

Picking of Hybridoma colonies from semi-solid agar using Pickolo™ on a Tecan EVO®

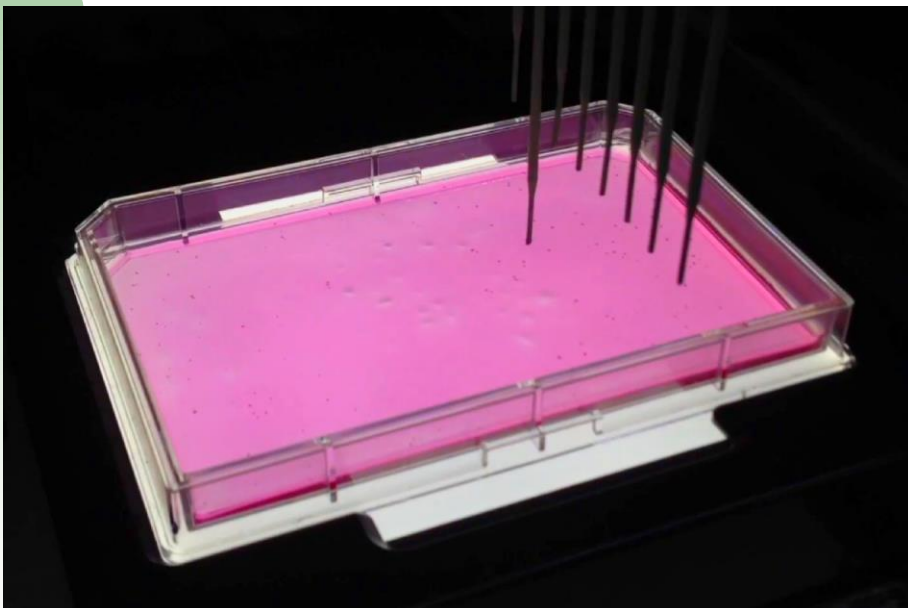
A large pharmaceutical company has successfully deployed Pickolo colony-picker for the picking of hybridoma mammalian cell colonies from semi-solid agar in a robust and efficient way

Background

Semi-solid agar is quickly becoming a popular method for cloning and isolation of monoclonal mammalian cell lines and for the production of Hybridomas, CHO cell lines and other cell lines. This technique lends itself readily for automation using commercial colony pickers but does present certain challenges compared with bacterial colony picking. Mammalian colonies grown in semi-solid agar are generally small and thus require good image analysis and high accuracy. Furthermore, the colonies are best aspirated gently and transferred to a liquid media. Therefore, the liquid-handling based picking solution such as the Pickolo on Tecan Evo is efficient, fast and robust solution for the Hybridoma picking task.

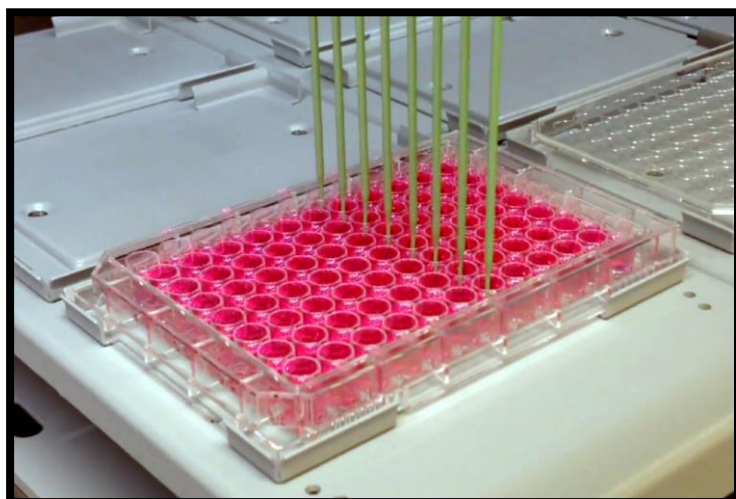
Assay requirements

A drug discovery lab in a large pharmaceutical company was looking to automate the task of picking thousands of hybridoma cell colonies from semi-solid agar into 96 well plates. Cell colonies are grown in SBS omni-plates for around 7 days where they generally sink to the bottom. Each omni-plate contains 100-300 colonies that are picked into 96 well SBS plates with fresh liquid media. The colonies should be aspirated using a small volume (5µl is commonly used) to minimize movement of nearby colonies and avoid pulling in free cells from the nearby agar. Two wells in each 96 well plate are left empty as negative controls. In a typical run around 10 source plates and 20 target plates are used and must be changed automatically to allow a long walk-away time (picking rate of ~2000 colonies within 3.5 hours). Colonies must be dispensed reliably and gently into the target well and are inspected by microscope to make sure they do not break down excessively from the treatment. They are also grown to verify viability.



Methods and results

Pickolo is a colony picking add on for Tecan platform. In Dec. 2015 it was installed in a drug discovery lab for the purpose of picking hybridoma colonies grown in semi-solid agar. EVO configuration chosen was EVO150 with a fixed tip LiHa and RoMa for changing plates. The picking protocol began with tip washing using Ethanol, bleach and water, then aspirating 40µl of fresh liquid media from the destination well, aspirating 5µl at the location of the colony slightly above the plate bottom and dispensing 40µl at the destination well. Source and destination plates were changed automatically from a plate carousel as needed.



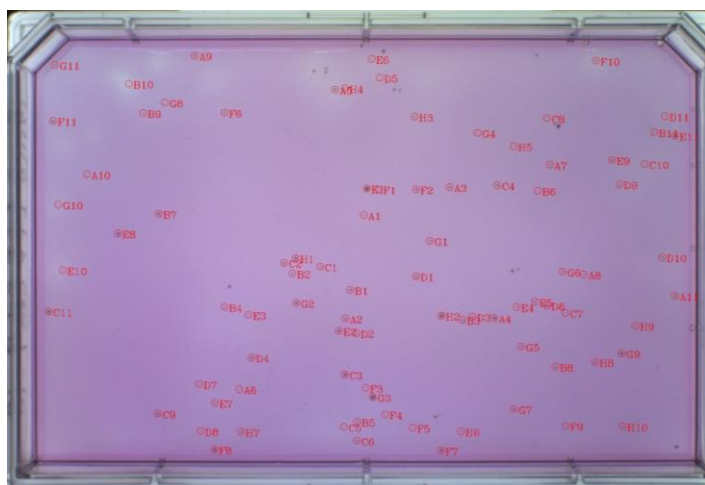
As a test run 1128 colonies were picked into 12 destination plates from 5 source plates. The test took 2 hours and 10 minutes averaging 54 seconds for a round of 8 colonies including plate changes and tip washing. The picking time could be further optimized to reduce washing time and thus shorten the overall test time.

Cell colonies were inspected in a microscope and were found intact in the destination wells. Destination plates were incubated and showed viability of picked colonies.

Summary

It was demonstrated that the Pickolo colony picker on a Tecan platform can be used to pick hybridoma cell colonies grown in semi-solid agar. Despite the small colony size and the requirement to aspirate only a small volume most colonies were successfully picked and transferred to the destination plate. Presumably this technique can be extended to other cell lines and help labs growing monoclonal mammalian cell lines to automate and streamline their processes everywhere.

In addition, several advantages of a combined liquid handling instrument and colony picker were demonstrated including high accuracy, high flexibility, good repeatability and ability to add further downstream treatment to colony picking workflows and other lab processes.



Documentation created by Pickolo during run. Each picked colony is marked on the image of the source plate with the well it was picked to

