Fig. 1

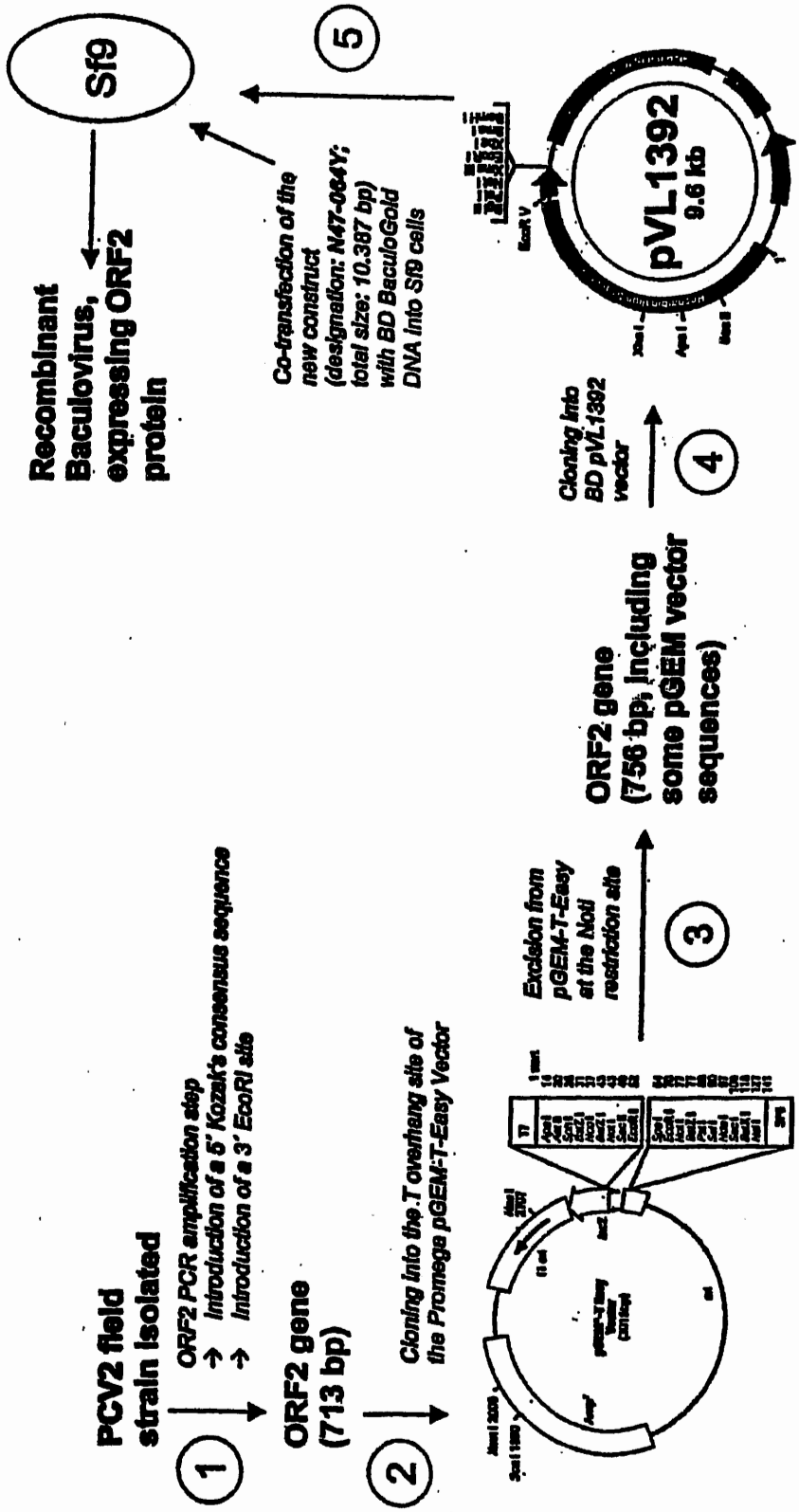

PCV2 field strain isolated
1
ORF2 PCR amplification step
→ Introduction of a 5' Kozak's consensus sequence
→ Introduction of a 3' EcoRI site
ORF2 gene (713 bp)
2
Cloning into the T overhang site of the Promega pGEM-T-Easy Vector
Excision from pGEM-T-Easy at the NotI restriction site
3
ORF2 gene (756 bp, including some pGEM vector sequences)
Cloning into BD pVL1392 vector
4
pVL1392 9.6 kb
5
Co-transfection of the new construct (designation: N47-064Y; total size: 10.387 bp) with BD BaculoGold DNA into Sf9 cells
Sf9
Recombinant Baculovirus, expressing ORF2 protein

Fig 2(a)

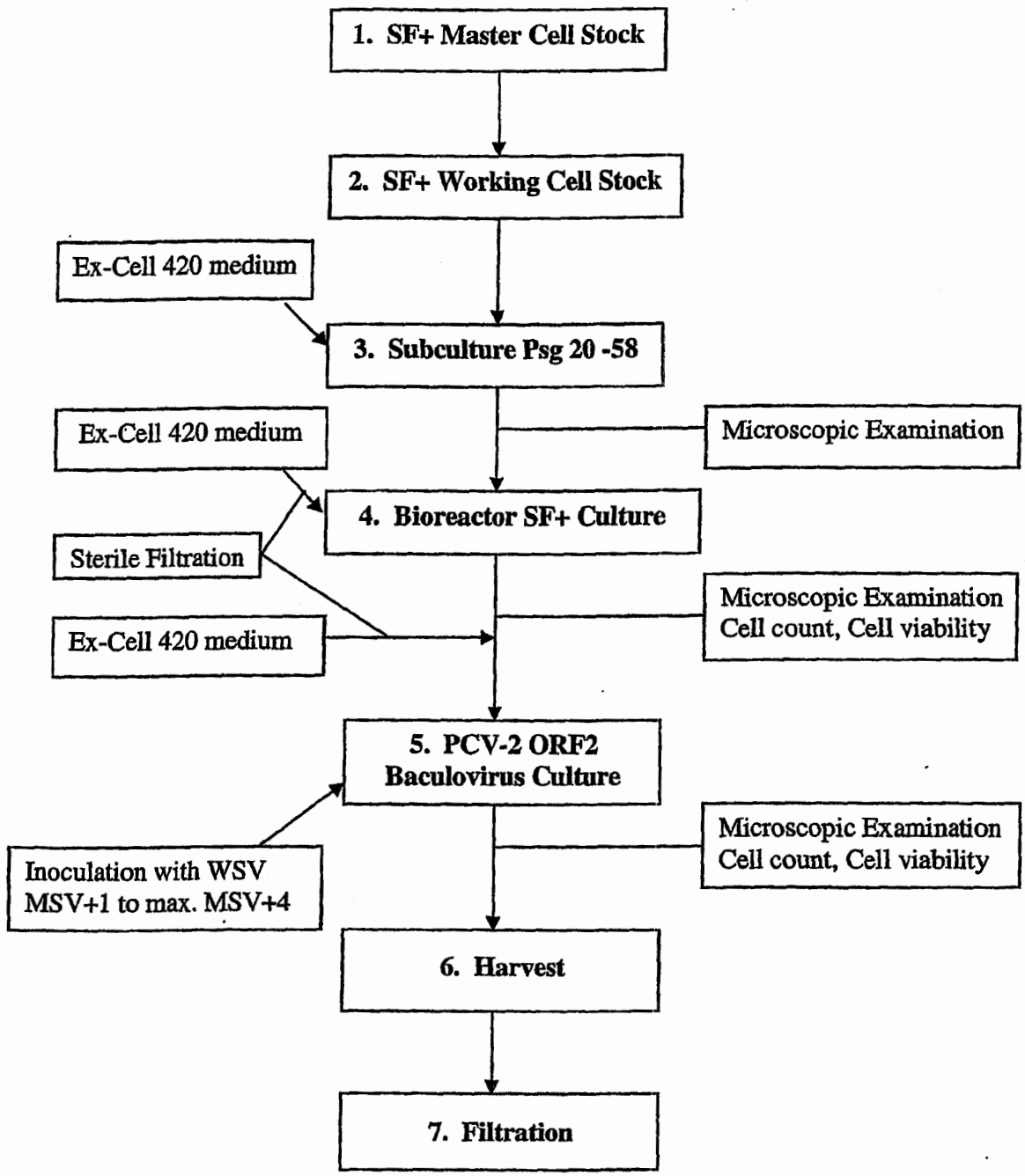
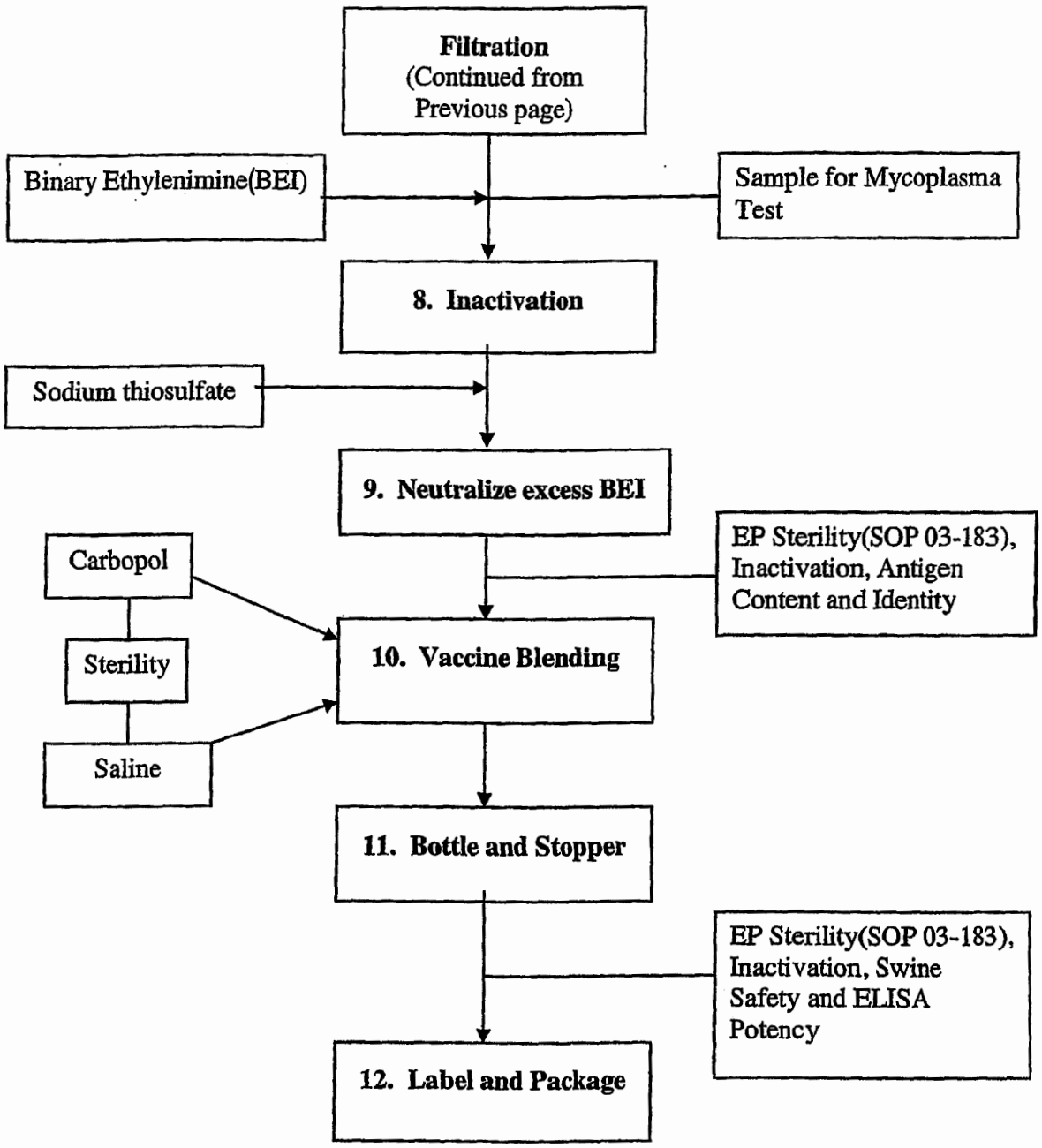

1. SF+ Master Cell Stock
2. SF+ Working Cell Stock
Ex-Cell 420 medium
3. Subculture Psg 20 -58
Ex-Cell 420 medium
Microscopic Examination
4. Bioreactor SF+ Culture
Sterile Filtration
Microscopic Examination
Cell count, Cell viability
Ex-Cell 420 medium
5. PCV-2 ORF2
Baculovirus Culture
Microscopic Examination
Cell count, Cell viability
Inoculation with WSV
MSV+1 to max. MSV+4
6. Harvest
7. Filtration

Fig 2(b)


Filtration
(Continued from
Previous page)
Binary Ethylenimine(BEI)
Sample for Mycoplasma
Test
8. Inactivation
Sodium thiosulfate
9. Neutralize excess BEI
Carbopol
EP Sterility(SOP 03-183),
Inactivation, Antigen
Content and Identity
Sterility
10. Vaccine Blending
Saline
11. Bottle and Stopper
EP Sterility(SOP 03-183),
Inactivation, Swine
Safety and ELISA
Potency
12. Label and Package