FIGURE 1

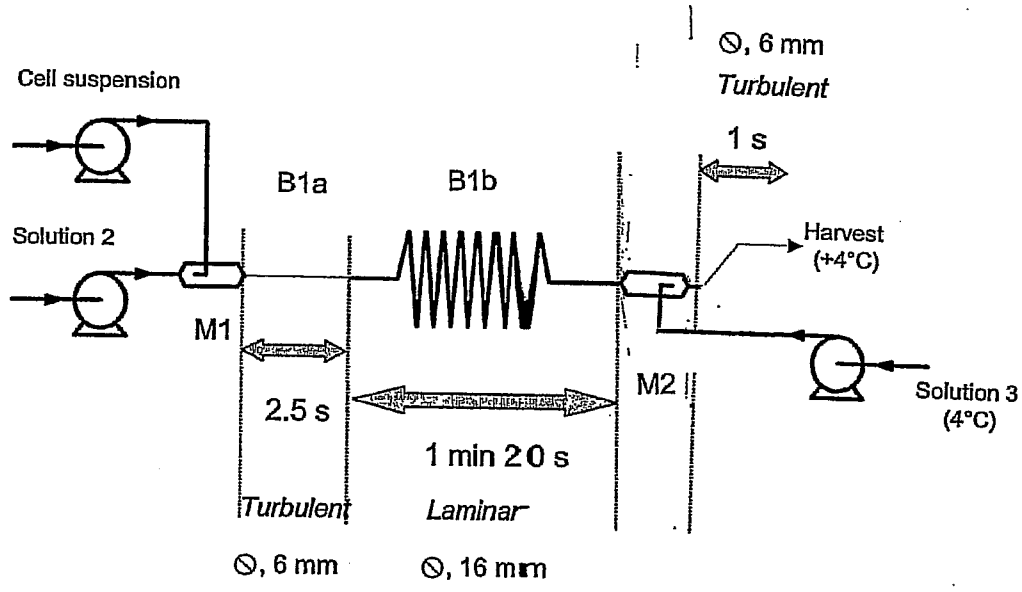

Cell lysis: continuous mode
Cell suspension
Solution 2
B1a
B1b
M1
2.5 s
Turbulent
⌀, 6 mm
1 min 20 s
Laminar
⌀, 16 mm
M2
⌀, 6 mm
Turbulent
1 s
Harvest
(+4°C)
Solution 3
(4°C)
> 10-fold less kDNA in continuous than in batch mode

FIGURE 2

General view of mixer M1

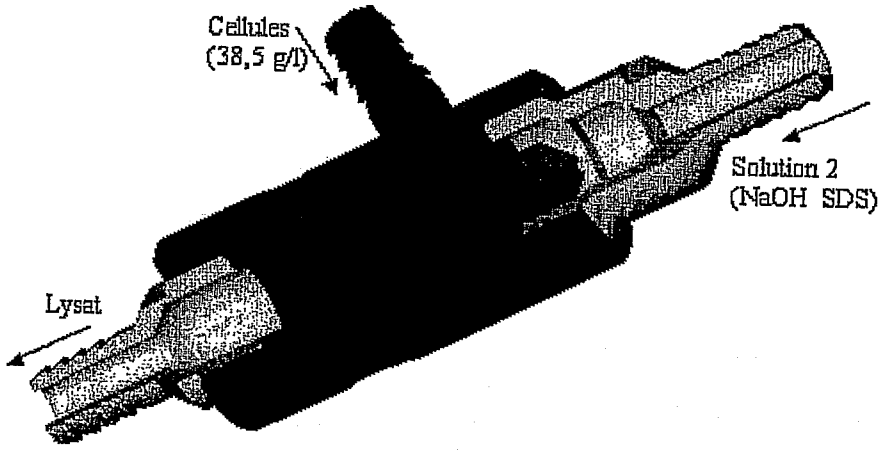


FIGURE 3

Sequence	Content of genomic DNA (%)	RNA (%)	Protein (%)	Endotox. (EU/mg of plasmid)	ccc form (%)	Comments
Lysis	500	> 1000	> 2000	> 100 000	≈ 95	
Anion exchange chromatography (AEC) [FractogelTMAE (HiCap)]	10 to 50	5 to 20	0.0002	20 to > 200	≈ 95	Very efficient protein removal (> 10^6-fold) Efficient endotoxin removal (1000-fold)
Anion exchange chromatography and Triple helix affinity chromatography (THAC-Sephacryl S1000)	0.3 to 1.0	0.002 to 0.01	< 0.0001	10 to 200	≈ 95	Very efficient RNA removal (1000-fold) Efficient genomic DNA removal (100-fold) Poor endotoxin removal (< 10-fold) No open circular plasmid form removal
Anion exchange chromatography and hydrophobic interaction chromatography (HIC) [Toyopearl Butyl (650s)]	0.2 to 0.6	< 0.01	ND	< 0.2	> 99	Very efficient oc plasmid form removal (10-fold) Efficient genomic DNA and RNA removal (100-fold) Efficient endotoxin removal (1000-fold)
Anion exchange chromatography, and Triple helix affinity chromatography and hydrophobic interaction chromatography in combination (AEC / THAC / HIC	< 0.00008	< 0.00002	< 0.00005	< 0.1	> 99	Very efficient removal of genomic DNA, RNA, protein, endotoxin, and oc plasmid form

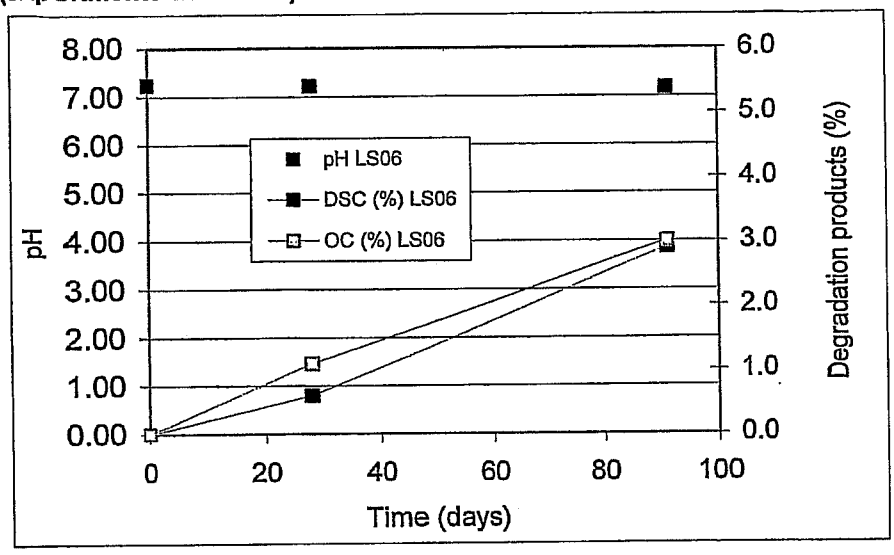
FIGURE 4

Methods of separation and purification of plasmid DNA	Genomic DNA (%)	RNA (%)	Protein (%)	Endotoxin (EU/mg)
$CaCl_2$ / AEC / AEC	< 1	< 0.1	< 2.2	< 1.5
HIC / HIC	< 1	< 1	< 1	< 5
AEC/HAC			< 0.1	< 0.1
AEC / SEC	< 1	< 0.2	< 1	< 100
UF / AEC	< 1	< 1	< 1	< 1
RPC / AEC	< 0.2 to 1.0	ND	< 0.1 to 1	< 5
UF / AEC	< 1	< 2	< 0.1	< 100
Heat lysis / AEC / RPC	3	< 1	< 1	2.8
Filtration/RNase/AEC	< 2	< 0.1	< 0.1	< 20
Detergent / precipitation / SEC	< 1	< 1	< 0.5	< 5
PEG / AEC / SEC Filtration (diatomaceous earth) / SEC / precipitation	< 1	ND	ND	< 100
THAC HAC / IEC or HAC / THAC	**< 0.01**			**< 10**
AEC / THAC / HIC	< 0.00008	< 0.00002	< 0.00005	< 0.1

Drawings
4/5

FIGURE 5

A) Formation of open circular and depurinated forms at +25°C (experiments with LS06).



B) Formation of open circular and depurinated forms at +5°C (experiments with LS06).

