ATCC

APPLICATION NOTE

A NOVEL NIH/3T3 CLONAL DERIVATIVE: NIH/3T3.2 RE-DEVELOPED AS A CELL MODEL FOR CONTACT INHIBITION RESEARCH

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ABSTRACT

NIH/3T3 (ATCC® CRL-1658™) is a spontaneously immortalized mouse fibroblast cell line utilized in cancer research and cell-based applications. The original NIH/3T3 cells were contact inhibited upon reaching confluency. Over time, the emergence of a sub-population of cells with the capability of cell transformation has enabled the NIH/3T3 cell line to lose contact inhibition, creating a multiclonal cell population. To restore the contact inhibition property, ATCC re-cloned our seed stock of NIH/3T3 (ATCC® CRL-1658™) and developed the novel clonal derivative cell line NIH/3T3.2 (ATCC® CRL-1658.2™).

INTRODUCTION

The NIH/3T3 cell line (ATCC® CRL-1658™) is one of the most commonly used cell lines in general cell biology and cancer research. It is a spontaneously immortalized non-cancerous fibroblast cell line that was developed in 1962 by George Todaro and Howard Green from an embryonic NIH/Swiss mouse.[1] NIH/3T3 was previously known for its strong contact inhibition and as such has been widely used in cell-based assays to determine the oncogenic potential of a gene.[2] However, over time, the emergence of a sub-clonal population of cells prone to cell transformation has created a multiclonal population, resulting in the loss of contact inhibition.[3,4] This loss of contact inhibition allows the fibroblast cells to proliferate continuously, forming growth foci that resemble tumors. In response, we aimed to generate a cell line with restored contact inhibition by using single-cell cloning to isolate and select for cell populations that do not produce growth foci.

RESULTS WITH MATERIAL AND METHODS

The NIH/3T3 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; ATCC® 30-2002™) supplemented with 10% Calf Bovine Serum (ATCC® 30-2031™) following published parameters and were monitored for sub-culturing below 75% confluency. The automated SH800 Cell Sorter (Sony®) was used to isolate individual cells into prepared 96-well plates. Each clone was grown for a 28-day period and was monitored daily and evaluated for growth foci formation capabilities. Differences in the presence of growth foci were observed between parental NIH/3T3 and clonal derivative cells following single-cell sorting. Clones were scored using a range from 1 to 3 with 1 as negative for growth foci and 3 representing high evidence of foci formation. Clones with negative growth foci (score of 1) were selected for further evaluation. The best selected clone was then developed as the new cell line NIH/3T3.2 (ATCC® CRL-1658.2™) (Figure 1).

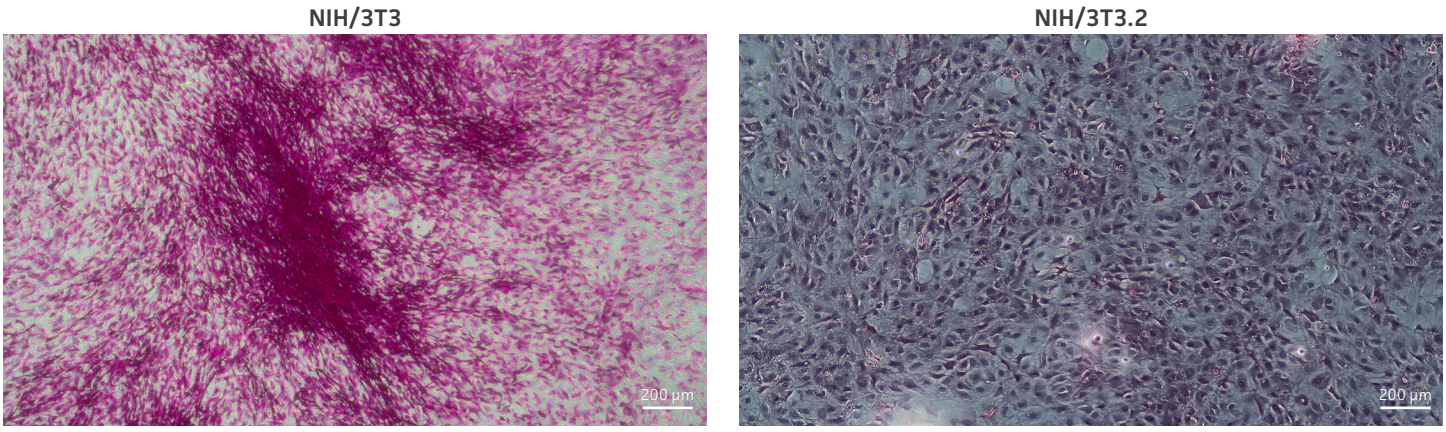

Figure 1: Evaluation of growth foci in parental and clonal derivative cells following carbol-fuchsin staining. The parental NIH/3T3 cell line demonstrated high evidence of foci formation. The NIH/3T3.2 clonal derivative presented here demonstrated negative growth foci.

The newly selected clonal derivative cell line was expanded into larger vessels and grown for several passages in calf serum–supplemented media with morphology and growth rate continuously monitored. The NIH/3T3.2 clonal derivative demonstrated morphology similar to that of the parental cell line with typical fibroblast-like adherent cells of spindle shape (Figure 2).

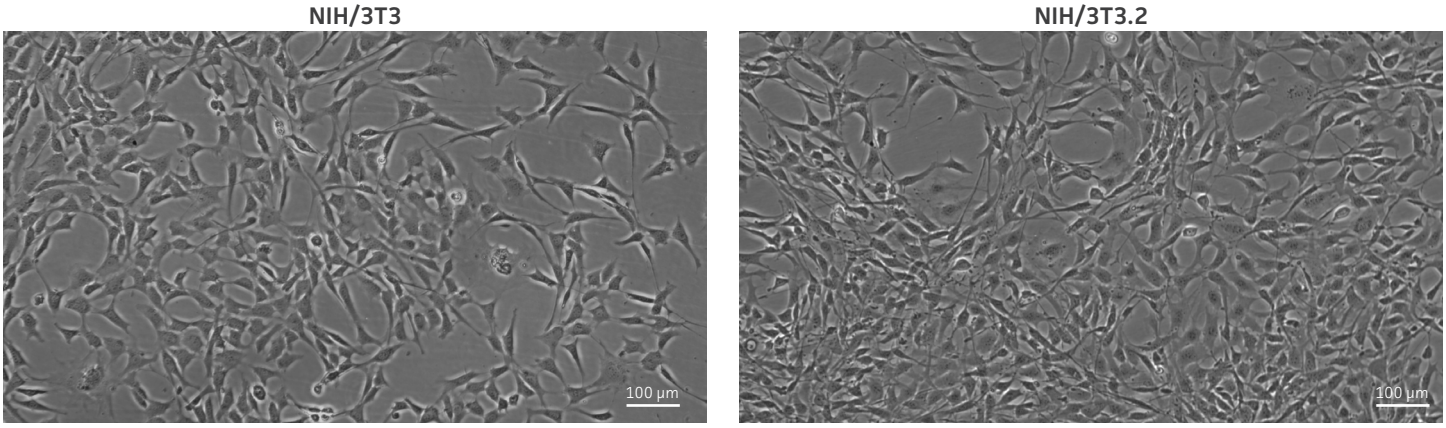

Figure 2: Morphology of the parental NIH/3T3 cells and NIH/3T3.2 clonal derivative cells at 70% confluency prior to sub-culture. Both cell lines showed fibroblast-like adherent cells of spindle or stellate shape.

The new clonal derivative was also assessed after cultivation in media supplemented with Fetal Bovine Serum (FBS; ATCC® 30-2021™). Since FBS is a more widely utilized serum for cell culture within the research community, this allowed for the new clone to be evaluated in a more widely used growth medium. Here, the BioSpa Cytation® 1 instrument (Agilent®) was used to evaluate growth rate (Figure 3) and morphology characteristics; the NIH/3T3.2 cell line showed minimal changes under the new conditions. Observance of growth foci was not seen in the selected clone at multiple passages or when grown in the new FBS culture conditions (Figure 4). These results show that the new clonal derivative does not have the capability to produce growth foci over multiple passages and is adapted for growth in FBS.

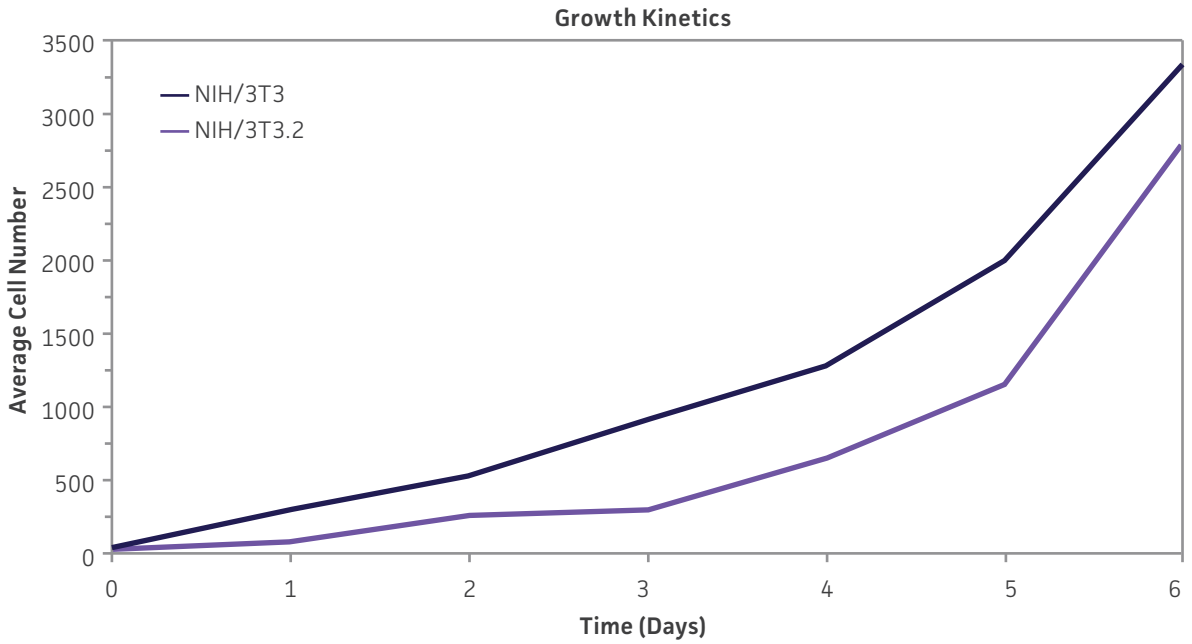

Figure 3: Growth kinetics following FBS adaption. Parental and clonal derivative cells demonstrated similar growth rates following FBS adaption.

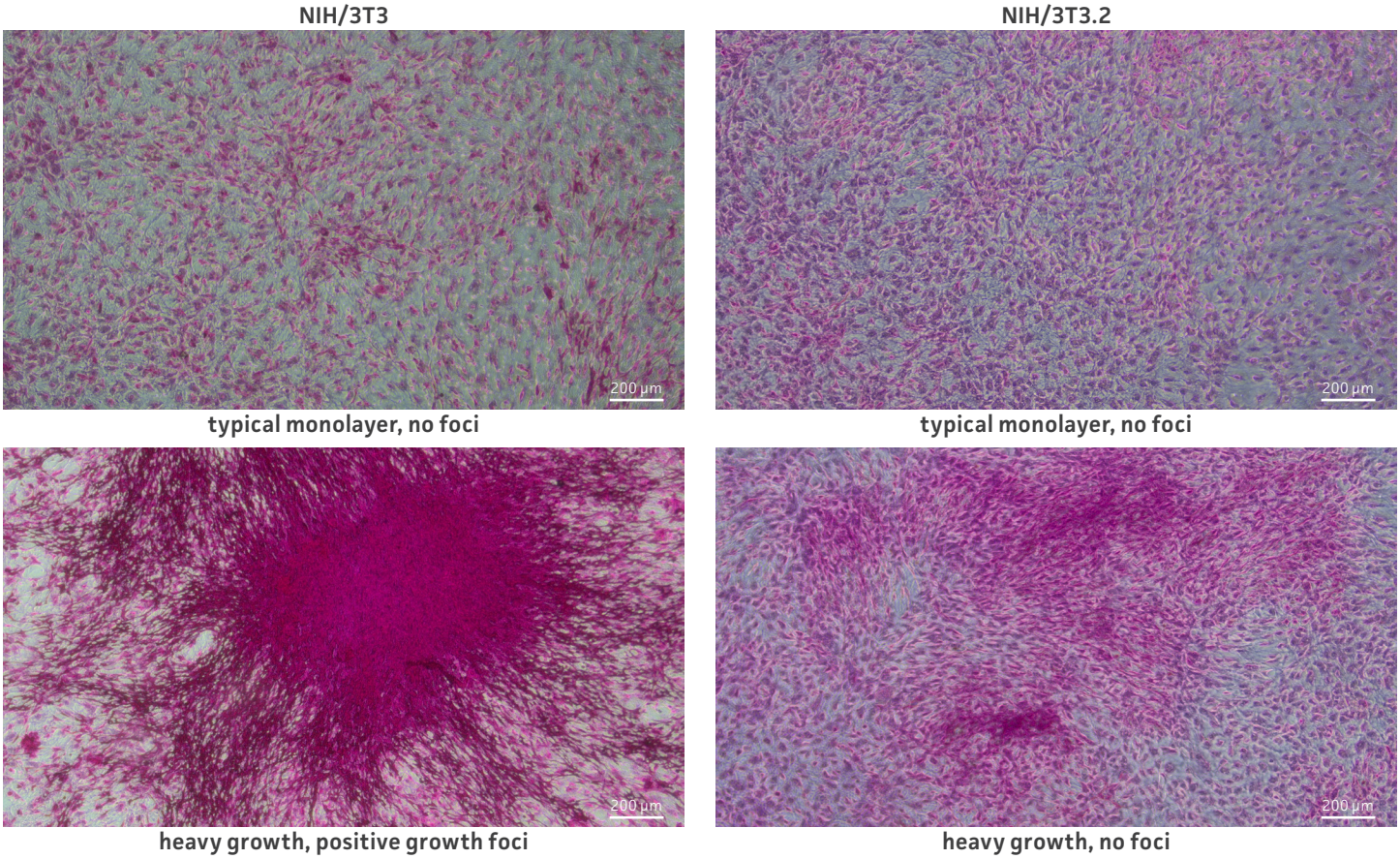

Figure 4: Contact inhibition testing comparison of the parental and clonal derivative. Typical monolayer growth areas can be seen in both cell lines (top). With heavier growth (bottom), the parental cell line demonstrates positive growth foci formation while the clonal derivative cell line demonstrates negative foci formation.

Fibroblasts contribute to the composition of connective tissue by forming stress fibers; this is particularly evident in cells expressing high levels of alpha-smooth muscle actin and F-actin.[5] To evaluate the expression of these proteins on the NIH/3T3.2 cell line, immunofluorescent staining was performed (Figure 5). The selected clone showed similar fluorescent staining expression of both alpha-smooth muscle actin and F-actin as the parental NIH/3T3 cell line.

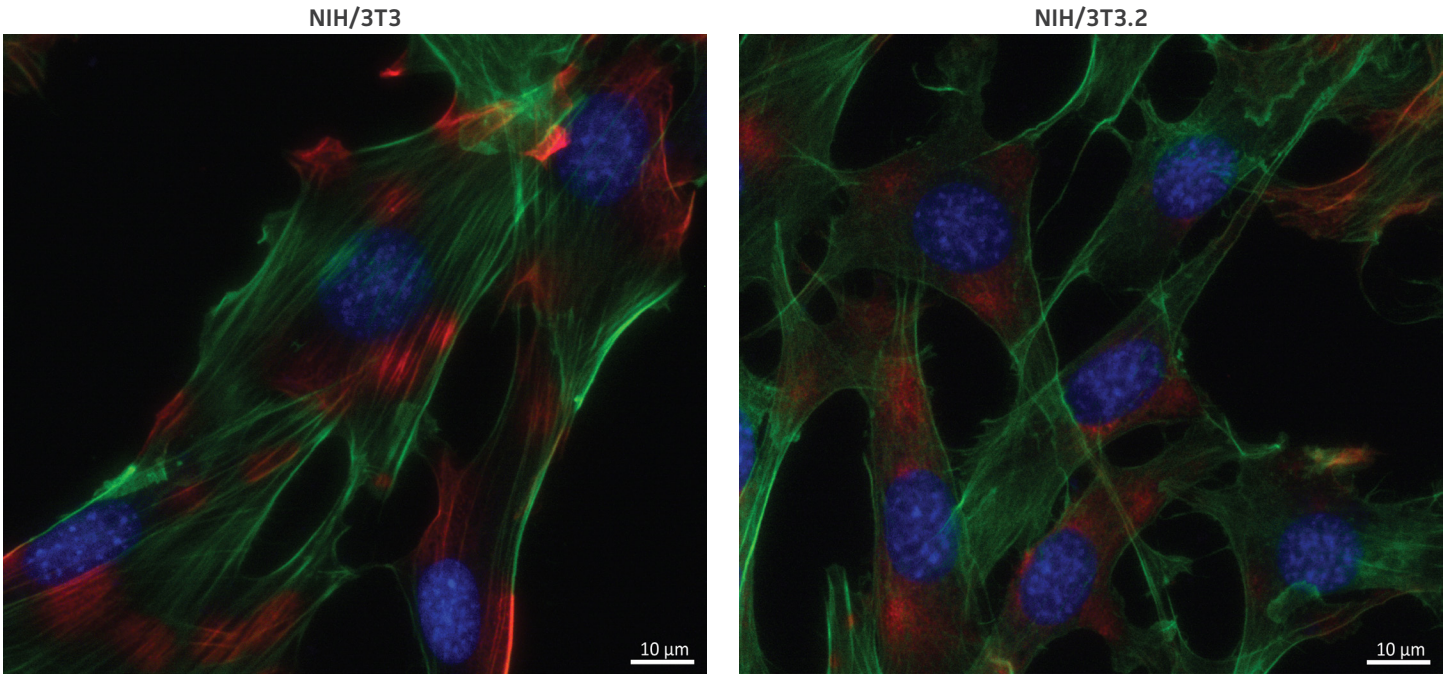

Figure 5: Immunofluorescent staining of alpha-smooth muscle actin and F-actin on parental and clonal derivative cells. The NIH/3T3.2 clonal derivative and NIH/3T3 parental cell lines demonstrated similar expression of alpha-smooth muscle actin (red) and F-actin (green).

CONCLUSION

NIH/3T3 is a standard fibroblast cell line utilized in many cell-based applications throughout general biology and cancer research. Though the NIH/3T3 cell line was originally known for its strong contact inhibition, which enabled its use in determining the oncogenic potential of a particular gene, the emergence of a multiclonal cell population has resulted in the loss of this property. Historical evidence of foci test failures and the inability to guarantee the contact inhibition trait of the NIH/3T3 cell line has led to an investigation into the possibility of ATCC's seed stock containing a sub-population of cells that have spontaneously transformed. This guided ATCC to develop a new clonal derivative with restored contact inhibition via single cell cloning of the NIH/3T3 parental cell line. Through this process, we were able to successfully generate a novel clonal derivative, NIH/3T3.2 (ATCC® CRL-1658.2™), that maintains the original contact inhibition characteristics associated with this cell line.

REFERENCES

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