Using Genetix ClonePix FL for hybridoma screening

Methods and results

Establish cells in semi solid culture (Using stable hybridoma line)

Purpose: To adapt cells to a semi-solid media and establish plating conditions for ClonePix FL screening. It is advisable to first establish conditions in a stable hybridoma line as this requires less optimization time than starting with a fresh fusion. Please anticipate a couple rounds of plating for this phase.

1. If frozen cells are to be used, thaw and passage for about 48 hours until viability reaches as least 85%, preferably above 90%.
2. On day of plating, spin to concentrate cells to 2 mL. Obtain a count of viable cells.
3. Practice plating cells at varying densities into media. It is critical to optimize the seeding densities to ensure successful colony selection and picking. Plate out 3 different densities: 50 cells/mL, 100 cells/mL, 150 cells/ mL.
   - CloneMatrix+ liquid hybridoma media concentrate (2X). CloneMatrix is the semi-solid concentrate. The liquid media should be a media known to support your hybridoma line.
   - CloneMedia Hybridoma. CloneMedia Hybridoma is an all-in-1 media which contains the semi solid concentrate + media that has been optimized to support hybridomas growth in the matrix. If the routinely used liquid media differs greatly in composition from CloneMedia Hybridoma, then a period of adaptation to the media may be required.
4. Add the fluorescent detection reagent, CloneDetect, to the media at the same time. This will add minimal volume to the final concentration (1 mL in 100 mL).

* Please refer to the "Starter Guide to Hybridoma" for specific protocols on plating and mixing.

5. Incubate plates with solid cultures at 37 °C and 5% CO_2, leaving undisturbed for at least 4 days.
6. Verify the presence of growing colonies at a suitable time point using a light microscope, usually ~10 days. Determine the approximate number of colonies/well. Ideally plates should obtain ~100 colonies/well.
7. If growth was not obtained, it may be necessary to add supplementation. For a stable line, we recommend initially trying:
   - FBS/FCS. Increase serum up to 20-30% final concentration. Our CloneMedia Hybridoma contains 20%.

Establish hybridomas screening using fresh fusion

Purpose: To optimize conditions in semi solid media using a fresh fusion. When plating fresh hybridoma fusions the optimal seeding density is highly dependent on the fusion efficiency as well as the kinetics and efficiency of selection. Cells may require a period of recovery prior to seeding in semi solid media. For the successful selection of hybrids, the selection pressure applied (*i.e.* concentration of HAT) may also require optimization.

1. Let the fusion rested for 24-48 hrs in liquid selection "HAT media" prior to plating in semi-solid media.
2. Using the same media optimized for the established hybridomas, plate to 100 mL final volume. Supplement with 1X HAT, preferred antibiotic, and L-Glutamine final concentration 8mM. (Recommend Invitrogen's GlutaMax, which is the more stable analogue.
3. On the day of plating, spin to concentrate cells to 2 mL. Obtain a count of total viable cells.
4. Plate cells at 4 different densities: 5000, 1X104, 1X105, 1X106 cells/mL.
5. Incubate plates for at least 7 days at 37°C and 5% CO_2, leaving undisturbed for at least 4 days.

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