

Reducing the timeline for isolating stable CHO DG44 clones producing high titers of monoclonal antibody

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Introduction

Isolating mammalian cell clones that stably produce high quantities of a desired protein of interest can be a time-consuming and costly process. A popular way to maximize expression is to use methotrexate (MTX), a high affinity folate antagonist, to amplify expression of a gene of interest in CHO DG44 cells. These cells, which are deficient in the dihydrofolate reductase gene (*dhfr*), are transfected with the gene of interest plus a rescue copy of *dhfr*. Positive transfectants are then selected in medium lacking hypoxanthine and thymidine (HT) which are essential for nucleotide synthesis via the alternative (salvage) pathway. MTX supplementation selects for amplification of the *dhfr* locus and the gene of interest, and thus significantly enhances the average expression level of the cell population. However, MTX escalation must be done slowly and commonly takes 6 months. Here we present a method for reducing MTX amplification timeline by early selection of cell candidates with innate tolerance of high MTX concentration.

Method

ClonePix FL™, an established technology for rapidly finding and isolating rare clones from large heterogeneous cell populations, was used to isolate high monoclonal antibody (mAb) secreting DG44 clones within 2 weeks of transfection. In the following 2 weeks, the selected high secretors were assessed for stability by using ClonePix FL to screen for homogeneity of protein secretion of daughter clones. These stable high producing isolates were more resistant to MTX and so could be exposed to a higher initial dose than would normally be considered.

Materials

Semi-solid media: CloneMedia-CHO (K8710)

Expansion media: XP Media-CHO (K8750)

Methylcellulose: CloneMatrix CHO (K8530)

Detection: CloneDetect anti-human IgG (K8200)

Media products are chemically defined but can be supplemented with serum if required.

Conclusions

Stable high producing clones isolated by ClonePix FL show higher resistance to MTX. Our hypothesis is that these cells already have a high copy number of *dhfr* and the gene of interest and/or may be more responsive to amplification. This substantially reduces the timeline for generating stable gene amplified clones.

CloneMedia-CHO supports DHFR/MTX selection

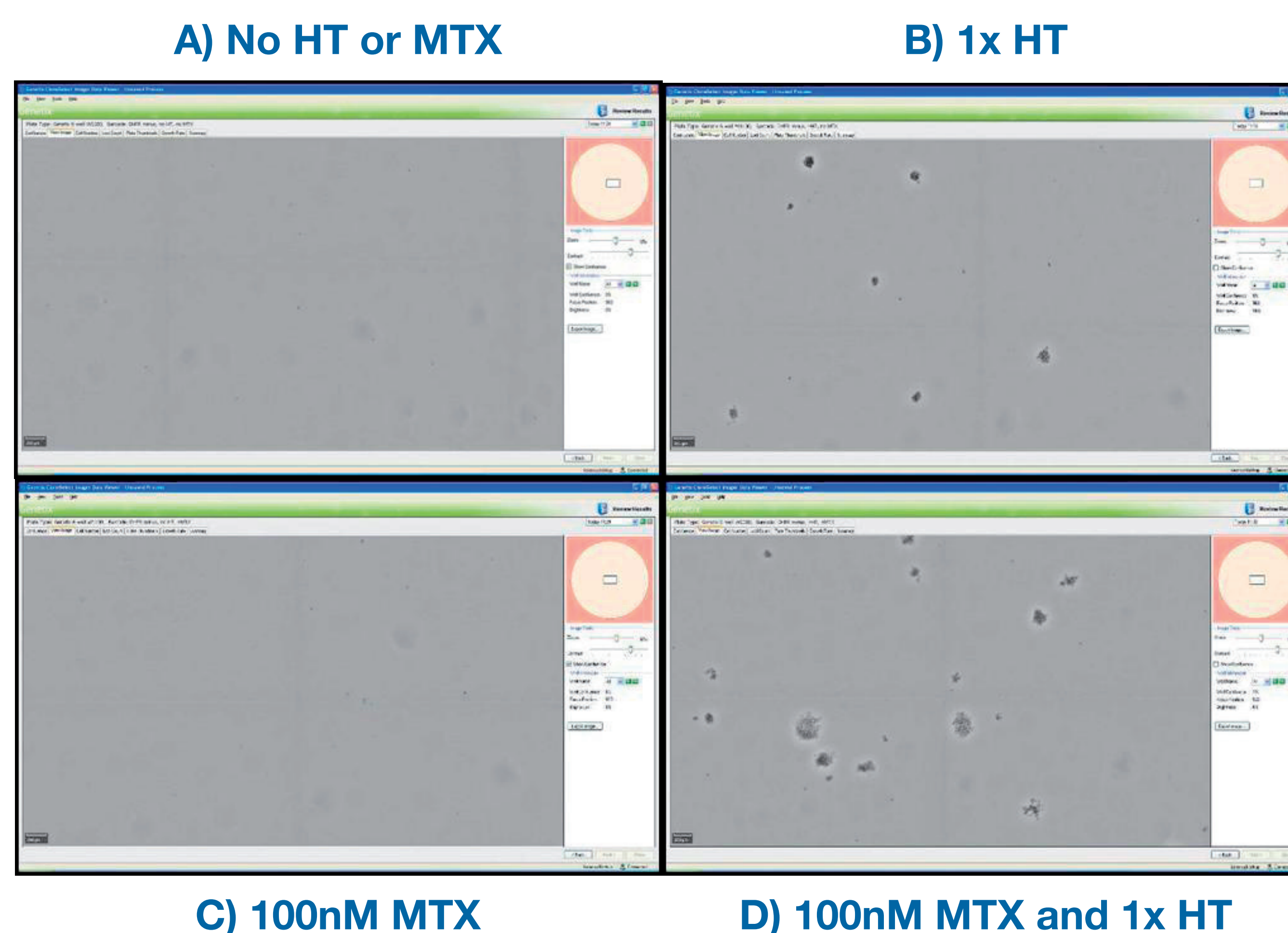


Figure 1: Images of DG44 colony growth in CloneMedia-CHO at day 6 with or without HT and MTX. Colony growth was evident only in the presence of 1x HT irrespective of the absence or presence of MTX. **A)** No colony formation. **B)** Compact colonies of about 32 cells are clearly visible and cells are showing expected level of growth. **C)** No colony formation. **D)** Cells have undergone at least 5 cell divisions.

High-expressers isolated rapidly post-transfection bypass the need for a step-wise increase in MTX concentration

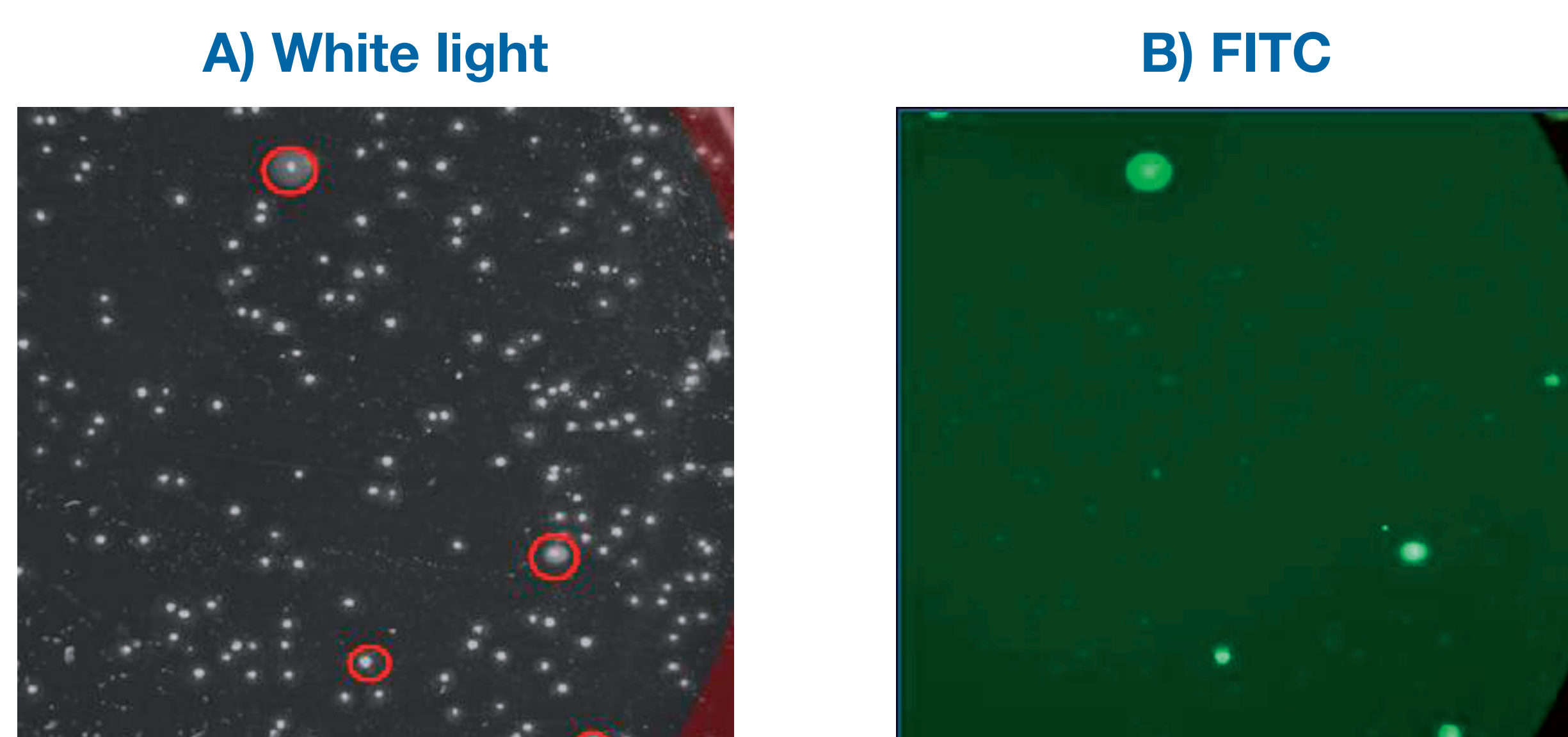


Figure 2: Mixed population of CHO DG44 in HT-selection: DG44 cells were transfected to express human IgG and then selected in HT-media prior to plating in CloneMedia-CHO in the presence of CloneDetect FITC anti-human IgG. High expressing MTX-resistant clones are circled in red on image A.



Figure 3: Rank Plot from showing the order of detected colonies from high to low fluorescence.

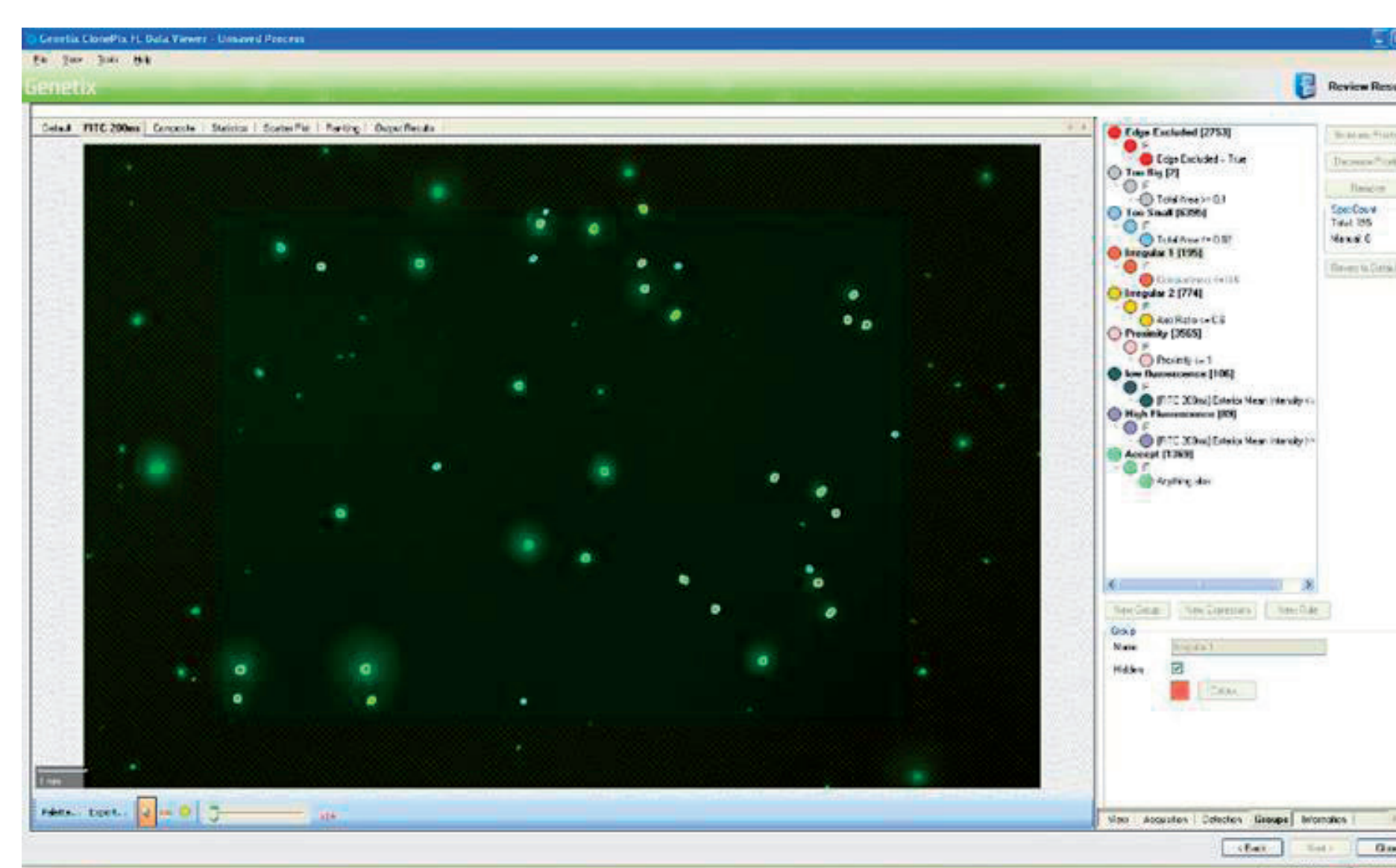


Figure 4: FITC image of the cell line shown in Fig 3 showing detected colonies.

Recommended Workflow

