5650 Ellis Avenue

May 28, 1952

Dr. Evelyn Witkin
Carnegie Institution
Cold Spring Harbor
Long Island, New York

Dear Witkin:

Once more we have to impose upon your generosity. Some time ago, starting from a lac-positive B/r, you prepared with U.V. lac-negative mutants. Twenty-five percent of these mutants spontaneously reverted to lac-positive, and you mentioned that you had spontaneous reversion rates between 10^{-10} and 10^{-7}.

We would like to study this spontaneous reversion in the chemostat and wondered whether you could send us some such lac-negative strains, preferably one which has a high reversion rate to lac-positive.

Am I correct in assuming that the lac-negative strains cannot grow on plates which have lactose as the only carbon source so that we can score the reverted specimen simply by plating on such plates?

If you saved your strains, would you be good enough to send us a few slants of the ones you judge most suitable? If you didn't save them, would you drop us a line giving us such advice and consolation as appears appropriate in the circumstances?

With kind regards,

Sincerely yours,

Leo Szilard

LS/sds

September 17, 1949

Mrs. E. M. Witkin
Carnegie Institute of Genetics
Cold Spring Harbor
Long Island, New York

Dear Witkin:

I wonder whether you can help me with the following: You sent us some time ago a slant of B/r which was marked B/r b. It is this B/r which we used in our experiments. Our survivor curves for ultra-violet are very different from those obtained with B/r by Demerec and Kelner, and I wondered whether the strain which you sent us is different from the strain which they used. Please let me know whatever information you have on this subject.

With kind regards.

Sincerely,

Leo Szilard

THE UNIVERSITY OF CHICAGO
Comptroller Form 47R
6-49-25M

PETTY CASH RECEIPT

No.

Date June 6, 1950

Pay to Western Union $

For Evelyn Witkin, Carnegie Inst. for Genetics, Cold Springs Harbor, New York

Charge Account Social Aspects of Atomic Energy E & E 4-5607-30-1000

Account Code				Document Number	Type of Charge	Amount	
L	Account	Sub	Fund				
4	5607	30	1000		27		
					27		
					27		

Signed Leo Szilard

Approved

Authorized Signature

Received above amount:

Signed

DOMESTIC SERVICE
Check the class of service desired; otherwise this message will be sent as a full rate telegram

FULL RATE TELEGRAM		SERIAL	
DAY LETTER	X	NIGHT LETTER	

WESTERN UNION

1206

W. P. MARSHALL, PRESIDENT

INTERNATIONAL SERVICE
Check the class of service desired; otherwise this message will be sent at the full rate

FULL RATE		DEFERRED	
CODE		NIGHT LETTER	

NO. WDS.-CL. OF SVC.	PD. OR COLL.	CASH NO.	CHARGE TO THE ACCOUNT OF	TIME FILED

Send the following message, subject to the terms on back hereof, which are hereby agreed to

June 6, 1950

Miss Evelyn Witkin
Carnegie Institute for Genetics
Cold Springs Harbor, New York

Would much appreciate your sending slants of your argininelessness, phenylalanineless, histidineless, and both methionineless strains. We shall test all of them for reversions before choosing one for use in chemostat. Regards--Leo Szilard, 6200 Drexel Avenue, Chicago.

CARNEGIE INSTITUTION OF WASHINGTON
DEPARTMENT OF GENETICS
COLD SPRING HARBOR, LONG ISLAND, N. Y.

June 8, 1952

Dr. Leo Szilard
Institute of Radiobiology
and Biophysics
5650 Ellis Avenue
Chicago, Ill.

Dear Dr. Szilard:

Sorry to be so long in answering your query about lac-negative stocks. I started a year's leave of absence a couple of weeks ago, and haven't had much opportunity to take care of things like this -- our second child is due next month, and I am going to be a plain mama for a year.

I have indeed saved my stocks, in hopes that they could be useful to someone. I have checked through my records, and selected a number of stocks that should suit your purposes, with one major qualification. Since I am not around the lab at all these days, I will not have a chance to test the stocks before having them sent to you, and therefore cannot guarantee that they are still what they used to be. These stocks have not been used for about a year, and have undergone several serial transfers since last tested. I send them without any real assurance that they are still good, and suggest that you test them before doing anything else with them. I have dozens of stocks, and if none of these is suitable, I will be glad to send others.

The stocks I am sending are all UV-induced lac$^-$ mutants, some of B and others of B/r, all of which had good clearcut reversion to lac+ when last tested by us. You can score reversions on synthetic medium with lactose as the sole carbon source, but you must be very wary of the dilution effect, as with auxotrophs. Our experience was that most of these strains arrive at the same final number of cells per plate regardless of inoculum plated, and the number of mutant colonies must be related to the final number of background cells, rather than to the number plated. If you take a culture of lac- cells, for example, and plate on lactose M9, using a series of dilutions so as to plate 10^8, 10^7, 10^6, 10^5, 10^4 and 10^3 cells, then wash the plates after a long incubation to determine the final growth (using plates having no mutant colonies), you find that all plates have close to 10^9 cells, and that the number of divisions the inoculum passes through is increased by about 3 divisions for each dilution step of 10. This happens commonly with many auxotrophs, and can be brought under control, as it is a very regular and predictable affair. In the case of lac$^-$ mutants, inoculum size plotted against residual divisions gives a perfect straight line. I mention all this because no quantitative work with reversions is possible without controlling residual growth of the parent strain.

May I suggest that in testing the stocks you plate them on EMB to make sure you get white colonies (many of which should develop dark red papillae after a week or so of incubation), and also plate 10^8 or more cells on a lactose synthetic medium (M9 or A, or any of the simple media used routinely should do -- add the lactose after autoclaving). You should get discrete colonies on these plates after 3 days -- 4 days is even better for accurate counts. The reversions should streak red on EMB.

I have been doing some work this year on temperature effects on mutations -- spontaneous and induced -- and have often wished that I might have a chance to see you to discuss my results. We find spontaneous mutations expressed as fluctuation of probability as If you should visit CSH any time in the near future, I hope you will let me know, as I believe you would be interested in what we have turned up in this work. I am at present writing a short paper, and will send you a copy of the manuscript when it is completed.

Please let me know if there is anything more I can do to be of help in connection with lac- mutants. I will have the stocks sent out as promptly as possible.

With kindest regards,

Sincerely,

Evelyn Witkin

Evelyn M. Witkin

5650 Ellis Avenue

June 12, 1952

Dr. Evelyn M. Witkin
Department of Genetics
Carnegie Institution of Washington
Cold Spring Harbor, L.I., New York

Dear Witkin:

Thanks for your letter of June 8th. I wonder whether your experience that the plates have invariably 10^9 cells irrespectively of the inoculum also holds if washed ager is used. I could understand it even with washed ager for lac-negative mutants because some of the ager might get decomposed in autoclaving, but I find it difficult to explain the phenomenon for auxotrophes requiring various amino acids. Or did you not use washed ager?

Please take your time answering this letter at your convenience. I, too, would like to be a plain mama for a year. How do I go about it?

With kindest regards,

Sincerely,

Leo Szilard

LS/sds

P.S. I shall try to contact you when I am in New York sometime between the 21st and 26th of June.