Manual extraction of plasmid DNA (from Chen Zhon et al., 1990):

Sample material:

Pellet from clones with appropriate inserts, harvested after overnight growth at 37° C in sterile liquid LB medium (5-10 ml in glass tubes, with appropriate antibiotics (which was used when you generated the clones). Store pellets at -20 °C until further processing.

Reagents:

- 3 M Sodium Acetate pH 5.2
- Ice cold EtOH (molecular grade)
- 70 % ice cold EtOH (molecular grade)
- TENS solution 100 ml:

-1 M TE Buffer	1 ml
-0,5 M EDTA	200 µl
-2 M NaOH	5 ml
-10 % SDS	5 ml
-Sterile MQ	88,8 ml

Procedure:

1) Add 300 µl TENS solution to the pellets in their eppendorf tubes. Vortex.
2) Add 150 µl 3 M sodium acetate (pH 5,2). Vortex.
3) Centrifuge for 2 min at < 13, 000 rpm.
4) Transfer the supernatant to a new sterile eppendorf tube and add 900 µl ice cold 96 % EtOH.
5) Centrifuge for 2 min at < 13, 000 rpm.
6) Remove the supernatant. Wash the pellet with ice cold 70 % EtOH (e.g. 1 ml).
7) Centrifuge for 10 min at < 13,000 rpm.
8) Remove the supernatant. Wash the pellet with ice cold 70 % EtOH (e.g. 1 ml).
9) Centrifuge for 10 min at ~ 13,000 rpm.
10) Remove the supernatant carefully. Vacuum centrifuge your pellet(s).
11) Dissolve your dried pellet in e.g. (depending on size of pellet) 30 µl sterile MQ or some appropriate buffer.
12) Store samples at +4 °C for shorter periods, or at -20°C for longer periods.