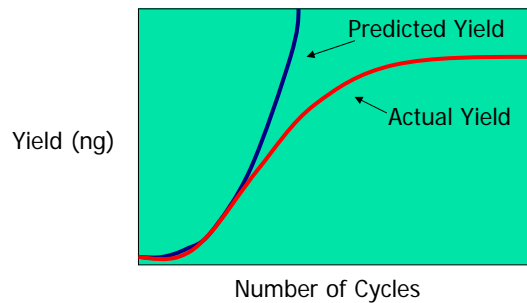
$$Y = 5.4X$$



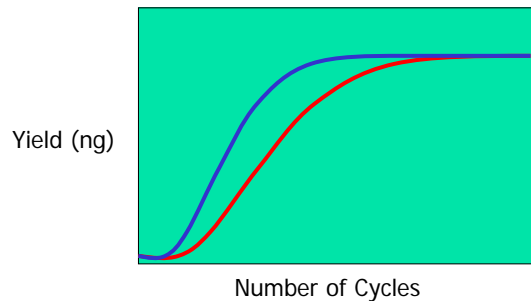


## Quantitative PCR

- Every round of PCR should double amount of DNA
  - Predict Exponential Growth in Yield



## How to estimate relative starting template.



Two estimate relative amounts of DNA compare yields during exponential Growth (i.e. prior to inflection point)

