

MEDICAL RESEARCH COUNCIL

MICROBIAL GENETICS RESEARCH UNIT

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HAMMERSMITH HOSPITAL,

DUCANE ROAD,

LONDON, W.12.

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Dear Dr. Liddigi,

Bob Pitcher has handed me your letter and I am enclosing three sub-cultures of *B. subtilis* 168.

(1) PROTOTROPH

(2) INDOLE^- . TYROSINE^- . These are two linked markers showing about 70% coincident transfer.

(3) INDOLE^- . ARG^- . These are two unlinked markers. With saturating concentrations of MMA the mbc of

Coincident transfer of the two markers should not be more than 5%.

For obtaining competent cells I would refer you to the method of Anagnostopoulos J. BACT. (1961) 81, 741.

I find it technically simpler to bubble air through cultures rather than mechanical shaking. The aeration must be very vigorous indeed. Another point to watch is the cleanliness of the glassware. Small amounts of detergent adsorbed by the glass interfere with the growth rate and completely inhibit transformation. The culture vessels and transforming tubes are therefore soaked in chromic acid and very thoroughly rinsed afterwards in

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tap water and then distilled water.

For preparing the smut I find Kirby's method the most effective and easiest. BIOCHEM. J. (1957) 66, 495.

Unless the experiments require purified smut it is not necessary to follow the whole of his procedure. PAS / phenol extraction

followed by 2-ethoxy ethanol extraction gives a fairly pure sample of smut with high biological activity and is quite sterile. I usually store smut at 4°C; dissolved in 0.015M NaCl + 0.0015M Sodium citrate and then made up to 1M NaCl; at a smut concentration of about 1mg/ml.

I am sending you a sample of protospore
mut, prepared by going through the whole
of Kirby's method. The mut concentration
is 2:1 in your part. If your first
experiments are not successful you
should be able to check with this
whether it is the mut preparation or growth
to competence which is going wrong.

If you have any problems or
need any further advice please do not
hesitate to write. Also if you need
any more mutants I shall be delighted
to send you any in my possession.

Yours sincerely,

Michael Kelly.