OCT 14, 2019

Insert + Vector DNA Ligation

Igem Dusseldorf¹

¹Heinrich-Heine Universität Düsseldorf

Igem Dusseldorf

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.76uhrew

Protocol Citation: Igem Dusseldorf 2019. Insert + Vector DNA Ligation.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.76uhrew>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: Oct 14, 2019

Last Modified: Oct 14, 2019

PROTOCOL integer ID:

28596

ABSTRACT

Pipette mix the following components:

	Double Digested DNA	ng/uL	size (kb)	bp ratio	1	3	5	I:V Ratio
	XXXX Vector, linearised + dephosphorylated			#VALUE!		#VALUE!		ng insert
	XXXX Gen, digested					#VALUE!		uL insert
	Volume (uL)	Ratio	Insert	Vector	T4 Ligase	Water	T4 Buffer	Total
	Ligation 1 Vector only	vector	0		1	17	2	20
	Ligation 2 Vector + Gene ligation	3 to 1	#VALUE!		1	#VALUE!	2	20

Nur die grün markierten felder müssen ausgefüllt werden. Die Formeln berechnen das optimale 3:1 verhältnis von Vektor zu gen. Check wie viel DNA insgesamt eingesetzt wird. Vektor ist bei mir immer so um 25-30 ng und totale DNA menge überschreitet nicht 100 ng dann sollten gute ergebnisse erzielt werden. Incubate 60 min at room temperature, and/or overnight at 16°C (*higher efficiency*). *Optional:* inactivate ligase by incubating for 5 min in 70°C heat block. *Transform into E. coli*