

J_H translocation junction cloning PCR protocol

adapted from Zhu C, Mills KD et al., Cell 02; and Megonigal MD, Rappaport EF et al., PNAS 98

I. DNA Digest:

Digest DNA to completion with EcoRI (quantitate by nanodrop) as follows:

2.4 µg DNA
4.8 µl 10X EcoRI digestion buffer
4.8 µl 10X BSA
30U EcoRI
H₂O to 48 µl
48 µl total volume

Leave at 37°C O/N

II. DNA Ligation

Ligation reaction to circularize DNA (important to be dilute to enrich for self-circularization versus ligation to other fragments)

10 µl EcoRI digested DNA from step 1 (= 0.5 µg)
1.4 µl 10X Ligase buffer (since 10µl of digested DNA already has 1X buffer)
11.6 µl H₂O
1 µl T4 DNA Ligase
24 µl total

Leave at 17°C O/N

III. PCR Reaction

5 µl ligated EcoRI digested DNA (100 ng)
5 µl 10X High Fidelity polymerase PCR buffer with Mg⁺²
(Roche)
1 µl dNTPs
1 µl 5' primer
1 µl 3' primer
36.5 µl H₂O
0.5 µl High Fidelity Polymerase mix
50 µl total

Per Reaction
X
No. of Samples

Also run unligated control for each- use 2 µl EcoRI digested
ng) instead

DNA (100

PCR conditions

94°C- 5 min

30 cycles of following:

94°C- 30 sec

55°C- 30 sec

72°C- 4 min

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72°C- 7 min

4°C- forever

Notes:

1. I have found that you must perform all steps sequentially to get good results- DNA seems to degrade if left either in -20°C or 4°C for more than a few days at any step
2. I only get good results when using a polymerase mix with proofreading activity- *Taq* has not worked in my reactions, probably because products are too big
3. Primer possibilities are listed in Zhu, et al. paper- I used a combination of these primers and my own to get specific products.