Seminar Minutes of 10-14-74

I. J.L.: "Discussion on computer"

This program uses the TBASIC DEBUGGER as a way to enter commands. One or more commands which may include most statements in the basic language may be entered. The program has already defined the variables U,V,W,X,Y,Z and will print their current values with the command. In addition the array A(100) is also defined. The most useful command is probably

PRINT <EXPRESSION> .

II. Ron: "Gene separation of RI-treated DNA by electrophoresis"

1. % survival of markers tested:

tryp-tyro	5%
his	10%
uracil	7%
methionine	100%
adenine	8%

2. Attempt was made to map these markers on gel. Tryp-tyro, his, and ura have been tested. There is a larger separation (~12 mm) between tryp-tyro and his; a smaller separation (~5mm) between his and ura.

3. Slicing technique has been improved by using a plastic "trough" which has grooves about 1 mm apart from each other. So one can easily cut the gel into 1 mm fragments accurately.

4. By rebanding technique, for tryp-tyro, more spreading is not obtained; activity still remains in 2-3 mm as before.But the specific activity (= (biological activity)/(physical activity)= (# transformants/ml)/(counts/min.)) was increased as follows;

tryp-tyro	.66 ⟶ .90
his	48 ⟶ 100*

* plates were over-grown and therefore result is not quantitative.

5. Recovery of material in using the rebanding technique is good.

6. To test the purity of the uracil and his markers, it is useful to employ a His^-Ura^+ double mutant as recipient. By comparing the His^-Ura^-, and the His^-Ura^+ transformants, one can know the relative % of his and ura obtained in the separation. But no Ura^-His^- mutant is available.

7. Suggestion:

His$^-$-Ura$^-$ mutants can be made by doing transformation in the reverse sense, i.e., looking for auxotrophs. Extract the DNA from His$^-$ cells, RI-treat, gel electrophoresis separate and cut out the his$^-$ fragment. Use the His$^+$-Ura$^-$ as recipient and looking for His$^-$-Ura$^-$ transformants.

III. Dusko: "Ectopic insertion"

1. Sheared DNA was used to compare linkage with that obtained from RI-treated DNA.

	tryp	his	tyro	primary selection
Nester's data				
([redacted]-DNA)	32%	8%	60%	tryp
Dusko's data				
(RI-DNA)	52%	8%	40%	tryp
	61%	10%	29%	aro2-tryp
(sheared DNA)	138 (77%)	18 (10%)	24 (13%)	aro2-tryp

In RI-treated DNA, when the recipients are transformed to His$^+$, the probability of also getting the tyro$^+$ is high. This may be due to integration of end piece. In the sheared DNA, the his-tyro linkage begins to disappear, because the cuttings by shearing force are at random sites, and so the end effect is not evident.

2. Attempts to link the tryp and his pieces to plasmids.

(A) To circularize pSC 101 with RI-digested DNA for transformation of E. Coli. The his piece can be transformed as well as the tryp, but the revertant frequency is high for his gene. Therefore,there is a need for a r$^-$ and His$^-$(deletion) mutant, which is not available at present.

(B) Ligation between pSC 101 and RI-DNA has been tested and did work nicely. The majority of the DNA formed circles.

	SC	SL	BC	BL	EC_{BC-}
tryp	96 (64%)	34 (22%)	6 (4%)	15 (10%)	
tryp	123 (65%)	24 (13%)	15 (8%)	26 (14%)	~ 34%
his	136 (65%)	33 (16%)	8 (3%)	33 (16%)	~ 20%

EC_{BC-} (efficiency of circularization of big circles)=BC/(BC+BL)

(i) Circularization stabilizes SC more than it does for BC.
$(T_m)_{SC}$ is greater than $(T_m)_{BC}$. ⟶ %SC is greater than %BC.

(ii) BL, compared to SL, are more difficult to move, so that the chances of the two ends meeting and circularizing are less for BL.
⟶ %SC is greater than %BC.

(iii) BL and BC are subject to more shearing stress, and thereby have the tendency to lose their sticky ends during cuttings.
⟶ %SL is increased.

(iv) Frequently, SL also tend to lose their sticky ends.
If SL with 1 sticky end left ⟶ %BL is increased.
If SL with 0 sticky ends left ⟶ %SL is increased.

(v) Efficiency of circularization of BC for the his gene (~20%) is less than that for the tryp gene (~34%). But this may not be real, more data is needed to establish the fact.

(iv) %SC (an intramolecular event) is much greater than %BC (an intermolecular event). It may be necessary to increase the DNA concentration to increase the absolute number of BC.

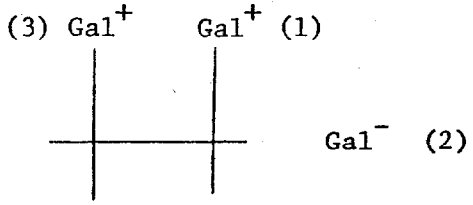
3. Tetracyclin marker on pSC 101.
Tc^r on pSC 101 may be used to test the competency of the recipient cells (in this case, E.coli). But it is not a good marker for differentiating between the pSC 101 plasmid circles and the circles made up of pSC 101 combined with RI-DNA fragments. One can use electrophoresis to eliminate the small circles that contain the pSC 101 only. Then select for Tc-resistant cells. But sometimes Tc-resistance is lost when a large piece of RI-DNA is integrated with the pSC 101. So other markers on RL-DNA should be used to select for co-transfer, e.g., tryp gene, but whether the tryp gene is working after being transformed to E.coli is not known yet.

4. Methionine (with 100% survival after RI-treatment) may be a good marker for investigating ectopic insertion. Use pooled DNA and looking for co-transfer, new linkages may thereby be established.

IV. Hela:
1. "Possible inhibition of Gal^+ by Gal^- cells"
B_6S. (SB 19,Gal^-) use galatose very slowly. When mixed in large amount ($> 10^6$) with Gal^+ (10^2) on min. plates having galactose, no growth of either type. This indicates that Gal^- may be inhibiting the growth of the Gal^+ cells.

Cross-streaked plates were made as suggested last week, inhibition was not evident. This may have been due to;

(i) the mechanical effect of the loop, it was suggested that the plates be streaked in the following order:



(ii) not enough Gal^- cells were present to make the inhibition evident.

2. "Attempt to establish recombination between B.S."

(i) Crossing was attempted between
B. S. (SB 863, $Lys^+ Aro2^- Tryp^- Tyro^- Str^r$) and
B. S. (SD1,2,8,$Lys^- Aro2^+ Tryp^+ Tyro^+ Str^s$).
Recombinants of the first 4 markers were selected.

(ii) After 48 hrs. of incubation, there were no recombinants. Plates were kept in incubator for further investigation.

(iii) Once recombinants are found, the str-marker will be used to distinguish the donor from the recipient.

3. "Set an upperlimit to the frequency with which lysogenized cells can be found after transfection"

(i) Transfection by P22 DNA of competent Salmonella rough cells was performed.

(ii) CElls were incubated for an additional 8 hrs on plate with no indicator bact. to let lysogeny occur.

(iii) The cells were then washed well to remove excess phages.

(iv) Mitomycin C was used to induce the lytic phase, then indicator bact. were added to measure the degree of lysogeny.

(v) Efficiency of Mitomycin C should be tested by using a known lysogenic cells.

4. Attempt to get new B. S. (SB 19) mutant that will use lactose as the only C-source, so that to combine with the sugar-fermentation mutants of E.coli for further experiments.

(i) B. S. (SB 19) in broth with 5% lactose (minus citrate) ——————— little growth.

(ii) A loopful of this inoculated broth was streaked on each of the following type plates;

Plates with 1/10 amount of citrate in Spiz	+ no sugar ———	+
----------	+ glucose ———	++++
----------	+ .5%lactose———	++
----------	+ 1% lactose———	+++

(iv) Hold the colonies until a medium without citrate is found for further investigation.

G.C.