



PROLUNG

BIOCONTAINMENT

TRYPTOPHAN REGULATORY SYSTEM

LAB BOOK 5

Im2 and Trp MiniPrep

Performed according to the QIAprep Spin Miniprep Kit protocol provided by Qiagen.

Im2 A: 58ng/ul

Im2 B: 48ng/ul

Im2 C: 48.4ng/ul

Trp A: 48.7ng/ul

Trp B: 38.6ng/ul

Trp C: 44ng/ul

Trp-YFP MiniPrep

Performed according to the QIAprep Spin Miniprep Kit protocol provided by Qiagen.

Trp-YFP: 43.7ng/ul

Trp-YFP digestion

Objective

Remove BFP in order to insert Im2

Procedure

Digestion

Mix all components on ice and add enzymes last.

	Trp-YFP
DNA	1ug
Xmal	1ul
Mfel	1ul
10x CutSmart	5ul
RNase-free water	To 50ul

37C/1h

PCR purification

Performed according to the QiAquick PCR Purification Kit protocol provided by Qiagen.

Discussion

The gel showed a band that did not match any of the expected outcomes. Later we found that the pSB4A5 backbone contains one of the restriction sites we were using, so the digestion did not work as expected.

Also, we had sent the Trp-YFP plasmid for sequencing, but the results came back with multiple mutations, so we decided to focus on the cumate system and stop working on the tryptophan system.