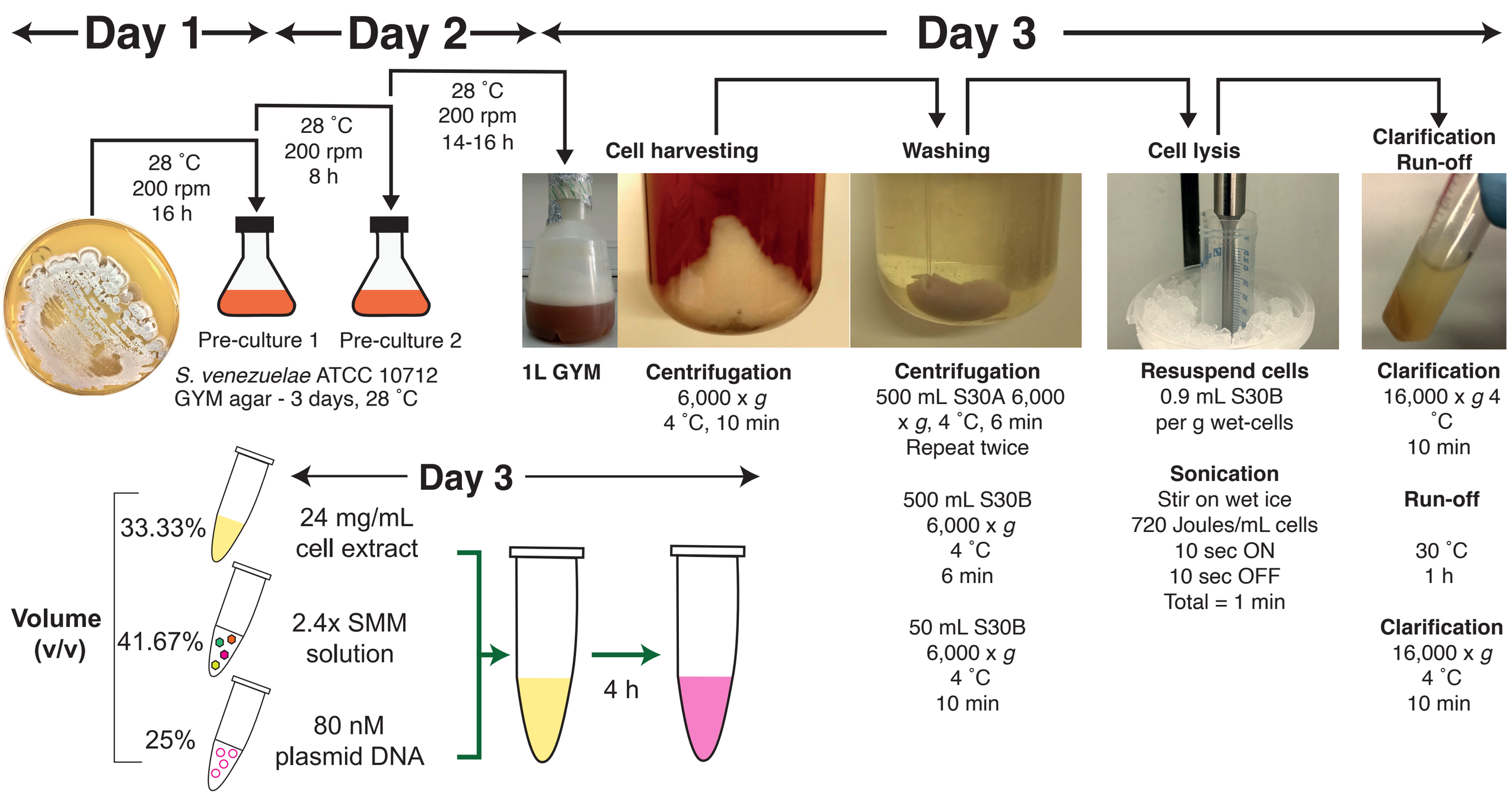
Day 1
Day 2
Day 3
28 ˚C
200 rpm
16 h
28 ˚C
200 rpm
8 h
28 ˚C
200 rpm
14-16 h
Pre-culture 1
Pre-culture 2
S. venezuelae ATCC 10712
GYM agar - 3 days, 28 ˚C
Cell harvesting
Washing
Cell lysis
Clarification
Run-off
1L GYM
Centrifugation
6,000 x g
4 ˚C, 10 min
Centrifugation
500 mL S30A 6,000
x g, 4 ˚C, 6 min
Repeat twice
500 mL S30B
6,000 x g
4 ˚C
6 min
50 mL S30B
6,000 x g
4 ˚C
10 min
Resuspend cells
0.9 mL S30B
per g wet-cells
Sonication
Stir on wet ice
720 Joules/mL cells
10 sec ON
10 sec OFF
Total = 1 min
Clarification
16,000 x g 4
˚C
10 min
Run-off
30 ˚C
1 h
Clarification
16,000 x g
4 ˚C
10 min
Day 3
Volume
(v/v)
33.33%
41.67%
25%
24 mg/mL
cell extract
2.4x SMM
solution
80 nM
plasmid DNA
4 h