

BAP

To prepare BAP from the ammonium sulfate suspension its sold as, spin out the precipitate for 5 min at 4 C in microfuge, resuspend in H₂O to a concentration of 0.1 units/ul, and centrifuge desalt on Sephadex G25 equilibrated in H₂O. Adjust the desalted BAP with Tris pH 8 to 0.1 M and store at 4 C stable essentially indefinitely.

To 10 ug DNA linearized plasmid or fragments dissolved in 45 æl H₂O. Add 5 ul CB 10 X BAP, preheat to 68 C and add 1 ul BAP (0.1 units/ul Worthington). Incubate at 68 C for 1/2 hr, add another aliquot of BAP (1 ul) and continue incubation for another 1/2 hr. Then add phenol and chloroform directly to 68 C reaction volume and vortex, do multiple extractions, add NaCl to 0.2 M and ethanol precipitate as usual.

CB 10 X BAP:

Tris 0.5 M pH 8.0

MgCl₂ 20 mM

ZnCl₂ 1 mM