

Improving Reproducibility of Recombinant Protein Expression



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

Issues with Reproducibility

- Did you do everything the same?
- Was the seeding density the same?
- The infection density?
- Was the MOI the same?
- Harvest time?
- Cell count and viability the same?



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Typical Explanations for Variability

- Operator error
- New lot of culture media
- It was the purification method/detection method
- **PASSAGE NUMBER**



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Hypothesis:

- The state of the cell cycle influences infectability and therefore productivity
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- Identifying the best time to infect would increase yield and reproducibility



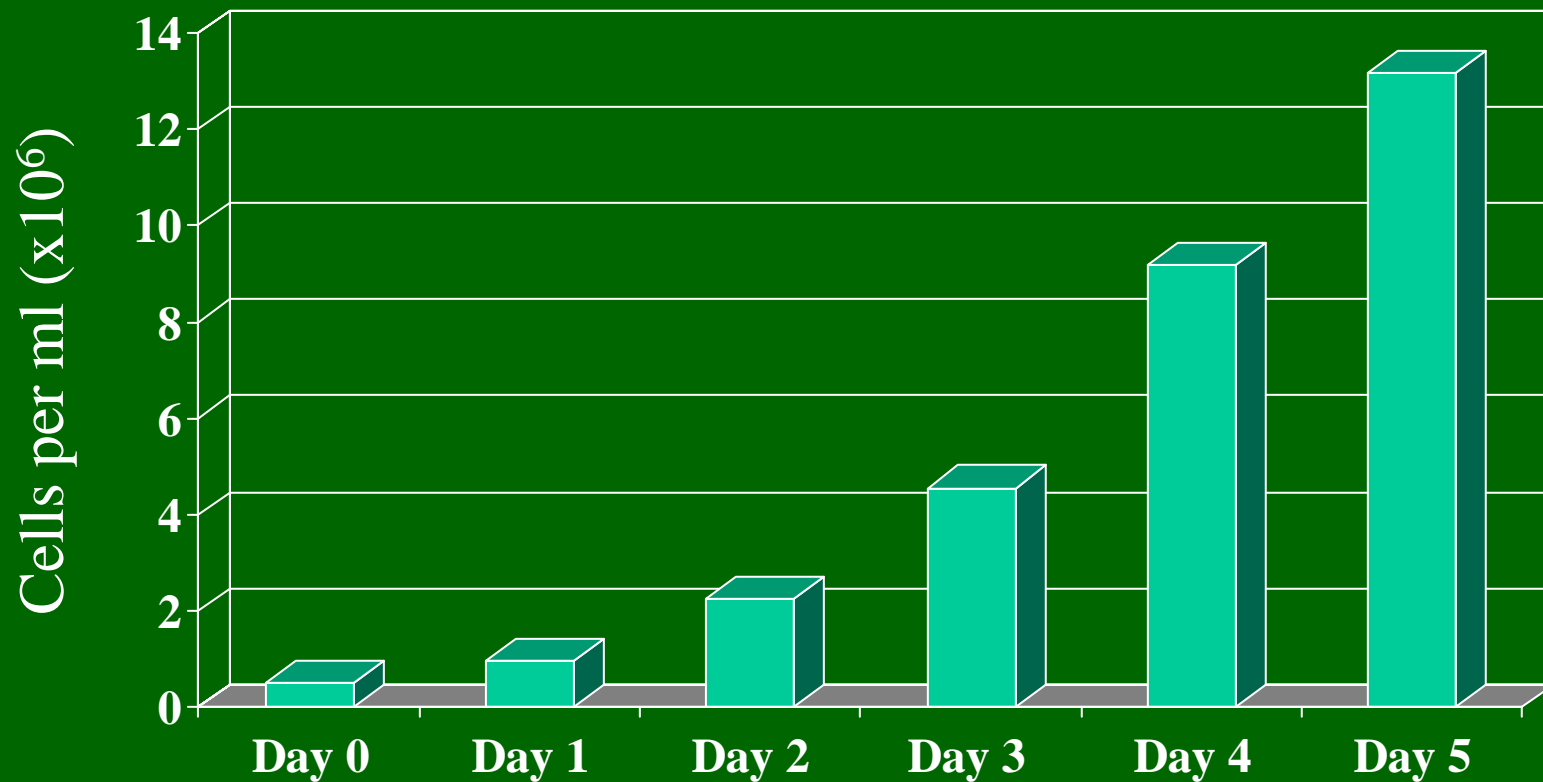
Experimental Design

- Cell cycle analysis
- Infectability (staining for gp64 expression)
- Virus binding
- Recombinant protein production



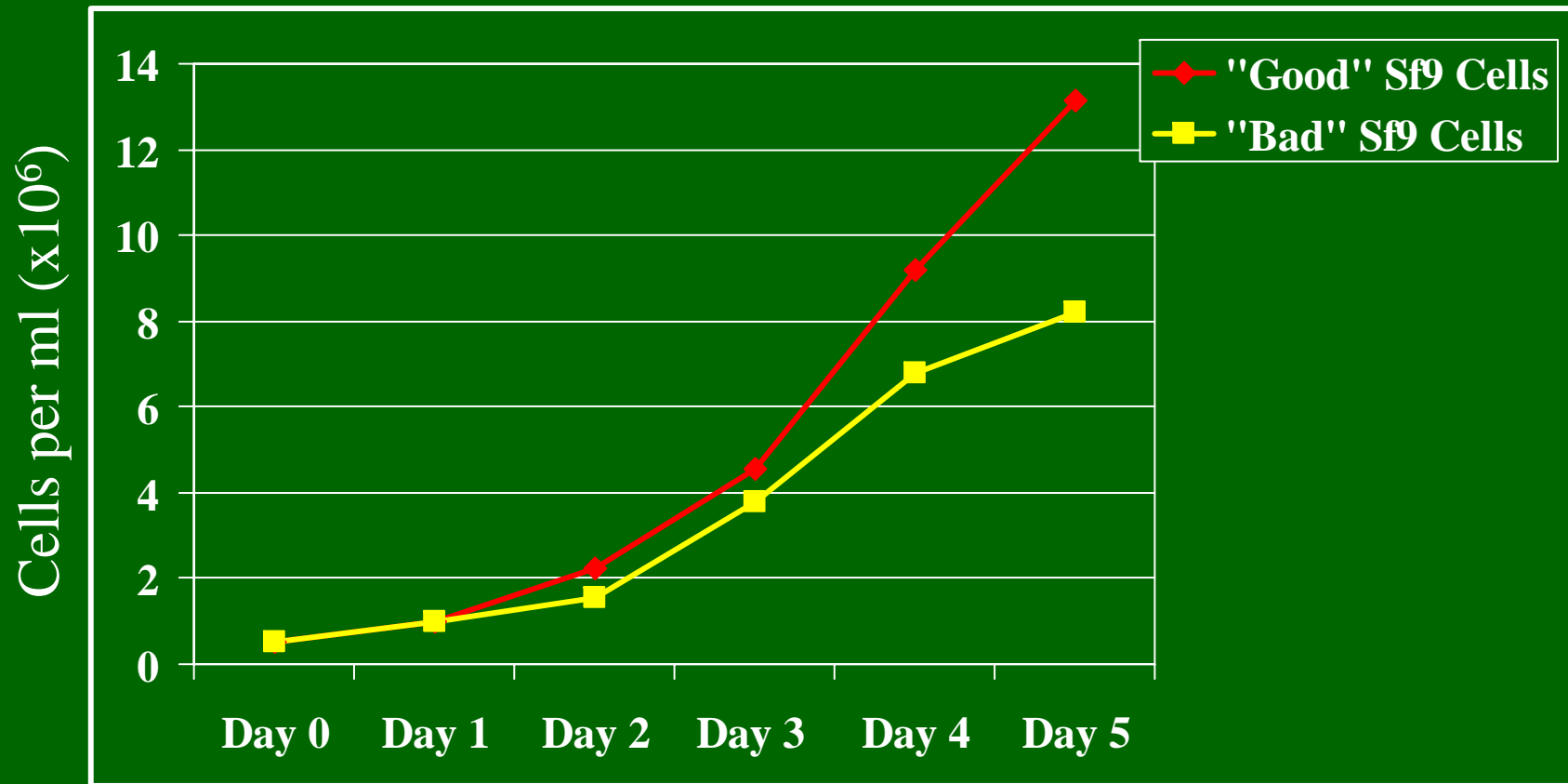
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Sf9 Cells in ESF921



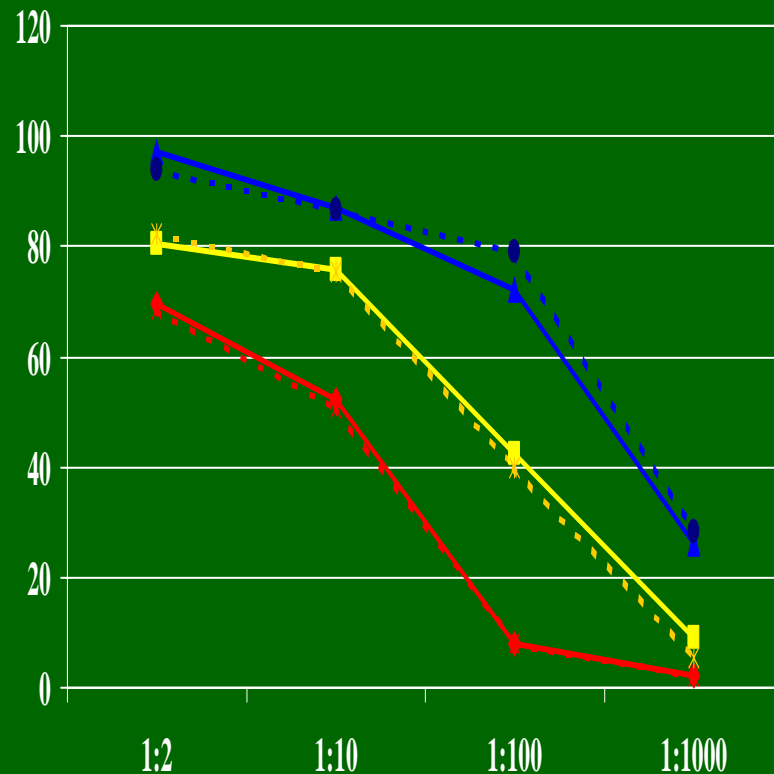
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Sf9 Cells in ESF921



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Comparing Infectability of “Good” and “Bad” Sf9 cells



- Cells are plated at 2×10^5 per well
- Virus is added in serial dilutions and incubated 18 hours
- Cultures are stained for gp64 expression as an indicator of infected cells



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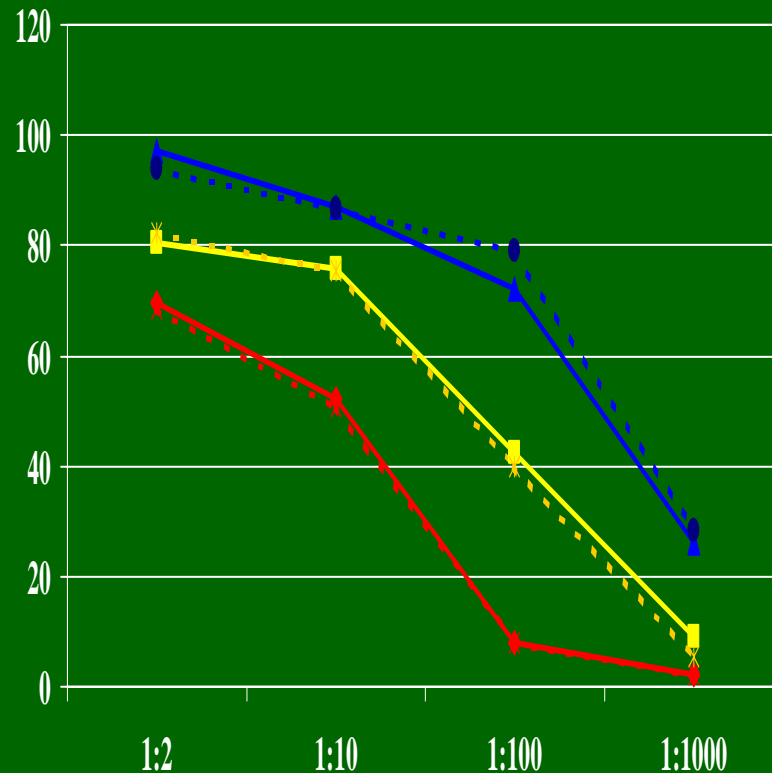
Comparing Infectability of “Good” and “Bad” Sf9 cells

Good: 2.75×10^6

Bad: 2.85×10^6

Good: 7.94×10^6

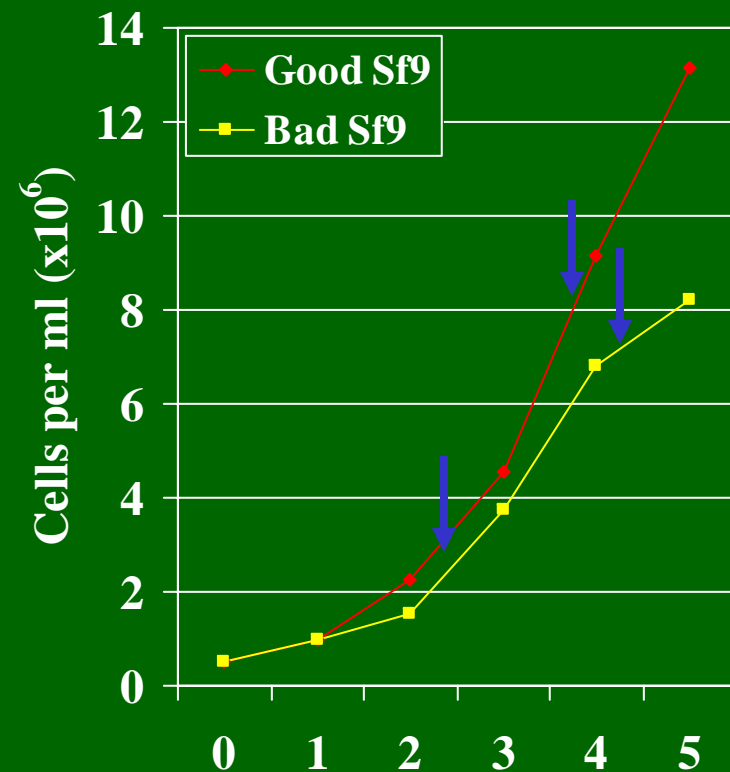
Bad: 7.24×10^6



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Hypothesis: The state of the cell cycle influences infectability

- It has been demonstrated that cells are infected best during mid-log phase
- The infectability of the subcultures was the same when infected at the lower conc....
- but differed as the cultures diverged for growth



Next Step: Look at the cell cycle over the growth curve to determine when infectability decreases

- Sample cultures over the growth curve
- Fix in cold 70% Ethanol
- Wash cells and transfer to 96-well plate
- Stain with propidium iodide in the presence of RNase

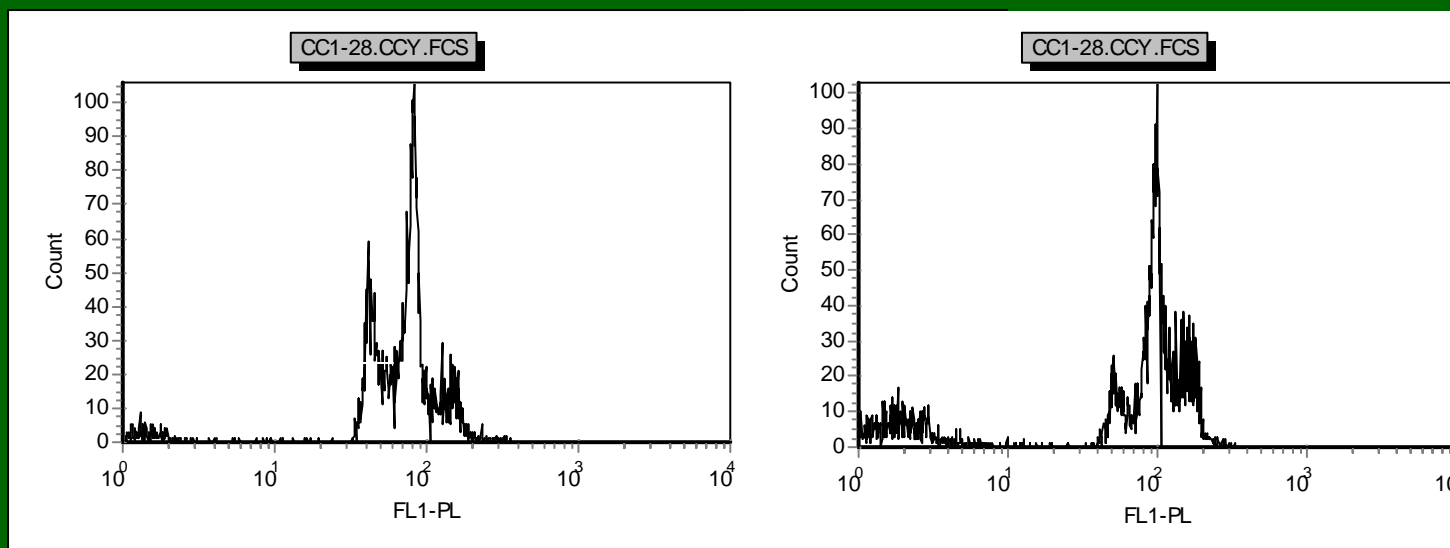


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Cell Cycle Profile of “Good” and “Bad” Cells

“Good” Sf9

“Bad” Sf9



M1= 22.0

M2= 56.8

M3= 16.7

M1= 10.5

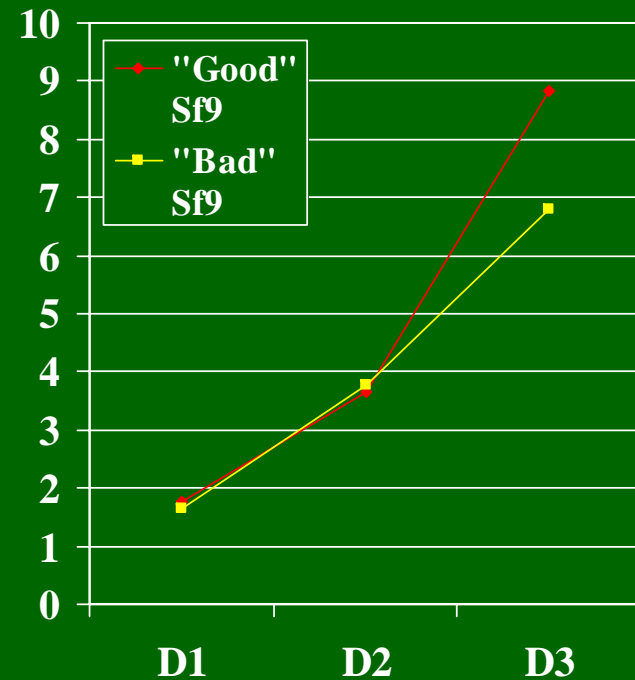
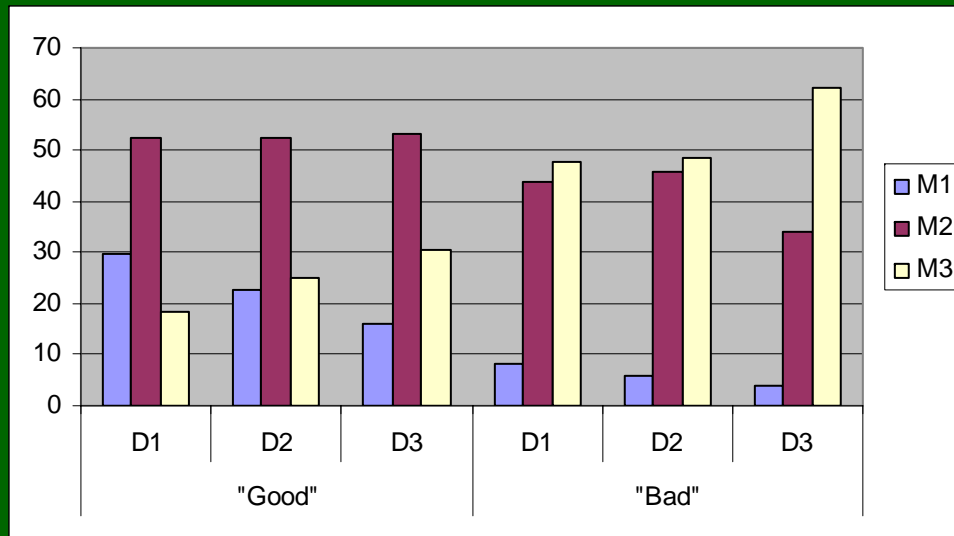
M2= 51.2

M3= 38.3



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Cell Cycle Profile Over Growth Curve



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Polyploidy correlates with reduced infectability and reduced growth capacity

- Jarman-Smith et al demonstrated chromosome instability of Sf9 cells with an accumulation of tetraploid cells occurring with increasing passage
- The authors hypothesized that the polyploidy state would have a negative effect upon recombinant protein expression
- Doverskog et al suggested that conversion to polyploidy is a result of cells entering lag phase

Jarman-Smith et al *Biotechnol. Prog.* 2002, 18: 623

Doverskog et al *Biotechnol. Prog.* 2000, 16: 837



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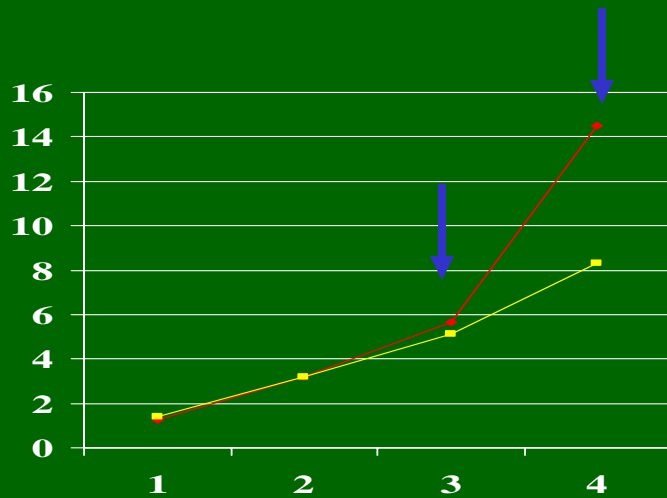
Expression in “Good” and “Bad” Sf9 Cells

Experiment:

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

Let grow overnight so they are in log phase

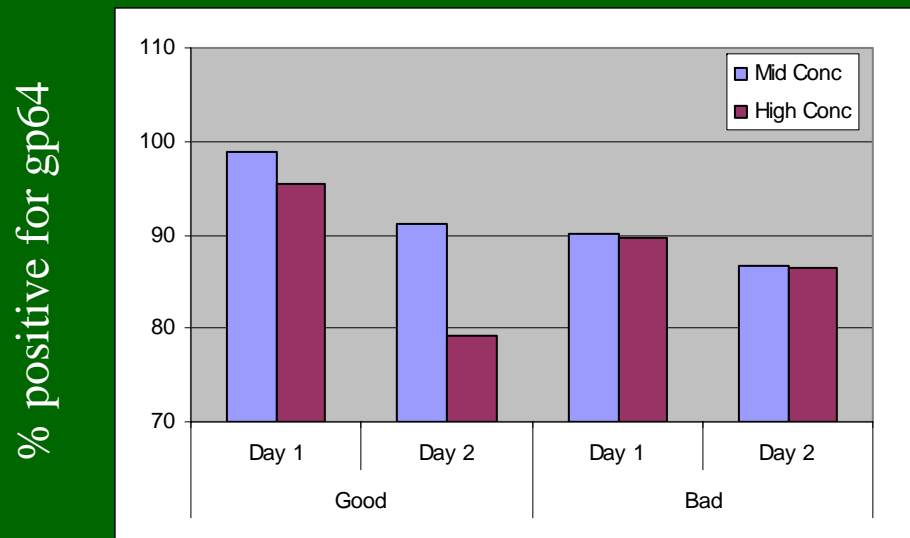
Infect at MOI of 1



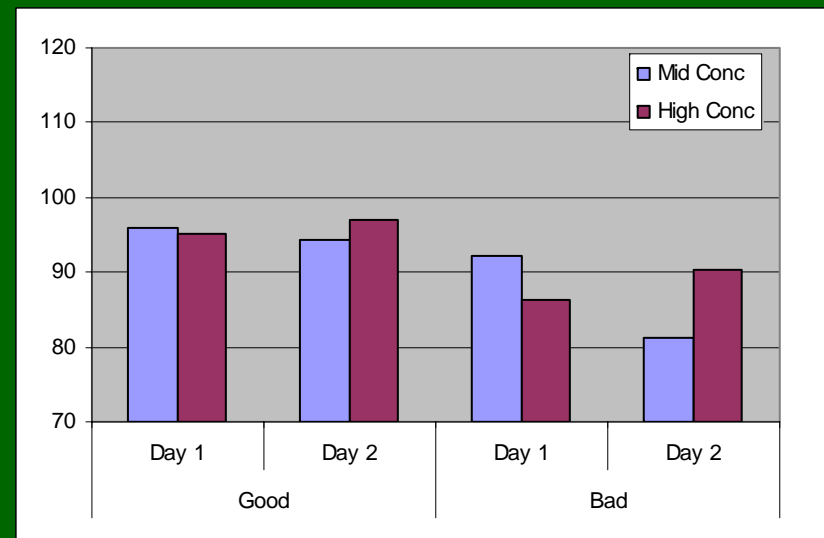
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Infectability of Sf9 Cells

Human transferrin



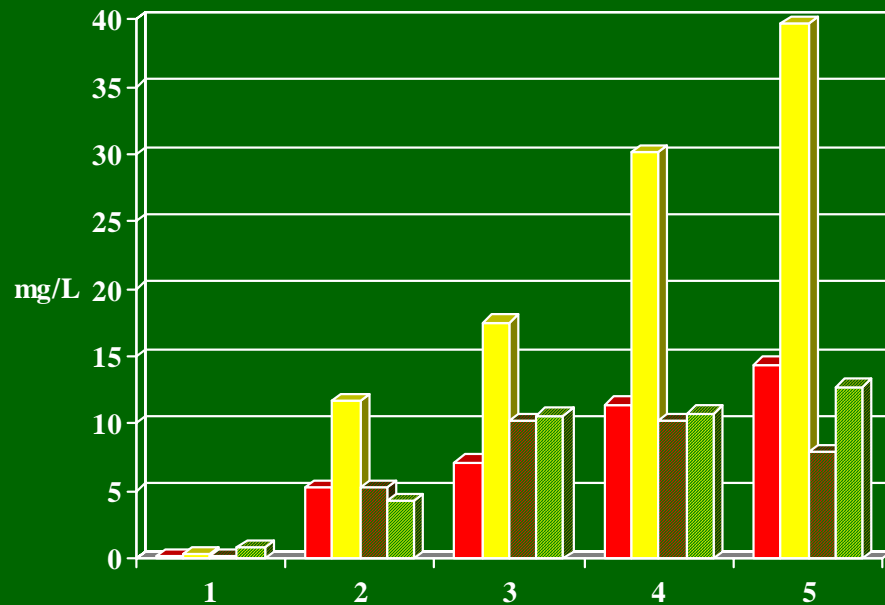
β -galactosidase



Samples were taken 24 and 48 hours post infection and stained for expression of gp64

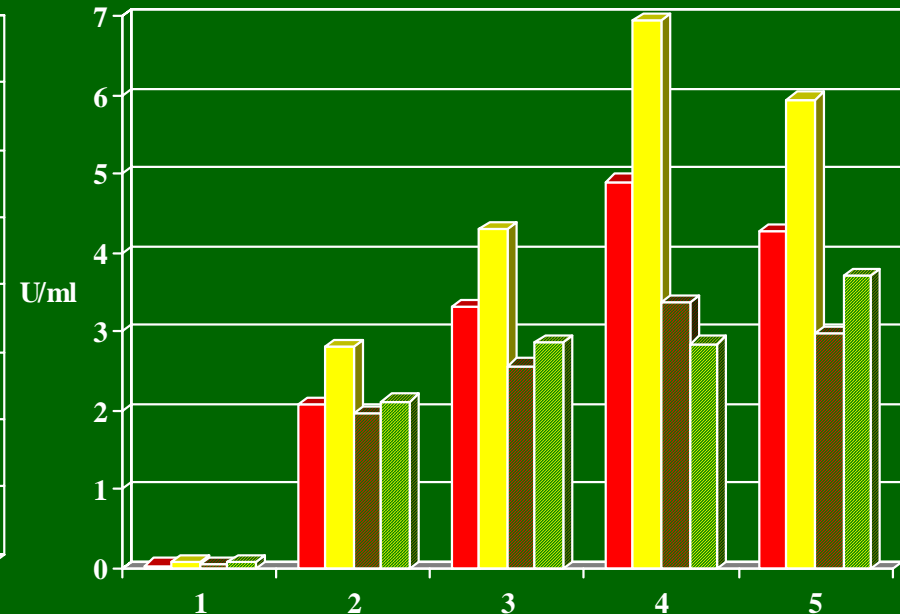
Expression of Recombinant Protein

Human transferrin



Good Sf9 Mid Conc
Bad Sf9 Mid Conc

Beta-galactosidase



Good Sf9 High Conc
Bad Sf9 High Conc



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Time to change the names of the Sf9 Subcultures?

- “Bad” Sf9 cells are less infectable than “Good” Sf9 cells
- “Bad” Sf9 cells have more tetraploid cells than “Good” Sf9 cells
- “Bad” Sf9 cells don’t grow to as high density as “Good” Sf9 cells
- “Bad” Sf9 cells produce more protein than “Good” Sf9 cells



Does Virus Binding Play a Role in Observations?

- Hypothesis: Cells will bind more virus if they are receptive to infection. (An extension of the observation that cells are more infectable in S phase; Lynn and Hink)
- Experiment: Bind virus to cells, fix, stain with propidium iodide and anti-gp64

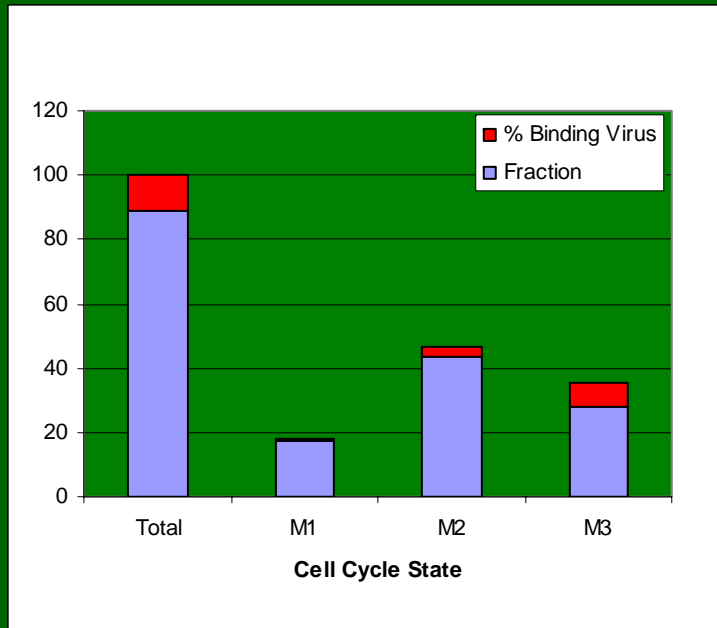
Lynn and Hink, *J. Inv. Pathol.* 1975, 32:1



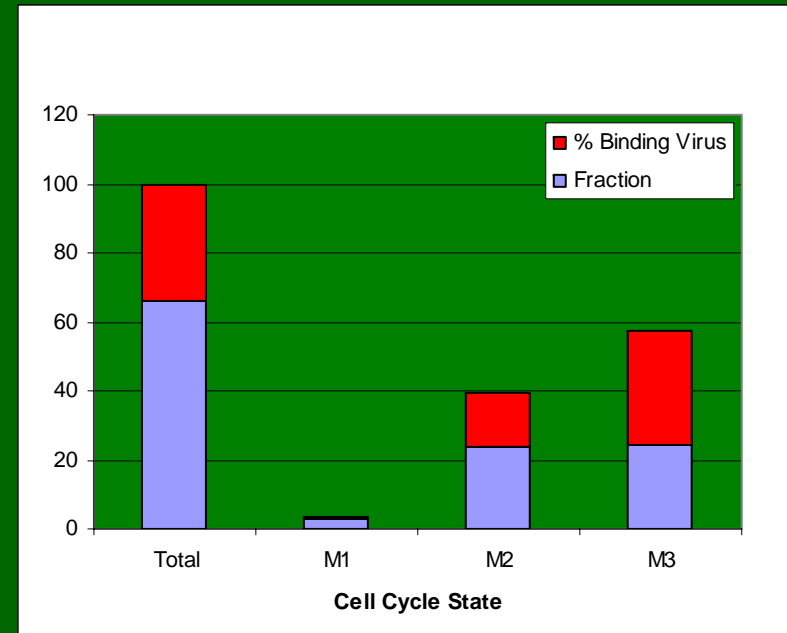
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Distribution of Virus Binding over Cell Cycle

Good Sf9



Bad Sf9



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Conclusion 1

- Virus binding is not distributed equivalently across the cell cycle
- Virus preferentially binds to G2 state cells
- This may be due merely to the size of the cells
- Large cells “mop up” virus, therefore altering the effective MOI when adding virus
- Increasing the MOI may be sufficient to compensate for affinity of virus to G2 cells although this may have practical limitations



Conclusion 2

- Passage number per se is likely not the problem; rather, it may be the genetic drift of the culture
- Culturing conditions can contribute to the accumulation of polyploid cells
- The seeding stock parameters can be crucial for reproducibility and optimization
- Cell cycle analysis and gp64 staining are a quick and easy way to test the reproducibility of the system



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Next Steps

- Expression Systems is in the process of subcloning the Sf9 cells
- These subclones will be characterized for growth, protein expression and virus propagation and transfection efficiency
- Tni PRO cells are being characterized also, although they do not appear to accumulate polyploid cells as the Sf9 cells do
- ES will continue to strive for a better understanding of the insect cell and its applications



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