QUICK GUIDE TO G-REX OPTIMIZATION

Definitions

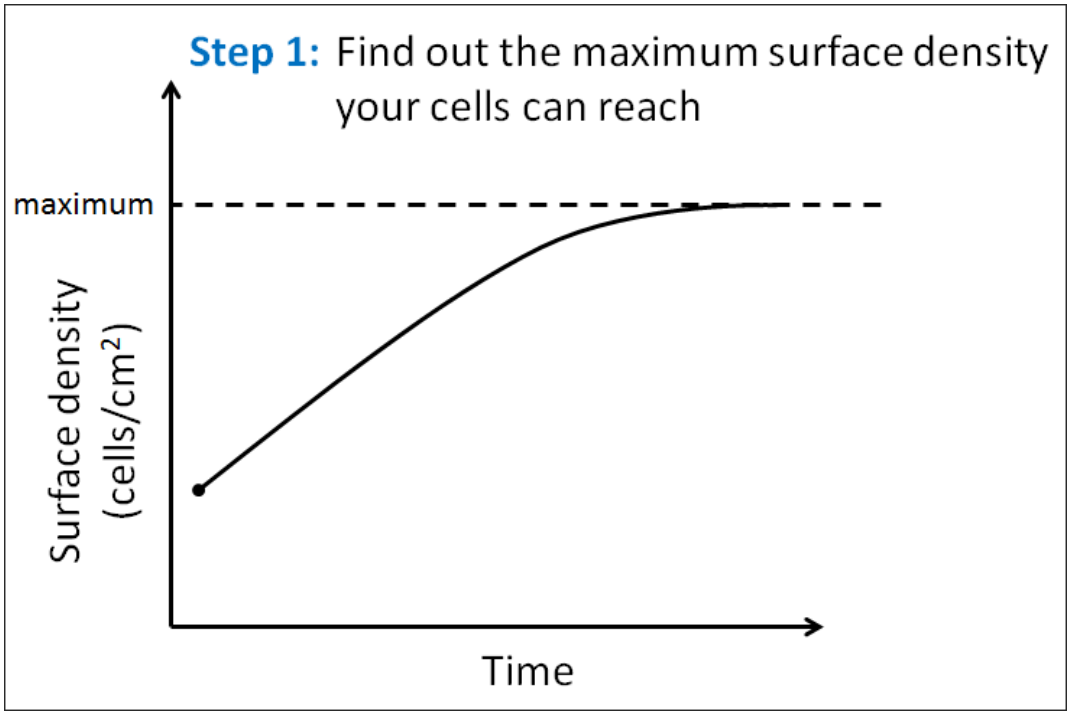
Surface Density: The number of cells residing on the gas permeable G-Rex bottom when normalized to units of cells/cm^2.

Maximum surface density: The maximum number of cells, normalized to cells/cm^2, that can reside on the gas permeable G-Rex bottom before a drop in viability occurs.

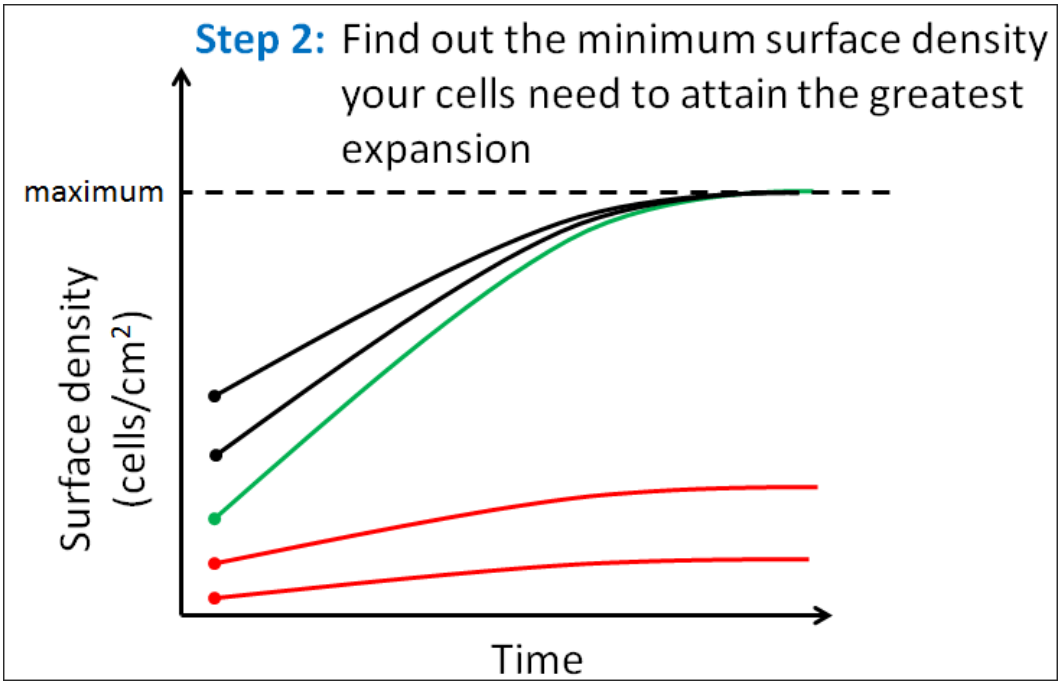
Minimum surface density: The minimum number of cells, normalized to cells/cm^2, that must reside on the gas permeable G-Rex bottom to initiate population expansion.

Fold expansion: The maximum surface density divided by the minimum surface density.

Background: The G-Rex10 is an excellent device for optimizing protocols for your cells. Whatever is learned in G-Rex10 scales directly to G-Rex100 and G-Rex100M. By using G-Rex10, you will use less media and counting cells is very easy (which is frequently required during optimization work).



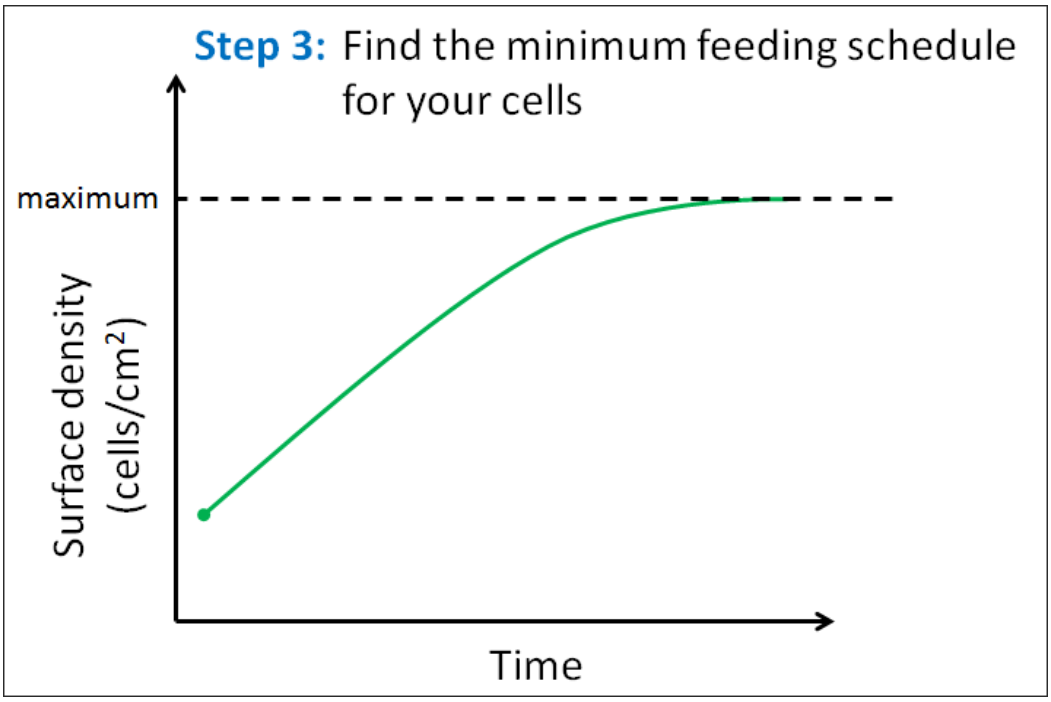
- Add 10 million cells (i.e. 1 x 10^6 cells/cm^2) and 40 ml of medium into each G-Rex10
- Every 3 days, remove and replace 30 ml of medium and count cells
- Continue until cells reach a plateau. That is the maximum cell density your cells can achieve.



Create an experimental matrix that determines the minimum surface density that is needed for your cells to begin growth. Add 40 ml of medium to each G-Rex10 devices along with the following number of cells:

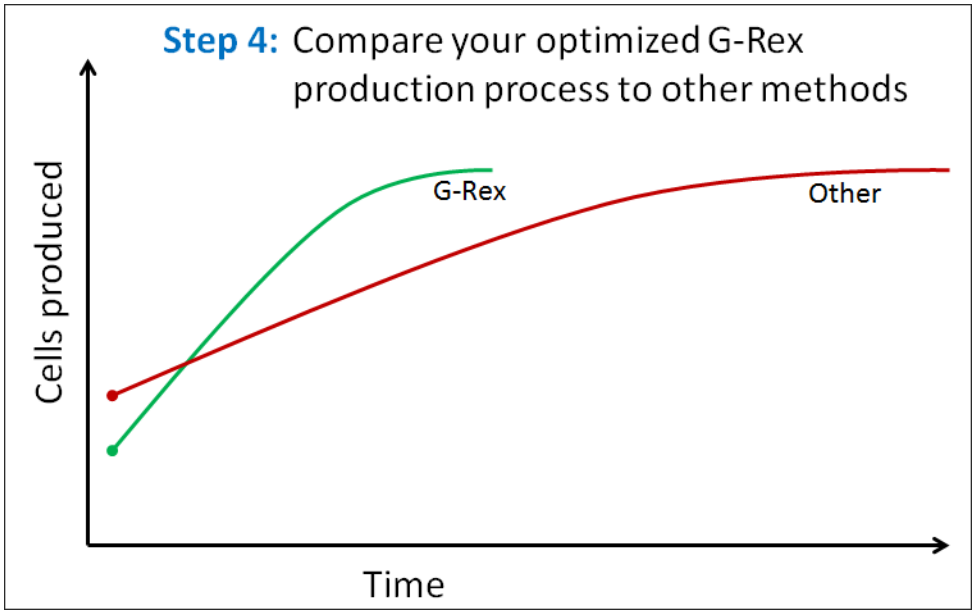
- 10 million (i.e. 1 x 10^6 cells/cm^2)
- 5 million (i.e. 0.5 x 10^6 cells/cm^2)
- 2.5 million (i.e. 0.25 x 10^6 cells/cm^2)
- 1.25 million (i.e. 1.25 x 10^6 cells/cm^2)
- 0.625 million (i.e. 0.0625 x 10^6 cells/cm^2)

About every 4 days, remove and replace 30 ml of medium and count cells to find out if cells are capable of expanding from the starting surface density. **Make sure you add IL2 (or other cytokines) at least every 2-3 days no matter how often you exchange the medium. Add the IL2 at a quantity that assumes it has all been depleted.** You can optimize IL2 frequency later, but we do not want to lack of adequate IL2 to affect your determination of the minimum surface density that is needed for your cells to begin growth.



- Start the cultures with the minimum surface density of Step 2 and with 40 ml of medium.
- Experiment with different feeding schedules to find out the minimum amount of medium needed to reach the maximum surface density of Step 1.
- **Make sure you add IL2 (or other cytokines) at least every 2-3 days no matter how often you exchange the medium. Add the IL2 at a quantity that assumes it has all been depleted.**

Note: Once the maximum and minimum surface density is found in G-Rex10, these values will scale 10-fold into the G-Rex100 and G-Rex100M. After you have determined the minimum feeding frequency, compare your optimized G-Rex production process to any other method.



- Feed G-Rex100 at the optimized schedule found in Step 3.
- Add 1 liter of medium into G-Rex100M at the onset of culture and do not feed. Periodically counts cells. **Make sure you add IL2 (or other cytokines) at least every 2-3 days until cells reach maximum surface density. This will probably occur in around 10 to 14 days.**

We encourage you to compare G-Rex to alternative production methods based upon the following criteria. We are confident a comparison will show the power of GRex to simplify and reduce the cost of your cell production process.

	Device Comparison		
Evaluation Criteria	**G-Rex**	**Bag**	**WAVE**
Quantified:			
Minimum starting surface density (cells/cm^2)			
Maximum final surface density (cells/cm^2)			
Media efficiency (ml per 10^6 cells)			
Cost of media per 10^9 cells			
Fold expansion			
Labor (minutes)			
Cost of labor per 10^9 cells			
Downstream processing			
Labor to separate cells from media			
Cost of labor per 10^9 cells			
Cost of centrifuge material per 10^9 cells			
Space required to perform production			
Characterized criteria:			
Practicality			
Scale up potential			
Skill level needed to perform production			
Commercialization practicality potential			
Functional Comparison			
Cell phenotyping comparison			
Cell functionality			