Q3
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INSTRUCTIONS FOR CROSSING BIOCHEMICAL MUTANTS OF ESCHERICHIA COLI K-12

Cultures W-6 and W-1177 will illustrate the procedure. W-6 is auxotrophic for methionine. W-1177 was derived in several steps, also from K-12, and carries the following "markers": T- L- B_1- (threonine-; leucine-; thiamin-requiring); Lac- Mal- Mtl- Xyl- Ara- (non-fermenter of lactose; maltose; mannitol; xylose; l-arabinose;--V_1^r;S^r (resistant to phage T1 & T5; resistant to streptomycin). Summarizing the markers, the cross can be symbolized as:

	M	T	L	B_1	Lac	Mal	Mtl	Xyl	Ara	V_1	S	
W-6	-	+	+	+	+	+	+	+	+	s	s	(F+)
												X
W-1177	+	-	-	-	-	-	-	-	-	r	r	(F-)

The first 4 (nutritional) markers are used to select for prototroph recombinants (M+ T+ L+ B_1+) by plating a mixture in minimal agar. The remaining 7 markers are free to recombine in accordance with the rules of segregation; if enough colonies are examined all of the 2^7 possible combinations (128) will be found.

Sexual compatibility(5). Until fairly recently, all strains of E. coli K-12 were believed to be mutually compatible. It has now been established that two "mating types" exist, designated F+ and F-, so that F- x F- is sterile, while F+ x F- and F+ x F+ are fertile. The wild type K-12 strain is F+, as is W-6, while W-1177 is F-. These traits are clonally stable, but mixed cultures of F- with F+ strain rapidly result in the conversion of the former to the F+ state.

These compatibility effects can be demonstrated with additional strains that will be furnished on request. W-2163 is the F- equivalent of W-6; W-1817 is the F+ equivalent of W-1177. The compatibility can also be demonstrated directly with the W-6 x W-1177 cross, by taking advantage of the fact that F+ cultures temporarily acquire F- behavior if they have been grown under conditions of vigorous aeration, (A). Thus W-6 (A) x W-1177 will be infertile, while W-6 x W-1177 (A) will be fertile.

PROCEDURE. Cultures are carried on plain nutrient agar slants, transferred often enough to maintain viability (3-4 month intervals). Cultures are grown overnight at 37° in any rich broth, preferably buffered. (For example, Difco Penassay Broth). The cultures should not be aerated or agitated, and should be harvested at the end of the phase of logarithmic growth or within a few hours. The cultures are washed in the usual way by sedimenting the cells in the centrifuge, and resuspending in sterile saline. Two sedimentations will usually suffice. The last pellet should be resuspended in a smaller volume to achieve a four- or five-fold concentration.

After washing, the suspensions of the two cultures are combined, and samples (.05 - .1 ml) of the mixture are spread on the surface of minimal agar plates (or poured in deep agar). The plates are then incubated at 37 C.

Prototroph colonies should appear in 24 to 48 hours, against a faint background of residual parental growth. Although some further tests can be carried out directly with these prototrophs, for careful work, it is essential to purify individual prototroph colonies by conventional streaking methods--the author finds EMB-lactose, etc., the most informative. The fermentation markers are best scored on EMB media containing the various carbohydrates. V_1 is scored by streaking the colonies suspended in 1 ml water across a streak of phage T1 or T5 on agar. S is scored similarly by a cross-streaking with a line-streak of a loopful of streptomycin solution, 1 mg/ml. Obviously, one can score simultaneously for S or V_1 and fermentation markers by using EMB or EMS agar.

MEDIA (See also Reference 2)

Minimal Agar (after Davis)
per liter

Glucose	1 gm.
K_2HPO_4	7
$K\ H_2PO_4$	2
Na_3 citrate $\cdot 5H_2O$	0.5
$MgSO_4 \cdot 7H_2O$	0.1
$(NH_4)_2SO_4$	1
Agar	15

(Glucose and agar should be autoclaved separately from salts)

Synthetic EMB (EMS)
per liter

Sodium succinate	5 gms.
Na Cl	1
Ammon Sulfate	5
$MgSO_4$	1
K_2HPO_4	2
Sugar	10 - 15
Agar	15
Eosin Y	0.4
Methylene Blue	0.065

REFERENCES

1. Papers in Microbial Genetics. 1951. (selected by J. Lederberg). University of Wisconsin Press. 2d printing, 1952. pp. 303.

2. J. L. 1950. Isolation and Characterization of Biochemical Mutants of Bacteria. Meth. in Med. Res. 3:5-22.

3. J. L. and Tatum, E. L. 1946. Gene Recombination in Escherichia coli. Nature 158:558.

4. J. L. and Tatum, E. L. 1954. Sex in bacteria: genetic studies, 1945-1952. A.A.A.S. Symposium, Sex in microorganisms, pp. 12-28. (held Dec., 1951). Also published 1953. Science 118:169-175.

5. J. L., L. L. Cavalli, and E. M. L. 1952. Sex compatibility in Escherichia coli. Genetics 37:720-730.

Joshua Lederberg
Professor of Genetics
University of Wisconsin
Madison, Wisconsin