

# Increasing Recombinant Protein Expression in Insect Cells



# EXPRESSION SYSTEMS

- An Insect and Mammalian Cell Culture Media Company
- Contract Manufacturing

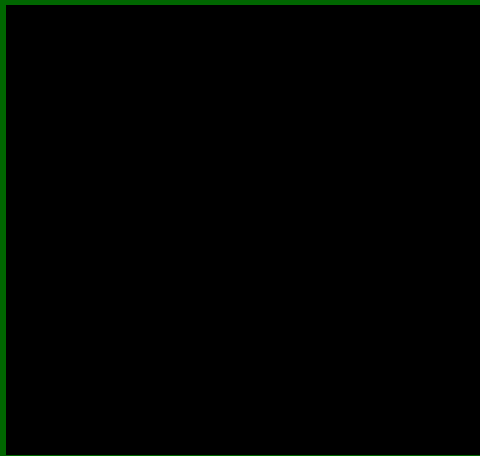
# Platform to Produce Recombinant Protein

Cells

Media

Vector

Culture Vessel



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# Cell Type

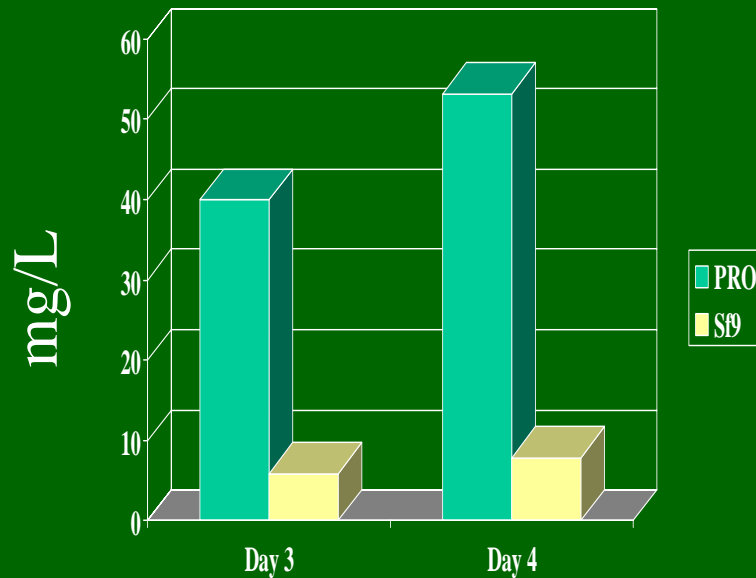
- Cell line choice is influenced by a number of factors
  - What is the goal?
    - ie virus production is typically done in Spodoptera cells
  - Is there a licensing fee?
  - How easy is it to grow?
  - Does it actually express my protein of interest?
  - Does the produced protein have the characteristics I want?



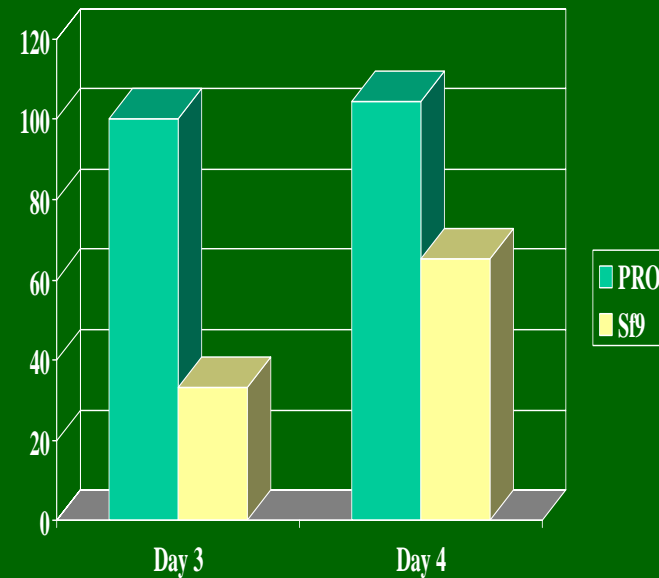
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# Trichoplusia vs Spodoptera

80 kD Protein



15 kD Protein



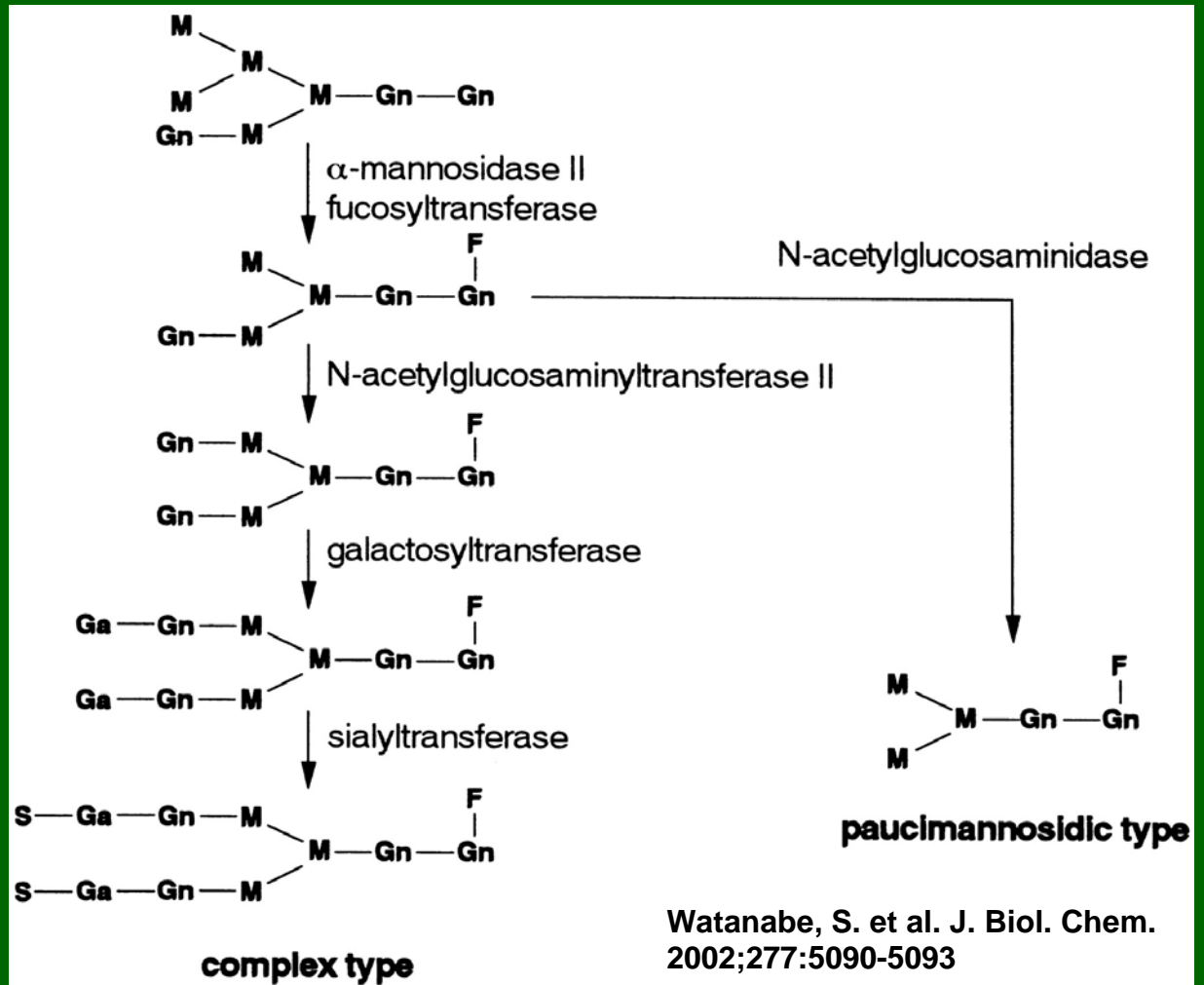
Expression of secreted proteins. Both cell lines were infected at an MOI of 1.



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# Do the Glycosylation Patterns Seen Suit My Protein?

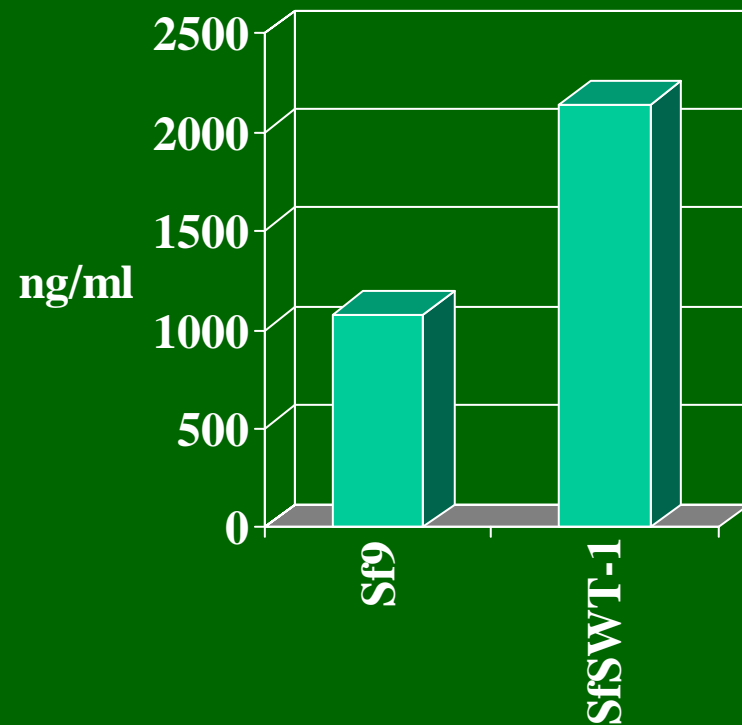
- Eukaryotic processing but not mammalian type
- This may or may not influence application
- Absence of terminal sialic acids is often unacceptable for therapeutics



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# Altering Glycosylation Patterns

- Transgenic insect cell lines were made by transforming with mammalian genes
- SfSWT-1 cells were transformed with 5 mammalian glycosyltransferases
- Expression of rTSH is enhanced compared to parental Sf9 cells



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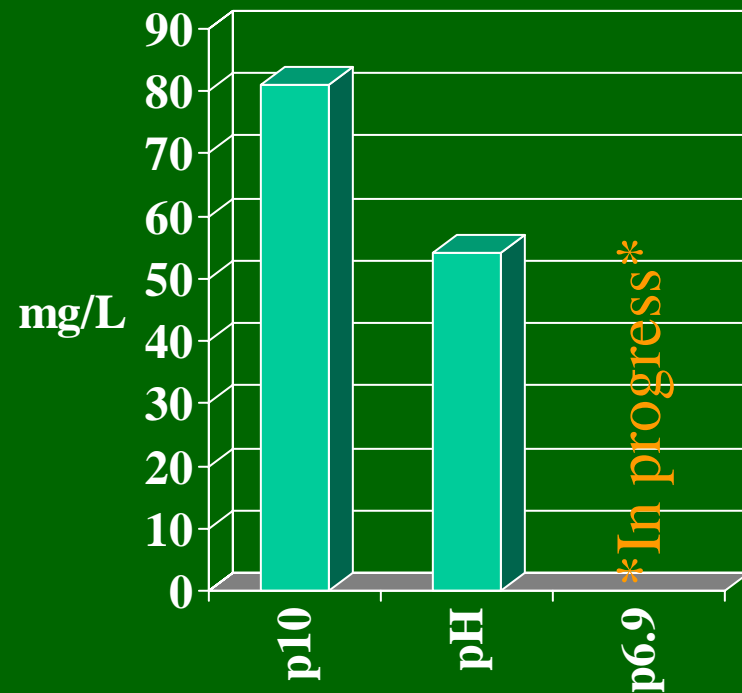
# Vector Choice

- Shuttle vectors allow for a variety of modifications
  - Targeting signals, purification tags, promoter choice, enhancers
- Vectors for stable cell line
- Basis of lytic vectors is AcMNPV with modifications of the backbone
  - Deletions, manipulations for ease of use



# Promoter Usage

- Expression of a secreted protein in PRO cells
- Secretion enhanced with honeybee mellitin signal
- Cells were infected at an MOI of 1 and harvested day 4



# Media Manipulations

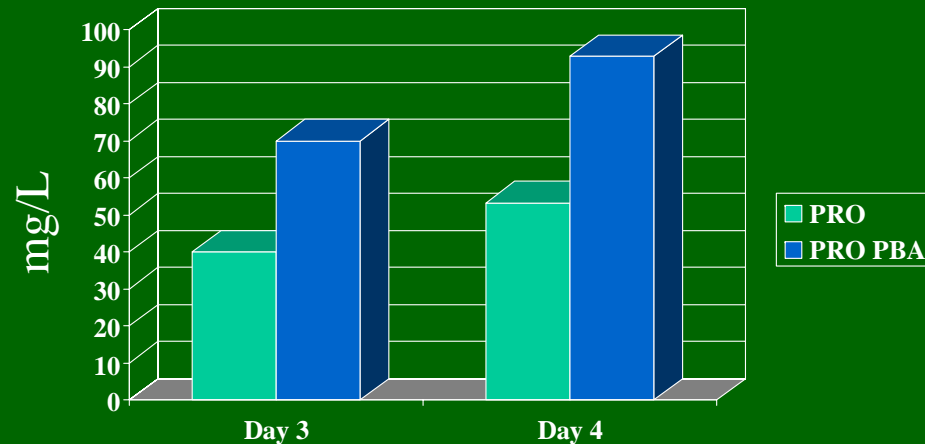
- Serum supplemented media
- Serum-free and protein-free formulations
- Additives
- Future Directions-
  - Chemically defined
  - Optimized for stable cells versus shorter term lytic production runs



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# Supplementation over course of production

## Secreted Protein



Cells were infected at an MOI of 1. Production Boost Additive was delivered as 5% of the total culture volume 18 hours post infection.



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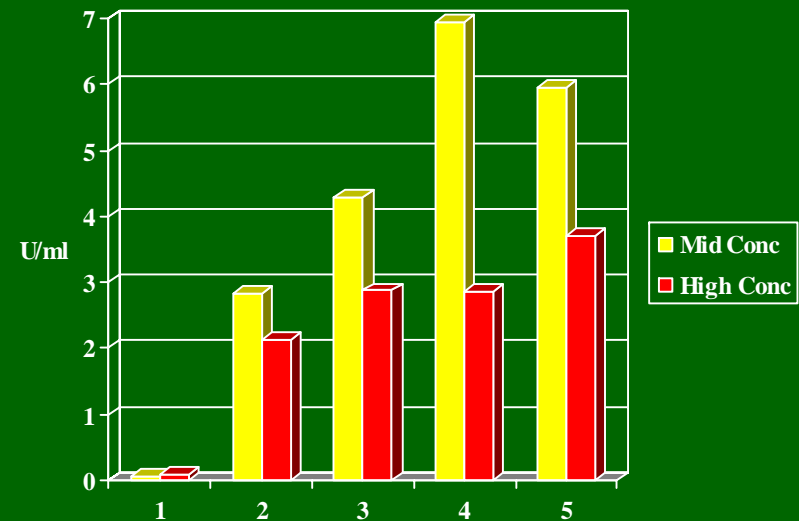
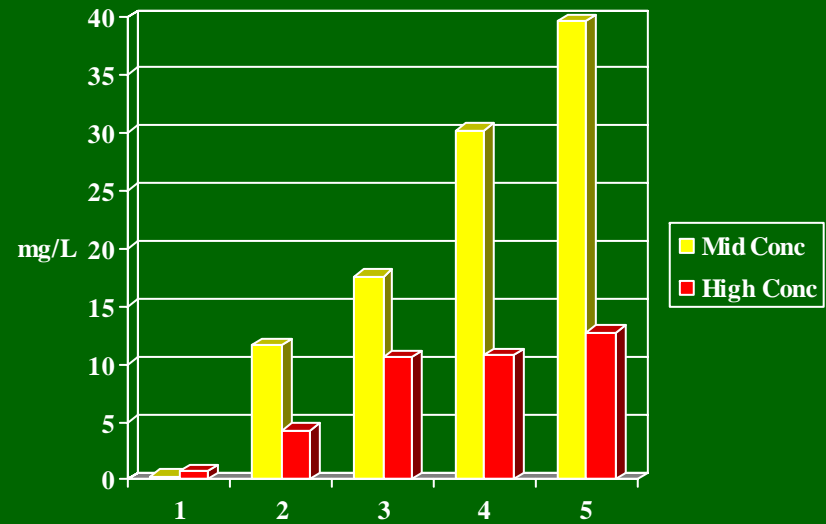
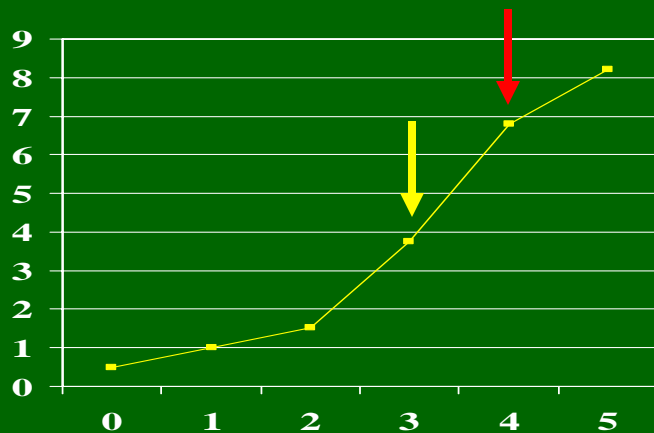
# Timing of Viral Infection

Experiment:

Take Sf9 cells from seed stock and inoculate flask at  $10^6$ /ml

Let grow overnight so they are in log phase

Infect at MOI of 1



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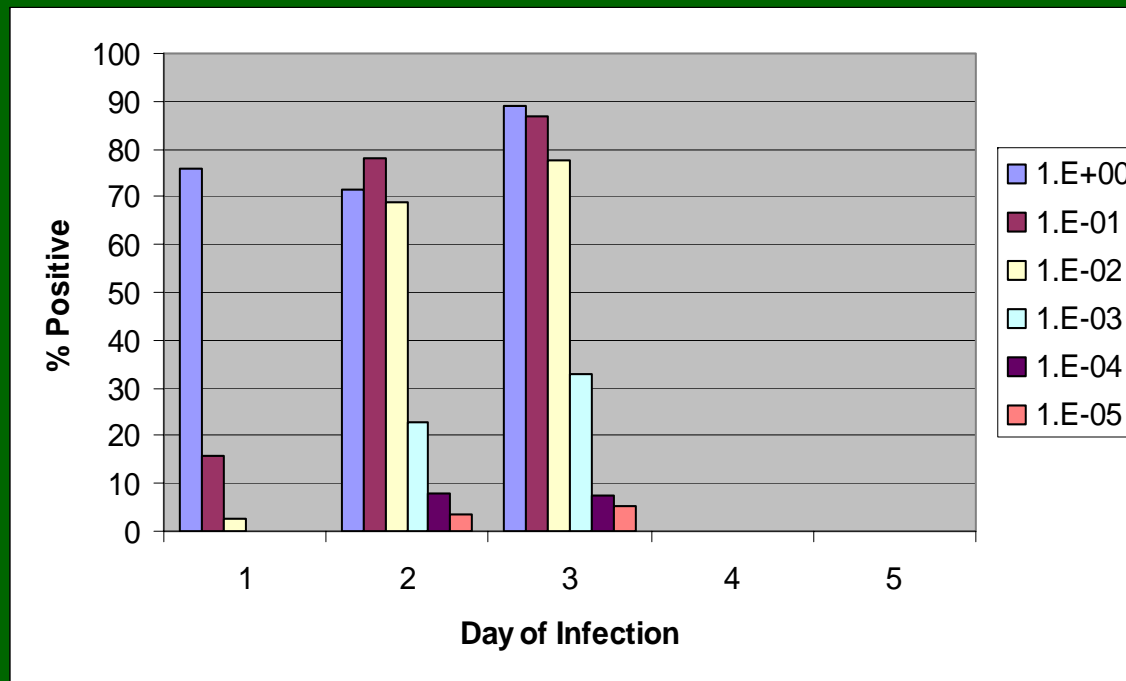
# Low MOI Experiment

- Hypothesis: Infecting with a low MOI may increase overall recombinant protein yield
- Sf9 cells were infected rBV expressing human transferrin as a model of a secreted protein
- Samples taken over timecourse to determine cell count, viability, spread of bv infection, and protein production



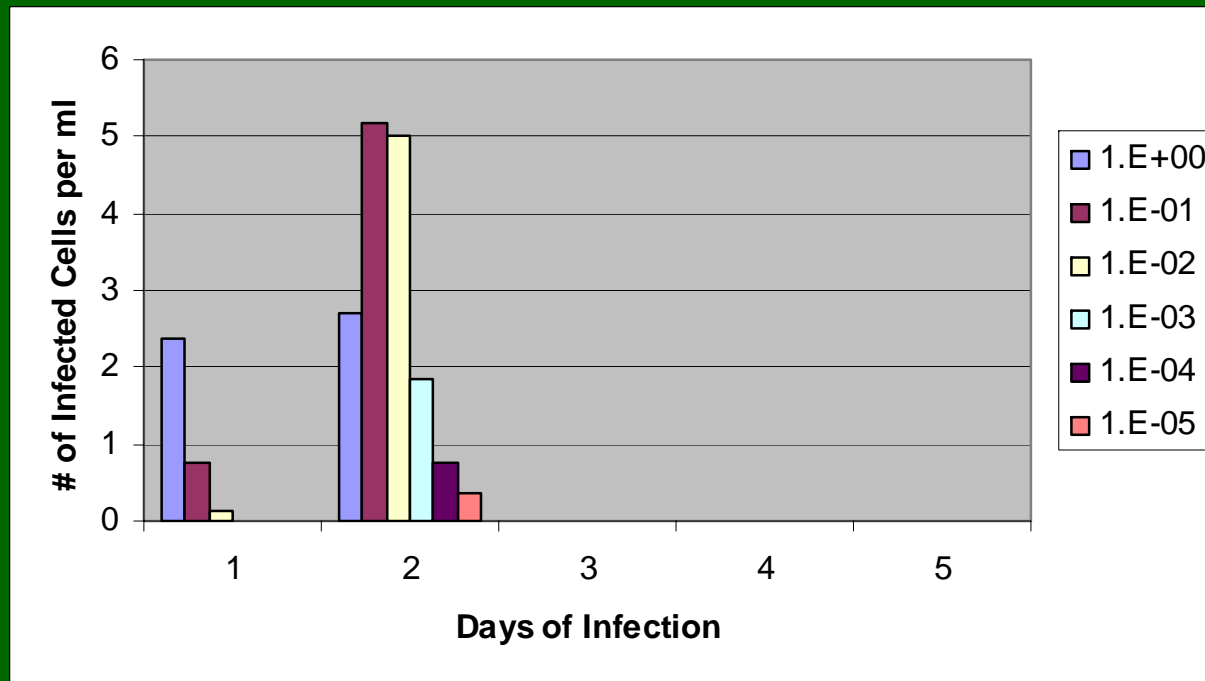
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# gp64 Staining Over Range of MOI



- gp64 expression demonstrates spread of virus

# Total Number of Infected Cells Increases with Lower MOI



- Uninfected cells do not undergo growth arrest leading to more targets for viral spread

# Viral Burst Size

- Cells grown in suspension in ESF 921
- Viral infection determined by gp64 staining
- Titers determined by flow cytometric method
- Assumes generation time of 24 hours
  
- Estimation based on low MOI expt with Sf9 cells:

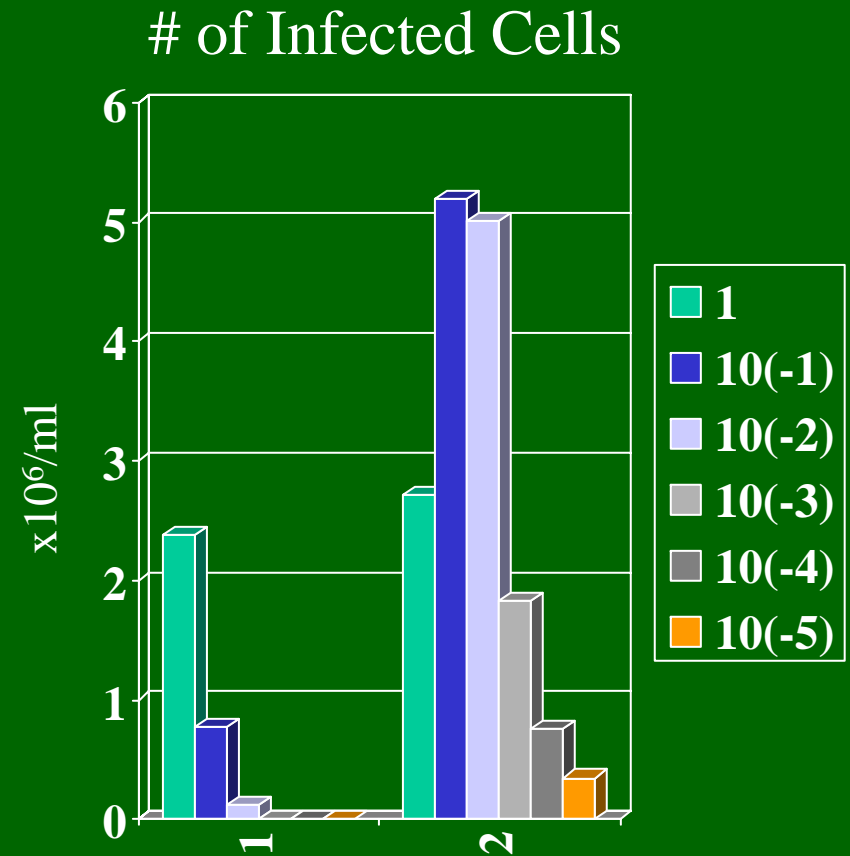
131 viruses per generation



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# What is the predicted amount of virus produced and resulting MOI?

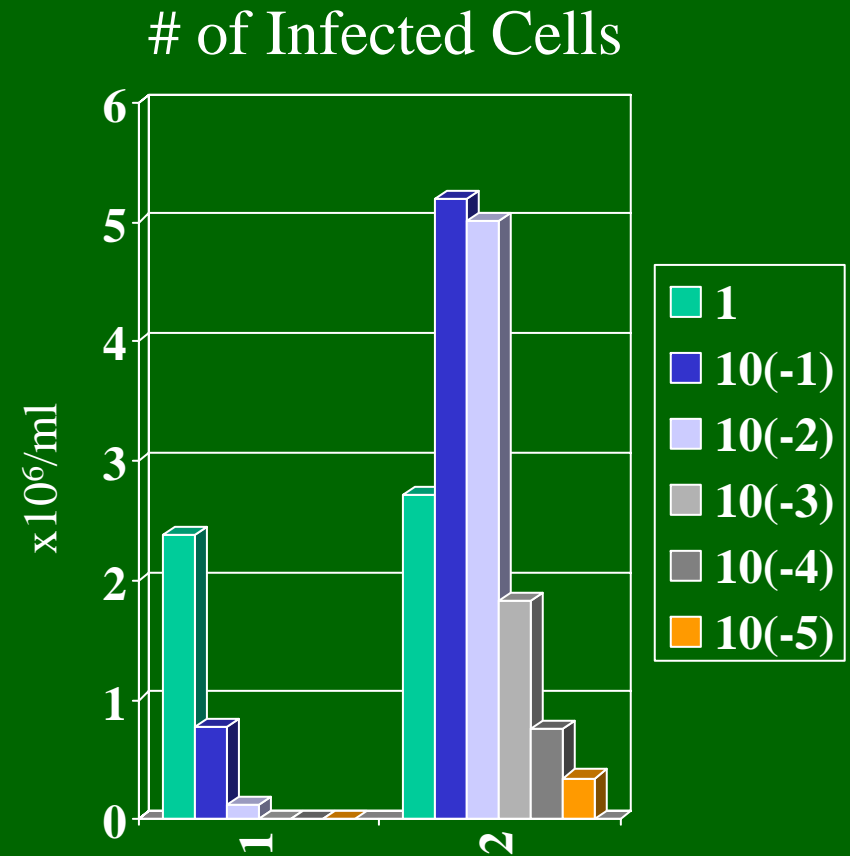
MOI	Virus Produced	# of targets	Effective MOI
1	$3.12 \times 10^8$	$3.15 \times 10^6$	
10(-1)	$1.01 \times 10^8$	$4.84 \times 10^6$	
10(-2)	$1.64 \times 10^7$	$4.48 \times 10^6$	
10(-3)			
10(-4)			
10(-5)			



EXPRESSION SYSTEMS

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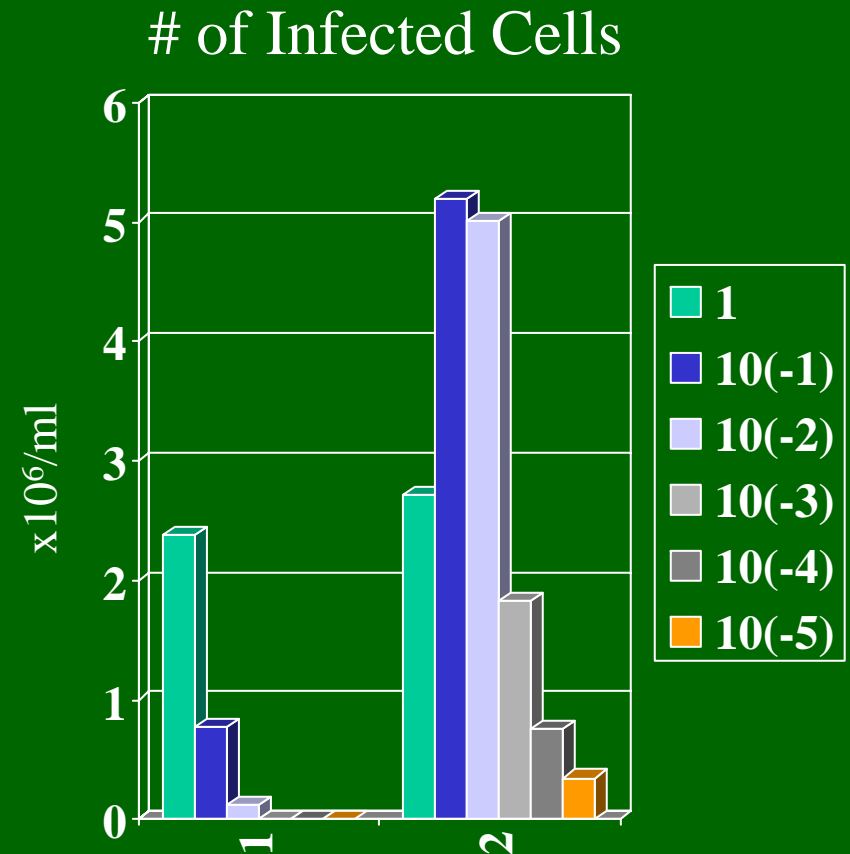
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EXPRESSION SYSTEMS

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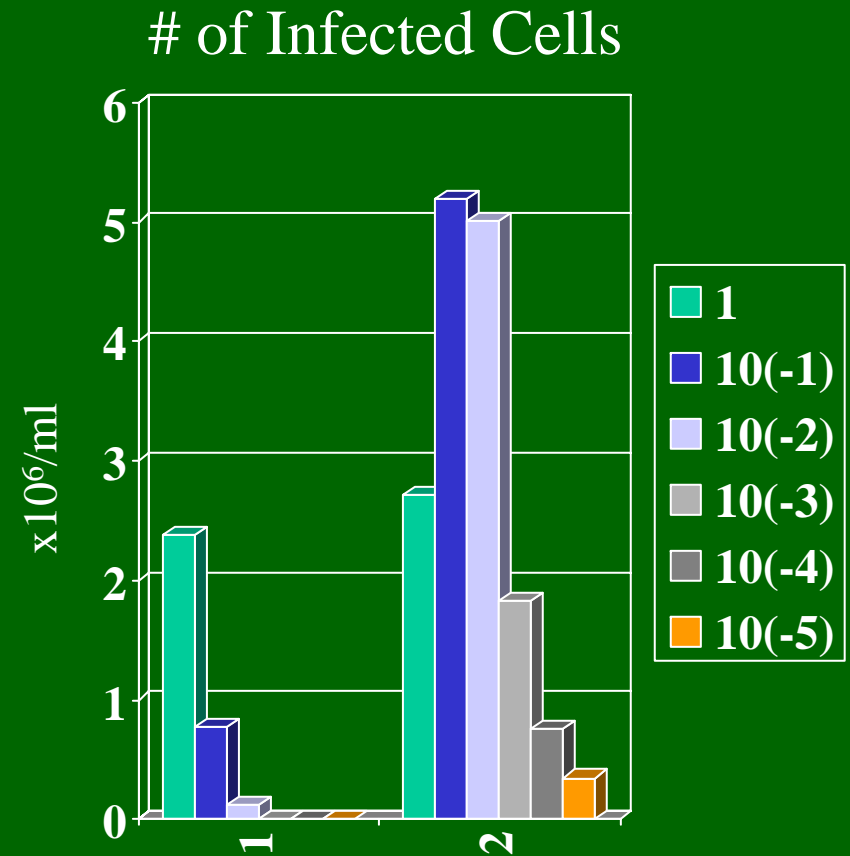
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EXPRESSION SYSTEMS

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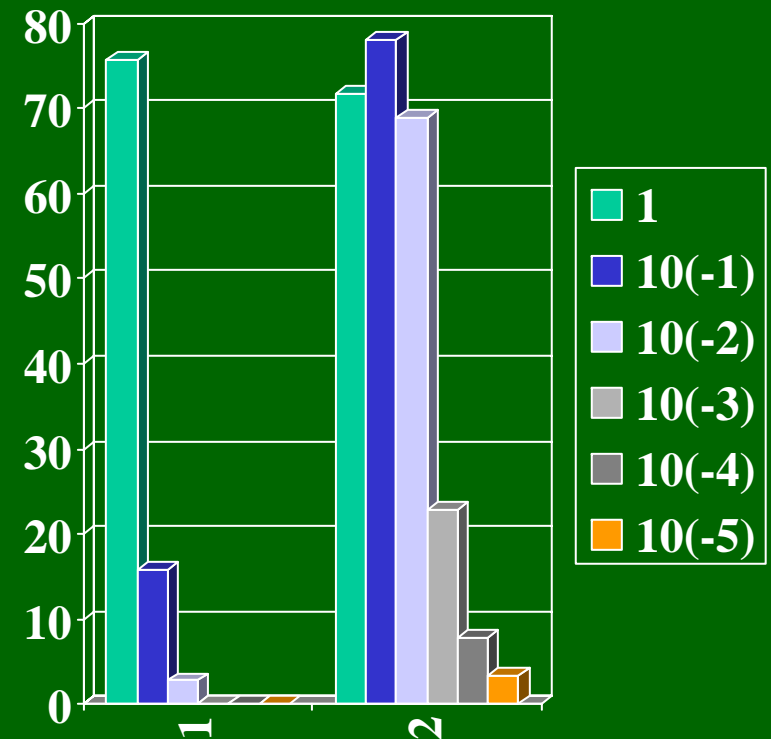


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# Does the estimated MOI meet our predictions?

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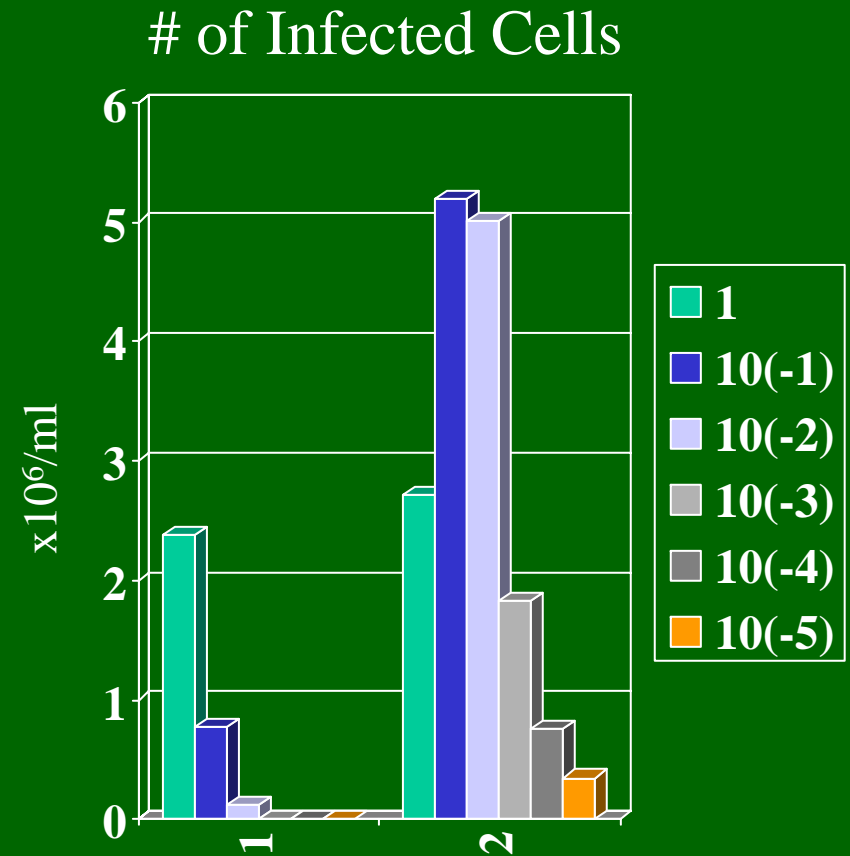
Percentage of Infected Cells



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# Taking it a step further- Predicting spread in the $10^{-3}$ culture

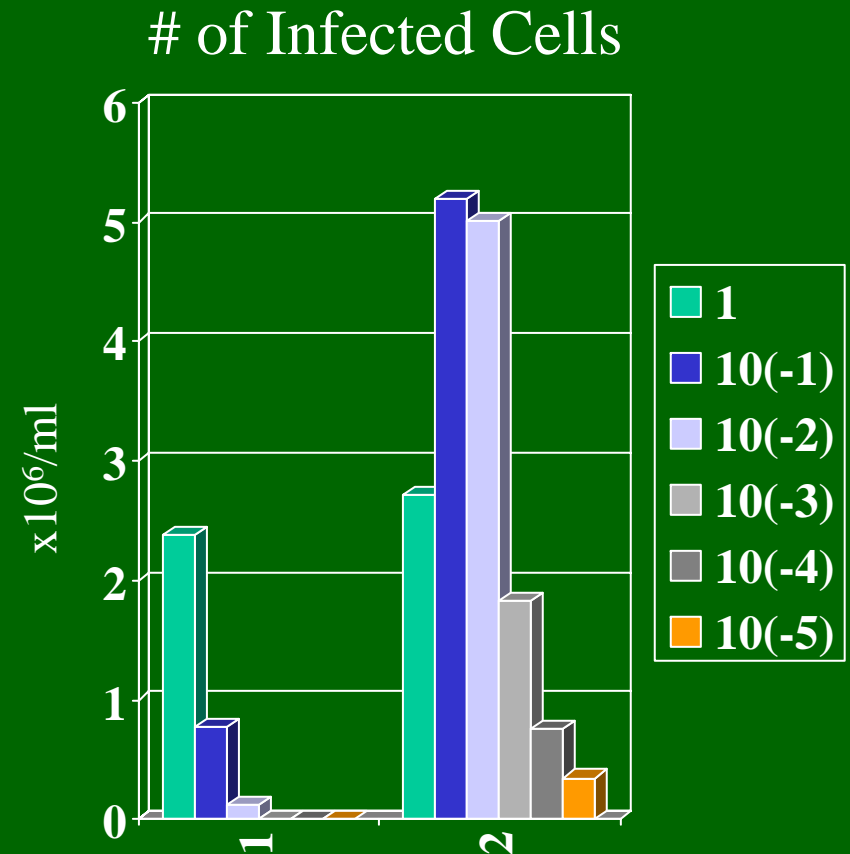
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$10(-3)$	Assume $1.64 \times 10^6$		
$10(-4)$			
$10(-5)$			



EXPRESSION SYSTEMS

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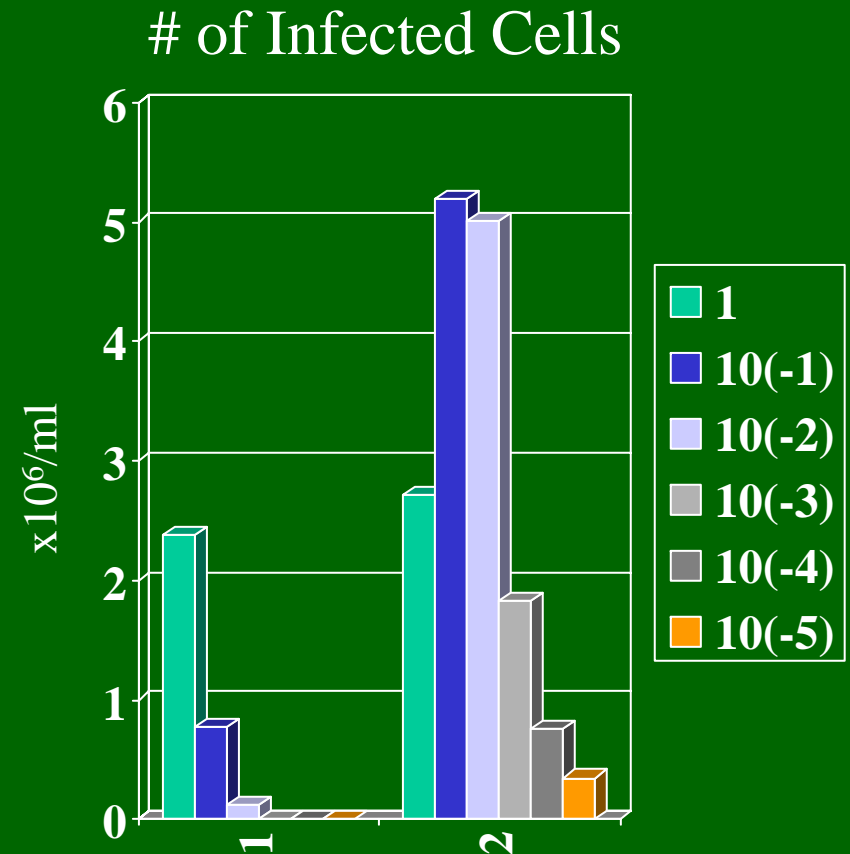
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10(-2)	$1.64 \times 10^7$	$4.48 \times 10^6$	3.7
10(-3)	Assume $1.64 \times 10^6$	$5.12 \times 10^6$	
10(-4)			
10(-5)			



EXPRESSION SYSTEMS

# Taking it a step further- Predicting spread in the $10^{-3}$ culture

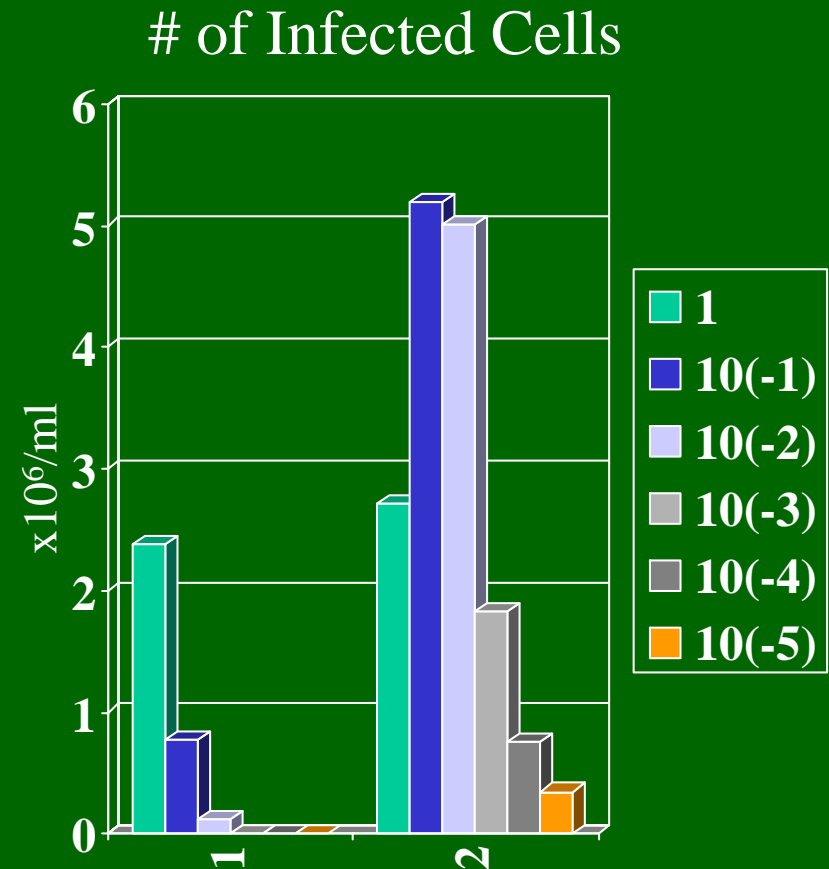
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$10(-2)$	$1.64 \times 10^7$	$4.48 \times 10^6$	3.7
$10(-3)$	Assume $1.64 \times 10^6$	$5.12 \times 10^6$	0.32
$10(-4)$			
$10(-5)$			



EXPRESSION SYSTEMS

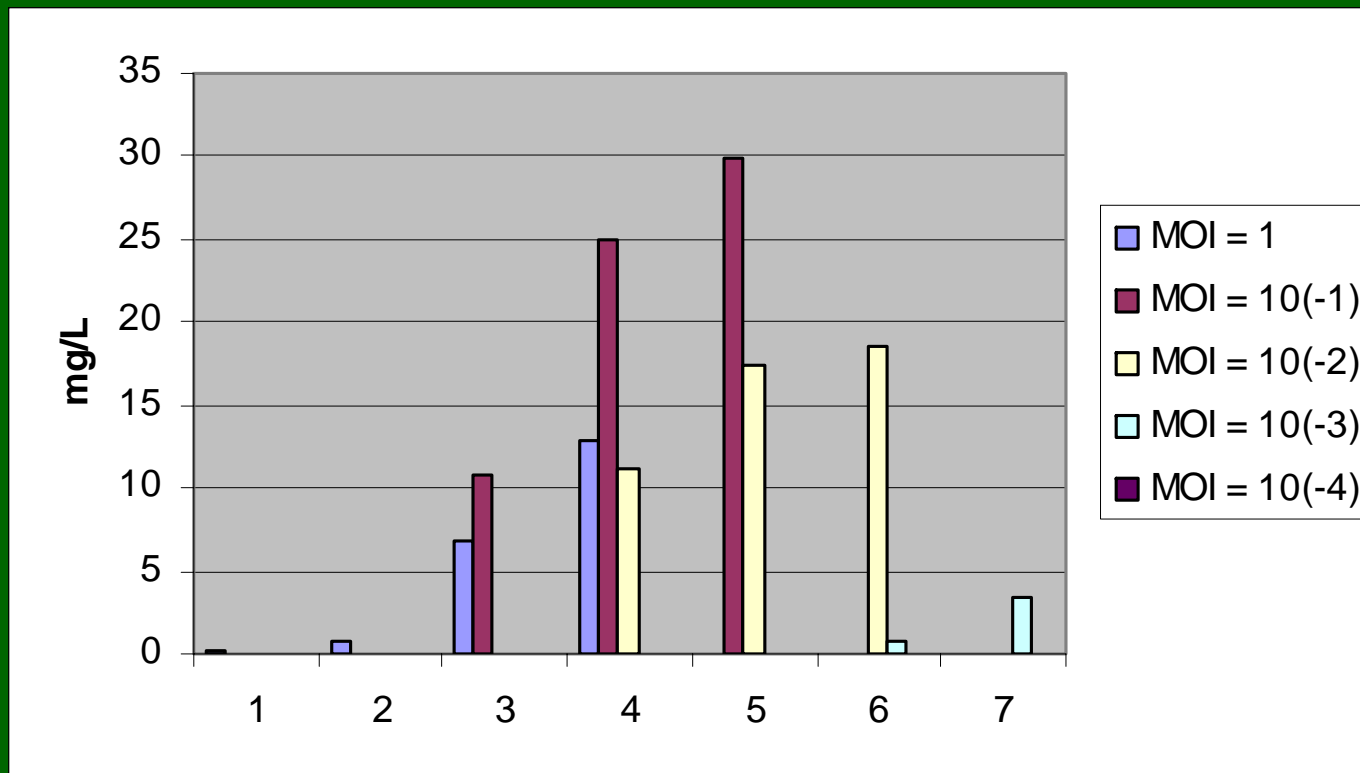
# Taking it a step further- Predicting spread in the $10^{-3}$ culture

- MOI of 0.32 times  $5.12 \times 10^6$  targets =  $1.64 \times 10^6$  infected cells/ml
- # of infected cells determined by cell counts and staining:
- $1.83 \times 10^6$



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# Production of human Transferrin



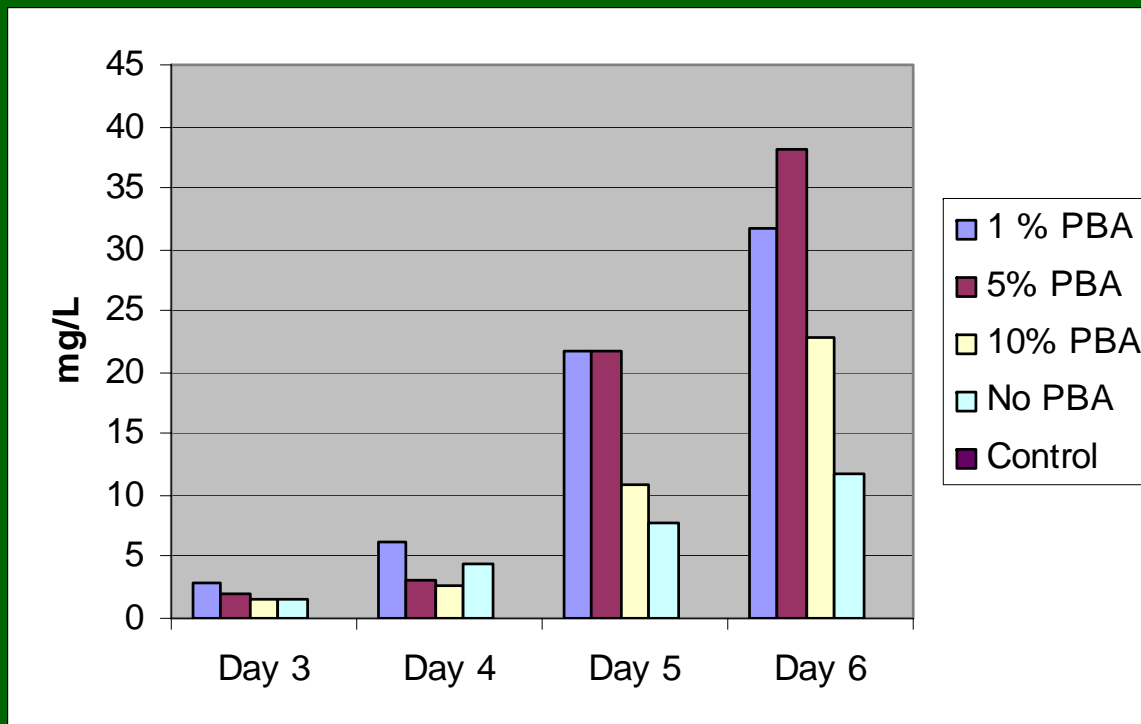
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## Low MOI with PBA

- Hypothesis: The high cell density that occurs when using a low MOI may benefit from a nutritive boost to maintain cell health
- Experiment: Infect Sf9 cells at an MOI of  $10^{-2}$ . Add PBA after the bulk of the cells are infected with baculovirus.



# Production of human Transferrin



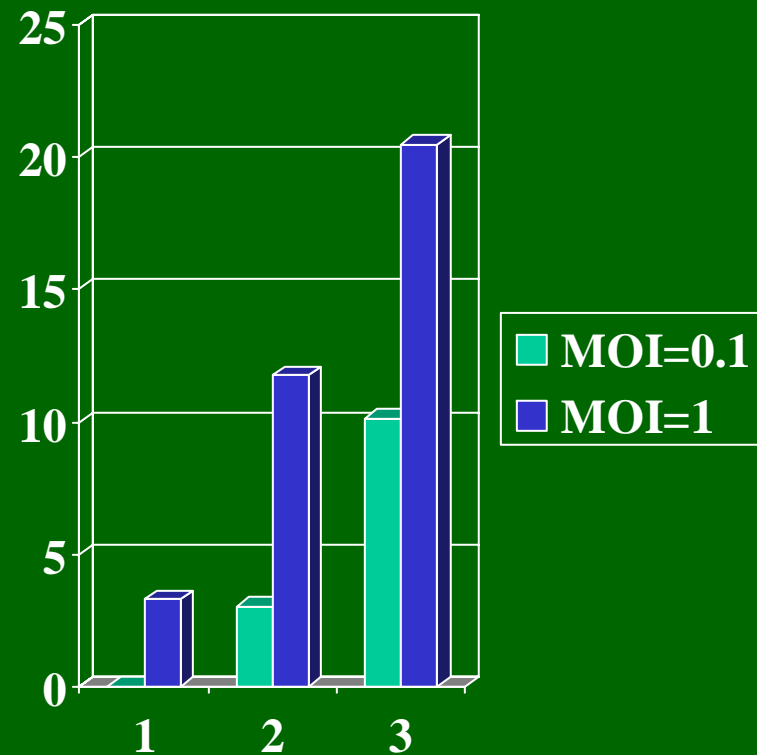
- PBA was added 36 hours after the addition of virus
- Cell count at the time of PBA addition was between  $10-12 \times 10^6$  cells/ml



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# Low MOI Expression in PRO cells

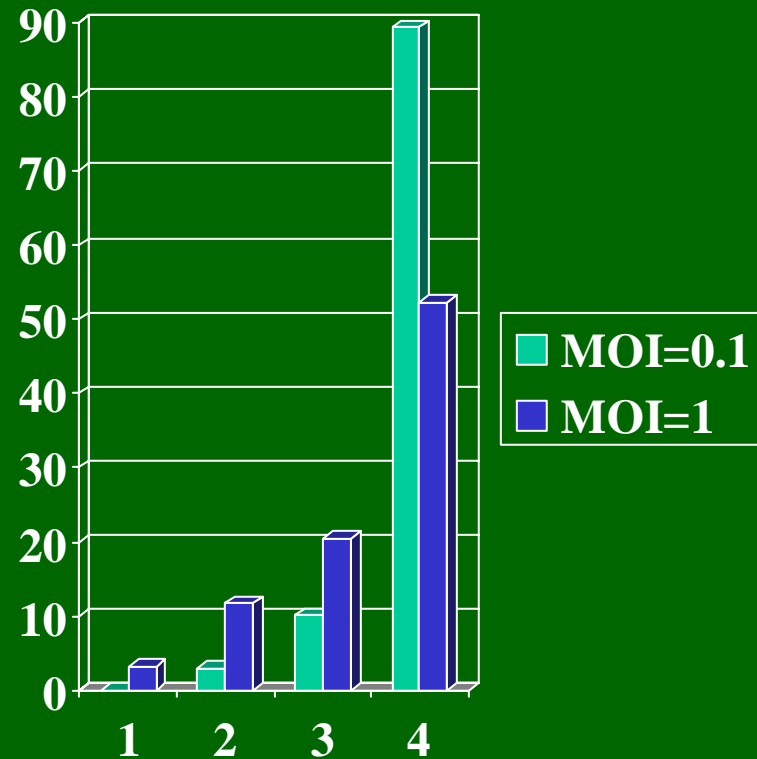
- PRO cells are derived from *T.ni* embryos
- Cells were infected at an MOI of 1 and 0.1
- 72 hour harvest shows higher MOI to be superior



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# Low MOI Expression in PRO cells

- PRO cells are derived from T.ni embryos
- Cells were infected at an MOI of 1 and 0.1
- 72 hour harvest shows higher MOI to be superior
- Waiting an extra day can have dramatic benefits



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# Platform-Build Your Own?

## Cells:

Sf9, Sf21,  
Tni, Ea4, S2,  
Bm4, Dpn1,  
etc etc

## Vectors:

Lytic-Bacmid Bacpak, etc  
Stable  
Transient  
Promoter choices

## Scale:

mls to Ls

## Culture Vessels:

Fermentor  
WAVE  
Fernbach  
Plates

## Media:

Serum-free  
Protein-free  
Animal-free  
Additives



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# High Throughput Screening

- Rapid screening to speed time to target
- Two cell lines to maximize possibility of detecting product
- Plaque purification of virus can wait until candidates are selected
- Small scale expressions (<1L) benefit from quality media but fine tuning not necessary



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# Production of Protein of Interest

- Pick cell line that creates product with most desirable characteristics
- Pick vectors that help streamline purification downstream
  - Purification tags, enhancement of solubility
- Pick stable vector, plaque purify, maintain low passage virus
- Maximize cell density during production
- Fine tune media formulation specific to protein



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# Acknowledgments

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