Mating Experiments

(1) Hfr H × F⁻ h56 (P⁺ transfer in Broth)

(2) Hfr K10 × F⁻ h56 (P⁺ transfer in Mg medium).

(3) P⁻ × P⁻ crosses in Broth.

Hfr S26	×	h56	
Hfr h56	×	S26	— 1
G5	×	S26	— 1
G5	×	h56	
S45	×	S26	
S45	×	h56	

for crosses with accurate differential rate recombinants at 100 mins.

(4) Making Z_1^- × Z_2^- - with Hfr H and the preser F⁻ Z^-.

(5) Making - Hfr $P_1^- Z_1^-$ × F⁻ $P_2^- Z_2^-$ (Hfr K10).

" " (Hfr H).

(Make necessary recombinants).

(6) Kahn's experiment

Hfr P_1^- × F^- P_2^- TrDNA - at high temperature & low temperature.

(Make - F^- P^- TsDNA)

(7) - $P^+ \rightarrow P^-$ transfer experiment with a rec$^-$ F^- strain.

(8) $P^+ \rightarrow P^-$ transfer with an F' strain

Recombinants

Strains

Make

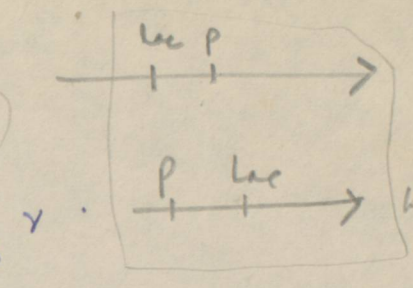
① Hfr H P^- Z^-

Hfr K10 P^- Z^-

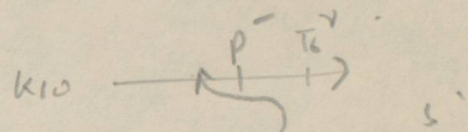
F^- lac 56 · Z^-

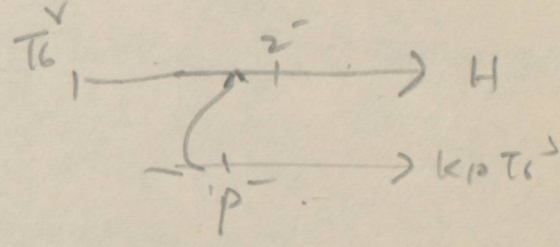
K10 P^- $T6^r$

Hfr Z^- $T6^r$



Simple





② TS DNA P^- strain

- Select → TL^+ from Hfr B TS DNA × F^- lac 56 TL^-
  " × F^- 526 TL^-
- Purify - 8 isolates from each cross - + Test on TS DNA on TG20 + S + B1.
- Streak TS DNA recombinants on TG + IP step B1 & spray with NPP.
- Cross white recombinant with K10 and plate 5×10^3 cells/plate on TG + Nalidixic acid
  Spray with NPP. +
  Pick strains giving all yellow recombinants.

DNA Synthesis in Fu56 TsDNA at 42°C and 36°C

Regrow Fu56 TsDNA to OD 0.2. Dilute 1ml → 4ml in flasks A and B

A. 4ml TG + 2oP + 0.01% Casamino acid
Calibrated at 36°C
Thymidine – 0.2 ml (lit. 0.5 μc
1 ml – Culture OD 0.2

B. 4ml TG+2oP+0.01% CA.
Calib at 42°C

Thymidine 0.2 ml (0.5 μc)
1 ml culture OD 0.2.

Take Samples from A and B

0.5 ml in 0.5 ml 10% chilled TCA.
wash with 5% TCA + 50 μg/ml thymidine
on millipore filter.
Dry at 80°C and add 10 ml
Scintillator – Count.

Time 0 mins, 10, 20 mins, 30, 60 m

	A (36°C	B (42°C)	
0	3.7	3.5	0
10	3.17	3.15	10
20	3.27	3.25	20
30	3.37	3.35	30
60	4.7	4.5	60