RpMatB His_6 C106A R286C Q457C K488A T303A: information and sequence

Plasmid

pTEV5_RpMatB_4

Usage

The plasmid is used to express Malonyl-coenzyme A synthetase from *Rhodopseudomonas palustris* (RpMatB), containing a His-tag and the mutations C106A, T303A, R286C, Q457C and K488A: (His6/C106A/R286C/Q457C/K488A/T303A)RpMatB.

The protein is modified for use as the scaffold of an ATP biosensor, Rho-RpMatB(T303A). The two cysteine mutations are for label attachment (R286C and Q457C); there is a mutation to prevent enzyme activity (K488A), a mutation to change the wild-type cysteine (C106A), a mutation that weakens ATP binding (T303A) and an N-terminal His-tag to aid purification.

When labelled with two 5-iodoacetamidotetramethylrhodamine (5-IATR) fluorophores, this adduct can be used as an ATP biosensor has a fluorescence intensity change of ~3-fold. With a Kd of ~50 μM, this adduct variant can be used to measure ATP formation in the range of up to ~100 μM, in the presence of millimolar ADP.

Publications

Vancraenenbroeck, R.; Webb, M. R., A fluorescent, reagentless biosensor for ATP, based on malonyl-coenzyme A synthetase. ACS Chem. Biol. 2015, 10, 2650-2657.

Vancraenenbroeck R, Kunzelmann S, Webb MR. Development of a range of fluorescent reagentless biosensors for ATP, based on malonyl-coenzyme A synthetase. PLOS ONE. 2017;12(6):e0179547.

Protocol

Separate file

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Protein sequence

MSYYHHHHHHDYDIPTSENLYFQGASMNANLFARLFDKLDDPHKLAIETAAGDKISYAELVARAGRVANVLVARG
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DIYK

Red is His-tag

Gene sequence

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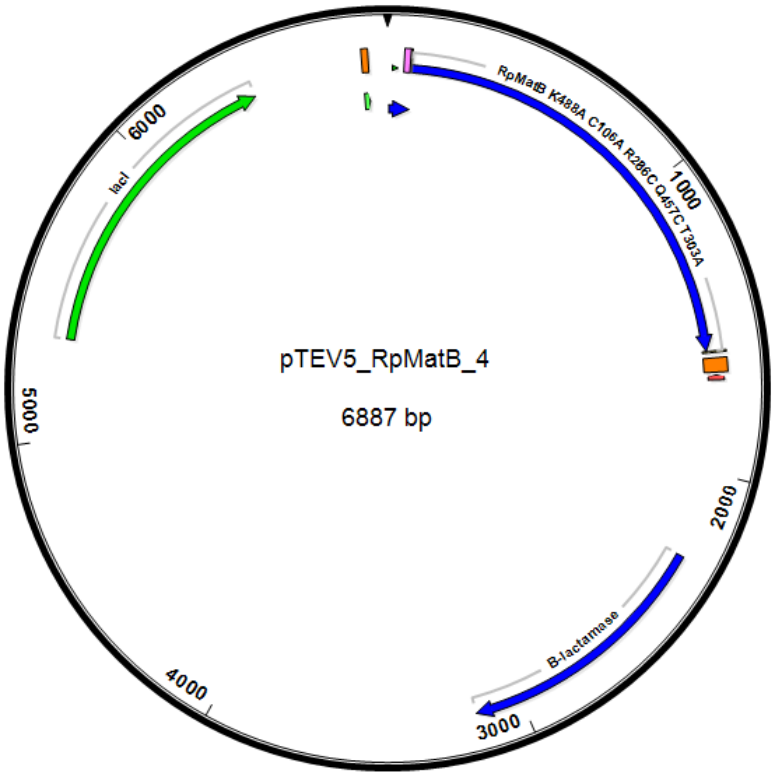
His-tag

Plasmid map:

Parent plasmid is pTEV5



Plasmid sequence

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