Subcloning of DT40 clones by limited dilutions

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1) Count the viable cells using Trypan blue.

2) Prepare three tubes containing 10 ml chicken medium each and add 300, 150 and 75 cells, respectively.

3) For each of the three dilutions add 100 microliter of the cell suspension to each well of a 96 well flat bottom microtiter plate.

4) Incubate the plates for 8 days without changing the medium. Subclones should be visible by then as round colonies.