

Evaluation of the stability of rCHOK1SV cells in the absence of selective pressure

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CHOK1SV

- a cell line developed by Lonza to facilitate production of recombinant protein therapeutics
- transfected by Hospira to produce model monoclonal antibodies

Why employ mammalian host cells?

- bacteria, yeasts, transgenic plant and insect cells may all be used to produce recombinant proteins
- most glycoproteins, including recombinant monoclonal antibodies, are produced using mammalian cells
- mammalian cells, are capable of performing post-translational modifications, such as glycosylation, required for appropriate bioactivity



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Why CHO cells?



- CHO cell line established over 50 years ago from cells isolated from the ovary of a Chinese hamster
- extremely robust and adaptable
- population doubling time of approximately 24 hrs
- safe hosts for production of recombinant biologics
- capable of producing glycoproteins with appropriate glycosylation profiles
- very amenable to stable genetic manipulation



Why CHOK1SV?

- grow in suspension
- adapted to chemically defined, animal-component-free growth medium
- high viable cell counts ($>1 \times 10^7/\text{mL}$) in shake flasks or bioreactors
- maintain high viability ($>95\%$) under suitable feed regime
- reported to produce more than 10 g/L recombinant protein under suitable growth conditions
- produce monoclonal antibodies with appropriate glycosylation profiles

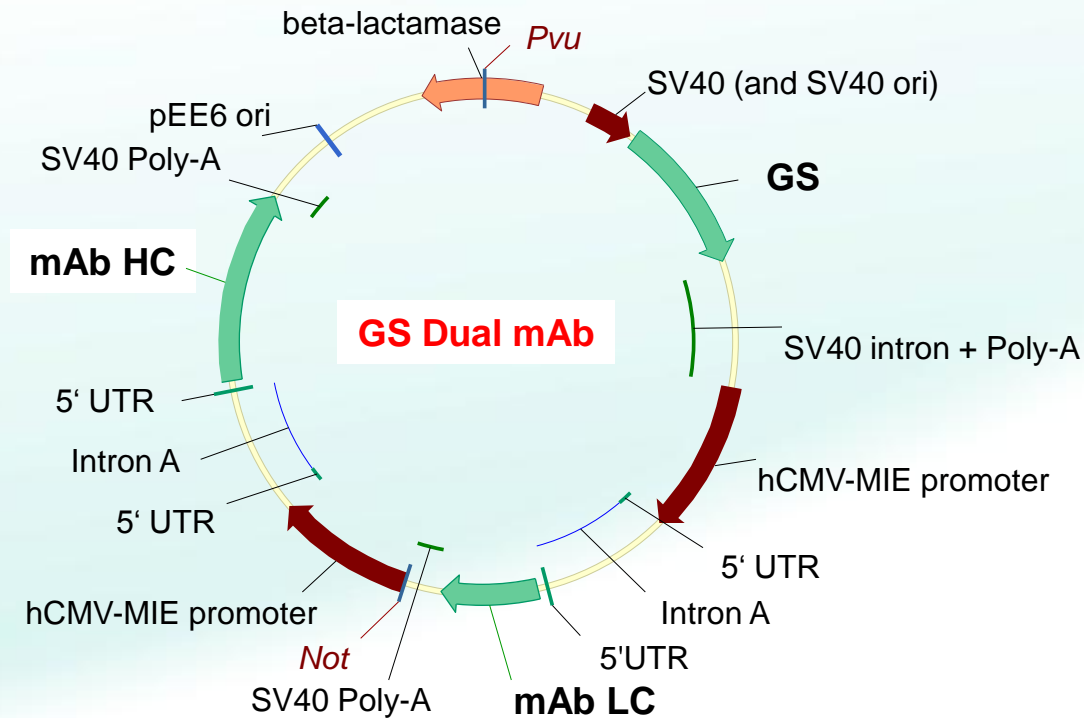


GS (Glutamine synthetase) Expression System:1

- developed and licensed by Lonza Biologics plc
- selection achieved via glutamine metabolism
- proven reliability
- well recognised by regulatory authorities:
 - recombinant therapeutics approved and in Stage III clinical trials
- enables rapid generation of high producing clones
- amplification not required to generate high producers

GS (Glutamine synthetase) Expression System:2

- strong promoter to drive expression of the gene of interest
- weak promoter driving GS cDNA
 - enables selection of clones where integration has occurred at efficient sites for transcription
- HC and LC genes on the same vector for co-expression





Glutamine synthetase (GS)

- catalyses biosynthesis of glutamine from glutamate and ammonia
- is the sole enzymatic pathway for glutamine synthesis by mammalian cells
- can be specifically inhibited by Methionine sulphoximine (MSX)



CHO cells and GS

- CHO cells inherently express sufficient GS to survive in glutamine-free growth medium
- addition of MSX to the medium at an appropriate concentration results in:
 - inhibition of endogenous GS activity and loss of viability of non-transfected clones
 - specific survival of GS transfected clones
- Lonza recommends that GS-CHOK1SV clones be grown in the presence of 25 μ M MSX to ensure genetic stability
- MSX adds to upstream and downstream costs of therapeutics
 - cost of MSX at scale
 - lot-release assay required to demonstrate removal of MSX from product



Aims

- to determine whether GS-CHOK1SV clones producing a model monoclonal antibody would maintain titre and product quality if grown in the absence of MSX
- to determine how many sub-cultivations were possible in the absence of MSX before titre and/or product quality were lost
- to determine whether titre and product quality could be regained by returning clones to MSX



Protocol: background

- Clones A & D, transfected to produce a model monoclonal antibody using the Lonza GS expression system
- Banks generated and frozen
- Clones thawed into a chemically-defined, animal-component-free growth medium supplemented with 25 μ M MSX as per Lonza protocol



Protocol: routine culture

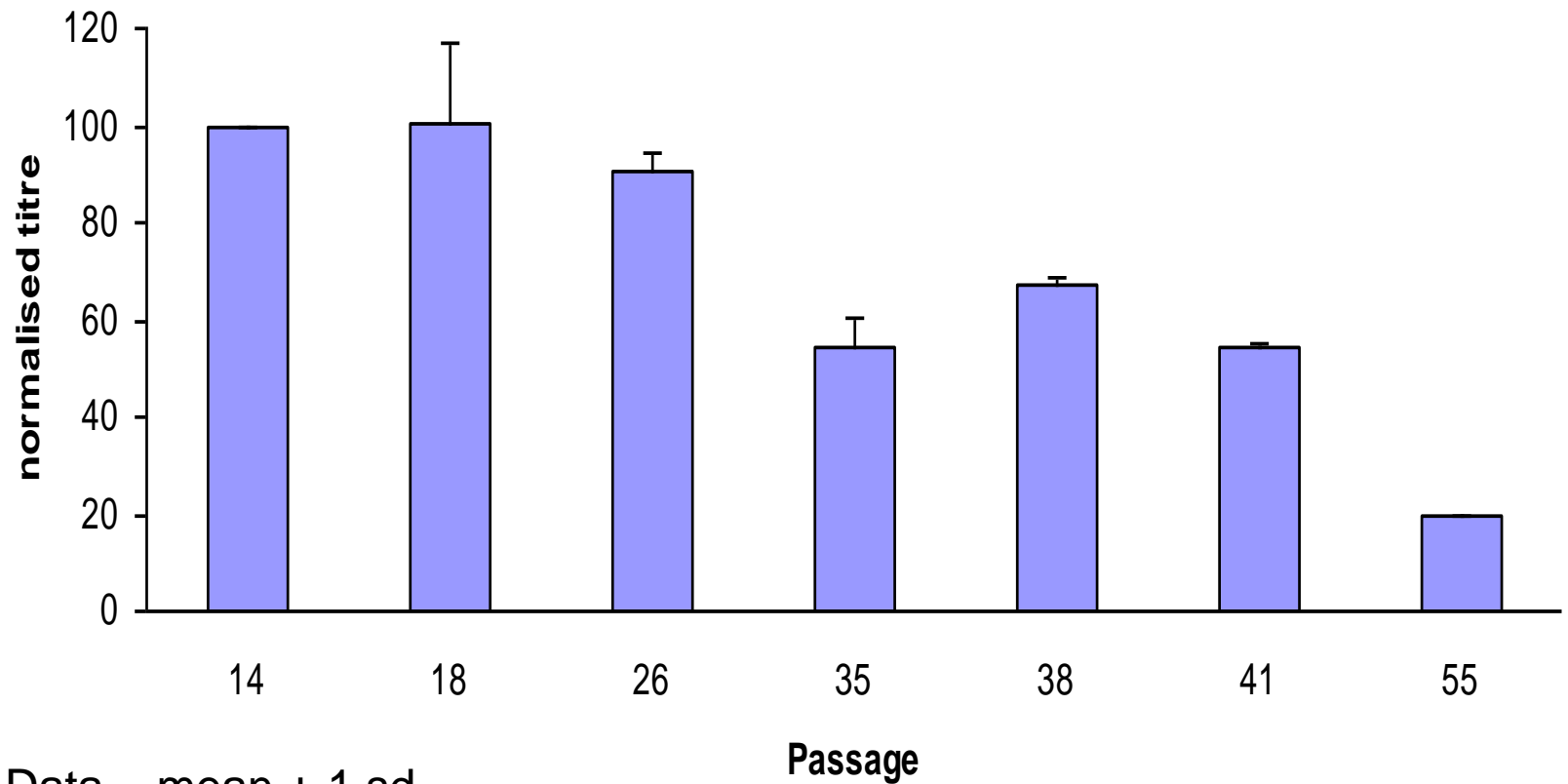
- clones passaged every 3 or 4 days
- seeded at $2-3 \times 10^5$ viable cells/mL
- after 3 passages in the presence of 25 μ M MSX, each culture split into two, and then grown in the presence or absence of MSX



Fed-batch assay 1

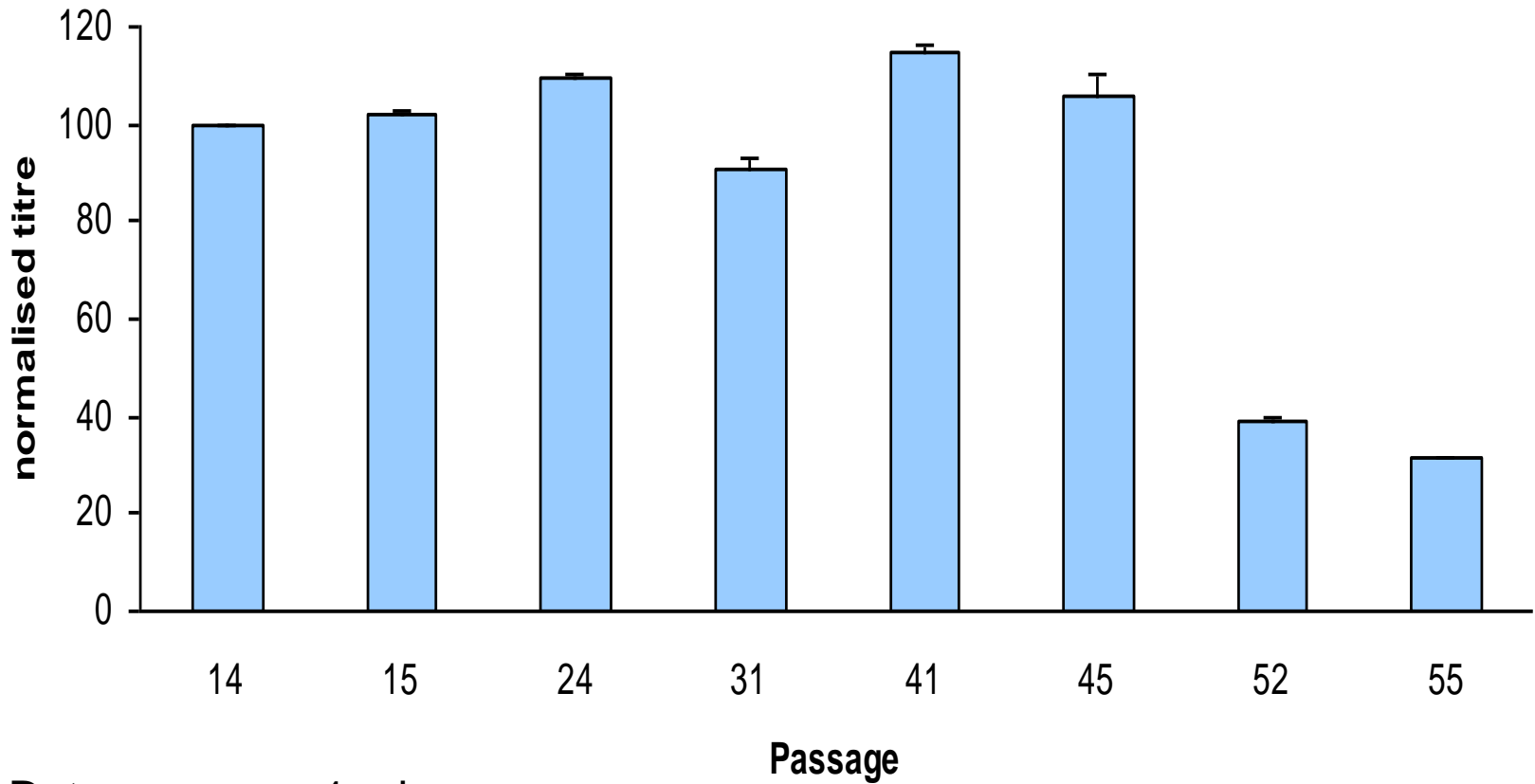
- Clones frozen after defined numbers of passage in the absence of MSX
- Thawed, then growth and productivity compared in fed-batch assays
- Day 0: seed at 5×10^5 viable cells/mL. 50 mL cultures in shake flasks (no MSX)
- Fed intermittently
- Harvest

Clone A: passage vs titre in the absence of MSX



Data = mean + 1 sd

Clone D: passage vs titre in the absence of MSX



Data = mean + 1 sd

Seed train



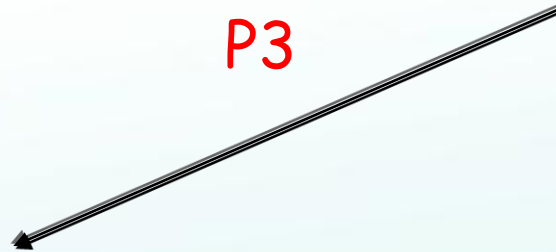
P1



P2



P3



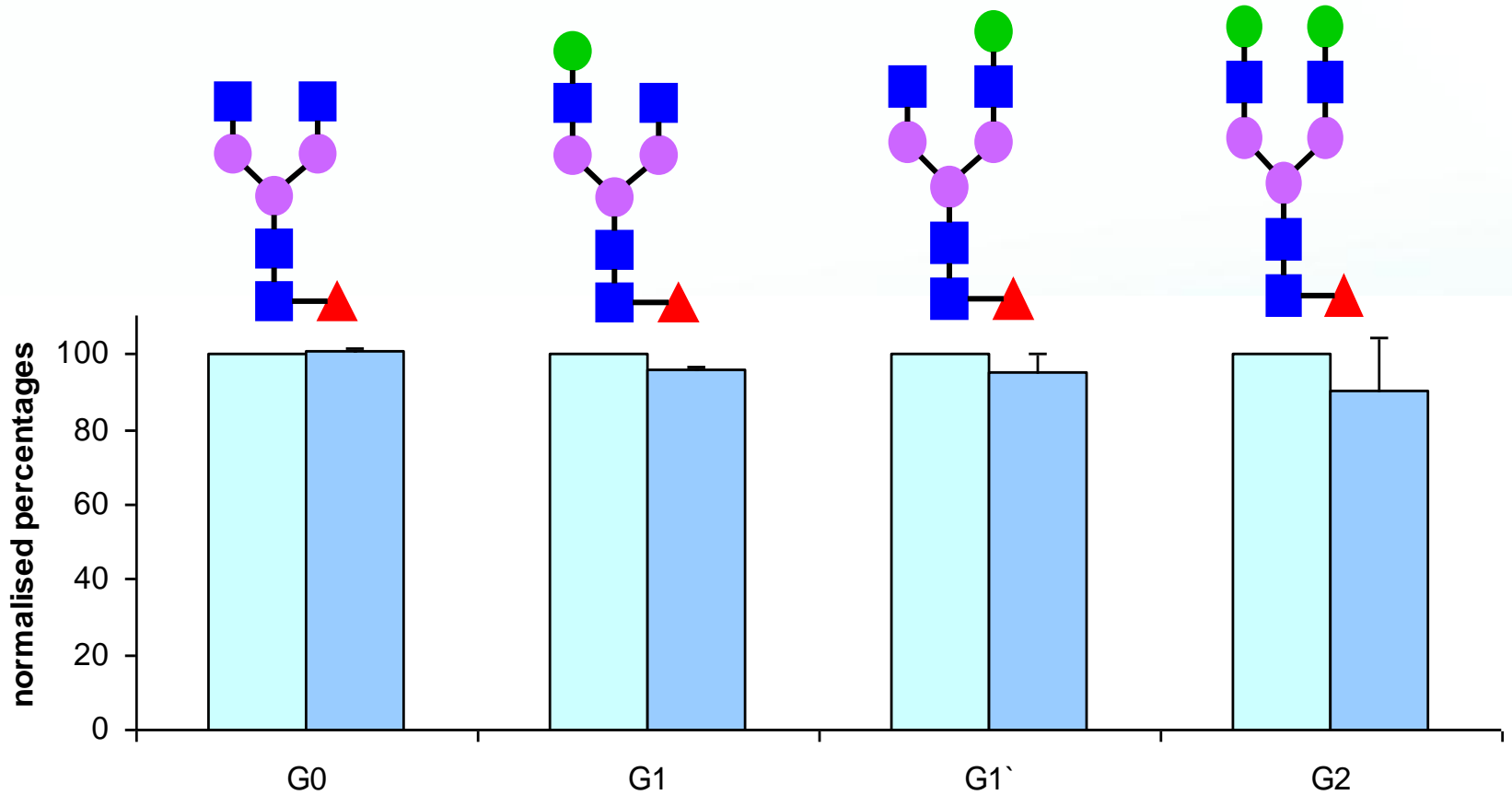
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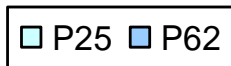
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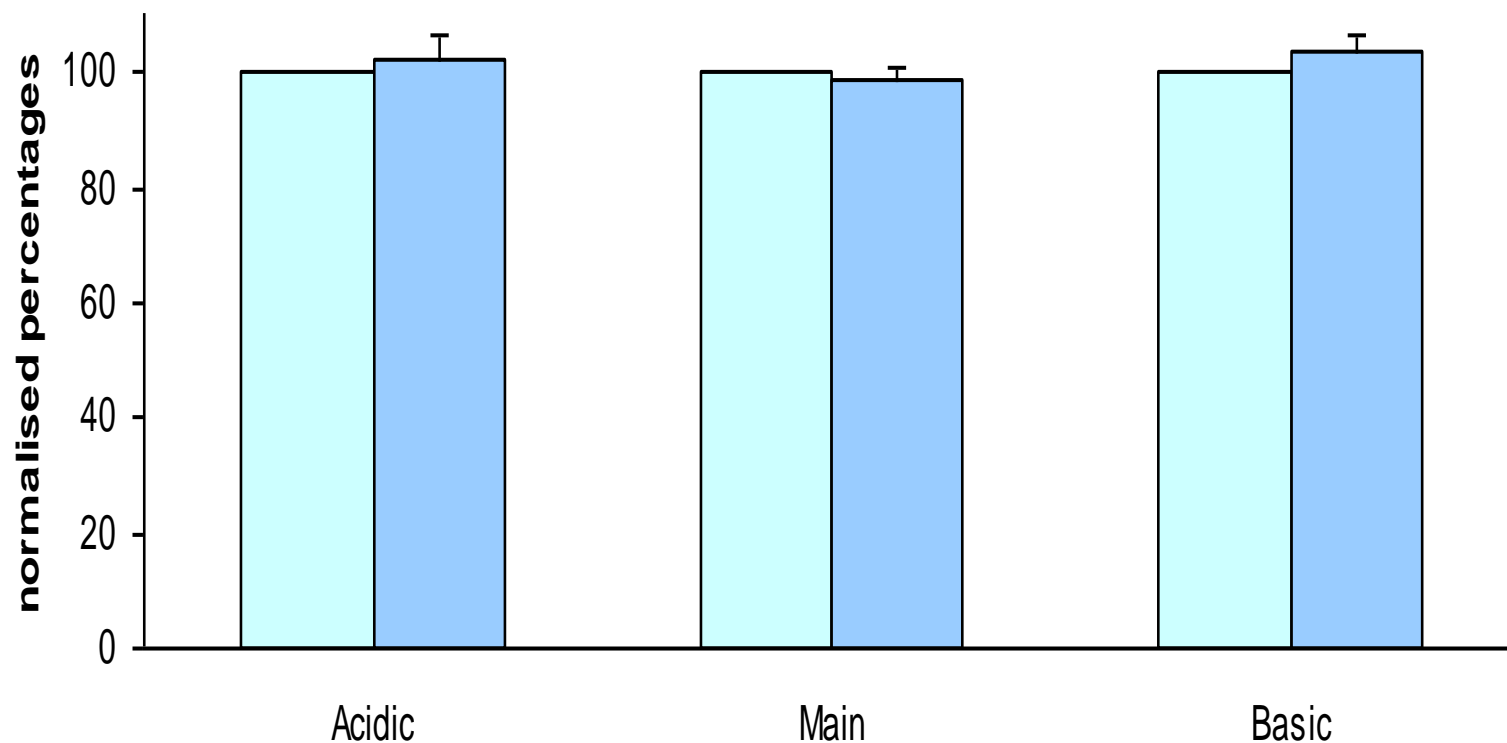
Clone D: effect of extended passage in absence of MSX on glycosylation



Data = mean + 1 sd



Clone D: effect of extended passage in absence of MSX on charge variants



Data = mean + 1 sd

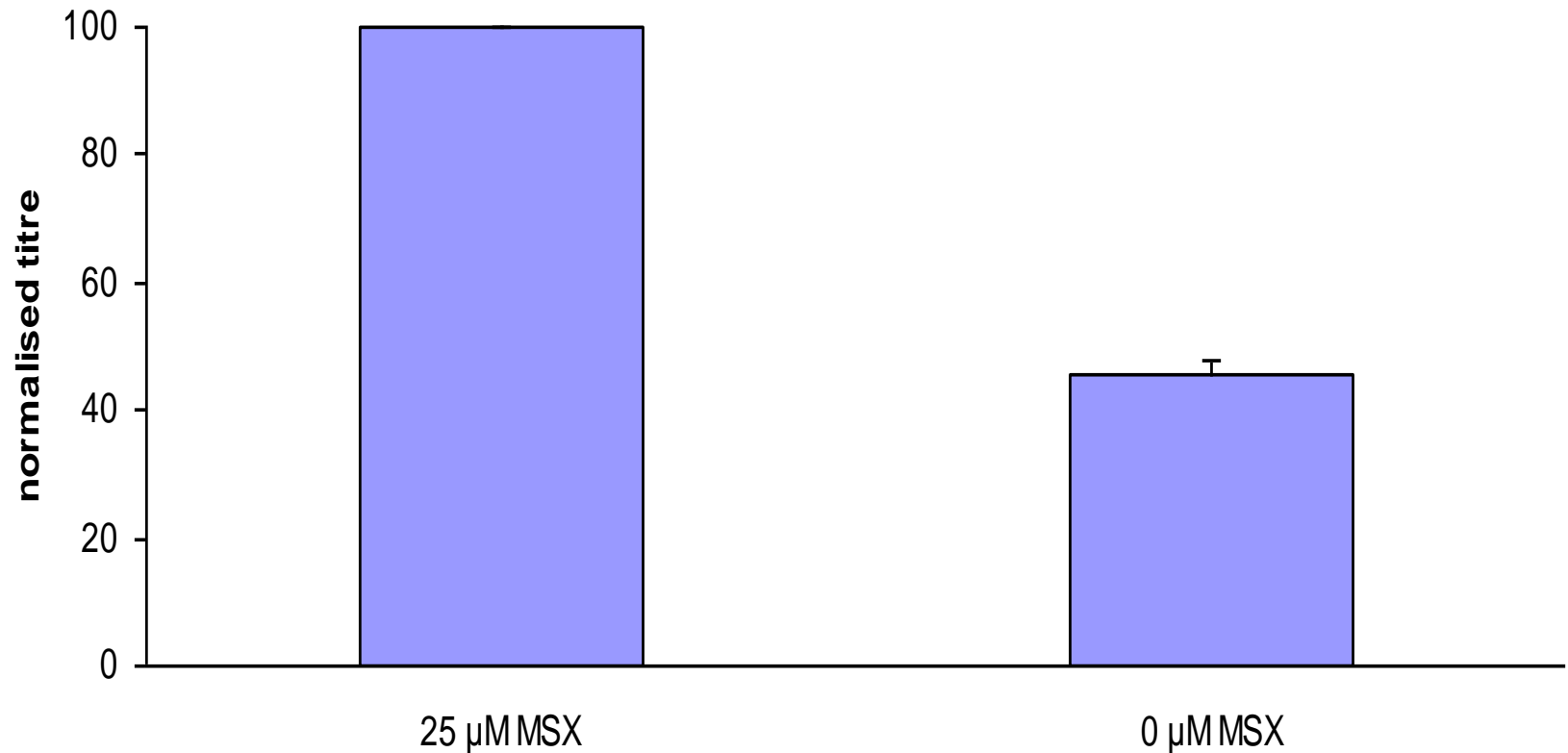




Fed-batch assay 2

- Clone A routinely grown in the presence or absence of 25 μM MSX from Passage 7 to Passage 35
- Day 0: seed at 5×10^5 viable cells/mL. 50 mL cultures in shake flasks (no MSX)
- Fed intermittently
- Harvest

Clone A: productivity after extended passage in the presence/absence of 25 μM MSX

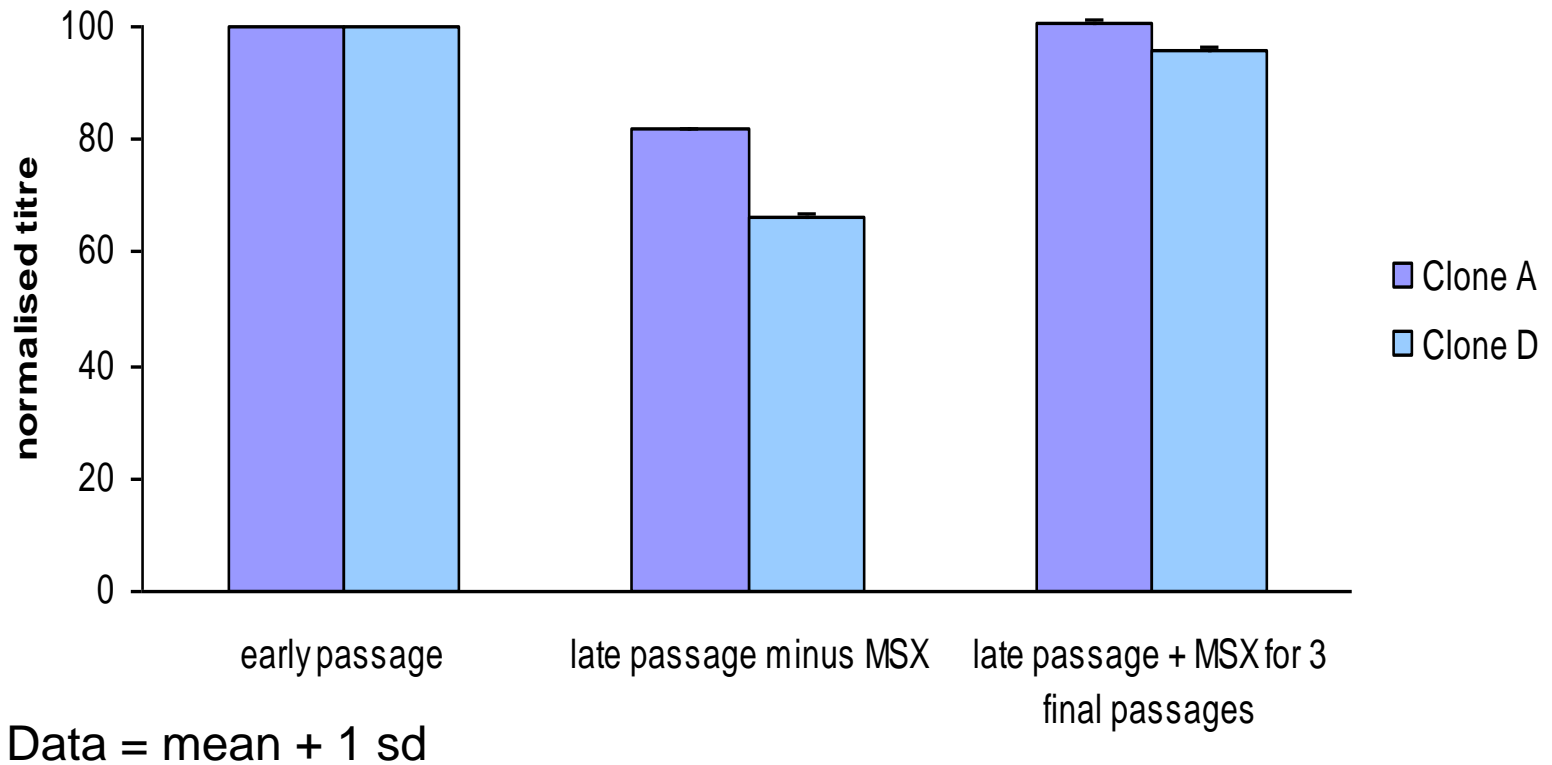




Fed-batch assay 3

- Clones extensively passaged in the presence or absence of 25 μM MSX
- Minus MSX culture split, and 25 μM MSX returned to one culture for 3 passages
- Day 0: seed at 5×10^5 viable cells/mL. 50 mL cultures in shake flasks (no MSX)
- Fed intermittently
- Harvest
- Assay culture supernatants for product titre and quality

Clones A & D: restored productivity after extended passage in the absence of 25 μ m MSX





Conclusions

- rCHOK1SV Clones A & D demonstrate a decrease in productivity, but not product quality, after extended passage in the absence of selective pressure
- Productivity was restored by returning 25 μM MSX to the growth medium
- Clones A & D maintain productivity in the absence of MSX over sufficient population doublings to justify excluding MSX from seed train
- If the presence of MSX in cultures is problematic, it is worthwhile investigating the stability of individual clones in its absence