

**SYNTHESIS PROGRAM FOR THE AUTOMATED ASSEMBLY OF
OLIGONUCLEOTIDE LIBRARIES IN THE ASM-800 DNA SYNTHESIZER,
USING Fmoc-TRINUCLEOTIDE PHOSPHoramidites**

Fragment: First

1. Gas open W1 W2
2. Valve open W2 Wst2
3. Wait T=0.3 sec
4. Valve close W2 Wst2
5. Valve open W2
6. Column scan
7. Pump in V=100 μ l F=50.0 μ l/sec
8. Column close
9. Valve close W2
10. Valve open W1b Wst2
11. Wait T=0.3 sec
12. Valve close W1b
13. Valve open W1a
14. Wait T=0.3 sec
15. Valve close W1a Wst2
16. Valve open W2
17. Column scan
18. Pump in V=40 μ l F=50.0 μ l/sec
19. Column close
20. Valve close W2
21. Gas close W1 W2
22. Gas open R2
23. Valve open R2
24. Column open 1 2 3 4 5 6 7 8
25. Pump in V=180 μ l F=50.0 μ l/sec
26. Column close 1 2 3 4 5 6 7 8
27. Column scan
28. Pump in V=35 μ l F=50.0 μ l/sec
29. Column close
30. Valve close R2
31. Gas close R2
32. Gas open Dbl
33. Valve open Dbl
34. Column open 1 2 3 4 5 6 7 8
35. Pump in V=125 μ l F=25.0 μ l/sec
36. Column close 1 2 3 4 5 6 7 8
37. Valve close Dbl
38. Cycle begin N=1
39. Valve open Dbl
40. Column scan
41. Pump in V=60 μ l F=25.0 μ l/sec
42. Column close
43. Valve close Dbl
44. Cycle end T=10.0 sec
45. Cycle begin N=3
46. Valve open Dbl
47. Column scan
48. Pump in V=50 μ l F=25.0 μ l/sec
49. Column close
50. Valve close Dbl
51. Cycle end T=10.0 sec
52. Gas close Dbl
53. Gas open W2
54. Cycle begin N=1
55. Valve open W2
56. Column scan
57. Pump in V=8 μ l F=50.0 μ l/sec
58. Column close
59. Valve close W2
60. Cycle end
61. Valve open W2 Wst2
62. Wait T=0.2 sec
63. Valve close
64. Valve open W1b
65. Wait T=0.2 sec
66. Valve close W1b
67. Valve open W1a
68. Wait T=0.2 sec
69. Valve close W1a
70. Valve open W1a W1b
71. Wait T=0.3 sec
72. Valve close W1a W1b
73. Wait T=0.1 sec
74. Valve close Wst2
75. Valve open W2
76. Column open 1 2 3 4 5 6 7 8

77. Pump in V=120 μ l F=50.0 μ l/sec
78. Column close 1 2 3 4 5 6 7 8
79. Column scan
80. Pump in V=40 μ l F=50.0 μ l/sec
81. Column close
82. Column scan
83. Pump in V=35 μ l F=50.0 μ l/sec
84. Column close
85. Valve close W2
86. Gas close W2
87. Gas open W1
88. Valve open W1 W1b Wst2
89. Wait T=0.4 sec
90. Valve close W1a W1b Wst2
91. Valve open W1b
92. Column scan
93. Pump in V=40 μ l F=50.0 μ l/sec
94. Column close
95. Valve close W1b
96. Valve open W1a
97. Column scan
98. Pump in V=50 μ l F=50.0 μ l/sec
99. Column close
100. Valve close W1a
101. Gas close W1
102. End
103.

Fragment: Main

1. Addition

2. Wait T=6.0 sec
3. Gas open W1 W2
4. Valve open W2 Wst2
5. Wait T=0.2 sec
6. Valve close W2
7. Valve open W1a W1b
8. Wait T=0.3 sec
9. Valve close W1a W1b
10. Valve close Wst2
11. Gas close W1 W2
12. Gas open CpA CpB
13. Column open 1 2 3 4 5 6 7 8
14. Valve open CpB
15. Pump in V=20 μl F=25.0 $\mu\text{l}/\text{sec}$
16. Valve open CpA
17. Pump in V=120 μl F=50.0 $\mu\text{l}/\text{sec}$
18. Valve close CpA CpB
19. Column close 1 2 3 4 5 6 7 8
20. Cycle begin N=1
21. Column scan
22. Valve open CpA CpB
23. Pump in V=55 μl F=50.0 $\mu\text{l}/\text{sec}$
24. Valve close CpA CpB
25. Column close
26. Cycle end T=12.0 sec
27. Cycle begin N=4
28. Column scan
29. Valve open CpA CpB
30. Pump in V=30 μl F=25.0 $\mu\text{l}/\text{sec}$
31. Valve close CpA CpB
32. Column close
33. Cycle end T=10.0 sec
34. Gas close CpA CpB
35. Gas open W2
36. Cycle begin N=1
37. Column scan
38. Valve open W2
39. Pump in V=10 μl F=50.0 $\mu\text{l}/\text{sec}$
40. Valve close W2
41. Column close
42. Cycle end T=10.0 sec
43. Valve open W2
44. Column open 1 2 3 4 5 6 7 8
45. Pump in V=130 μl F=50.0 $\mu\text{l}/\text{sec}$
46. Column close 1 2 3 4 5 6 7 8
47. Column scan
48. Pump in V=40 μl F=50.0 $\mu\text{l}/\text{sec}$
49. Column close
50. Valve close W2
51. Gas close W2
52. Gas open W1
53. Valve open Wst2
54. Valve open W1a
55. Wait T=0.2 sec
56. Valve close W1a
57. Valve open W1b
58. Wait T=0.2 sec
59. Valve close W1b
60. Valve open W1a W1b
61. Wait T=0.3 sec
62. Valve close W1a W1b
63. Valve close Wst2
64. Gas close W1
65. Gas open Oxd
66. Valve open Oxd
67. Column open 1 2 3 4 5 6 7 8
68. Pump in V=150 μl F=50 $\mu\text{l}/\text{sec}$
69. Column close 1 2 3 4 5 6 7 8
70. Valve close Oxd
71. Cycle begin N=1
72. Valve open Oxd
73. Column scan
74. Pump in V=40 μl F=50.0 $\mu\text{l}/\text{sec}$
75. Column close
76. Valve close Oxd
77. Cycle end T=10.0 sec
78. Cycle begin N=1
79. Valve open Oxd
80. Column scan
81. Pump in V=10 μl F=50.0 $\mu\text{l}/\text{sec}$
82. Column close
83. Valve close Oxd
84. Cycle end T=10.0 sec
85. Gas close Oxd
86. Gas open W2
87. Cycle begin N=2
88. Valve open W2
89. Column scan
90. Pump in V=8 μl F=50.0 $\mu\text{l}/\text{sec}$

91. Column close
92. Valve close W2
93. Cycle end T=7.0 sec
94. Gas open W1
95. Valve open W2 Wst1
96. Column open 1 2 3 4 5 6 7 8
97. Wait T=1.0 sec
98. Column close 1 2 3 4 5 6 7 8
99. Column scan
100. Wait T=1.4 sec
101. Column close
102. Valve close W2 Wst1
103. Valve open W1b Wst2
104. Wait T=0.2 sec
105. Valve close W1b
106. Valve open W1a
107. Wait T=0.2 sec
108. Valve close W1a
109. Valve open W1a W1b
110. Wait T=0.4 sec
111. Valve close W1a W1b Wst2
112. Valve open W2
113. Column scan
114. Pump in V=50 μ l F=50.0 μ l/sec
115. Column close
116. Valve close W2
117. Valve open W2
118. Column scan
119. Pump in V=40 μ l F=50.0 μ l/sec
120. Column close
121. Valve close W2
122. Deblock
123. Gas open W2
124. Valve open W2 Wst2
125. Wait T=0.3 sec
126. Valve close W2 Wst2
127. Valve open W2
128. Column open 1 2 3 4 5 6 7 8
129. Pump in V=120 μ l F=50.0 μ l/sec
130. Column close 1 2 3 4 5 6 7 8
131. Column scan
132. Pump in V=40 μ l F=50.0 μ l/sec
133. Column close
134. Column scan
135. Pump in V=40 μ l F=50.0 μ l/sec
136. Column close
137. Valve close W2
138. Gas close W2
139. Gas open W1
140. Valve open W1b Wst2
141. Wait T=0.2 sec
142. Valve close W1b
143. Valve open W1a
144. Wait T=0.3 sec
145. Valve close W1a
146. Valve open W1a W1b
147. Wait T=0.4 sec
148. Valve close W1a W1b
149. Valve close Wst2
150. Cycle begin N=3
151. Valve open W1b
152. Column scan
153. Pump in V=50 μ l F=50.0 μ l/sec
154. Column close
155. Valve close W1b
156. Cycle end T=0.0 sec
157. Gas close W1
158. End

Fragment: Deblock

1. Gas open R2
2. Column open 1 2 3 4 5 6 7 8
3. Valve open R2
4. Pump in V=180 μl F=50.0 $\mu\text{l}/\text{sec}$
5. Valve close R2
6. Column close 1 2 3 4 5 6 7 8
7. Valve open R2
8. Column scan
9. Pump in V=30 μl F=50.0 $\mu\text{l}/\text{sec}$
10. Column close
11. Valve close R2
12. Gas close R2
13. Gas open Dbl
14. Column open 1 2 3 4 5 6 7 8
15. Valve open Dbl
16. Pump in V=125 μl F=25.0 $\mu\text{l}/\text{sec}$
17. Valve close Dbl
18. Column close 1 2 3 4 5 6 7 8
19. Cycle begin N=1
20. Valve open Dbl
21. Column scan
22. Pump in V=60.0 μl F=25.0 $\mu\text{l}/\text{sec}$
23. Column close
24. Valve close Dbl
25. Cycle end T=10.0 sec
26. Cycle begin N=3
27. Valve open Dbl
28. Column scan
29. Pump in V=50 μl F=25.0 $\mu\text{l}/\text{sec}$
30. Column close
31. Valve close Dbl
32. Cycle end T=10.0 sec
33. Gas close Dbl
34. Gas open W2
35. Cycle begin N=1
36. Valve open W2
37. Column scan
38. Pump in V=7 μl F=50.0 $\mu\text{l}/\text{sec}$
39. Column close
40. Valve close W2
41. Cycle end T=7.0 sec
42. Gas close W2
43. Gas open W1
44. Valve open W1a W1b Wst2
45. Wait T=0.4 sec
46. Valve close W1a W1b Wst2
47. Valve open W1b
48. Column scan
49. Pump in V=50 μl F=25.0 $\mu\text{l}/\text{sec}$
50. Column close
51. Valve close W1b
52. Valve open W1a
53. Column scan
54. Pump in V=50 μl F=25.0 $\mu\text{l}/\text{sec}$
55. Column close
56. Valve close W1a
57. Gas close W1
58. End

Fragment : Final

1. Gas open W1
2. Valve open W1a W1b Wst2
3. Wait T=0.3 sec
4. Valve close W1a W1b Wst2
5. Wait T=0.1 sec
6. Gas close W1
7. Gas open W2
8. Column scan
9. Valve open W2
10. Pump in V=140 μ l F=50.0 μ l/sec
11. Valve close W2
12. Column close
13. Valve open Wst1
14. Column open 1 2 3 4 5 6 7 8
15. Wait T=1.0 sec
16. Column close 1 2 3 4 5 6 7 8
17. Valve close Wst1
18. Gas close W2
19. End
- 20.

Fragment : A (C, G or T)

1. Gas open Mon1 W1
2. Valve open Wst2
3. Valve open W1b
4. Wait T=1.0 sec
5. Valve close W1b
6. Valve open W1a
7. Wait T=1.0 sec
8. Valve close W1a
9. Valve open W1a W1b
10. Wait T=1.0 sec
11. Valve close W1a W1b
12. Valve close Wst2
13. Valve open Act1
14. Column open 1 2 3 4 5 6 7 8
15. Pump in V=15 μ l F=25.0 μ l/sec
16. Column close 1 2 3 4 5 6 7 8
17. Column scan
18. Pump in V=15 μ l F= 25.0 μ l/sec
19. Column close
20. Valve close Act1
21. Column open 1 2 3 4 5 6 7 8
22. Valve open A (C, G or T)
23. Pump in V=10 μ l F=25 μ l/sec
24. Valve close A (C, G or T)
25. Column close 1 2 3 4 5 6 7 8
26. Cycle begin N=1
27. Column scan
28. Cycle begin N=8
29. Valve open A (C, G or T)
30. Pump in V=5 μ l F=25.0 μ l/sec
31. Valve close A (C, G or T)
32. Valve open Act1
33. Pump in V=5 μ l F=25.0 μ l/sec
34. Valve close Act1
35. Cycle end T=4.0 sec
36. Column close
37. Cycle end T=32.0 sec
38. Valve open W1a
39. Column scan
40. Pump in V=6 μ l F=50.0 μ l/sec
41. Column close
42. Valve close W1a
43. Gas close Mon1 W1
44. End

Fragment: 5 (6, 7 or 8)

1. Gas open R1
2. Column open 1 2 3 4 5 6 7 8
3. Valve open R1
4. Pump in V=200 μl F=50.0 $\mu\text{l}/\text{sec}$
5. Column close 1 2 3 4 5 6 7 8
6. Valve close R1
7. Wait T=4.0 sec
8. Cycle begin N=6
9. Valve open R1
10. Column scan
11. Pump in V=100 μl F=12.5 $\mu\text{l}/\text{sec}$
12. Column close
13. Valve close R1
14. Cycle end T=9.0 sec
15. Gas close
16. Gas open W2
17. Valve open W2 Wst2
18. Wait T=1.0 sec
19. Valve close W2 Wst2
20. Valve open W2
21. Column open 1 2 3 4 5 6 7 8
22. Pump in V=150 μl F=50.0 $\mu\text{l}/\text{sec}$
23. Column close 1 2 3 4 5 6 7 8
24. Column scan
25. Pump in V=100 μl F=50.0 $\mu\text{l}/\text{sec}$
26. Column close
27. Column scan
28. Pump in V=100 μl F=50.0 $\mu\text{l}/\text{sec}$
29. Column close
30. Valve close W2
31. Gas close W2
32. Gas open W1
33. Valve open W1b Wst2
34. Wait T=1.0 sec
35. Valve close W1b
36. Valve open W1a
37. Wait T=1.0 sec
38. Valve close W1a
39. Valve open W1a W1b
40. Wait T=1.0 sec
41. Valve close W1a W1b
42. Valve close Wst2
43. Cycle begin N=3
44. Valve open W1b
45. Column scan
46. Pump in V=100 μl F=50.0 $\mu\text{l}/\text{sec}$
47. Column close
48. Valve close W1b
49. Cycle end T=0.0 sec
50. Gas open Mon1 Mon2 W1
51. Valve open Wst2
52. Valve open W1a
53. Wait T=1.0 sec
54. Valve close W1b
55. Valve open W1a
56. Wait T=1.0 sec
57. Valve close W1a
58. Valve open W1a W1b
59. Wait T=1.0 sec
60. Valve close W1a W1b
61. Valve close Wst2
62. Valve open Act1
63. Column open 1 2 3 4 5 6 7 8
64. Pump in V=15 μl F=25.0 $\mu\text{l}/\text{sec}$
65. Column close 1 2 3 4 5 6 7 8
66. Column scan
67. Pump in V=15 μl F=25.0 $\mu\text{l}/\text{sec}$
68. Column close
69. Valve close Act1
70. Column open 1 2 3 4 5 6 7 8
71. Valve open m5
72. Pump in V=8 μl F=25.0 $\mu\text{l}/\text{sec}$
73. Valve close m5
74. Column close 1 2 3 4 5 6 7 8
75. Cycle begin N=1
76. Column scan
77. Cycle begin N=8
78. Valve open m5
79. Pump in V=5 μl F=25.0 $\mu\text{l}/\text{sec}$
80. Valve close m5
81. Valve open Act1
82. Pump in V=5 μl F=25.0 $\mu\text{l}/\text{sec}$
83. Valve close Act1
84. Cycle end T=3.0 sec
85. Column close
86. Cycle end T=30.0 sec
87. Valve open Act1
88. Column scan
89. Pump in V=15 μl F=25.0 $\mu\text{l}/\text{sec}$
90. Column close

91. Valve close Act1
92. Column open 1 2 3 4 5 6 7 8
93. Valve open A (C, G or T)
94. Pump in V=10 μ l F=25.0 μ l/sec
95. Valve close A (C, G or T)
96. Column close 1 2 3 4 5 6 7 8
97. Cycle begin N=1
98. Column scan
99. Cycle begin N=8
100. Valve open A (C, G or T)
101. Pump in V=5 μ l F=25.0 μ l/sec
102. Valve close A (C, G or T)
103. Valve open Act1
104. Pump in V=5 μ l F=25.0 μ l/sec
105. Gas close Act1
106. Cycle end T=3.0 sec
107. Column close
108. Cycle end T=30.0 sec
109. Valve open W1a
110. Column scan
111. Pump in V=6 μ l F=50.0 μ l/sec
112. Column close
113. Valve close W1a
114. Gas close Mon1 Mon2 W1
115. End

Fragment: 9 (10, 11 or 12)

1. Gas open R1
2. Column open 1 2 3 4 5 6 7 8
3. Valve open R1
4. Pump in V=200 μl F=50.0 $\mu\text{l}/\text{sec}$
5. Column close 1 2 3 4 5 6 7 8
6. Valve close R1
7. Wait T=4.0 sec
8. Cycle begin N=6
9. Valve open R1
10. Column scan
11. Pump in V=100 μl F=12.5 $\mu\text{l}/\text{sec}$
12. Column close
13. Valve close R1
14. Cycle end T=9.0 sec
15. Gas close R1
16. Gas open W2
17. Valve open W2 Wst2
18. Wait T=1.0 sec
19. Valve close W2 Wst2
20. Valve open W2
21. Cycle begin N=3
22. Column scan
23. Pump in V=100 μl F=50.0 $\mu\text{l}/\text{sec}$
24. Column close
25. Cycle end T=0.3 sec
26. Valve close W2
27. Gas close W2
28. Gas open W1
29. Valve open W1b Wst2
30. Wait T=1.0 sec
31. Valve close W1b
32. Valve open W1a
33. Wait T=1.0 sec
34. Valve close W1a
35. Valve open W1a W1b
36. Wait T=1.0 sec
37. Valve close W1a W1b
38. Valve close Wst2
39. Cycle begin N=3
40. Valve open W1b
41. Column scan
42. Pump in V=100 μl F=50.0 $\mu\text{l}/\text{sec}$
43. Column close
44. Valve close W1b
45. Cycle end T=0.0 sec
46. Gas open Mon1 W1
47. Valve open Wst2
48. Valve open W1b
49. Wait T=1.0 sec
50. Valve close W1b
51. Valve open W1a
52. Wait T=1.0 sec
53. Valve close W1a
54. Valve open W1a W1b
55. Wait T=1.0 sec
56. Valve close W1a W1b
57. Valve close Wst2
58. Valve open Act1
59. Column open 1 2 3 4 5 6 7 8
60. Pump in V=15 μl F=25.0 $\mu\text{l}/\text{sec}$
61. Column close 1 2 3 4 5 6 7 8
62. Column scan
63. Pump in V=15 μl F=25.0 $\mu\text{l}/\text{sec}$
64. Column close
65. Valve close Act1
66. Column open 1 2 3 4 5 6 7 8
67. Valve open A (C, G or T)
68. Pump in V=10 μl F=25.0 $\mu\text{l}/\text{sec}$
69. Valve close A (C, G or T)
70. Column close 1 2 3 4 5 6 7 8
71. Cycle begin N=1
72. Column scan
73. Cycle begin N=8
74. Valve open A (C, G or T)
75. Pump in V=5 μl F=25.0 $\mu\text{l}/\text{sec}$
76. Valve close A (C, G or T)
77. Valve open Act1
78. Pump in V=5 μl F=25.0 $\mu\text{l}/\text{sec}$
79. Valve close Act1
80. Cycle end T=3.0 sec
81. Column close
82. Cycle end T=30.0 sec
83. Valve open W1a
84. Column scan
85. Pump in V=6 μl F=50.0 $\mu\text{l}/\text{sec}$
86. Column close
87. Valve close W1a
88. Gas close Mon1 W1
89. End