DCS-Blue MRF' Tet Supercompetent Cells

Storage: Supercompetent cells must be placed immediately at the bottom of a –80°C freezer directly from the dry ice shipping container. Do not store the cells in liquid nitrogen.

BACKGROUND

DCS-Blue MRF´ Tet Genotype: Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac [F´ proAB lacIqZΔM15Tn10 (Tetr)]. (Genes listed signify mutant alleles. Genes on the F' episome, however, are wild-type unless indicated otherwise.)
DCS-Blue MRF´ Tet cells are tetracycline resistant. The DCS-Blue MRF´ Tet (Minus Restriction) strain is a restriction minus (McrA–, McrCB–,McrF–, Mrr–, HsdR–) derivative. DCS-Blue MRF´Tet cells are deficient in all known restriction systems [Δ(mcrA)183, Δ(mcrCB-hsdSMR-mrr)173], and are endonuclease (endA), and recombination (recA) deficient. The hsdR mutation prevents the cleavage of cloned DNA by the EcoK endonuclease system, and the recA mutation helps ensure insert stability. The endA1 mutation greatly improves the quality of miniprep DNA. DCS-Blue MRF´Tet cells contain the lacIqZΔM15 gene on the F´ episome, allowing bluewhite screening for recombinant plasmids.

TRANSFORMATION PROTOCOL 1 (high transformation rates)

1. Thaw the supercompetent cells on ice.
2. Add 0.1–50 ng of the experimental DNA to one aliquot of cells. Swirl the tubes gently.
3. Incubate the tubes on ice for 30 minutes.
4. Heat-pulse the tubes in a 42°C water bath for 30 seconds. The duration of the heat pulse is critical for maximum efficiency.
5. Incubate the tubes on ice for 2 minutes.
6. Add 0.45 ml of preheated SOC medium and incubate the tubes at 37°C for 1 hour with shaking at 225–250 rpm.
7. Plate ≤200 µl of the transformation mixture on LB agar plates containing the appropriate antibiotic (and containing IPTG and X-gal if color screening is desired)
11. Incubate the plates at 37°C overnight (at least 17 hours for blue-white color screening). Colonies containing plasmids with inserts will remain white, while colonies containing plasmids without inserts will be blue. The blue color can be enhanced by incubating the plates for two hours at 4°C following the overnight incubation at 37°C.

TRANSFORMATION PROTOCOL 2 (fast protocol)

1.Thaw competent cells carefully on ice
2.Incubate the DNA with the competent cells for 1 min on ice
3.Incubate for 30-40 sec. at 42°C - heat shock -
4.Spread the cells on prewarmed plates with the appropriate selection marker
5.Incubate plates over night at 37°C

This procedure works excellent with ampicillin as a selection marker. With kanamycin or streptomycin transformation rates are ten times lower.With chloramphenicol as a selection marker this method failed in our hands.

For more information see:

Brian Pope and Helen M. Kent
High efficiency 5 min transformation of Escherichia coli
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