Ligation Efficiency Test Report

G108-G: Safe-Green™

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Purpose

To investigate if Safe-Green™ has any negative effect on ligation reactions compared to using Ethidium Bromide.

Procedure

1) Linearize 6 µg of vector using XhoI.
2) Run 3 µg of the linearized vector on a gel with Ethidum Bromide + regular DNA Loading dye, and run 3 µg of the linearized vector on a gel with no Ethidum Bromide + Safe-Green as loading dye.
3) Gel extraction with a Column-Pure DNA Gel Extraction Kit and elute vector in 25 µl elution buffer.
4) Set up a ligation reaction using 4 µl of purified linearized vector and T4 DNA Ligase [also set up a no ligase control to assess level of uncut vector background] as below:

Component	Test Sample (EB or SG)	No Ligase Control
Linearized Vector	4 µl	4 µl
5X T4 DNA Ligase Buffer	2 µl	2 µl
T4 DNA Ligase	1 µl	-
ddH_2O	3 µl	4 µl
Total	10 µl	10 µl

5) Transform and plate onto LB + ampicillin plates to select for colonies containing the desired recircularized plasmid. Compare colony counts the following day.

Results

There was no difference observed on ligation efficiency when using Safe-Green compared to Ethidium Bromide.