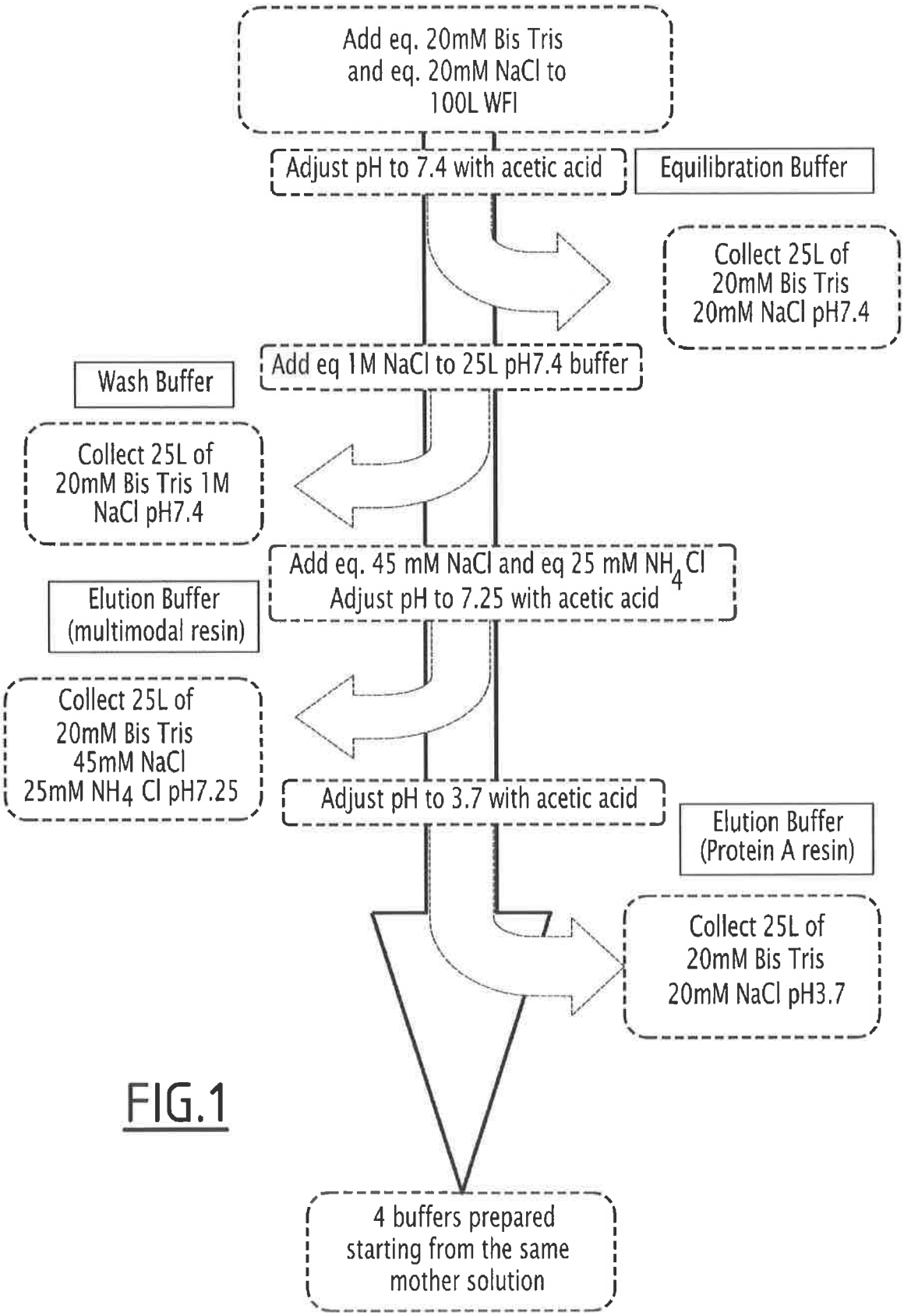

Add eq. 20mM Bis Tris and eq. 20mM NaCl to 100L WFI
Adjust pH to 7.4 with acetic acid
Equilibration Buffer
Collect 25L of 20mM Bis Tris 20mM NaCl pH7.4
Add eq 1M NaCl to 25L pH7.4 buffer
Wash Buffer
Collect 25L of 20mM Bis Tris 1M NaCl pH7.4
Add eq. 45 mM NaCl and eq 25 mM NH4Cl Adjust pH to 7.25 with acetic acid
Elution Buffer (multimodal resin)
Collect 25L of 20mM Bis Tris 45mM NaCl 25mM NH4 Cl pH7.25
Adjust pH to 3.7 with acetic acid
Elution Buffer (Protein A resin)
Collect 25L of 20mM Bis Tris 20mM NaCl pH3.7
4 buffers prepared starting from the same mother solution

FIG.1

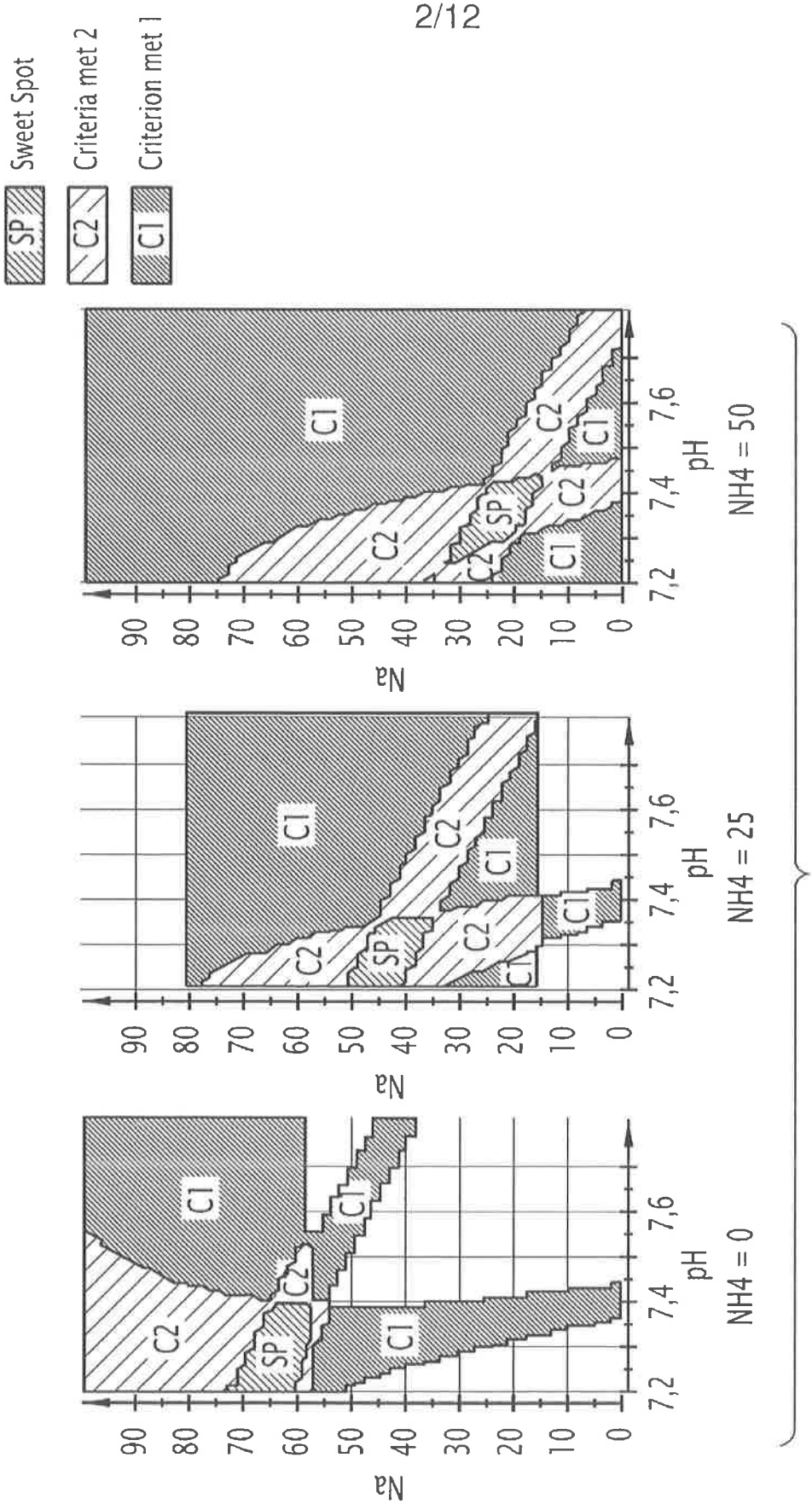

Sweet Spot
Criteria met 2
Criterion met 1
SP
C2
C1
Na
pH
NH4 = 0
NH4 = 25
NH4 = 50

FIG.2

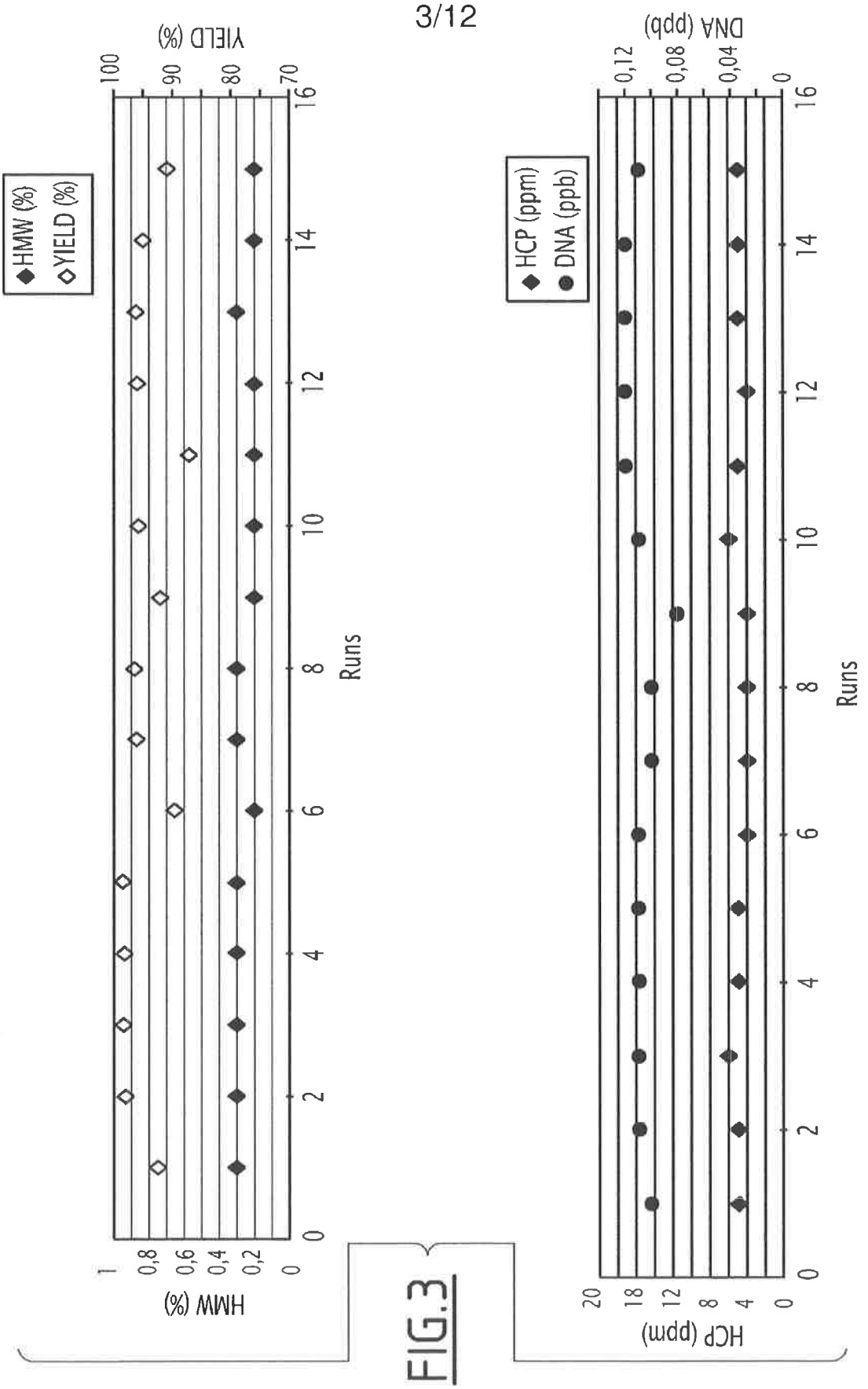


FIG.3

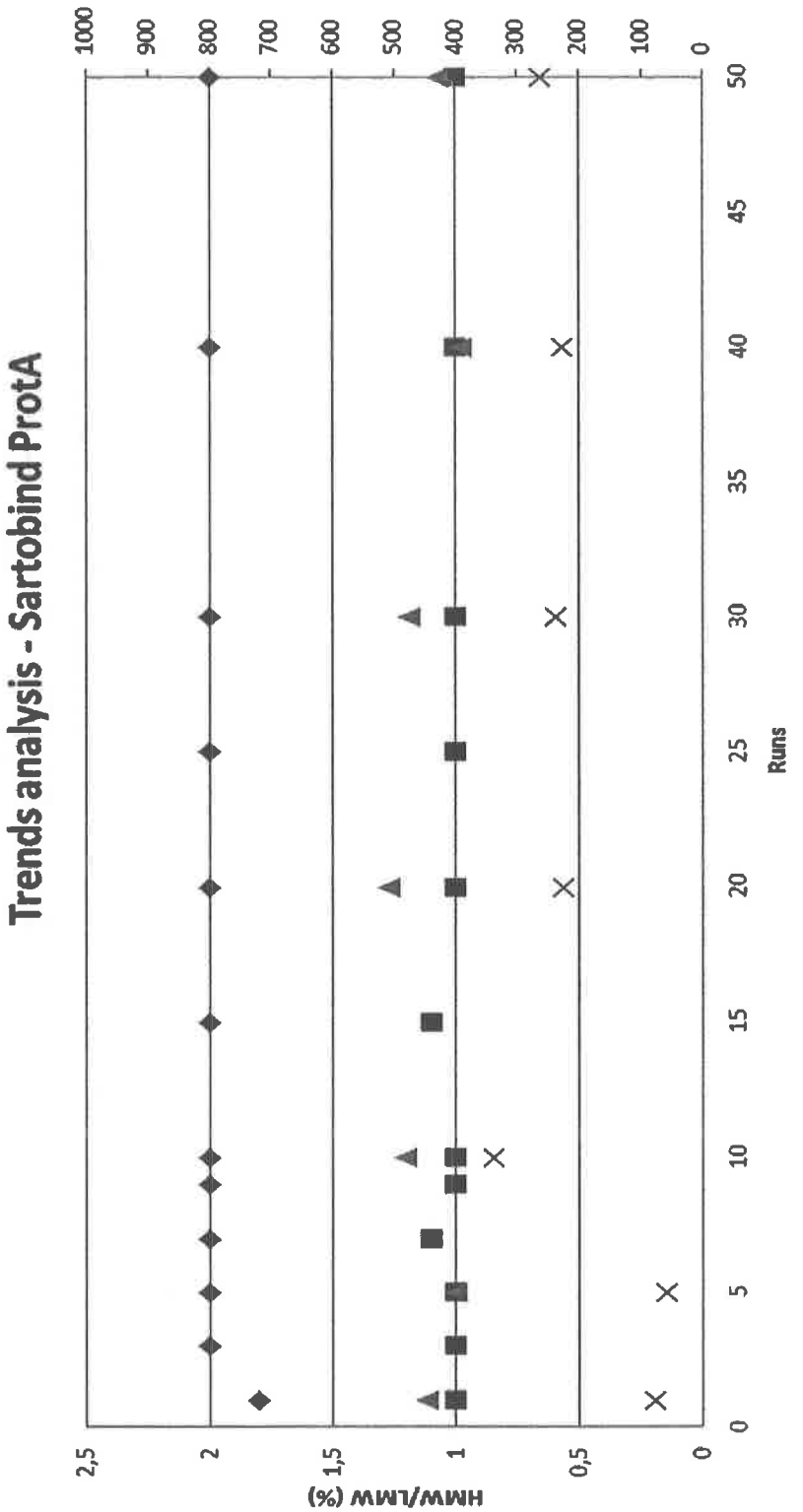

Trends analysis - Sartobind ProtA
HMW
LMW
HCP
DNA
HMW/LMW (%)
Runs
2,5
2
1,5
1
0,5
0
1000
900
800
700
600
500
400
300
200
100
0
50
45
40
35
30
25
20
15
10
5
0

FIG.4

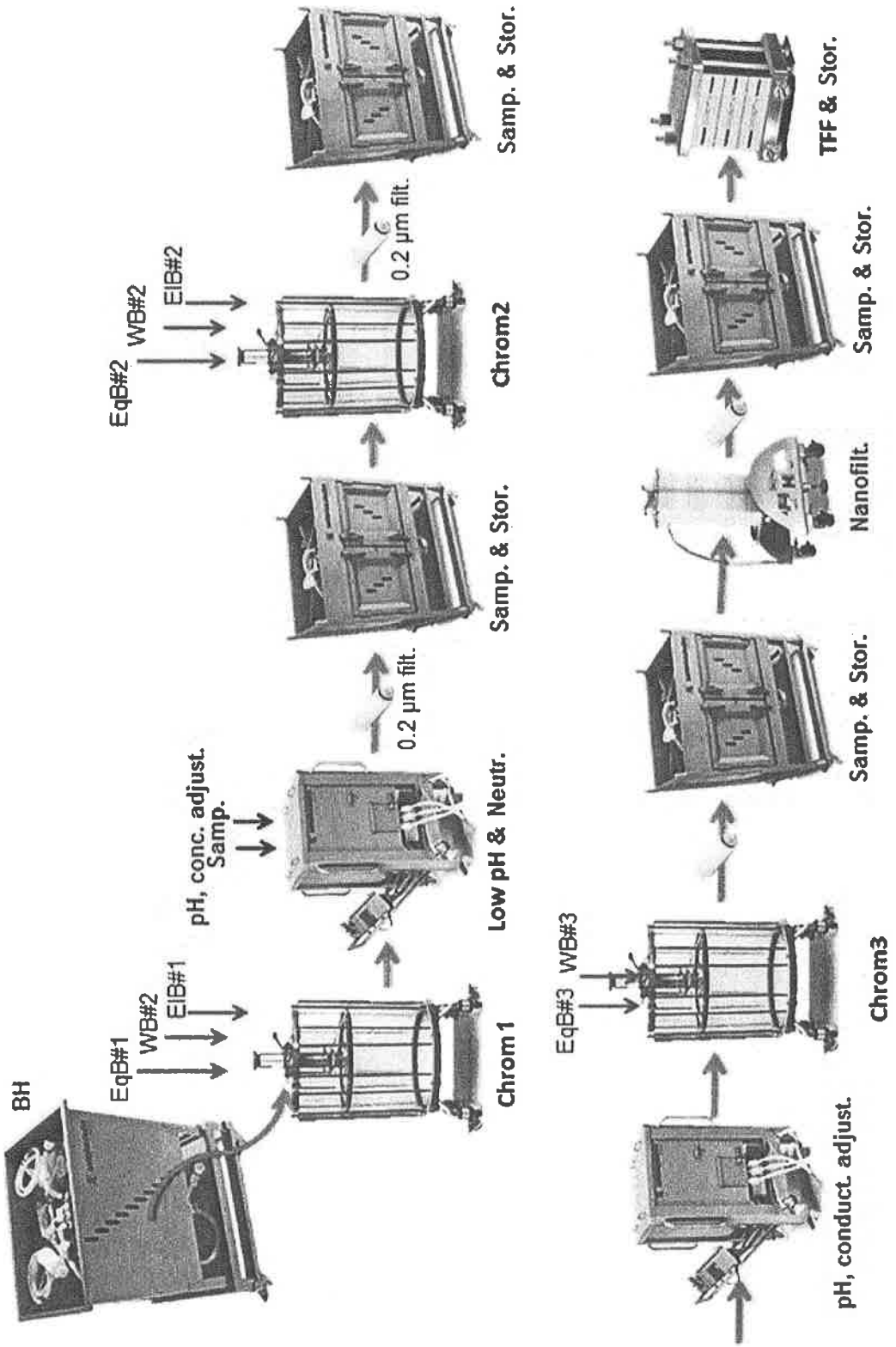

BH
EqB#1
WB#2
ElB#1
Chrom 1
pH, conc. adjust.
Samp.
Low pH & Neutr.
0.2 µm filt.
Samp. & Stor.
EqB#2
WB#2
ElB#2
Chrom2
0.2 µm filt.
Samp. & Stor.
EqB#3
WB#3
pH, conduct. adjust.
Chrom3
Samp. & Stor.
Nanofilt.
Samp. & Stor.
TFF & Stor.

FIG.5

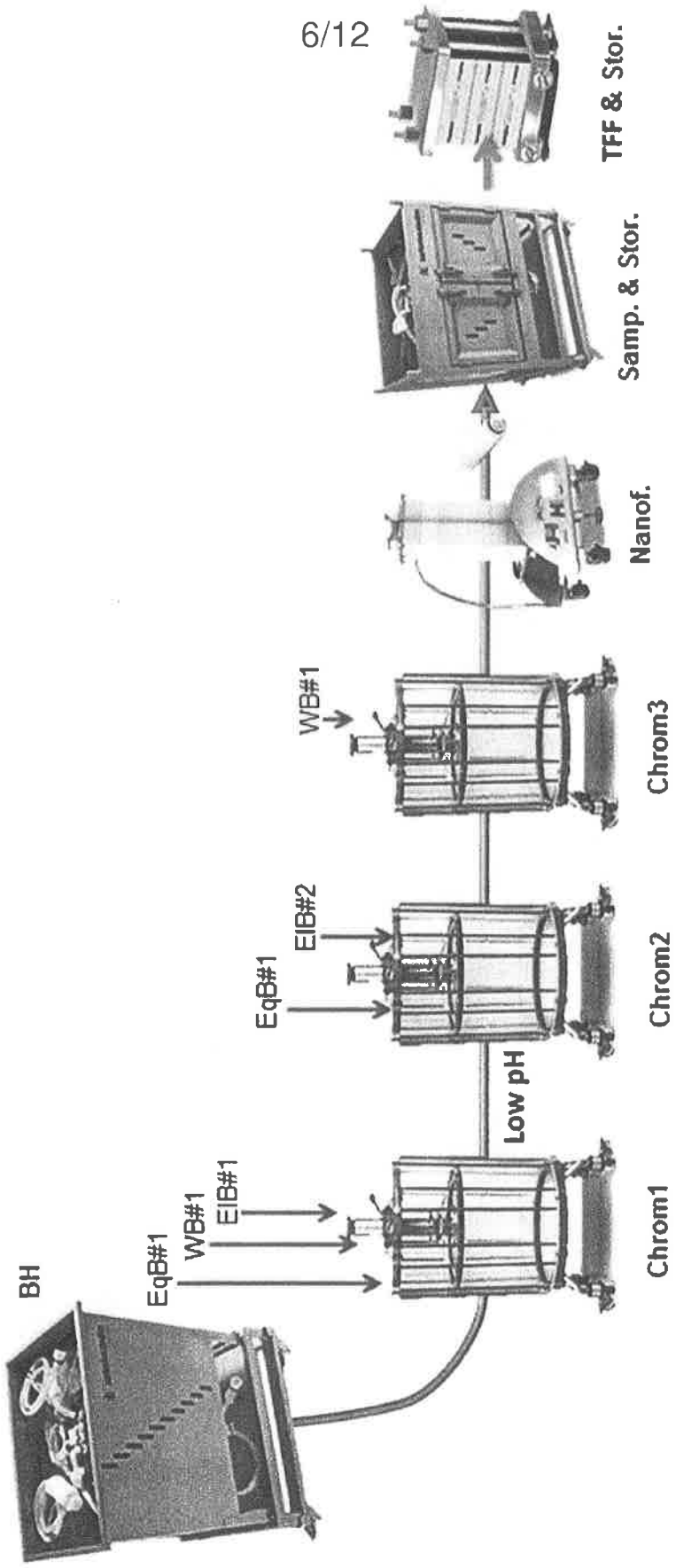

BH
EqB#1
WB#1
EIB#1
Chrom1
Low pH
EqB#1
EIB#2
Chrom2
WB#1
Chrom3
Nanof.
Samp. & Stor.
TFF & Stor.

FIG.6

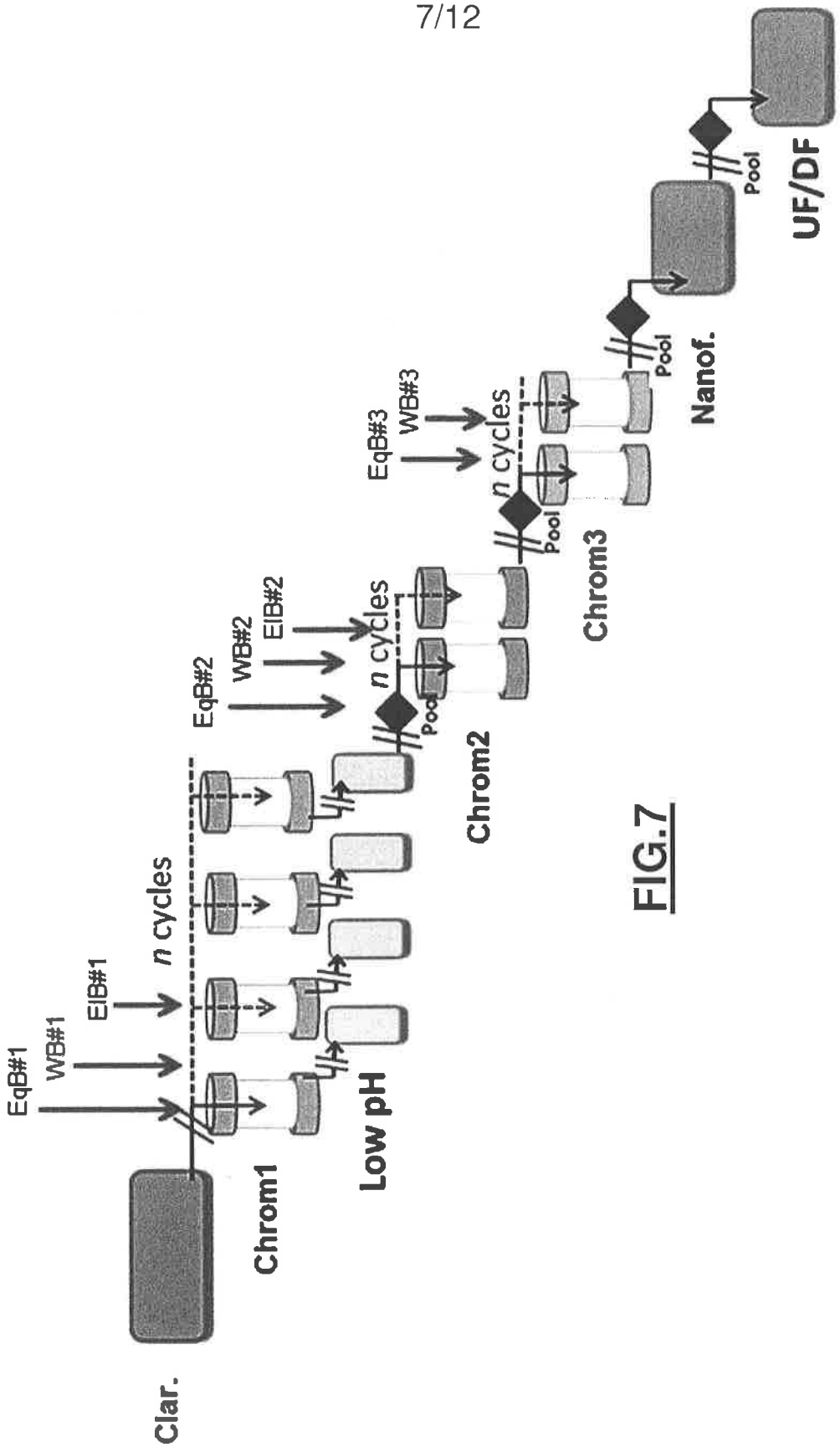

Clar.
EqB#1
WB#1
EIB#1
n cycles
Chrom1
Low pH
EqB#2
WB#2
EIB#2
n cycles
Pool
Chrom2
EqB#3
WB#3
n cycles
Pool
Chrom3
Pool
Nanof.
Pool
UF/DF

FIG.7

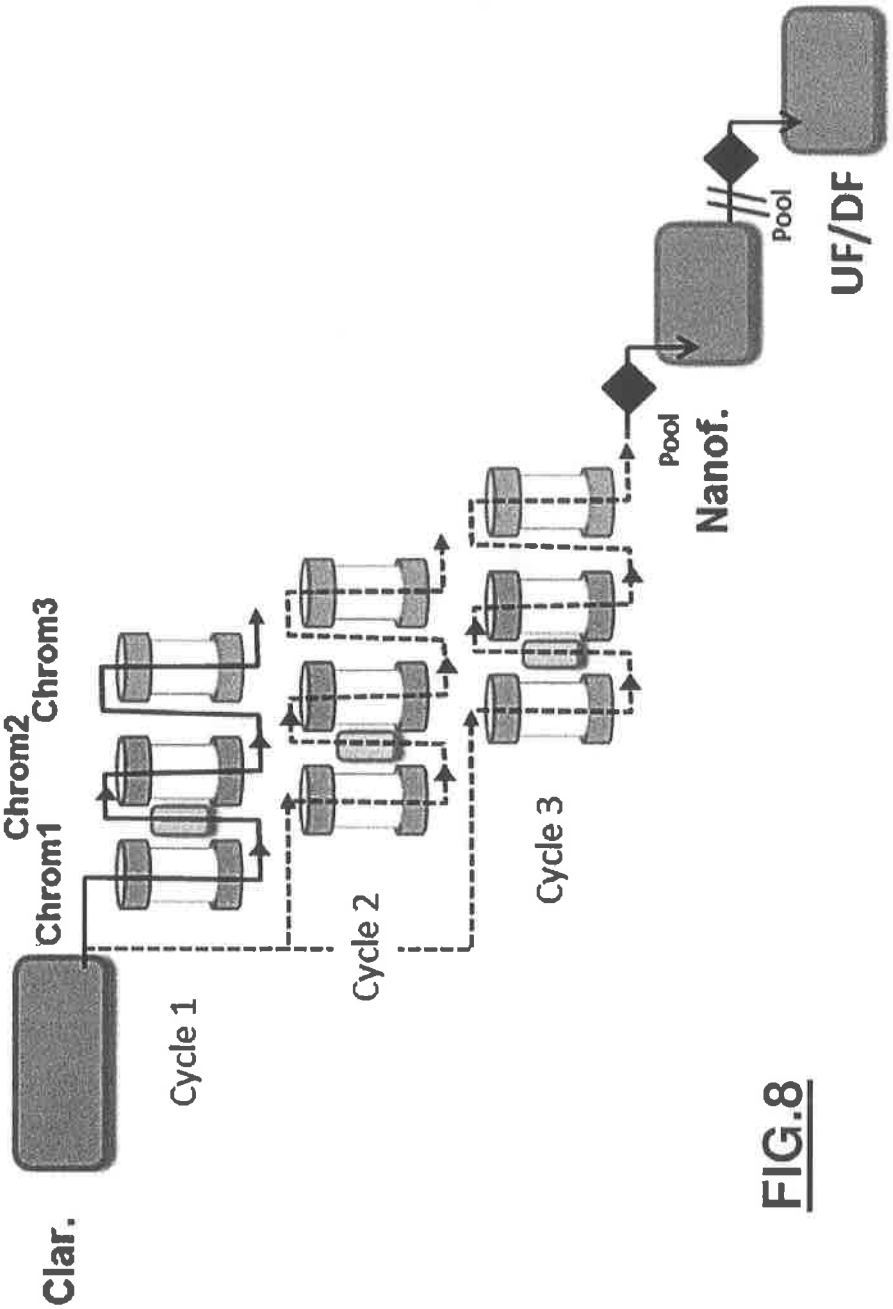

Clar.
Chrom1
Chrom2
Chrom3
Cycle 1
Cycle 2
Cycle 3
Pool
Nanof.
Pool
UF/DF

FIG.8

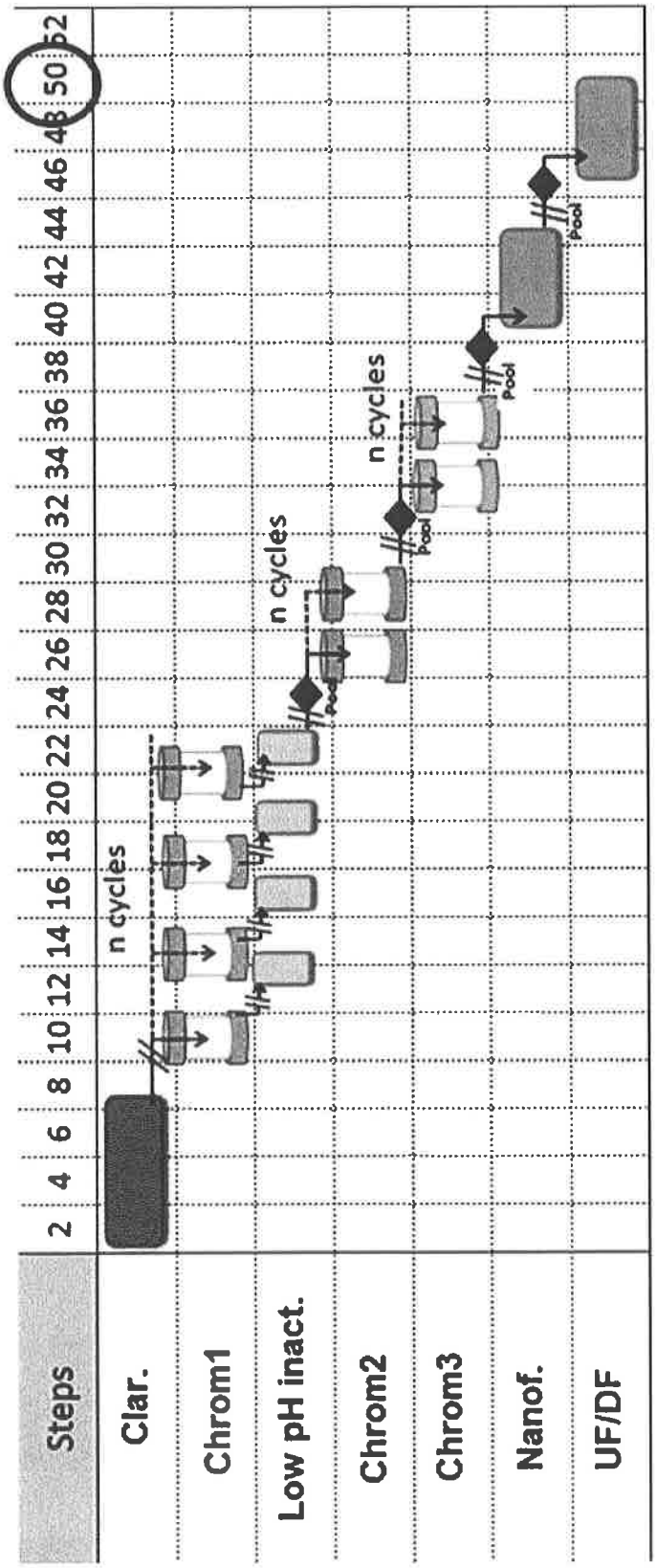

Steps
Clar.
Chrom1
Low pH inact.
Chrom2
Chrom3
Nanof.
UF/DF
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52
n cycles
n cycles
n cycles
Pool
Pool
Pool
Pool

FIG.9

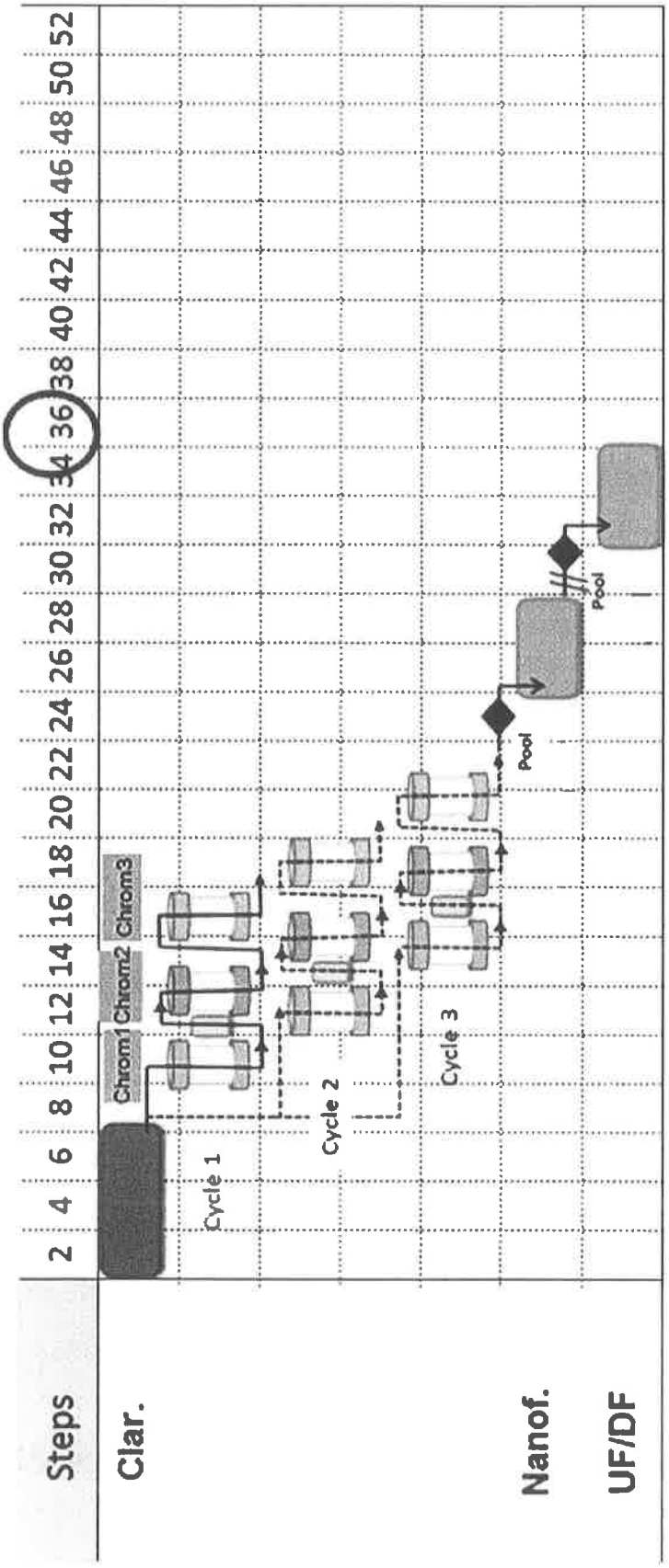

Steps
Clar.
Nanof.
UF/DF
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
52
Chrom1
Chrom2
Chrom3
Cycle 1
Cycle 2
Cycle 3
Pool
Pool

FIG.10

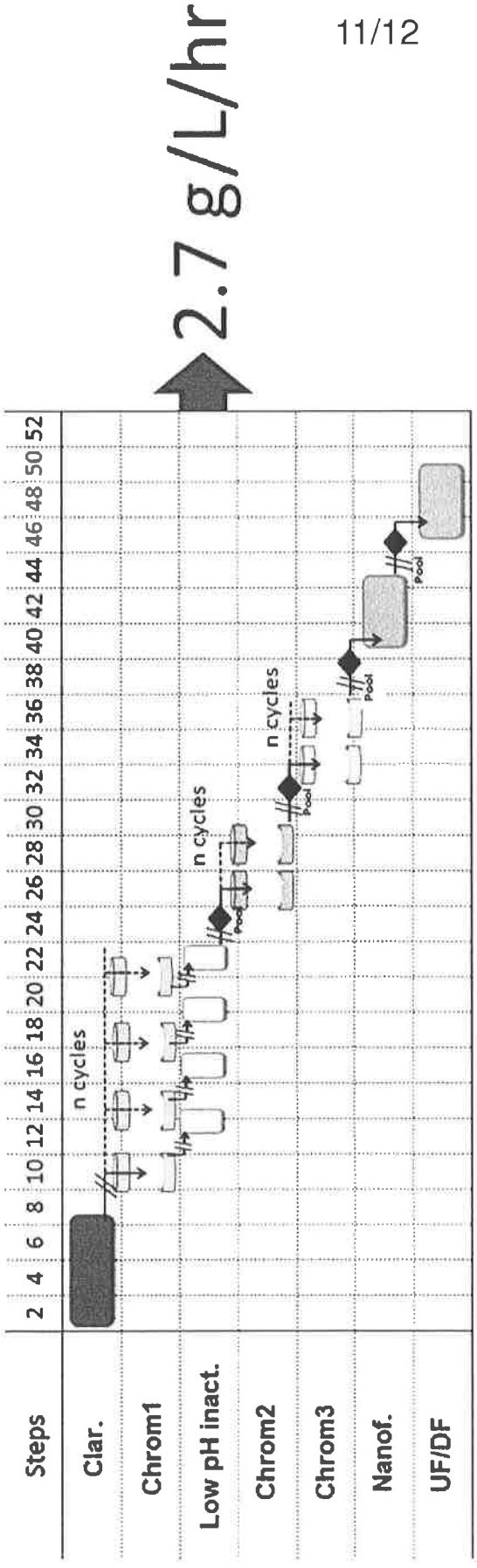

2.7 g/L/hr
Steps
Clar.
Chrom1
Low pH inact.
Chrom2
Chrom3
Nanof.
UF/DF
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52
n cycles
n cycles
n cycles
Pool
Pool
Pool
Pool

FIG.11

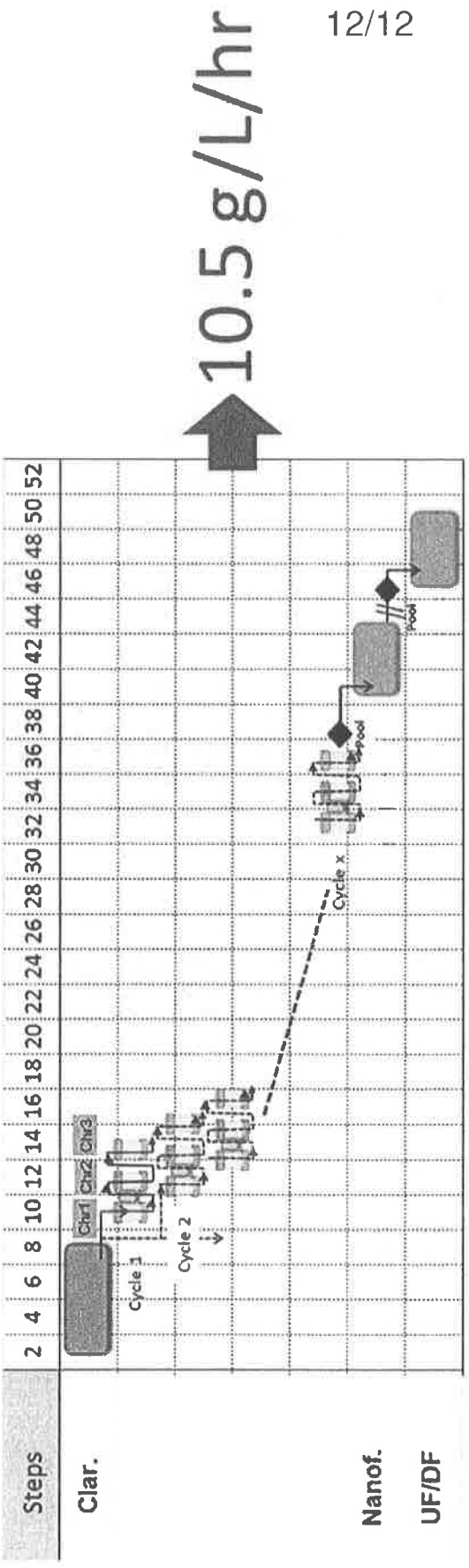

10.5 g/L/hr
Steps
Clar.
Nanof.
UF/DF
Chr1
Chr2
Chr3
Cycle 1
Cycle 2
Cycle x
Pool
Pool
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52

FIG.12