Expression Systems

Cell Adaptation Techniques

Researchers are often hesitant to change reagents used in their established laboratory processes. This resistance to change is understandable, but with advances in cell culture technology, researchers may be missing out on the enhanced performance delivered by modern media formulations such as those produced by Expression Systems. Although the basic composition of cell culture media is similar across all manufacturers, advanced cell culture media are more nutrient rich and formulated with compounds that are unique to each formulation. When switching to an advanced cell culture medium, simply seeding cells directly into the new medium can stress cells due to differences in formulation. Although cells may eventually grow and appear to recover from the initial shock, far better results may be achieved if the cells undergo an adaptation process.

GROWTH CURVES

Before initiating the adaptation process, Expression Systems recommends establishing a reference growth curve for the cell line that is to be adapted. Seed a culture at the standard passaging density and collect cell count and viability data daily. Continue collecting data until the viable cell count has plateaued. This growth curve will serve as a reference to gauge the progress of adaptation. Figure 1 depicts a standard growth curve with three phases of growth in cell culture; lag phase, exponential phase, and stationary phase. Do not use the growth curve culture for further adaptation as the cells will be allowed to reach peak density and enter stationary phase.

PROCEDURE OVERVIEW

Successful adaptation is achieved through sequential adaptation of cells to an increasing ratio of the new medium to old medium. For a typical adaptation, cells will be cultured in the following ratios of new medium to old medium: 50:50, 75:50, 87.5:12.5, and finally 100:0. Think of the whole process as a series of individual adaptations to different mixtures of media until the cells are adapted to 100% new medium. The cells must be fully adapted to one mixture of media before moving to the mixture with a higher percentage of new medium. There is no set number of times that a culture should be passaged in a particular mixture of media before increasing the

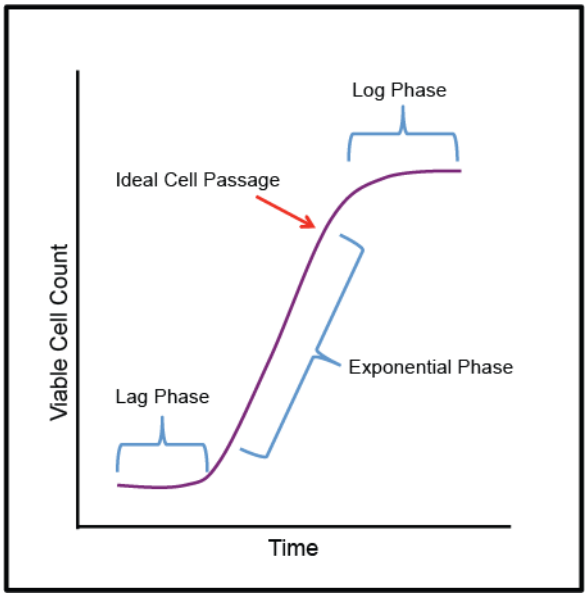

Figure 1. A typical growth curve illustrating the three growth phases observed in cell culture.

percentage of new medium, the culture should be passaged until it is fully adapted to that mixture. The reference growth curve can be used to gauge the progress of an adaptation culture. The objective is for the growth curve of the adaptation culture to match the reference growth curve. Once the adaptation culture's growth kinetics match the original culture's in terms of length of lag after passage, growth rate in the exponential growth phase, and maximum cell density, they may be considered adapted and shifted to the next mixture with a higher percentage of new medium.

When cells are first introduced to a new mixture of media, they will typically exhibit an extended lag phase after seeding, slower maximum growth rate, and lower maximum density. With continued passaging in the same combination of media the lag phase should shorten, maximum growth rate increase, and maximum cell density increase. Figure 2 illustrates a series of typical growth curves for different passages of an adaptation culture. By passage 5, the cells are exhibiting the same grow kinetics in the new medium mixture as they were in the original medium. At passage 6, the cells have maintained these

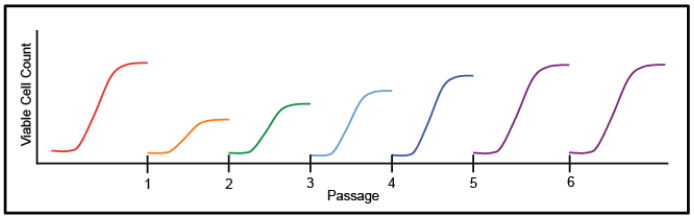

Figure 2. Graphic illustration of how growth curves may change during the course of an adaptation.

growth kinetics and may be seeded into a mixture of media with a higher percentage of new media. Keep in mind that Figure 2 does not represent the complete adaptation process; it only illustrates adaptation to one distinct ratio of media. Figure 2 is only an example and the actual number of passages required for adaptation will vary based on the cell line and the composition of the original and new media.

PASSAGE SCHEDULE

Another important point of consideration is the passage schedule for the adaptation culture. In short, there is no passage schedule. Expression Systems advocates maintaining a strict passage schedule for all established cell cultures in order to achieve consistent results with all downstream cell culture processes. The passage interval for the adaptation culture may not integrate with your standard passage schedule, and a passage schedule should not be imposed on the adaptation culture. It is beneficial to allow cell cultures to reach their full potential before passaging. During the adaptation, cultures will likely require a longer interval between passaging to allow them to approach the end of the exponential growth phase.

Creating a growth curve is an effective way to gain a better understanding of the culture's growth potential. The culture used for establishing the growth curve should be prepared in parallel to the primary adaptation culture because the growth curve culture will be allowed to reach maximum density and enter stationary phase, and therefore should not be used for further adaptation. During the adaptation process, it is recommended to seed cultures at a higher density than typically used for passaging. Cells that are generally passed to $0.5x10^6$ cells/ml should be passaged to $1.0x10^6$ cells/ml during adaptation, for instance. Typically, cultures should be split when the cells are approaching their maximum density, but before exiting the exponential growth phase. Figure 3 demonstrates why a standard passage schedule may be inappropriate for the adaptation culture. At the time when the adapted cells may be passaged as part of a regular schedule, the adaptation culture is still relatively early in the exponential growth phase and will benefit from continued incubation until it is closer to the maximum density.

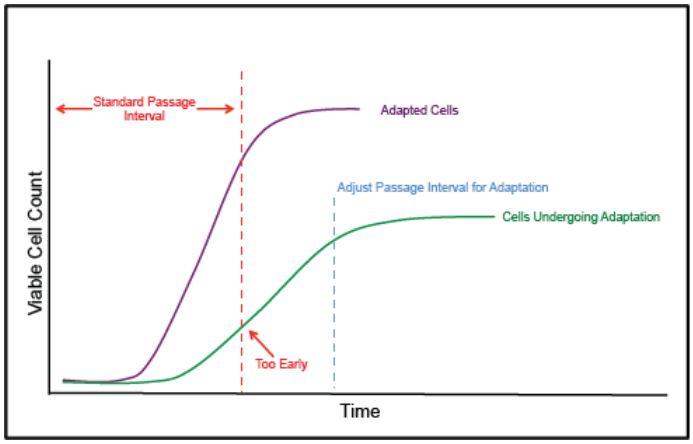

Figure 3. A longer passage interval may be required during adaptation.

CONFIRMING ADAPTATION

Once the cells are growing in 100% new medium, and the growth rate and maximum cell density meet or exceed expected values, aliquots of the culture should be cryopreserved in liquid nitrogen. Cells can be considered fully adapted once it has been demonstrated that they can be frozen and thawed into the new medium while maintaining a high viability.

For technical assistance or documentation, such as Certificates of Analysis or Safety Data Sheets, email support@expressionsystems.com

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