Figure 1

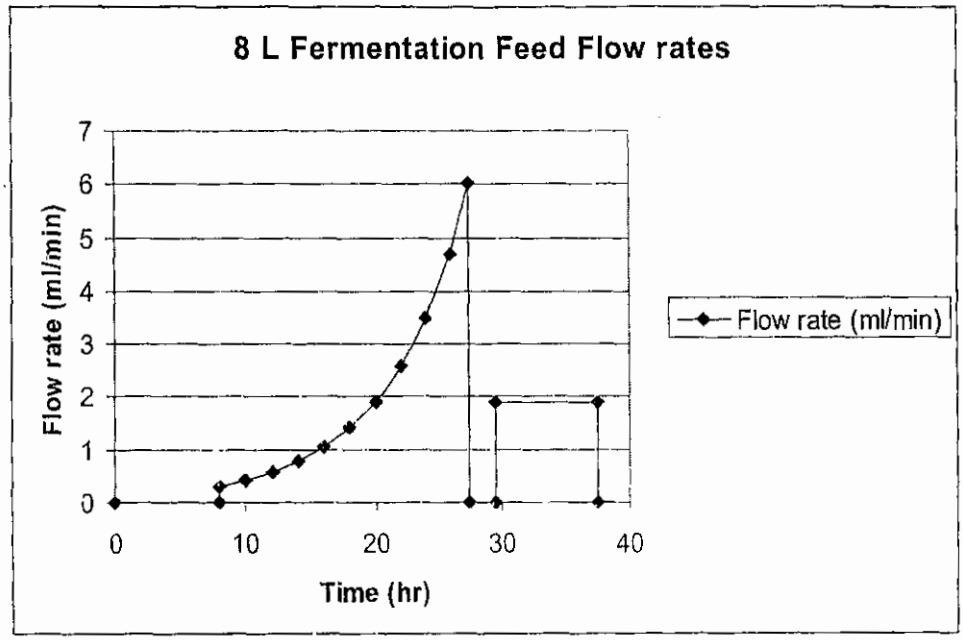
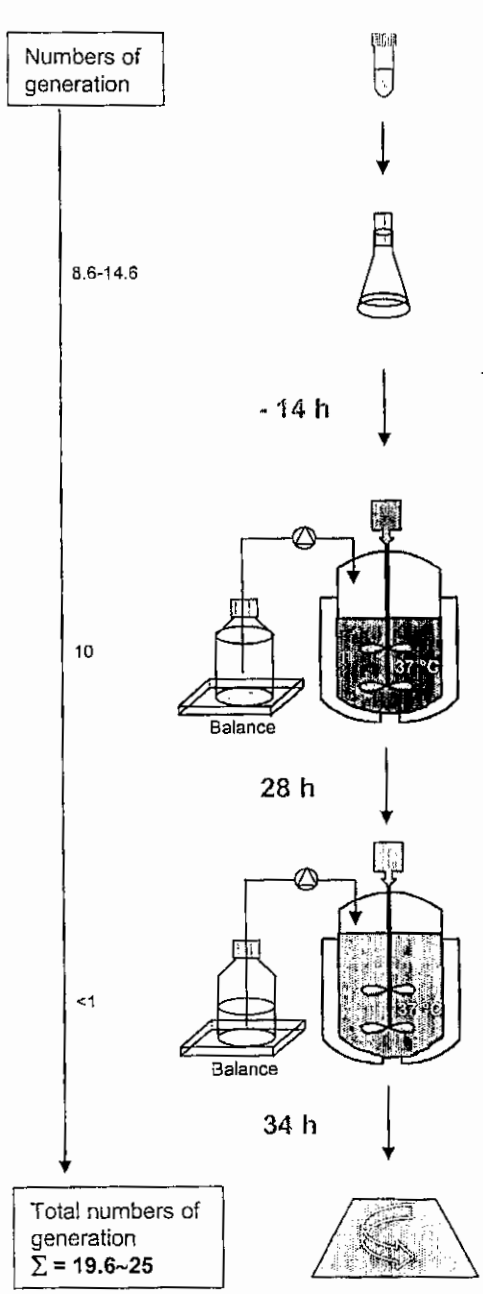

8 L Fermentation Feed Flow rates
Flow rate (ml/min)
0
1
2
3
4
5
6
7
0
10
20
30
40
Time (hr)
Flow rate (ml/min)

Figure 2

The Ambrx Upstream Process on 5-l Scale



I) Production cells
- *E. coli* glycerol stock (OD_{600} 1?)
- Using 20, 100, 500 µl

II) Pre-culture in 3 shake flasks
- Pre-culture in 0.5-l shake flask
- 100 ml batch medium with 7.5 g glucose l^{-1}
- Incubate at 37 °C and 250 RPM until OD_{600} = 4 – 5
- Residual glucose in the pre-culture medium should be more than 2 g l^{-1}

III) Main fed-batch culture
- Medium: Batch medium starting volume 2 l
- Inoculate the fermentor to an initial OD_{600} 0.2
- Approx. 8 h batch phase until pO_2 spikes
- 20 h fed-batch phase with the feeding profile:
  S_f = 322 g glucose l^{-1} ?
  F_0 = 9.6 ml h^{-1} (1.5 g glucose l^{-1} h^{-1} ?)
  μ_{set} = 0.15 h^{-1} for 20 h
  F_{max} = 192.8 ml h^{-1} (19.3 g glucose l^{-1} h^{-1} ?)

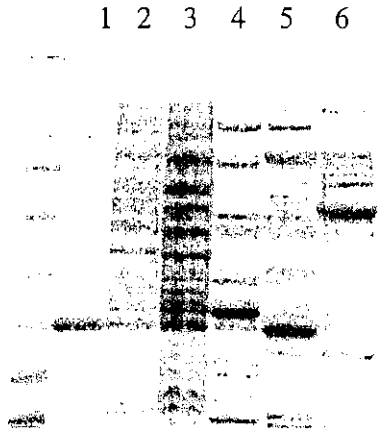
IV) Induction
- The culture reaches an OD_{600} about 120.
- At 2 h b.i. start YE feed.
- At 1 h b.i. add 200 ml bolus 40% glycerol and decrease glucose feed to maintain pO_2 at 30%.
- At the time of induction, **a**) stop glucose feed, **b**) add 14 ml pAF, and **c**) induce with 4 ml bolus of 20% arabinose
- At 2 h a.i. start 40% glycerol feed at 114 ml h^{-1}
- Induce for 4 h.

V) Harvest
- After 4 h induction
- The culture remains at about 120 OD_{600}
- Cool the fermentor to 10 °C
- Centrifuge 2 x 200 ml of culture cells
- Pack and freeze cells in plast bag at -80 °C
- Ship 200 ml cells to Ambrx by World Courier

Figure 3

hGH Periplasmic Release vs. Homogenization

A. Periplasmic Release



B. Lysis by Microfluidizer

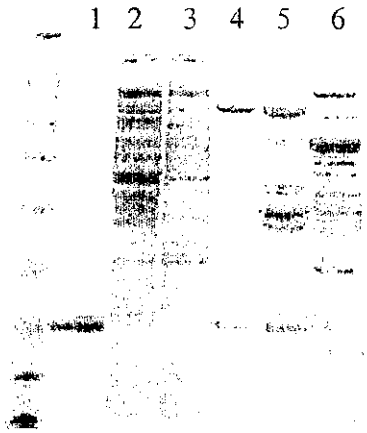


Figure 4

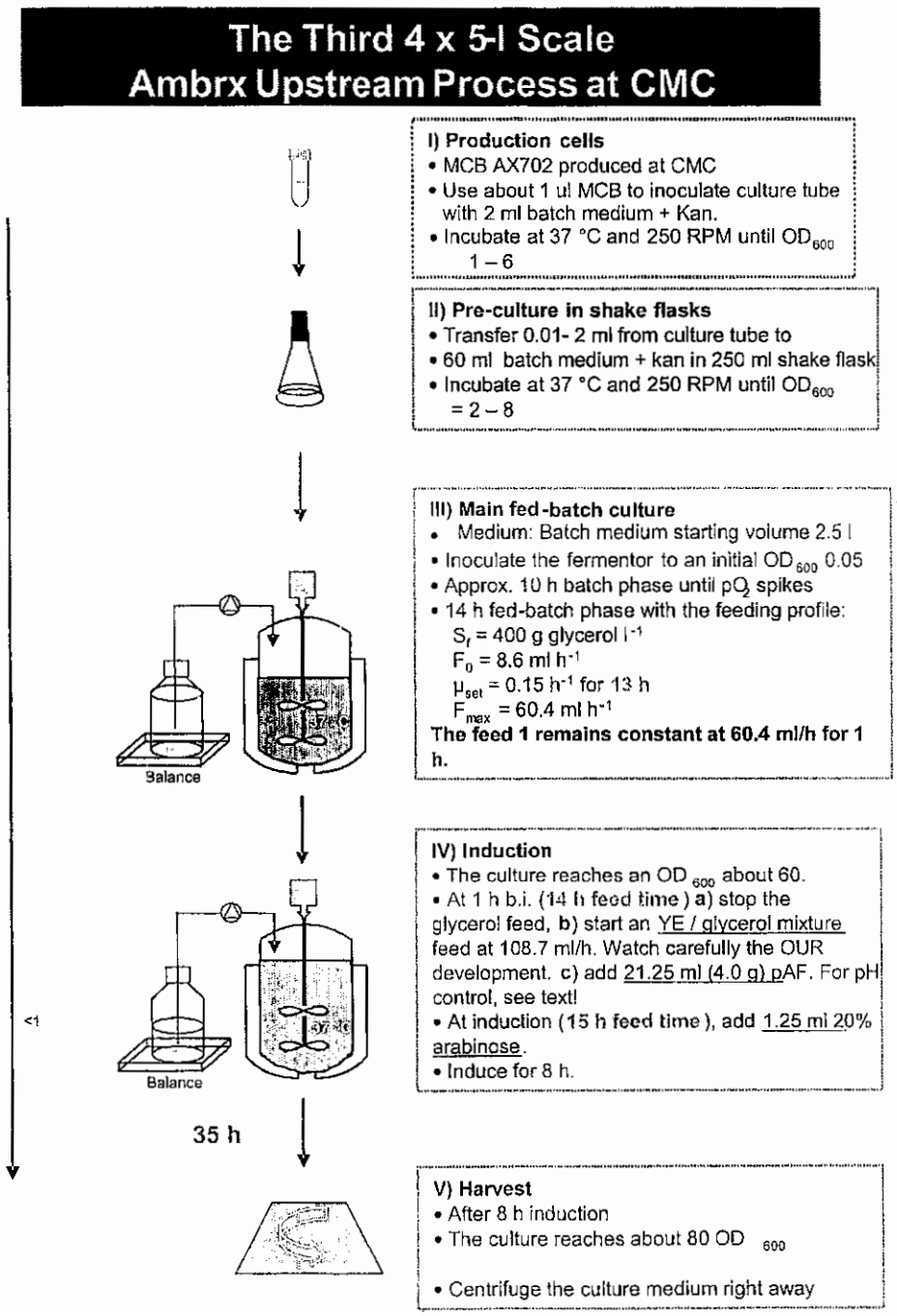
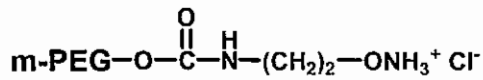

The Third 4 x 5-l Scale
Ambrx Upstream Process at CMC
I) Production cells
• MCB AX702 produced at CMC
• Use about 1 ul MCB to inoculate culture tube with 2 ml batch medium + Kan.
• Incubate at 37 °C and 250 RPM until OD600 1 – 6
II) Pre-culture in shake flasks
• Transfer 0.01- 2 ml from culture tube to
• 60 ml batch medium + kan in 250 ml shake flask
• Incubate at 37 °C and 250 RPM until OD600 = 2 – 8
III) Main fed-batch culture
• Medium: Batch medium starting volume 2.5 l
• Inoculate the fermentor to an initial OD600 0.05
• Approx. 10 h batch phase until pO2 spikes
• 14 h fed-batch phase with the feeding profile:
Sf = 400 g glycerol l-1
F0 = 8.6 ml h-1
μset = 0.15 h-1 for 13 h
Fmax = 60.4 ml h-1
The feed 1 remains constant at 60.4 ml/h for 1 h.
Balance
IV) Induction
• The culture reaches an OD 600 about 60.
• At 1 h b.i. (14 h feed time) a) stop the glycerol feed, b) start an YE / glycerol mixture feed at 108.7 ml/h. Watch carefully the OUR development. c) add 21.25 ml (4.0 g) pAF. For pH control, see text!
• At induction (15 h feed time), add 1.25 ml 20% arabinose.
• Induce for 8 h.
Balance
35 h
V) Harvest
• After 8 h induction
• The culture reaches about 80 OD 600
• Centrifuge the culture medium right away

Figure 5



linear, 30 kDa monomethoxy-poly(ethylene glycol)-2-aminooxy ethylamine carbamate hydrochloride