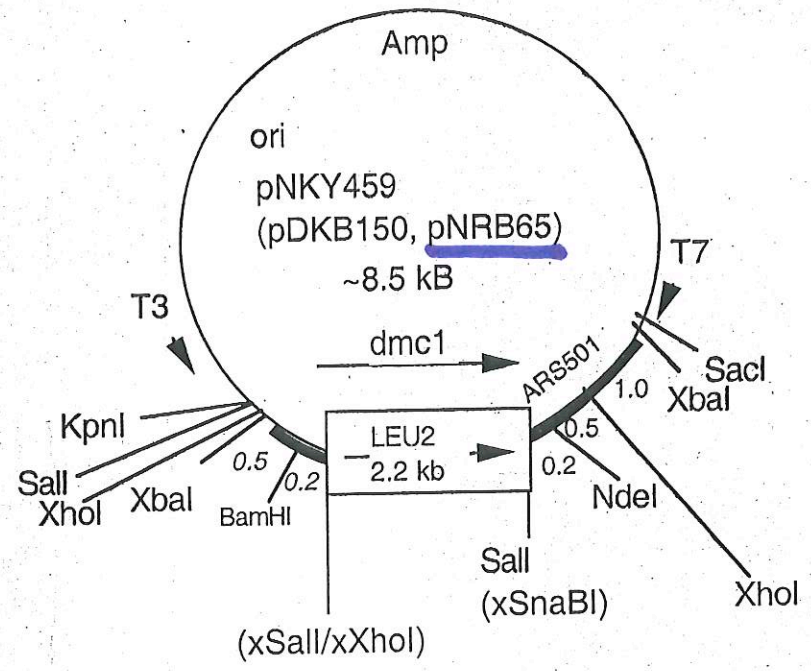
Plasmid for generating *dmc1Δ::LEU2* alleles by single step transplacement.



XbaI insert of pNKY1179 cloned into XbaI site of pNB8(3 kB). A description of these plasmids and a detailed description of the deletion/dispruption endpoints are given in Bishop et al. Cell 69, 439-456.

A XbaI digest will release a fragment for single step transplacement (about 5.2 kb).

This plasmid contains ARS501(Ferguson et al. Cell 65, 507-515) which has been tentatively mapped between the SnaBI and NdeI sites 3' of DMC1. If an XbaI digest is used to target deletion/disruption of DMC1 only 1/50 transformants are true transplacements. Most other transformants are autonomous circles with or without a single XbaI site.

One way to get a correct transplacement is to make a dmc1:LUK mutant first and then transform the mutant with digested pNKY459 (Leu+) and screen transformants for Ura-. When we did this 3/3 had the correct structure. A second way is to mate transformants to a dmc1Δ strain and screen the resulting diploids for Spo- using the UV plate test or microscopy.

Use a fragment of pNKY1173 as probe for Southerns. Genomic DNA is digested with XbaI as detailed in the paper cited above.

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