Figure 1

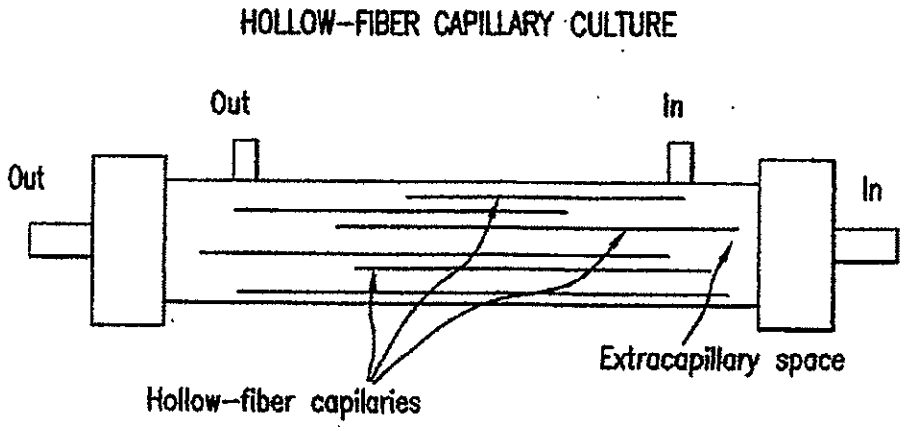

HOLLOW-FIBER CAPILLARY CULTURE
Out
In
Out
In
Extracapillary space
Hollow-fiber capilaries

Figure 2

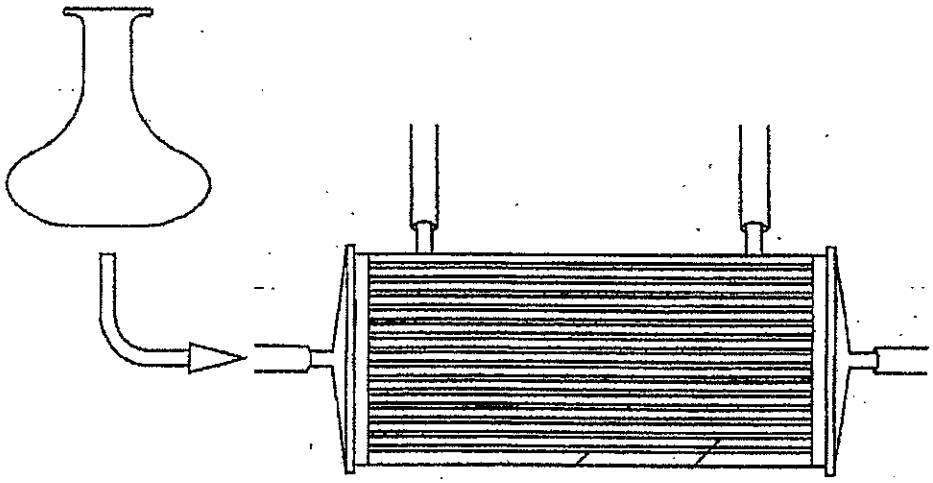


Figure 3A

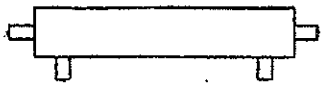


Figure 3B

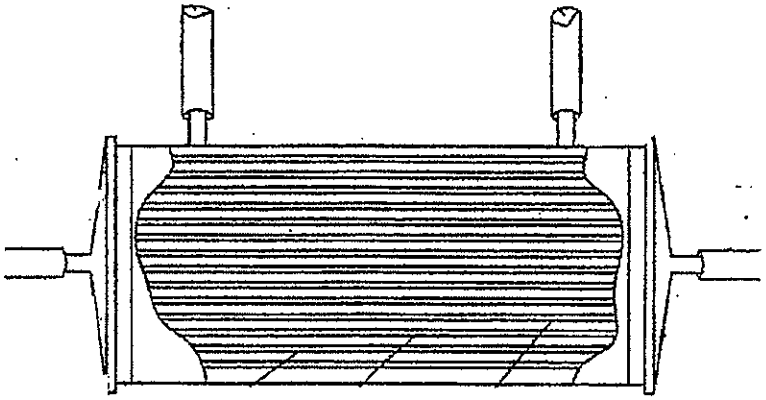


Figure 4

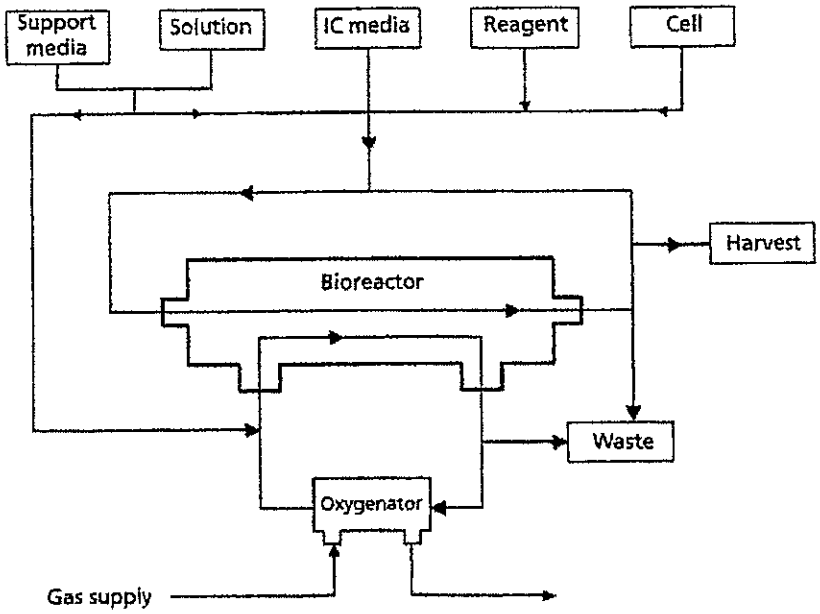

Support media
Solution
IC media
Reagent
Cell
Harvest
Bioreactor
Waste
Oxygenator
Gas supply

Figure 5

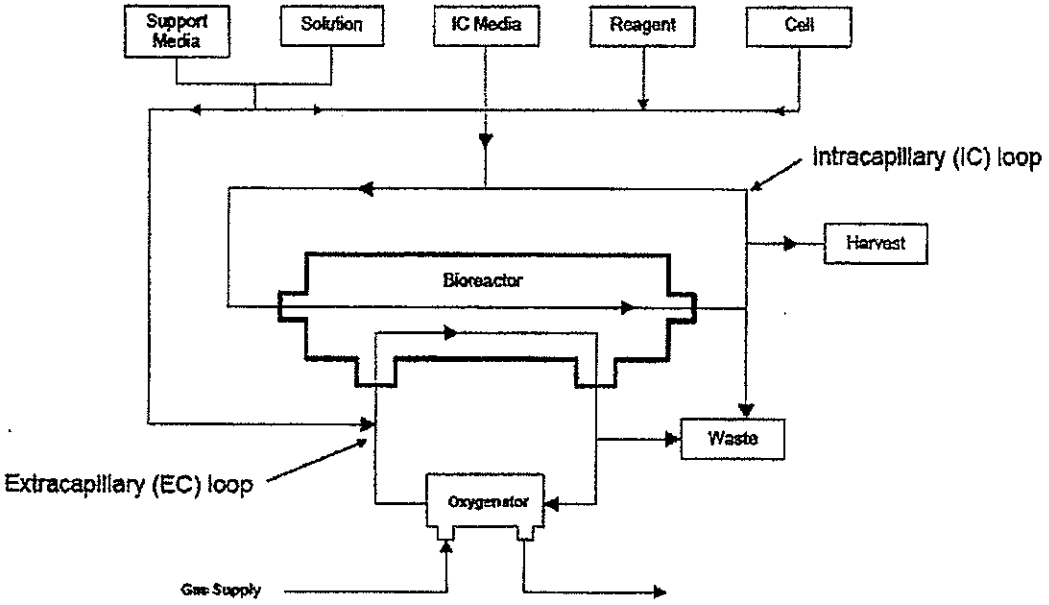

Support Media
Solution
IC Media
Reagent
Cell
Intracapillary (IC) loop
Harvest
Bioreactor
Waste
Extracapillary (EC) loop
Oxygenator
Gas Supply

Figure 6

Isolation and expansion for 3 different donors

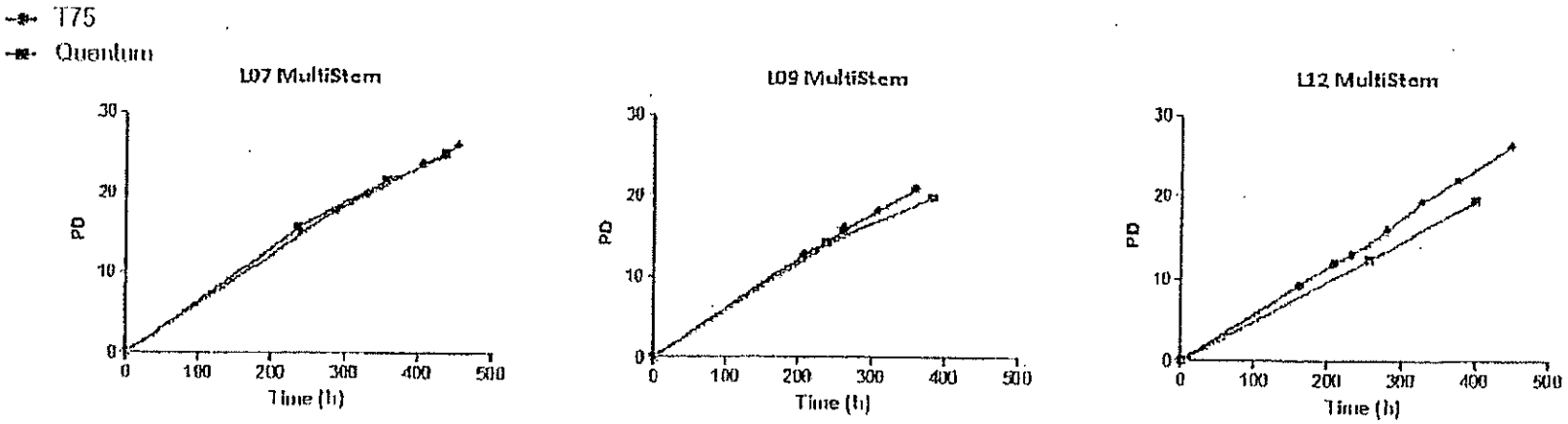
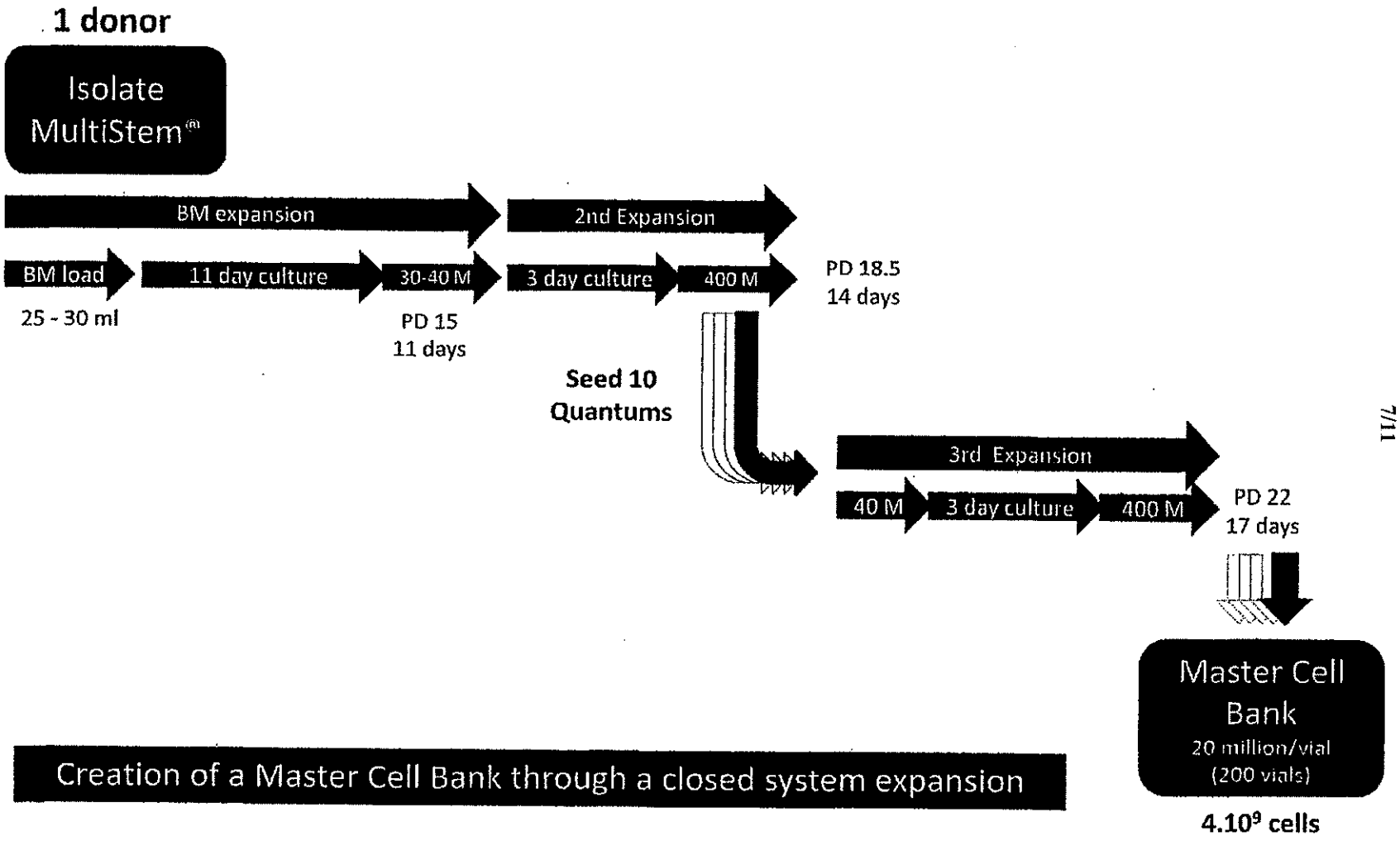


Figure 7

Master Cell bank creation



Creation of a Master Cell Bank through a closed system expansion

Figure 8

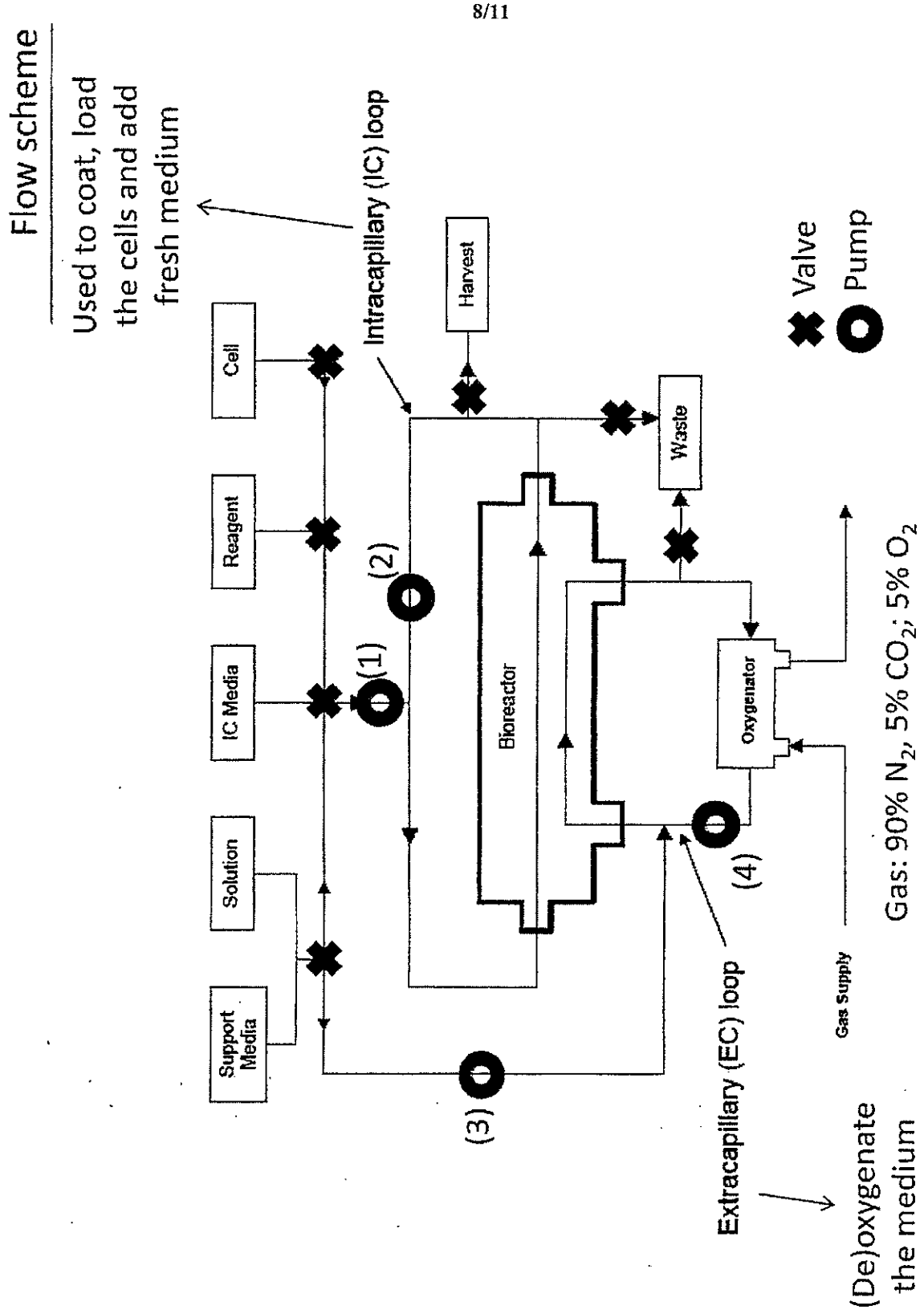

Flow scheme
Used to coat, load the cells and add fresh medium
Intracapillary (IC) loop
Cell
Reagent
IC Media
Solution
Support Media
(1)
(2)
(3)
(4)
Harvest
Waste
Bioreactor
Oxygenator
Gas Supply
Valve
Pump
Gas: 90% N_2, 5% CO_2; 5% O_2
Extracapillary (EC) loop
(De)oxygenate the medium

Figure 9

Procedure

- Software provides automated procedures
- Setup
  - Load and prime disposable set
  - Coat the bioreactor membrane with fibronectin (overnight coating)
  - Replace PBS in both loops by media
  - Medium conditioning
  - Load the cells and let them attach (overnight attachment)
  - Perform a washout to remove non adherent cells
  - Continuously feed cells with medium
  - Adjust feed rate according to lactate generation and glucose consumption
  - Harvest with Trypsin/EDTA
- Workload
  - 1 run takes 6 working hours
    - An estimate of 2 to 3 hours if everything would already be available in bags

Figure 10

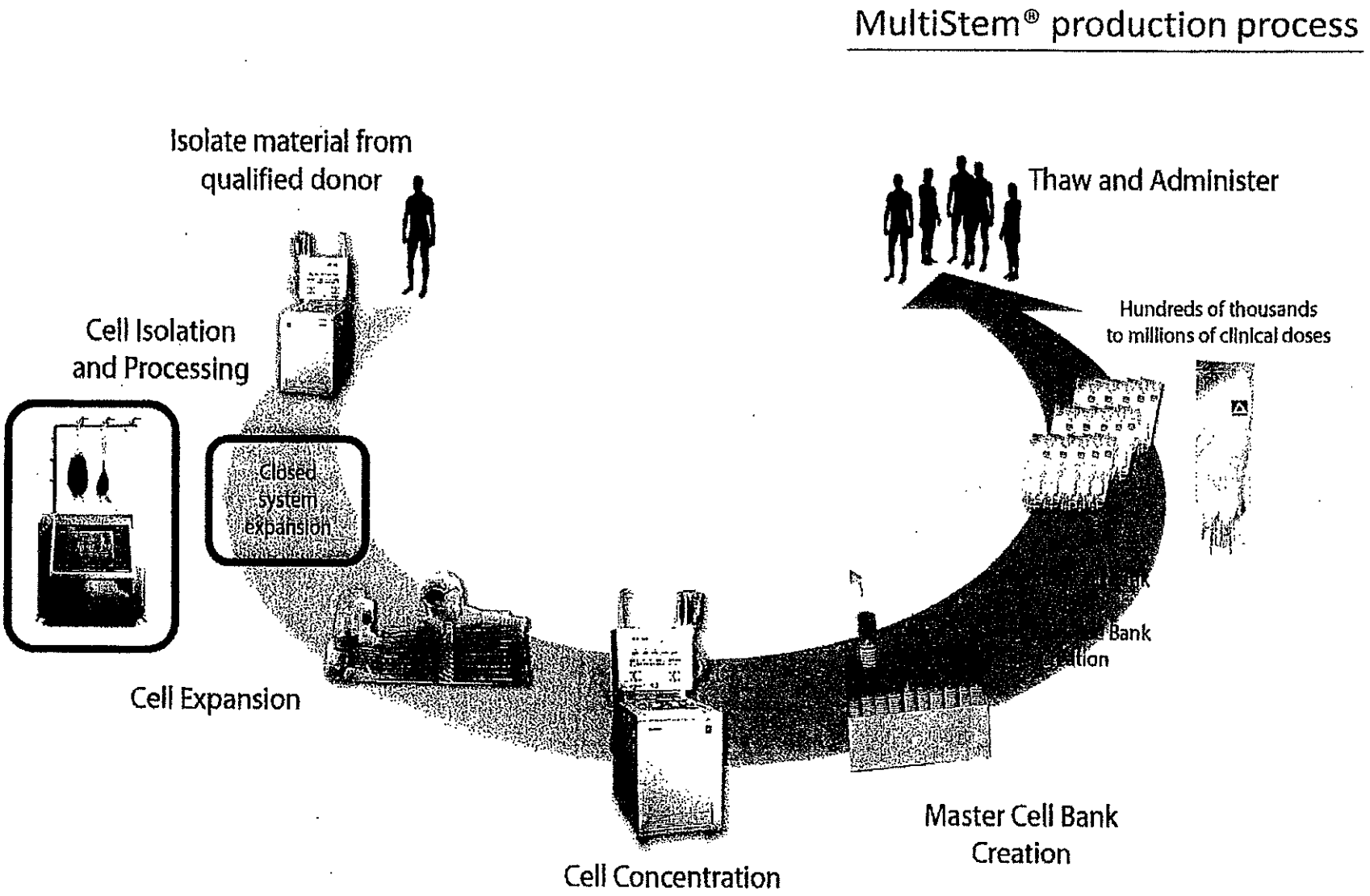
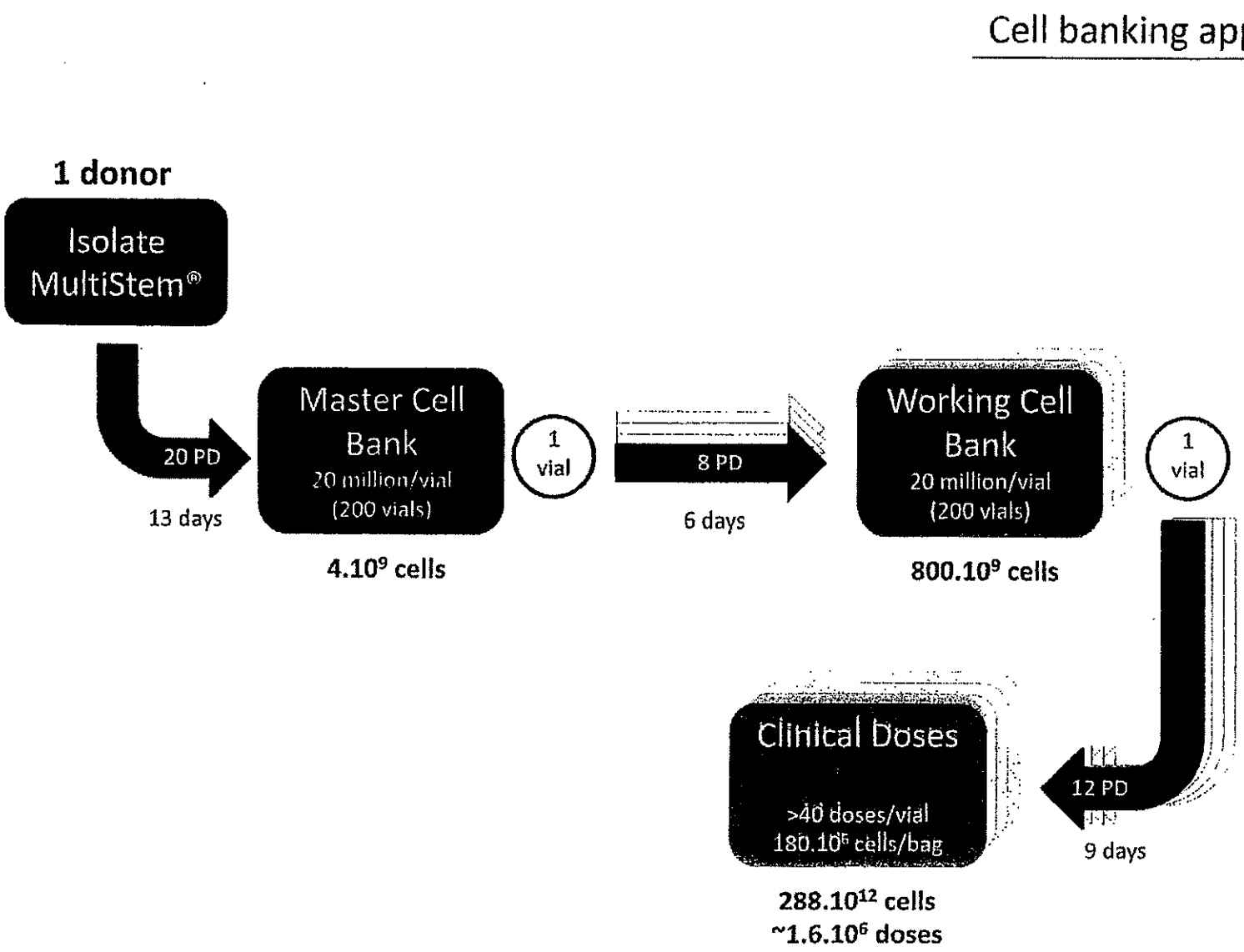

MultiStem® production process
Isolate material from qualified donor
Thaw and Administer
Cell Isolation and Processing
Hundreds of thousands to millions of clinical doses
Closed system expansion
Cell Expansion
Cell Concentration
Master Cell Bank Creation

Figure 11


Cell banking approach
1 donor
Isolate MultiStem®
20 PD
13 days
Master Cell Bank
20 million/vial
(200 vials)
4.10⁹ cells
1 vial
8 PD
6 days
Working Cell Bank
20 million/vial
(200 vials)
800.10⁹ cells
1 vial
12 PD
9 days
Clinical Doses
>40 doses/vial
180.10⁶ cells/bag
288.10¹² cells
~1.6.10⁶ doses