done with very little added time. In ad-
samples, and additional samples can be
DNA on a gel is about 1 h for 96
tal time from picking a colony to load-
running samples on a gel by 6–16 h. To-
we have been successful in reducing to-
lished quick miniprep methods (3–9),
plasmid miniprep method to other pub-
cells are required. In comparing our
suspension and lysis of the bacterial
tion, no pelleting of the bacteria or re-
(PCR) or colony hybridization. In addi-
ally require an overnight incubation pe-
period for bacterial cultures (2–9). When a
large pool of transformants has to be
screened, it is important to use a proto-
lar colony from an agarose plate,
the method described here, recircular-
prep method you chose to use. Using
which is done no matter which mini-
ral colony from an agarose plate,
procedure is sterile isolation of a bacte-
most time-consuming step of this
procedure is sterile isolation of a bacte-
colonies containing recombinant
DNA is a routine procedure in molecu-
lar biology. Usually recombinants are
identified by restriction enzyme analysis
of plasmids that were isolated using
minipreparation protocols. However,
current methods for preparing mini-
preparations and performing restriction
enzyme digestion can be time-consum-
ing, expensive and cumbersome. Even
the newer “quick” methods and kits usu-
ally require an overnight incubation pe-
riod for bacterial cultures (2–9). When a
large pool of transformants has to be
screened, it is important to use a proto-
cluster that is rapid and cost effective. The
method described here is cheaper and
faster than currently used screening
methods that involve boiling, alkaline
lysis, lithium, polymerase chain reaction
(PCR) or colony hybridization. In addition,
no pelleting of the bacteria or res-
suspending and lysis of the bacterial
cells are required. In comparing our
plasmid miniprep method to other pub-
lished quick miniprep methods (3–9),
we have been successful in reducing to-
tal time spent from picking a colony to
running samples on a gel by 6–16 h. To-
tal time from picking a colony to load-
ing DNA on a gel is about 1 h for 96
samples, and additional samples can be
done with very little added time. In ad-
dition, the cost of a 96-well microplate
($1.80) is less than 96 microcentrifuge
tubes ($2.40), and no restriction en-
yzmes, Taq DNA polymerase or ra-
dioisotopes are needed. Thus, our plas-
mid miniprep protocol is a more rapid
and cost-effective alternative to other
routinely followed miniprep procedures.

In the protocol described here, we
have modified the procedure by Akada
(1), in which plasmid isolation is per-
formed directly from the bacterial
growth media (LB broth). However,
our method eliminates the 4-h to
overnight incubation of the cells in LB
broth, using instead a microplate for-
atise for ease in isolation of multiple
samples at one time. This method uses
phenol:chloroform to liberate the nu-
cleic acids and RNase A to degrade
RNA. Nunc® 96-well, heat-resistant
polypropylene plates (Nalge Nunc In-
ternational, Rochester, NY, USA) were
used because they are resistant to
degradation by the organic solvents
used in this method and also because
they facilitate ease in sample handling.
The most time-consuming step of this
procedure is sterile isolation of a bacte-
rial colony from an agarose plate,
which is done no matter which mini-
prep method you chose to use. Using
the method described here, recircular-
ized vector can easily be distinguished
from a recombinant (insert-containing)
vector. In addition, since size differ-
cences of circularized recombinants can
be easily differentiated using agarose
gel electrophoresis (5), vectors with in-
serts of different sizes can be identified
by their relative electrophoretic mobili-
ties (Figure 1).

When single culture tubes are used
at any step, substantial effort is put into
labeling, sorting, and opening and clos-
ing of the tubes at each step, thereby in-
creasing the time involved and potential
for accidental mixing of samples. The
96-well plate format significantly de-
creases the time spent on sample han-
dling, the chance of mislabeling or in-
correct sorting, the loss of sample
tables and accidental mixing of sam-
nes. Using the described method, up to
384 samples can be analyzed with just
one centrifuge step. Thus, the efficien-
cy of this procedure is because of re-
duced sample handling and small sam-
ple size.

We have used this method success-
fully with a high-copy-number plasmid
[pCR-Script™ Amp SK (+) (Strata-
gene, La Jolla, CA, USA), which can
have up to 150 copies/cell in Epicurian
Coli® XL-1 Blue cells (Stratagene)]
and low/mid-range-copy-number plas-
mids [pGEX-2T, pGEX-4T-3 and
pGEX-1AT (all from Amersham Phar-
macia Biotech, Piscataway, NJ, USA)],
which normally have 15–20 copies/cell
in XL-1 Blue cells or DH10B™ cells
(Life Technologies, Gaithersburg, MD,
USA)]. These data suggest that our pro-

Figure 1. Agarose gel electrophoresis of plasmid samples prepared by the rapid miniprep method.
Samples were prepared following the described method and analyzed on a 0.7% agarose gel (Ultra Pure
DNA Grade Agarose; Bio-Rad, Hercules, CA, USA) containing 0.1 µg/mL ethidium bromide. Lanes 1–3
show DNA from colonies containing a pGEX-4T-3 (Amersham Pharmacia Biotech) vector with insert,
totaling 5.53 kb, in DH10B cells. Lanes 4–6 show DNA from DH10B colonies transformed with a
pGEX-1AT vector containing an insert of 1580 bp, making the construct 6.53 kb. Lanes 7–9 show plas-
mid pGEX-2T with no insert (4.95 kb) in XL-1 Blue cells. Lanes marked M are 1-kb molecular weight
markers (Life Technologies), and the molecular weight is shown for several of the markers. The actual
size of the recombinants shown was confirmed by restriction enzyme digestion (not shown).
**Table 1. Protocol for Rapid Preparation of Plasmid DNAs for Screening**

1. Add 30 µL of LB broth to each well you wish to use in a heat-resistant, 96-well microplate. It is important to use heat-resistant plates in this method; we found Nunc V-96 polypropylene microplates worked well (Nalge Nunc International). A Brinkmann Instruments repeating pipetter (Transferpette® Pipetter; Westbury, NY, USA) is useful for aliquoting LB into the wells.

2. Inoculate each well with a single colony using a sterile toothpick. Pick colonies that are 0.5 mm or larger (we found that larger colonies often give more plasmid DNA for visualization). Immediately transfer the same toothpick to a marked LB agar Petri dish with the appropriate antibiotic so that you have the correct colony to go back to after you find a recombinant.

3. Add 30 µL of a 1:1:1 mixture of phenol:chloroform:loading buffer [20% Ficoll® 400, 100 mM EDTA, pH 8.0, 1% sodium dodecyl sulfate (SDS), 0.25% bromophenol blue and 0.5 mg/mL RNase A] to each well. Mix using a multichannel pipetter by pipetting up and down for a minimum of 5×. Add the loading buffer to the phenol:chloroform mixture right before adding to your samples. **Caution:** phenol and chloroform should be used in a fume hood and contact should be avoided.

4. Cover the plates with Titer Tops™ (USA/Scientific Plastics, Ocala, FL, USA) or seal well with Parafilm®. The low sample volume and covering of the plate eliminates cross-contamination between wells.

5. Spin the 96-well plates at room temperature for 10 min at 3250 rpm in a Model GS-6R Centrifuge with a GH-3.8 Rotor and Microplate Carrier Adapters (all from Beckman Instruments, Fullerton, CA, USA).

6. Load 15 µL of the aqueous phase from each sample onto a 0.7% agarose gel containing 0.1 µg/mL ethidium bromide. Take care to avoid aspirating the phenol:chloroform layer with the sample. In addition, loading circularized plasmid control DNA sample without insert is advised to verify the size of recombinants. Run the gel and visualize it under UV light. As suggested by Mohler et al. (7), the use of an adjustable 8- or 12-channel Transferpette Pipetter can aid in ease of loading samples. Since small sample sizes are being loaded, we recommend using a comb size of about 3 × 1.5 mm.

