Gateway BP recombination of attB tailed PCR products into pDONR/zeo (5 µl assay) Version 2

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Abstract

This is a slightly modified version of Thermo Fisher's gateway BP protocol which uses less enzyme and is therefore more economical.

Citation: Johannes Debler Gateway BP recombination of attB tailed PCR products into pDONR/zeo (5 µl assay). **protocols.io**
dx.doi.org/10.17504/protocols.io.g5rby56
Published: 07 Feb 2017

Protocol

Calculate amount of attB PCR product needed

Step 1.

ng of attB PCR product = size of attB tailed PCR product in bp * 0.0165

eg. 778 bp * 0.0165 = 12.9 ng

If your purified PCR product has a concentration of 25 ng/ul you would use 0.5 µl in the next step.

NOTES
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Your attB PCR product should be purified either by gel purification or with a PCR clean-up kit

Combine in 0.2 ml PCR tube

Step 2.

attB PCR product	x µl (as calculated in step 1)
pDONR/zeo (150 ng/µl)	0.5 µl
TE buffer pH 8.0	to a total volume of 4 µl

BP Clonase II enzyme mix

Step 3.

Remove from freezer and vortext for 2 seconds.

add BP Clonase II

Step 4.

Add **0.5 µl** of BP Clonase II enzyme mix and mix well.

Incubate at room temperature

Step 5.

Incubate for **1 hour** at **room temperature** (2-3 hours or overnight for more colonies).

DURATION

01:00:00

Stop reaction with Proteinase K

Step 6.

Add **0.5 µl** Proteinase K, mix well and incubate for **10 min at 37°C.**

DURATION

00:10:00

Transform into Omnimax 2 E.coli

Step 7.

Combine **1 µl** BP product with **50 µl** Omnimax 2 competent *E.coli* cells in 1.5 ml tube.

Incubate on ice

Step 8.

DURATION

00:10:00

Heat shock

Step 9.

30 seconds at **42°C**

Reco on ice

Step 10.

DURATION

00:02:00

Add SOC

Step 11.

Add **500 µl** SOC.

Incubate

Step 12.

1 hour at **37° C** at **250 rpm** on shaking incubator.

DURATION
01:00:00

Plate out

Step 13.

Plate **20 ul** (or more) on LB plates containing **50 ug/ml** zeocin and incubate at **37° C over night.**

NOTES
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I have observed that the older your BP Clonase II enzyme mix is, the more of the BP reaction you need to plate out. You can therefore concentrate your cells by centrifuging at 1000 x g for a couple of minutes, decant most of the supernatant and resuspend the cell pellet in about 50-100 µl of the leftover supernatant. Then plate all of that on the LB + zeocin plate.