Figure Legends

Figure 1. Process flow sheet describing two alternative routes for the intermediate purification of plasmid DNA prior to preparative hydrophobic interaction chromatography (HIC). The precipitation-based process concentrates and pre-purifies pDNA by precipitation with isopropanol and ammonium sulphate respectively, while the ATPS-based process uses a single extraction step (Abbreviations: ATPS – aqueous two-phase system, pp-precipitation).

Figure 2. Phase diagram with binodal (■) for the PEG 600-$(NH_4)_2SO_4$ system at room temperature. Systems were prepared with compositions corresponding to four different tie-lines with lengths (w/w) equal to 38.13 % (●), 49.38 % (▲), 59.01 % (▼) and 65.89 % (○).

Figure 3. Analytical HIC-HPLC analysis of (a) neutralised lysate, (b) bottom phase obtained after aqueous two-phase extraction of pDNA from the alkaline lysate (35% w/w PEG 600, 6% w/w $(NH_4)_2SO_4$, 38.16 % w/w tie-line length, phase ratio 6.2 v/v and 20% w/w lysate load) and (c) bottom phases obtained after performing a control (no lysate) aqueous two-phase extraction with a similar ATPS. The sharp peak at 0.76 min corresponds to pDNA. Sample volume 20 μl. UV absorbance at 260 nm was used to monitor the chromatography runs.

Figure 4. Agarose gel electrophoresis analysis of pDNA-containing samples. Lane 1: alkaline lysate; lane 2: supernatant obtained after ammonium sulphate precipitation; lane 3: bottom phase obtained after aqueous two-phase extraction; lane 4: pDNA pool obtained after processing the ammonium

sulphate supernatant by preparative HIC; lane 5: pDNA pool obtained after processing the salt-rich bottom phase by preparative HIC. M: molecular weight markers.

Figure 5. Preparative hydrophobic interaction chromatography purification of pDNA pre-purified by (a) aqueous two phase extraction and (b) sequential precipitation with isopropanol and clarification with ammonium sulphate. ATPS composition: PEG 600-34 % w/w, $(NH_4)_2SO_4$ - 7% w/w; tie-line length: 38.16 % (w/w); phase ratio: 6.2 v/v; lysate load 20% (w/w). Lysate composition: [Protein]=421.8 μg/ml, [Plasmid]= 24 μg/ml, [Endotoxin]=209 EU/ml. The absorbance of the eluate was recorded at 260 nm and is shown by the full line. Ammonium sulphate concentration in the eluate is shown by the dashed line.

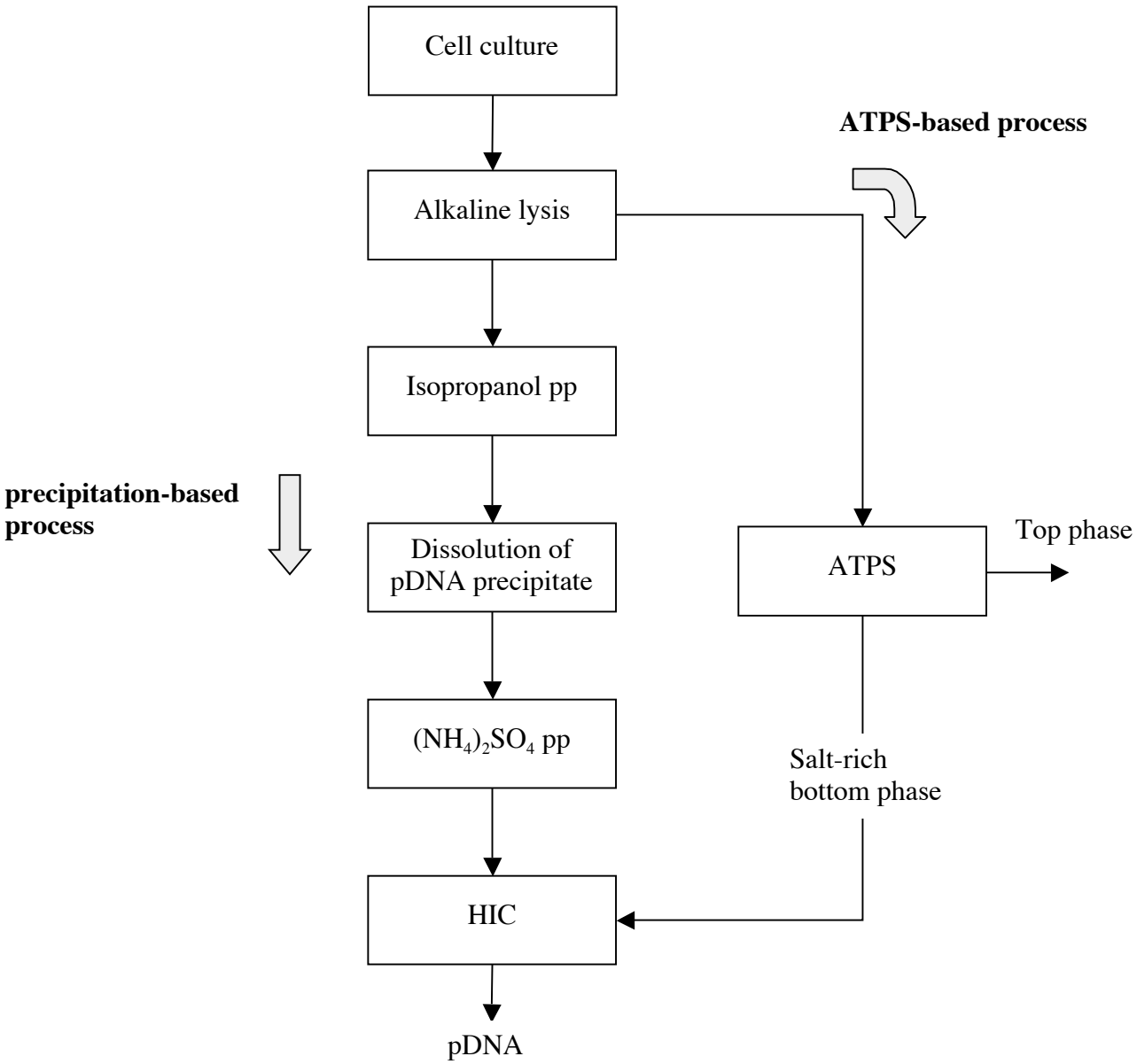

Cell culture
ATPS-based process
Alkaline lysis
Isopropanol pp
precipitation-based process
Dissolution of pDNA precipitate
ATPS
Top phase
$(NH_4)_2SO_4$ pp
Salt-rich bottom phase
HIC
pDNA

Figure 1

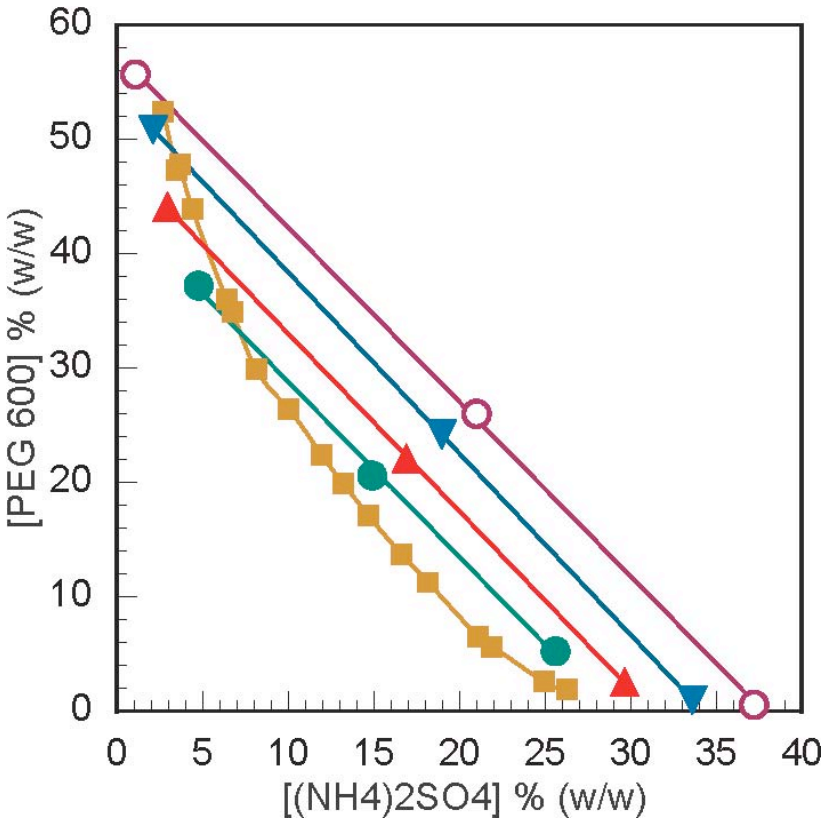


Figure 2

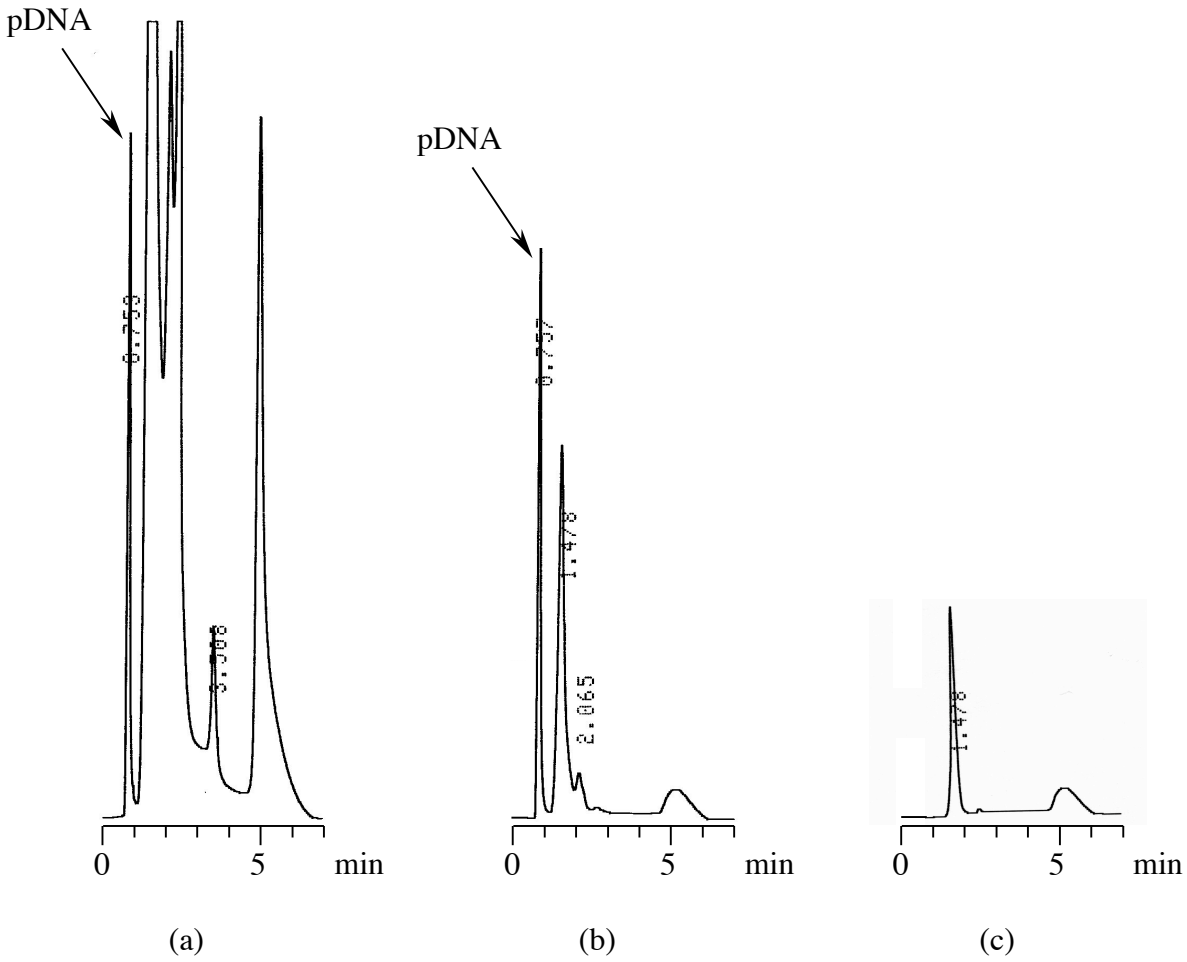

pDNA
0.759
3.008
0
5
min
(a)
pDNA
0.757
1.478
2.065
0
5
min
(b)
1.478
0
5
min
(c)

Figure 3

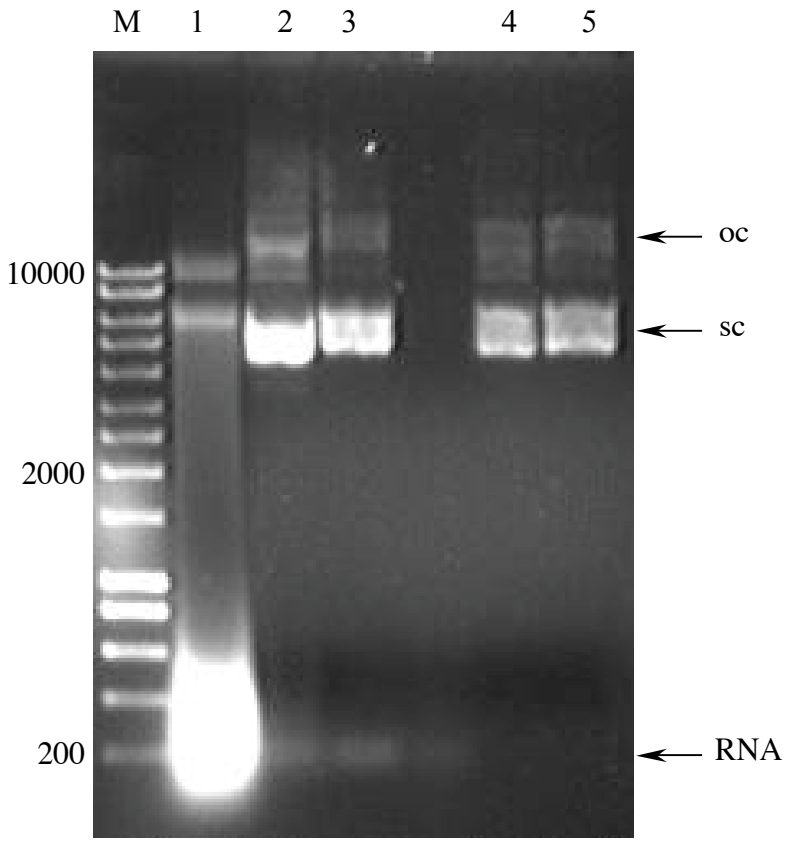
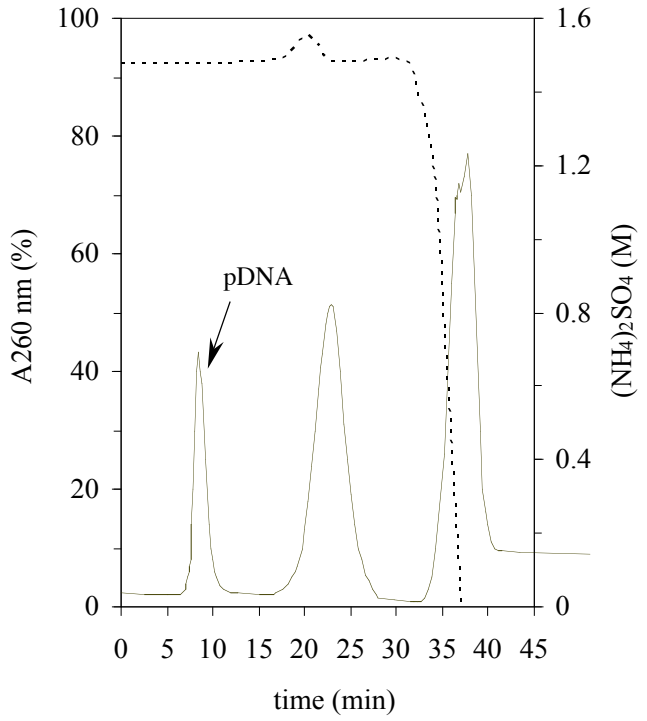


Figure 4

(a)


100
80
60
40
20
0
1.6
1.2
0.8
0.4
0
pDNA
A260 nm (%)
(NH4)2SO4 (M)
0 5 10 15 20 25 30 35 40 45
time (min)

(b)

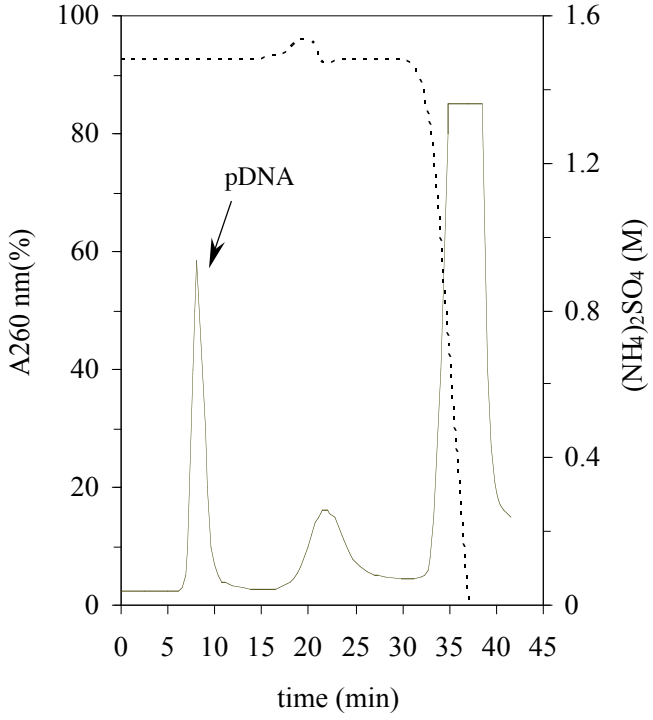

100
80
60
40
20
0
1.6
1.2
0.8
0.4
0
pDNA
A260 nm(%)
(NH4)2SO4 (M)
0 5 10 15 20 25 30 35 40 45
time (min)

Figure 5