# Rapid and reliable cloning of PCR products.

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# Rapid and Reliable Cloning of PCR Products

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The cloning of PCR products is often desirable, especially when characterizing heterogenous product populations. To achieve this, restriction sites are frequently included at the 5′ end of each primer to allow direct cloning. However, low cloning frequencies are sometimes encountered, presumably due to incomplete digestion or *Taq* polymerase carryover.[1,2] Adaptor and linker cloning strategies can be employed to circumvent these problems but standard protocols include several time-consuming purification steps. Several members of our laboratory have experienced difficulty in obtaining PCR clones. The following protocol routinely generates high cloning efficiencies ($10^6$ transformants/μg of vector) from previously recalcitrant PCR products. Self-ligation of linear PCR products generates multimeric DNA substrates for complete cleavage by restriction enzymes.[1] The concurrent incubation of Klenow, T4 polynucleotide kinase, and T4 DNA ligase employed here effectively creates concatemeric DNA substrates by polishing, phosphorylating, and ligating PCR termini in a single step (Fig. 1). A variation for linker and adaptor cloning requiring minimal manipulation is also shown. The linker protocol facilitates directional cloning when only a single restriction site is

## KLENOW–KINASE–LIGASE (KKL)

1. Phenol-chloroform extract PCR reaction; ethanol-precipitate.
2. Dissolve in double-distilled $H_2O$; add 2 μl of 10x ligase buffer (300 mM Tris-HCl, pH 7.8, 100 mM $MgCl_2$, 100 mM DTT, 5 mM ATP).
3. Add dNTPs to 0.2 mM, 5 units of Klenow, 4 units of T4 polynucleotide kinase, 2 units of T4 DNA ligase; 25 μl final volume.
4. Incubate at 25°C, 1–2 hr.
5. Heat-inactivate enzymes (e.g., 70°C, 10 min); dilute to double volume in restriction enzyme buffer and cut with 40 units of restriction enzyme for 2 hr at appropriate temperature.
6. Heat-inactivate (or extract and ethanol-precipitate), ligate to vector, and transform host *Escherichia coli* cells.[6]
7. PCR screen colonies by scraping small amounts of cells with a pipette tip, and inoculating, 50 μl $H_2O$; boil for 3 min, add PCR ingredients (e.g., 20 mM Tris-HCl [pH 8.3 at 20°C], 1.5 mM $MgCl_2$, 25 mM KCl, 100 μg/ml gelatin, 50 μM of each dNTP and 0.25 μM of each primer) and run for 25 cycles (e.g, 94°C, 55°C, and 72°C, each at 1 min). Check insert length on agarose gel.

## LINKER AND ADAPTOR CLONING

1. Phenol-chloroform extract PCR reaction; ethanol-precipitate.
2. Dissolve in double-distilled $H_2O$, add 1 μl 10x ligase buffer, dNTPs to 0.2 mM, 5 units of Klenow, and 4 units of kinase; 10 μl final volume.
3. Incubate at 37°C, 10 min; heat-inactivate.
4. Add a 50-fold molar excess of linkers/adaptors, 1 μl 10x ligase buffer, and 2 units of T4 ligase; 20 μl final volume.
5. Incubate at 16°C, 4 hr; heat-inactivate.
6. Add 5 μl 10x restriction enzyme buffer and cut linkers with 40 units of restriction enzyme in a final volume of 50 μl for 3 hr at the appropriate temperature.
7. Remove excess linkers/adaptors (e.g., glass beads[7]).
8. Dissolve in double-distilled $H_2O$ and add 2 μl 10x ligase buffer. Phosphorylate adaptored fragments with 4 units of kinase at 37°C, 10 min in 20 μl; heat-inactivate.
9. Ligate to vector, transform, and screen as above.



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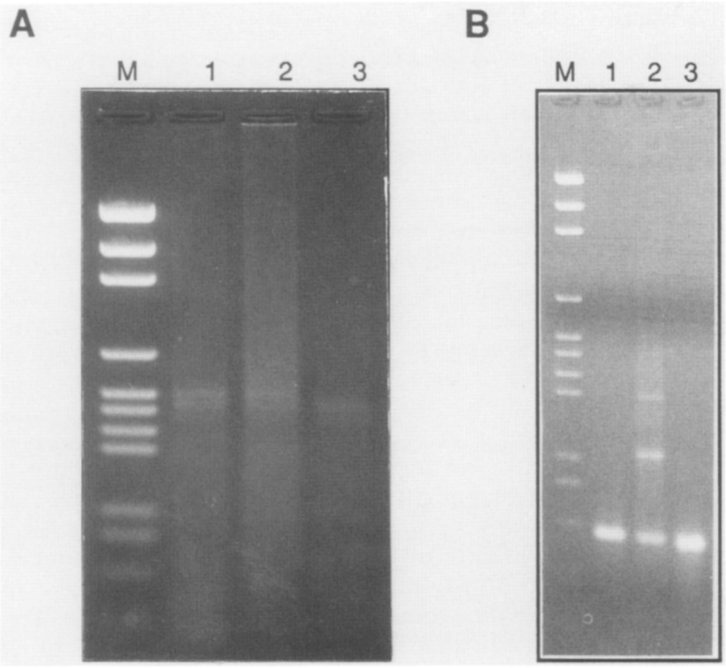


**FIGURE 1** Cloning of PCR products with the Klenow–kinase–ligase protocol. (*A*) Anchor PCR products from salmon hypo-thalamus cDNA using a gonadotropin-rleasing hormone specific primer[3] were cloned using the Klenow–kinase–ligase (KKL) protocol with a single restriction enzyme (*Cla*I) located in the anchor.[4] (Lane *1*) Anchor PCR products; (lane *2*) concatemeric PCR products following KKL ligase incubation; (lane *3*) *Cla*I-cut products; (lane *M*) pGEM DNA marker (Promega). All clones analyzed contained inserts of which most represented the two major bands at 450 and 500 bp. (*B*) Specific PCR products from salmon hypothalamus cDNA cloned by the KKL protocol using *Bam*HI and *Eco*RI sites located in the primers.[5] (Lane *1*) PCR products; (lane *2*) concatemeric PCR prod-ucts following KKL incubation; (lane *3*) *Bam*HI- and *Eco*RI-cut products; (lane *M*) pGEM DNA marker (Promega). All clones analyzed contained the 100-bp insert.

available, whereas adaptor cloning completely avoids the use of restriction enzymes.

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