LAB INSTRUCTION

Transposon induced mutants

Preliminary Version

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1 Background

Transposable elements, colloquially known as jumping genes, are elements that can change their position within a genome. This creates insertions, deletions, inversions, chromosomal fusion mutations and thus forms the basis for genetic diversity, so that they occur in all genomes. The simplest transposons are IS elements (insertion elements). They only have a short inverted repeat sequences at the ends (inverted repeats) and an open reading frame for the transposase. In most cases Transposons can contribute a further genetic information to the coding of the IS elements, e. g. an antibiotic resistance. The transposon Tn5 is one of the composite transposon which is flanked by IS elements. In this case, one of the two IS elements is mutated, so that no intact transposase can be expressed. Tn5 is a "cut and paste" transposon whose mutation is said to be very stable, because it rarely comes back to the spin-off of the transposon. It is resistant to kanamycin, streptomycin (low concentration) and bleomycin. Since transposons do not replicate autonomously, you will need a vector, in this case a pSUP5011 plasmid. This plasmid is a pBR325 derivative and shows resistances to ampicillin, kanamycin as well as chloramphenicol. It can be mobilized in gram-negative *Enterobacteriaceae*. In order for the plasmid to conjugate, a mob-site has been cloned to the transposon. The plasmid pSUPP5011-Tn5::mob is now approximately 10 kbp in size.

2 Task

The aim of this experiment is to transmit the transposon Tn5 from the donor *Escherichia coli* through conjugation into *Ralstonia eutropha* (resistant to streptomycin in high concentration), where it cannot replicate. Conjugated *Ralstonia eutropha* can only be cultivated on a medium containing streptomycin and kanamycin when the transposon has jumped into the genome of the recipient. Defects in the poly-(3HB)-biosynthesis, the central carbon metabolism or the amino acid biosynthesis metabolism can be identified among the transconjugants.

3 Material

3.1 mediums

DSMZ 1/Strep/Kana/X-Gal/IPTG:	peptone	0,5 % (w/v)
	meat extract	0,3 % (w/v)
	streptomycin	0,05 % (w/v)
	kanamycin	0,016 % (w/v)
	X-Gal	0,004 % (w/v)
	IPTG	0,002 % (w/v)
DMSZ 381/Kana:	NaCl	1 % (w/v)
	tryptone	1 % (w/v)
	yeast extract	0,5 % (w/v)
	kanamycin	0,016 % (w/v)
micronutrient solution (SL6):	H_3BO_3	0,003 % (w/v)
	$CoCl_2 \times 6\ H_2O$	0,002 % (w/v)
	$ZnSO_4 \times 7\ H_2O$	0,001 % (w/v)
	$NaMoO_4$	0,0003 % (w/v)
	$MnCl_2 \times 4\ H_2O$	0,0003 % (w/v)
	$NiCl_2 \times 6\ H_2O$	0,0002 % (w/v)
	$CuCl_2 \times 6\ H_2O$	0,0001 % (w/v)

mineral salt medium:	Na_2HPO_4 x 12 H_2O	0,9 % (w/v)
	KH_2PO_4	0,15 % (w/v)
	$MgSO_4$ x 7 H_2O	0,02 % (w/v)
	$CaCl_2$ x 2 H_2O	0,002 % (w/v)
	Fe(III)NH_4-citrate	0,00012 % (w/v)
	SL6	0,01 % (v/v)
Nile Red mineral salt medium	fructose	0,5 % (w/v)
	NH_4Cl	0,02 % (w/v)
	Nile Red	0,0005 % (w/v)
Na-gluconate mineral salt medium	Na-gluconate	0,5 % (w/v)
	NH_4Cl	0,1 % (w/v)
Na-succinate mineral salt medium	Na-succinate	0,5 % (w/v)
	NH_4Cl	0,1 % (w/v)
Saline solution	NaCl	0,9 % (w/v)
Nile Red solution	Nile Red dissolved in acetone	0,01 % (w/v)

3.2 Culture

One-Day Culture (24 hours, 120 rpm, 30 °C)
- *Ralstonia eutropha* HF39 (DSMZ 15444) in DSMZ 1/Strep
- *Escherichia coli* pSUP5011::Tn5-mob (DMSZ 5167) in DSMZ 381/Kana

4 Instructions

One-Day-Cultures are provided by us.

4.1 Mating

Harvest 8 ml of the bacterial culture by centrifugation at 3000 x g for 15 min at 4 °C. Decant the supernatant and remove all remaining medium. Resuspend the pelleted cells in 200 µl of saline solution. Pipette 200 µl of donor suspension (DSMZ 5167) and 200 µl of acceptor suspension (DSMZ 15444) of a DSMZ 1 plate. Incubate at 30 °C for 2 days.

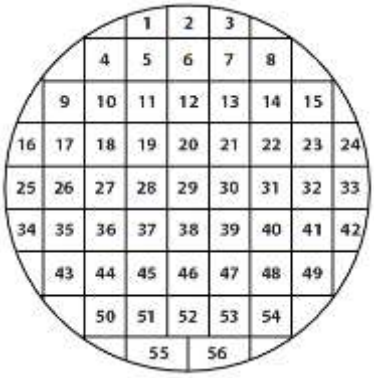
4.2 Selection

Resuspend the microbial biomass in 2 ml of the saline solution using the inoculating loop. Spread 100 µl of the indicated dilution using a Drigalski scoop. Incubate two days at 30°C.

	dilution	
DSMZ 1/X-Gal/IPTG	3 plates of 10^{-7}	3 plates of 10^{-8}
DSMZ 1/Strep/Kana	3 plates of 10^{-1}	3 plates of 10^{-2}

Donor cells have Tn5, thus have a streptomycin resistance. However, this gene is expressed only weakly. Consequently, donor cells containing Tn5 die when exposed to a high concentration of streptomycin. Only conjugated acceptor cells can grow on the NB/Strep/Kana-medium, since only those have sufficient high resistance.

Pick the colony with a sterile pipette tip and plate the microbial biomass in correct order Nile Red mineral salt medium, Na-gluconate mineral salt medium, Na-succinate mineral salt medium and DSMZ 1/Strep/Kana according to this scheme. Incubate two days at 30°C.



Cover the Nile Red mineral salt plate with 5-10 ml Nile Red solution and let this evaporate under the extractor hood.

4.3 Data Analysis

Calculate the yield of conjugation and give an answer where the Tn5 is jumped.