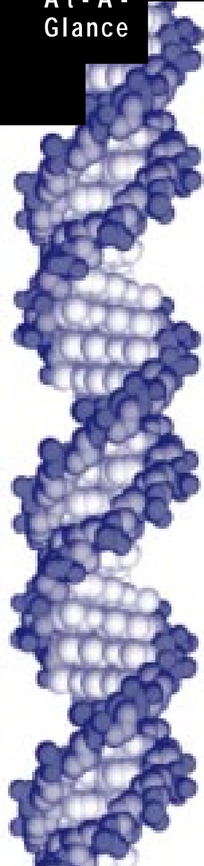
Protocol At-A-Glance



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AdvanTAge™ PCR Cloning Kit Protocol-at-a-Glance (PT3067-2)

Please read the *User Manual* before using this abbreviated protocol.

PCR Amplification

1. Generate PCR products using your own protocol. Be sure to finish with a final 7–10 min extension step.
2. Analyze 10 µl of each PCR sample by agarose/EtBr gel electrophoresis.
3. Quantify the amount of DNA for each product.

Cloning into pT-Adv

1. For each desired ligation reaction, mix:

Sterile H_2O	5 µl
10X Ligation buffer	1 µl
pT-Adv (25 ng/µl)	2 µl
Fresh PCR product (~10 ng)	1 µl
T4 DNA ligase	1 µl
Total	10 µl

2. Incubate ligation reaction mixture at 14°C for at least 4 hr (preferably overnight).
3. Centrifuge ligation reaction mixture briefly and place on ice.

Transformation

1. Thaw appropriate number of tubes of TOP10F' *E. coli* competent cells on ice.
2. Pipet 1–2 µl of each ligation reaction into the competent cells. Mix by gently tapping.

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AdvanTAge™ PCR Cloning Kit Protocol-at-a-Glance, *continued*

Transformation *continued*

3. Incubate the tubes on ice for 30 min.
4. Heat shock for *exactly* 30 sec in a 42°C water bath. Do not mix.
5. Place the tubes on ice for 2 min.
6. Add 250 µl of SOC medium to each tube.
7. Incubate tubes at 37°C for exactly 1 hr with shaking (225 rpm).
8. Place the tubes on ice.

Analysis

1. Plate 50 µl and 200 µl from each transformation on separate LB/X-Gal/IPTG plates containing 50 µg/ml of either ampicillin or kanamycin.
2. Incubate at 37°C for at least 18 hr.
3. Shift plates to 4°C for 2–3 hr for color development.
4. Analyze white transformants for the presence and orientation of inserts by restriction mapping or sequencing.

The PCR process is covered by patents owned by Hoffmann-La Roche, Inc., and F. Hoffmann-La Roche, A. G.

Products covered by U.S. Patents #5,487,993 & #5,827,657 and European Patent #0550693.

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