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Screening of pUC plasmid clones for inserts based on growth rate (without X-gal)

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pUC plasmids are the most widely used plasmid cloning vectors nowadays. Work with them has been facilitated by placing polylinkers into a truncated lacZ gene whose product complements the M15 deletion of the host (1). Clones without inserts form blue colonies on indicator plates containig the substrate X-gal (5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside) together with the inducer IPTG (isopropyl-ß-D-thiogalactopyraoside), whereas clones with inserts form white colonies in most cases. The drawback of this system are the indicator plates. X-gal is expensive and not stable. Occasionally colonies develop which (depending on age and humidity of the plates) are all white or blue irrespective of inserts (cf. 2).

Hosts generally used for pUC plasmids are JM strains (e.g. JM101, JM105, JM109...). These carry the M15 deletion making -complementation possible and the $lacI^q$ allele providing for repression of the lac promoter in the absence of an inducer. In our experiments, we have used conventional recA strains without $lacI^q$ (DH1 and HB101) as hosts for pUC18 and pUC19 constructs. After streaking colonies on master plates (LB medium with 50µg/ml ampicillin) we have observed an interesting and useful phenomenon: colonies were clearly of two types exhibiting different sizes. Comparing them with results obtained from plasmid screening we found that the large colonies contained plasmids with inserts and the small colonies the vector without an insert. We now use this test routinely for looking for recombinant plasmids after ligating DNA fragments into pUC vectors. This procedure works just as well as the classical X-gal assay with the following limitations: short inserts with proper ORFs produce small colonies and inserts coding for products lethal for E. coli would escape. Colonies should be monitored for size not on original plates but after re-streaking on new plates with ampicillin (this procedure might be inconvenient in cases of very low cloning efficiencies). Large colonies from these plates can be directly used for mini-scale plasmid preparation (3,4).

A possible cause of the described phenomenon might be that a constitutive transcription from the lac promoter interferes with plasmid replication and/or with transcription of the ß-lactamase gene. In DH1 and HB101, lac repressor is titrated out by operators on the multicopy plasmid.

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