Oct 03, 2019

CDO expression into OnePot PURE

Forked from CDO expression into OnePot PURE

DOI
https://dx.doi.org/10.17504/protocols.io.7v3hn8n

Dana Mozaffari[1]

[1]EPFL - EPF Lausanne

iGEM EPFL

Dana Mozaffari

DOI: https://dx.doi.org/10.17504/protocols.io.7v3hn8n

Protocol Citation: Dana Mozaffari 2019. CDO expression into OnePot PURE. **protocols.io** https://dx.doi.org/10.17504/protocols.io.7v3hn8n

Protocol status: In development
We are still developing and optimizing this protocol

Created: October 02, 2019

Last Modified: October 03, 2019

Protocol Integer ID: 28315

Keywords: OnePot PURE, CDO, Catechol, Cell Free, cdo expression into onepot pure, deoxygenase, onepot pure cell, catechol, cdo expression, hydroxymuconate semialdyhyde, enzyme, onepot pure, cdo, free transcription, colorless substrate

Abstract

This protocol explains the procedure for expressing the catechol degrading enzyme *Catechol-2,3-deoxygenase* (*CDO*) into a "homemade" OnePot PURE cell-free transcription/translation system.

Once expressed, this enzyme degrades catechol, a colorless substrate, into a yellow colored one, 2-hydroxymuconate semialdyhyde (2-HMS), providing a colorimetric signal that can be easily implemented in another protocol.

Guidelines

We used a CDO plasmid with A T7 promoter added to it.

Materials

	Materials µl	CDO expression (1)	Control without plasmid (2)	Control without catechol (3)
	Energy solution	2	2	2
	Ribosomes	0.9	0.9	0.9
	Proteins	0.65	0.65	0.65
	CDO Plasmid	25 ng	-	25 ng
	Catechol 10mM	0.5	0.5	-
	Water	up to 5 µl	up to 5 µl	up to 5 µl
	Total	**5**	**5**	**5**

Before start

Preheat the incubator at 37°C

1 Label 3 PCR tubes according to the reactions

2 In each tubes add the Energy solution, proteins and ribosomes.

3 Add the catechol, CDO and water as needed according to the materials chart.

4 Make a quick spin in the centrifuge to have all the liquid in the bottom.

5 Incubate one hour at 37°C