

## Hirt Protocol for Isolating Small DNA Molecules from Mammalian Cells

*Lan Zhou 2/7/03*

Spin down cells into 1.5ml tube;  
Add 0.4ml SDS/EDTA for 15mins;  
Add 0.1ml 5M NaCl;  
Tumble them in cold room gently for 4 –5 hrs;  
Spin down @4C for 4mins @top speed;  
Get supernatant carefully;  
PC-8;  
Ppt;  
Wash X2;  
Add ddH<sub>2</sub>O to resuspend pDNA  
Fetch some + 10ul DH10B, zap @1.8KV  
Plate them into LB/AMP  
PCR screen  
See positive rate

Notes:

0.6%(w/v)/10mM EDTA

prep: 0.2 ml 0.5M EDTA + 0.3ml 20% SDS + 9.5ml ddH<sub>2</sub>O-----10ml SDS/EDTA.