Technical Info

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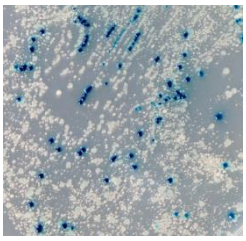

Blue-White Selection / α-Complementation

During processes of genetic engineering, the so called Blue-White-Selection is used for evaluation of recombinant bacteria. Thus, it not only provides identification of those bacteria carrying the desired plasmid, but also helps to determine whether the recombination procedure has proceeded correctly.

Those vectors (plasmids) are suitable for blue-white selection carrying a β-galactosidase gen (*lacZ*) at the insertion site (MCS, Multiple Cloning Site). Following insertion of the required DNA fragment into the MCS, based on this plasmid a shortened, or inactive galactosidase is expressed.

As bacterial ‚host' for the plasmid, a bacterial strain is used for transformation which doesn't have an operational galactosidase gen by itself (for instance, *E. coli* strains JM109, DH5α and XL1-Blue). In the mutant galactosidase gen of these strains (*lacZΔM15* mutant, originating from *E. coli* M15) amino acids 11 to 41 have been deleted, hence inhibiting formation of the enzymatically active homotetramer. Bacteria carrying both, the mutant galactosidase *and* successfully recombined plasmids, are not able to form functionally active galactosidase molecules.

➔ Bacteria carrying successfully recombined vectors are forming white colonies.

Which is quite different to the situation in those bacteria carrying a non-recombinant plasmid. In these cells, a short peptide of the galactosidase (α-peptid) is expressed, which is able to replace the mutated genomic peptide sequence, forming a functionally active galactosidase (α-complementation) with the ‚ω-peptid'.

Bacteria carrying non-recombined vectors are forming blue colonies.

➔ Bacteria which have not been transformed, hence carry no plasmid (with some antibiotic resistance-gen) at all, are removed by antibiotics.

In order to perform this α-complementation, two reagents are added to a standard agar plate: IPTG for induction of the mechanism, and X-β-Gal being an efficient colour substrate of the galactosidase.

Carl Roth GmbH + Co. KG
Labware · Life Science · Chemicals
Schoemperlenstraße 3–5 · D-76185 Karlsruhe
Phone +49 721 5606-0 · Fax +49 721 5606-149
P.O.Box 100121
76231 Karlsruhe
info@carlroth.com
www.carlroth.com

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Mechanism

Being a galacto pyranoside, IPTG inhibits the *lac* repressor, therefore leading to the induction the *lac* promotor. Subsequently, using the plasmid as matrix the C-terminal 146 amino acid fragment of the bacterial β-galactosidase is expressed, which functions as so called α-donor and complements the C-terminally deleted bacterial β- galactosidase. The then functional β-galactosidase restricts the galactoside X-Gal, resulting in a blue-coloured dye - therefore, these *lac*+ clones are coloured dark blue.

Since the C-terminal part of the α-donor is located **upstream** and the N-terminal part is located **downstream** of the multi cloning site of the plasmid, inserted DNA normally leads to frame shift or at least to major chain elongation, resulting in an unfunctional α-donor and no functional galactosidase - recombinant clones are white. The appearance of light blue clones either results from very short insertions or from the fact that in some plasmids the C-terminal fragment cloned upstream of the MCS is sufficient for α-complementation and restriction of a small amount of X-Gal.

Protocol

Stock solution

IPTG: 0.5 M (1 g /8.4 ml) in water, freeze sterile filtered and aliquoted

X-β-Gal: 100 mg/ml (240 mM) in DMF, store aliquoted and in the dark at -20 °C

Approach

Add 10 µl IPTG solution + 10 µl X-β-Gal solution per 25 ml autoclaved, cooled Agar and pour plates.

Alternatively, mix 10 µl IPTG and X-β-Gal solution with approx.100 µl sterile water and plate onto a hardened 9 cm agar petri dish.

IPTG, BioScience-Grade	Ord. No. 2316
X-β-Gal, BioScience-Grade	Ord. No. 2315

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76231 Karlsruhe
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