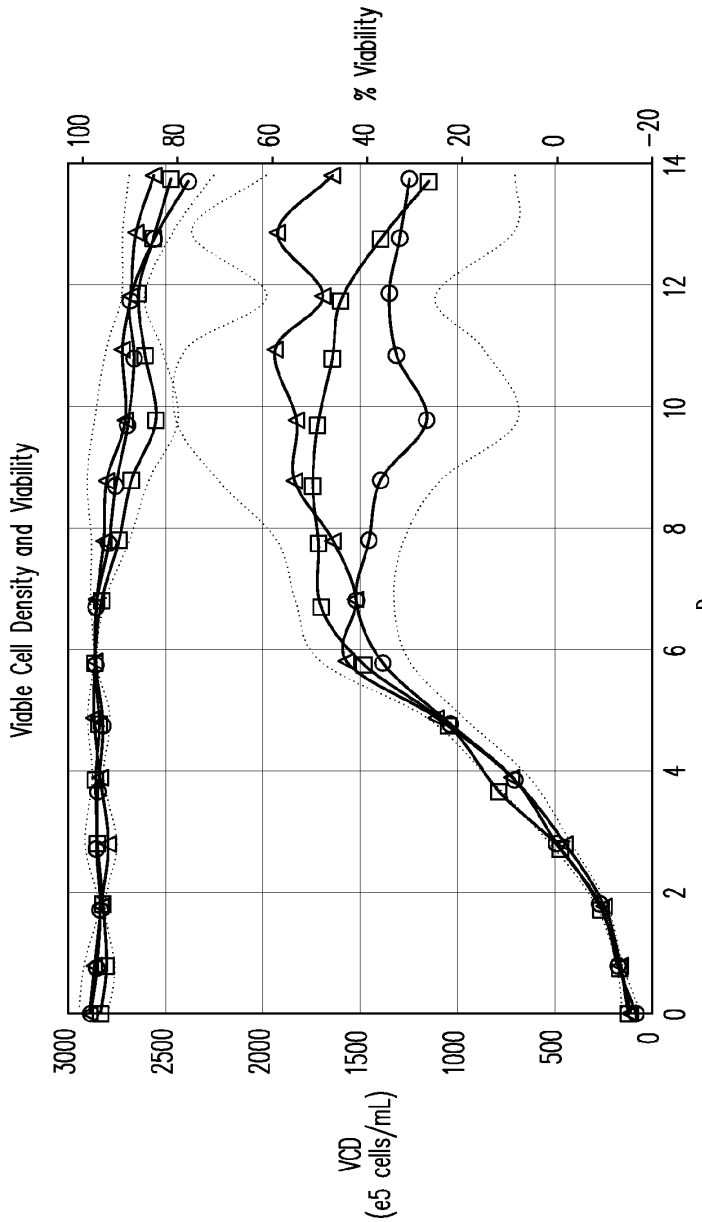

Viable Cell Density and Viability
VCD
(e5 cells/mL)
% Viability
Day
3000
2500
2000
1500
1000
500
0
100
80
60
40
20
0
−20
0
2
4
6
8
10
12
14

FIG. 1

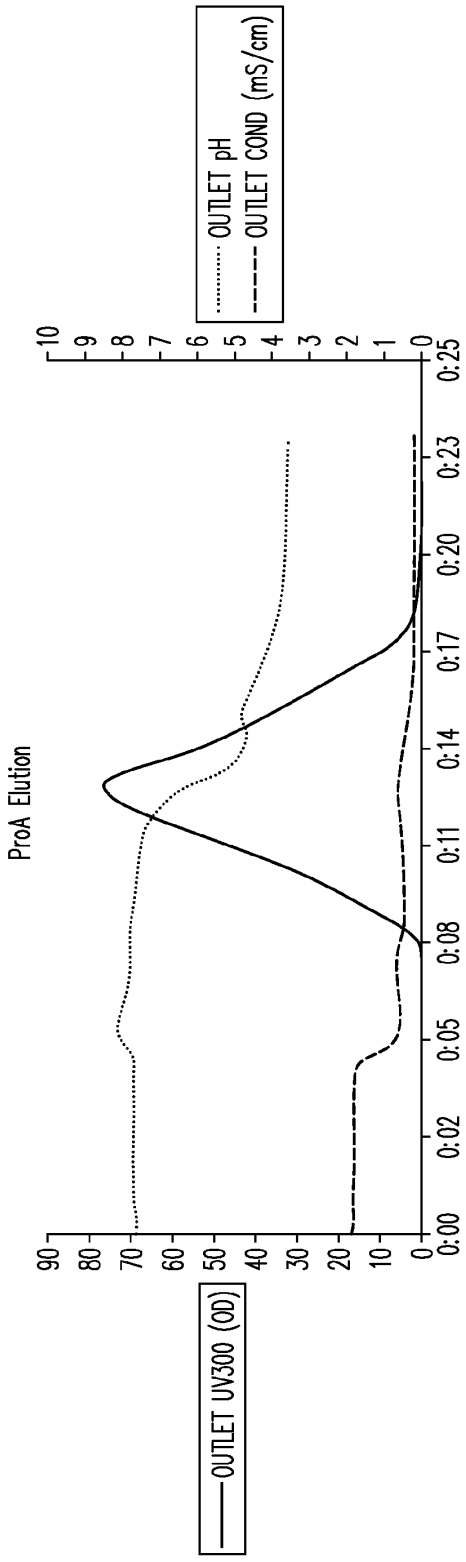

OUTLET pH
OUTLET COND (mS/cm)
OUTLET UV300 (OD)
ProA Elution
NORMALIZED TIME (hr:mm)
0:00
0:02
0:05
0:08
0:11
0:14
0:17
0:20
0:23
0:25
0
10
20
30
40
50
60
70
80
90
0
1
2
3
4
5
6
7
8
9
10

FIG. 2

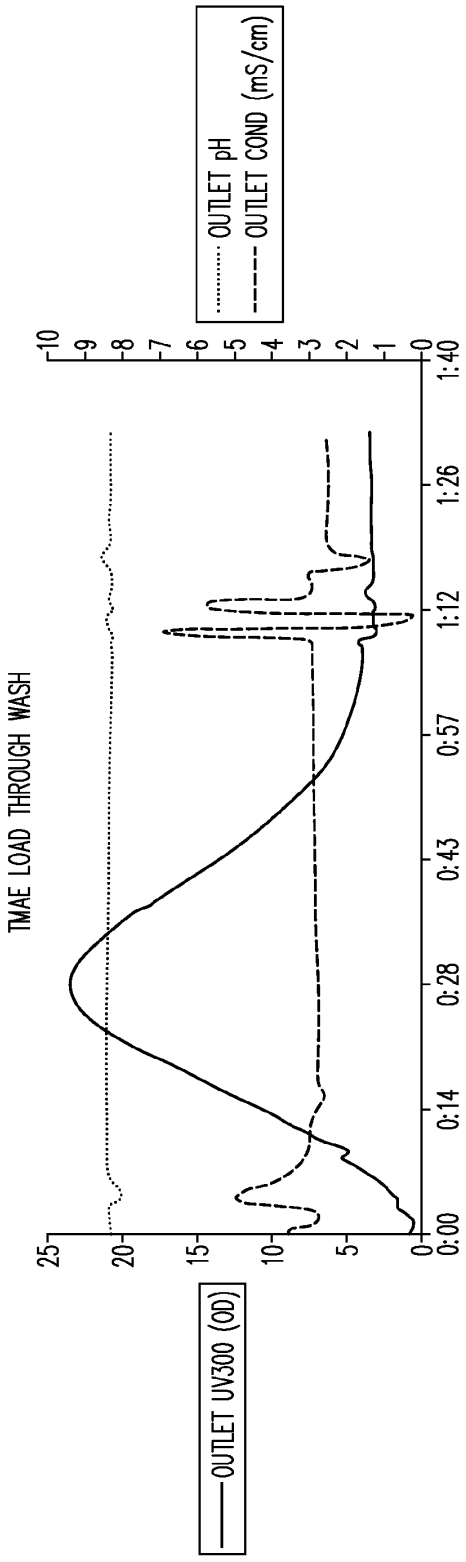

OUTLET pH
OUTLET COND (mS/cm)
OUTLET UV300 (OD)
TMAE LOAD THROUGH WASH
NORMALIZED TIME (hr:mm)
0:00
0:14
0:28
0:43
0:57
1:12
1:26
1:40
0
5
10
15
20
25
0
1
2
3
4
5
6
7
8
9
10

FIG. 3

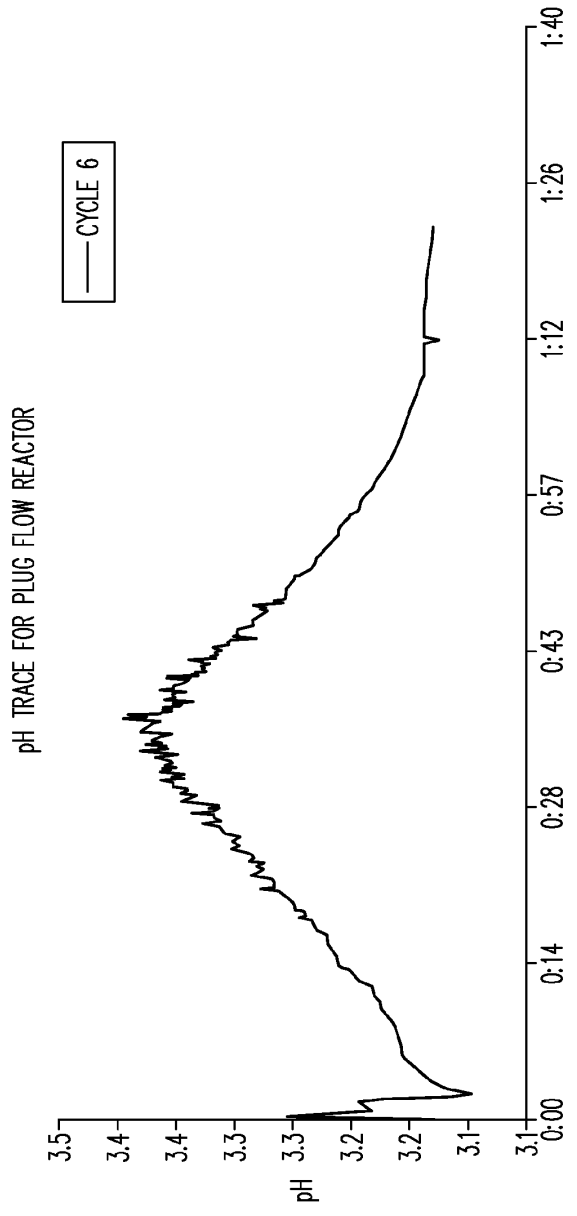

pH TRACE FOR PLUG FLOW REACTOR
CYCLE 6
pH
3.5
3.4
3.4
3.3
3.3
3.2
3.2
3.1
3.1
0:00
0:14
0:28
0:43
0:57
1:12
1:26
1:40
NORMALIZED TIME (hr:mm)

FIG. 4

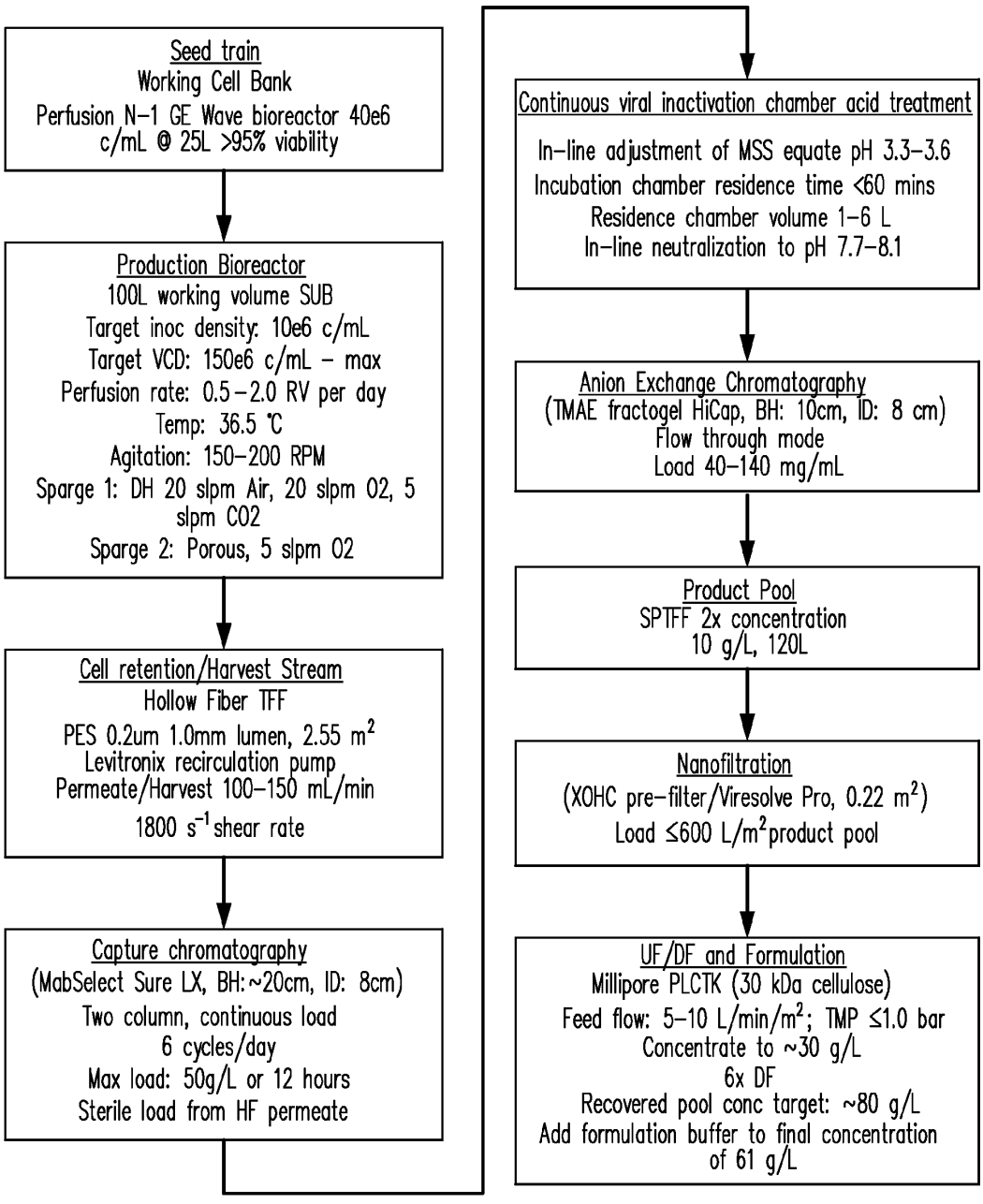

Seed train
Working Cell Bank
Perfusion N-1 GE Wave bioreactor 40e6 c/mL @ 25L >95% viability
Production Bioreactor
100L working volume SUB
Target inoc density: 10e6 c/mL
Target VCD: 150e6 c/mL – max
Perfusion rate: 0.5 – 2.0 RV per day
Temp: 36.5 °C
Agitation: 150–200 RPM
Sparge 1: DH 20 slpm Air, 20 slpm O2, 5 slpm CO2
Sparge 2: Porous, 5 slpm O2
Cell retention/Harvest Stream
Hollow Fiber TFF
PES 0.2um 1.0mm lumen, 2.55 m2
Levitronix recirculation pump
Permeate/Harvest 100–150 mL/min
1800 s-1 shear rate
Capture chromatography
(MabSelect Sure LX, BH:~20cm, ID: 8cm)
Two column, continuous load
6 cycles/day
Max load: 50g/L or 12 hours
Sterile load from HF permeate
Continuous viral inactivation chamber acid treatment
In-line adjustment of MSS equate pH 3.3–3.6
Incubation chamber residence time <60 mins
Residence chamber volume 1–6 L
In-line neutralization to pH 7.7–8.1
Anion Exchange Chromatography
(TMAE fractogel HiCap, BH: 10cm, ID: 8 cm)
Flow through mode
Load 40–140 mg/mL
Product Pool
SPTFF 2x concentration
10 g/L, 120L
Nanofiltration
(XOHC pre-filter/Viresolve Pro, 0.22 m2)
Load ≤600 L/m2 product pool
UF/DF and Formulation
Millipore PLCTK (30 kDa cellulose)
Feed flow: 5–10 L/min/m2; TMP ≤1.0 bar
Concentrate to ~30 g/L
6x DF
Recovered pool conc target: ~80 g/L
Add formulation buffer to final concentration of 61 g/L

FIG. 5

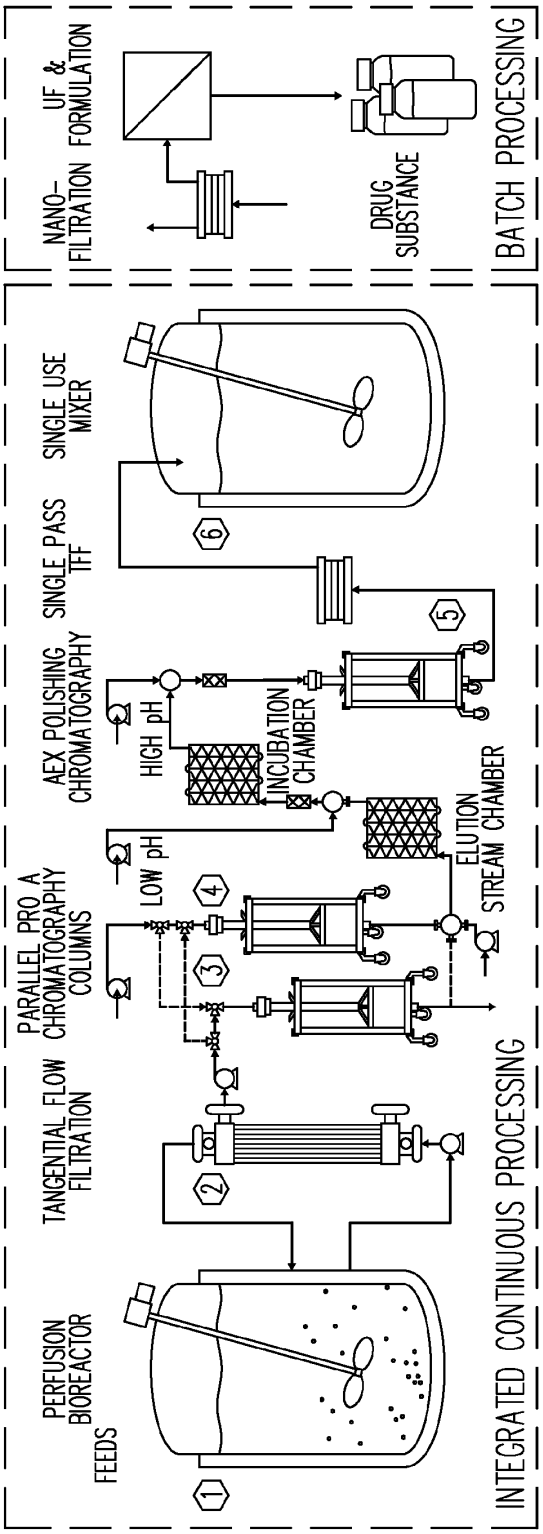

INTEGRATED CONTINUOUS PROCESSING
BATCH PROCESSING
PERFUSION BIOREACTOR
FEEDS
TANGENTIAL FLOW FILTRATION
PARALLEL PRO A CHROMATOGRAPHY COLUMNS
LOW pH
HIGH pH
AEX POLISHING CHROMATOGRAPHY
INCUBATION CHAMBER
ELUTION STREAM CHAMBER
SINGLE PASS TFF
SINGLE USE MIXER
NANO-FILTRATION
UF & FORMULATION
DRUG SUBSTANCE
1
2
3
4
5
6

FIG. 6

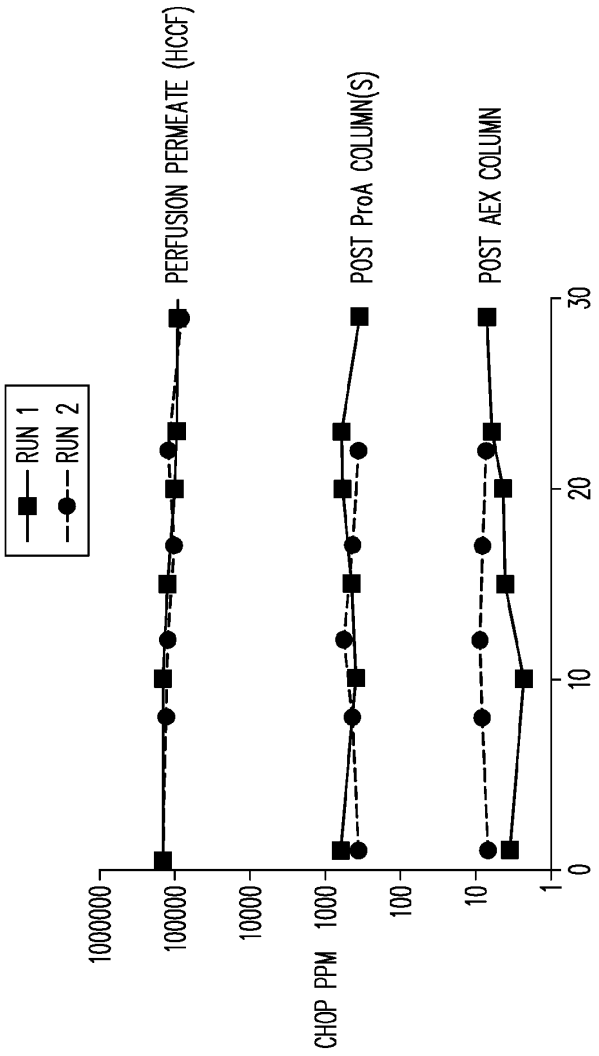

RUN 1
RUN 2
PERFUSION PERMEATE (HCCF)
POST ProA COLUMN(S)
POST AEX COLUMN
CHOP PPM
1000000
100000
10000
1000
100
10
1
0
10
20
30
CYCLE #

FIG. 7

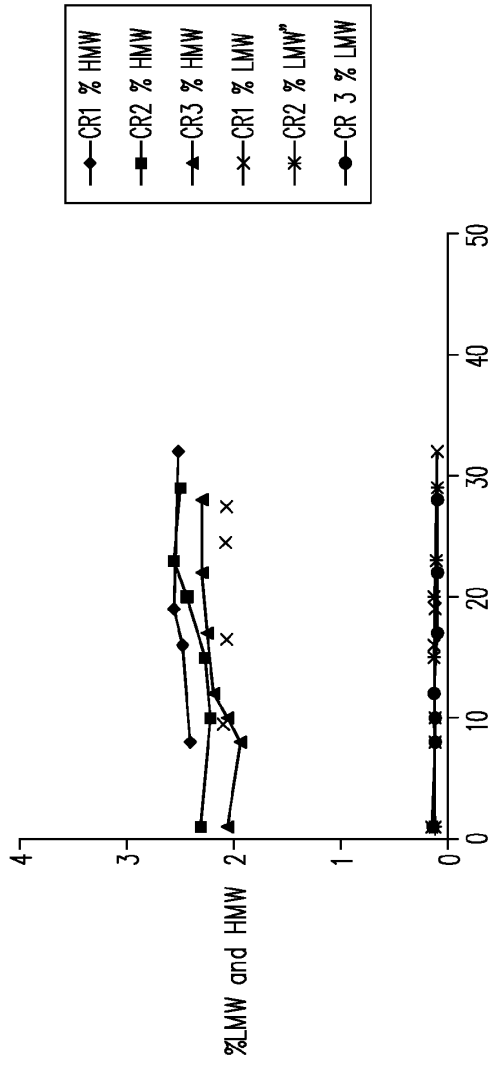

CR1 % HMW
CR2 % HMW
CR3 % HMW
CR1 % LMW
CR2 % LMW"
CR 3 % LMW
%LMW and HMW
Cycle #
0
1
2
3
4
0
10
20
30
40
50

FIG. 8

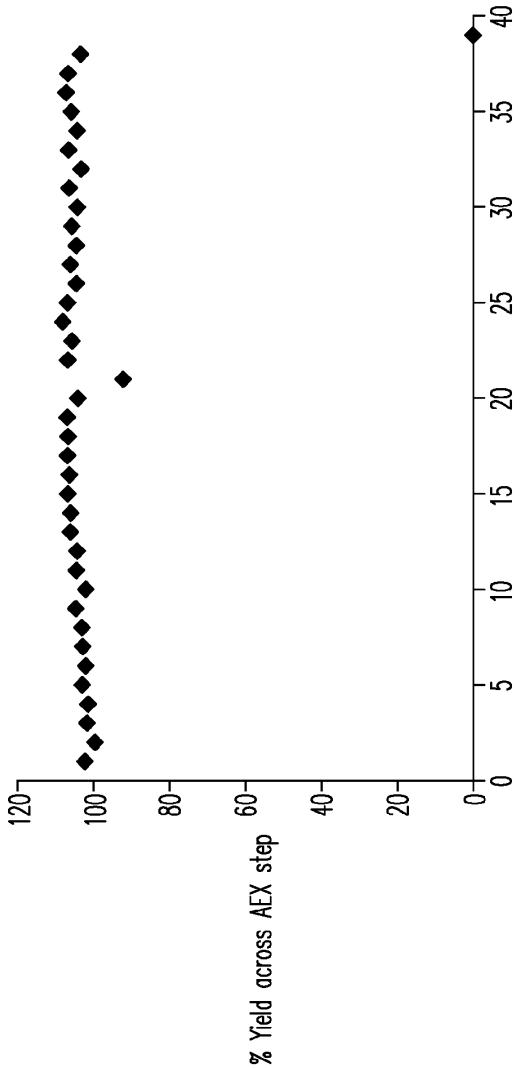

% Yield across AEX step
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
Cycle

FIG. 9