

# This Week's Citation Classic

**Puck T T, Marcus P I & Cieciura S J.** Clonal growth of mammalian cells in vitro: growth characteristics of colonies from single HeLa cells with and without a "feeder" layer. *J. Exp. Med.* **103**:273-83, 1956.  
[Dept. Biophysics, Florence R. Sabin Labs., Univ. Colorado Med. Ctr., Denver, CO]

**A simple method is described for quantitative growth of single mammalian cells in tissue culture so that each cell forms a discrete colony. A feeder layer of X-irradiated cells can stimulate growth in suboptimal nutrient medium. The procedure permits mutant selection, and other genetic studies on somatic cells. [The SC<sup>1</sup> indicates that this paper has been cited in over 660 publications since 1961.]**

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"This paper describes experiments designed to furnish a new approach to the study of human genetics which would be free of the complications of human mating. Human genetics had been a weak science before 1956 because of the long human generation time and the impossibility of carrying out particular matings which would answer specific questions in human genetics. It occurred to me that a genetics based on somatic cells instead of germ cells, utilizing tissue culture techniques and applying the concepts of microbial genetics, would solve these problems. It became necessary to develop techniques for: taking biopsies of somatic cells from any person; producing reliable growth of such cells into large, genetically stable populations; and a rapid, quantitative, routine method for growing of single cells into discrete, macroscopic colonies. Such an approach would bypass the need for human mating and permit genetic experiments on somatic cells *in vitro* that should have all the simplicity and power of microbial genetics.

"I was influenced by the experiments of K.K. Sanford<sup>1</sup> and her colleagues, who had cloned occasional single mammalian cells though not in quantitative fashion, by Earle's concept of the need for cells to condition the primitive growth media available in those days, and by the simplici-

ty of the culture techniques of Enders and his co-workers.

"I began experiments with Roshan Christensen, a postdoctoral fellow. We achieved reliable growth only with inocula of 500 cells or more. Philip Marcus then joined me as a graduate student. We considered ways of feeding single cells with nutrients from a mass culture. Leo Szilard, who was visiting the laboratory at this time, suggested suspending the single cells above a massive culture in the same dish. However, the problem was not solved until Marcus and I conceived of an X-irradiated feeder layer.<sup>2</sup> At first we separated the single cells from the feeder layer by a glass plate in the same dish, but then incorporated both cell types in a single layer. A simple quantitative operation suitable for genetic experiments resulted. Subsequently, we eliminated the feeder layer by improving the nutrient medium. However, the X-irradiated feeder layer has since found application in the induction of continuing growth of differentiated cells.

"Simple routine methods for initiating long-term cultures with stable karyotypes from any person were developed and single-cell survival curves were introduced as a means for study of the effects of physical, chemical, and biological agents on the reproduction of single cells.<sup>3</sup> Survival curve analysis has had many applications including initiation of quantitative mammalian cell radiobiology which contributed to a more effective radiotherapy and demonstrated the role of cell turnover in the mammalian radiation syndrome.<sup>4</sup> The human chromosomes were characterized and, with the aid of a study group which we organized in Denver, the classification system for the human chromosomes still used today was devised.<sup>5</sup> Subsequent developments in somatic cell genetics included preparation and characterization of single gene mutants,<sup>4</sup> cell hybridization,<sup>6</sup> development of recombinant DNA approaches, production of monoclonal antibodies, and mapping of the human genes.<sup>7</sup> Somatic cell genetics is now making important contributions to medicine and to the understanding of differentiation and development.

"The work in this paper initiated the discipline of somatic cell genetics which has become the principal mode of study of human genetics and its applications to medicine and human molecular biology."

1. **Sanford K K, Earle W R & Likely G E.** The growth in vitro of single isolated tissue cells. *J. Nat. Cancer Inst.* **9**:229-46. 1948.  
[Citation Classic. *Current Contents/Life Sciences* **24**(29): 19. 20 July 1981.]
2. **Puck T T & Marcus P I.** Rapid method for viable cell titration and clone production with HeLa cells on tissue culture: the use of X-irradiated cells to supply conditioning factors. *Proc. Nat. Acad. Sci. US* **4**:432-7. 1955.
3. .... Action of X-rays on mammalian cells. *J. Exp. Med.* **103**:653-66, 1956.
4. **Puck T T.** *The mammalian cell as a microorganism: genetic and biochemical studies in vitro.* San Francisco: Holden-Day. 1972. 219 p.
- 5 **Book J A, Lejeune J, Levan A P, Chu E H Y, Ford C E, Fracarro M, Harnden D G, Esu T C, Huogerford D A, Jacobs P A, Makino S, Puck T T, Robinson A, Tjio J H, Calchside D G, Muller H J & Stern C.** A proposed standard system of nomenclature of human mitotic chromosomes. *J. Amer. Med. Assn.* **174**:159-62. 1960.
6. **Harris H.** *Cell fusion.* Cambridge, MA: Harvard University Press. 1970. 108 p.
7. **Gusella J F, Jones C, Kao F T, Housman D & Puck T T.** Genetic fine structure mapping in human chromosome 11 by use of repetitive DNA sequences. *Proc. Nat. Acad. Sci. US* **79**:7804-8, 1982.
8. **Puck T T & Kao F T.** Somatic cell genetics and its application to medicine. *Annu. Rev. Genet.* **16**:225-71. 1982.