Ligase-Assisted Selection for the Enrichment of Responsive Ribozymes (LigASERR)

1. TRT reaction – Transcription of DNA into RNA using T7 RNA polymerase (in presence/absence of ligand), then reverse transcription of RNA into cDNA
2. Purification of cDNA using solid-phase reversible immobilisation (SPRI) beads
3. Digestion of RNA by incubation at 37°C for 30 mins with 0.1 mg ml^{-1} RNase A
4. Annealing of purified cDNA to 1 µM Splint, with 1 µM Adapter F (or Adapter C) and 1x NEB Taq DNA Ligase Reaction Buffer
5. Ligation reaction
    - Reaction transferred to ice (cool plate), NEB Taq DNA Ligase added to final concentration of 1.6 U $µl^{-1}$
    - Reaction incubated at 51.7°C for 30 mins to ligate full-length cDNA (or 46.4°C for 30 mins to ligate cleaved cDNA)
    - Ligation reaction quenched with 20 mM EDTA (final concentration)
6. Dynabeads™ MyOne™ Streptavidin C1 magnetic beads binding for cDNA selection
    - Binding step
    - NaOH washing step to remove unligated material
        - Ligated DNA immobilised and washed with NaOH using Dynabeads according to the manufacturer's instructions

TRT reaction

Reagents:

Master TRT reaction mix (w or w/out ligand)	Reservoir-1	A1
DNA library	Reservoir-1	A2

Steps:

1. Pick up tip.
2. Pre-wet tip with master TRT reaction mix.
3. Transfer 80 µl master TRT reaction mix from reservoir-1 A1 to column 1 of thermocycler plate, mix before 3 times.
4. Blow out into destination well.
5. Discard tip.
6. Pick up tip.
7. Pre-wet tip with DNA library.
8. Transfer 80 µl DNA library from reservoir-1 A2 to column 1 of thermocycler plate, mix before 3 times, mix after 3 times.
9. Blow out into destination well.
10. Put tip back to tip rack.
11. Turn on thermocycler (37°C for 30 mins, 65°C for 20 mins).
12. Pick up tip (same tips form step 12).
13. Transfer 70 µl TRT reaction from column 1 of thermocycler plate to column 1 of 96-well plate, mix before 3 times.
14. Blow out into destination well.
15. Discard tip.

Purification of cDNA with SPRI beads

Reagents:

Washed SPRI beads in buffer	Reservoir-1	A3
70% ethanol	Reservoir-1	A4
SPRI elution buffer	Reservoir-1	A5
Liquid waste destination	Reservoir-2	A12

Steps:

1. Pick up tip.
2. Pre-wet tip with SPRI beads solution.
3. Transfer 126 µl SPRI beads solution from reservoir-1 A3 to column 1 of 96-well plate, mix before 3 times, mix after 10 times.
4. Blow out into destination well.
5. Incubate at room temperature for 5 mins.
6. Transfer 150 µl reaction solution from column 1 of 96-well plate to column 1 of plate on magnetic block, mix before 3 times.
7. Blow out into destination well.
8. Discard tip.
9. Engage magnetic module.
10. Leave for 2 mins for SPRI beads to settle.

11. Pick up tip.
12. Transfer 130 µl reaction solution from column 1 of plate on magnetic block to reservoir-2 A12.
13. Discard tip.
14. Pick up tip.
15. Pre-wet tip with 70% ethanol.
16. Transfer 180 µl 70% ethanol from reservoir-1 A4 to column 1 of plate on magnetic block, air gap 10 µl.
17. Blow out into destination well.
18. Incubate at room temperature for 30 sec.
19. Transfer 180 µl supernatant from column 1 of plate on magnetic block to reservoir-2 A12, air gap 10 µl.
20. Discard tip.
21. Pick up tip.
22. Pre-wet tip with 70% ethanol.
23. Transfer 180 µl 70% ethanol from reservoir-1 A4 to column 1 of plate on magnetic block, air gap 10 µl.
24. Blow out into destination well.
25. Incubate at room temperature for 30 sec.
26. Transfer 180 µl supernatant from column 1 of plate on magnetic block to reservoir-2 A12, air gap 10 µl.
27. Discard tip.
28. Disengage magnetic module.
29. Pick up tip.
30. Pre-wet tip with SPRI elution buffer.
31. Transfer 180 µl SPRI elution buffer from reservoir-1 A5 to column 1 of plate on magnetic block, mix after 10 times.
32. Transfer 180 µl reaction solution from column 1 of plate on magnetic block to column 2 of 96-well plate.
33. Blow out into destination well.
34. Put tip back to tip rack.
35. Incubate at room temperature for 2 mins.
36. Pick up tip (same tips form step 32).
37. Transfer 150 µl reaction solution from column 2 of 96-well plate to column 2 of plate on magnetic block, mix before 10 times.
38. Blow out into destination well.
39. Discard tip.
40. Engage magnetic module.
41. Leave for 1 min for SPRI beads to settle.
42. Pick up tip.
43. Pre-wet tip with supernatant.
44. Transfer 100 µl supernatant from column 2 of plate on magnetic block to column 2 of thermocycler plate.
45. Blow out into destination well.
46. Discard tip.
47. Disengage magnetic module.

RNA digestion

Reagents:

RNase A	Reservoir-1	A6

Steps:

1. Pick up tip.
2. Pre-wet tips with RNase A solution.
3. Transfer 50 µl RNase A from reservoir-1 A6 to column 2 of thermocycler plate, mix after 3 times.
4. Blow out into destination well.
5. Put tip back to tip rack.
6. Turn on thermocycler (37°C for 30 mins).
7. Pick up tip (same tips form step 5).
8. Transfer 55 µl reaction from column 2 of thermocycler plate to column 3 of thermocycler plate.
9. Blow out into destination well.
10. Discard tip.

Annealing and Ligation reaction for cDNA

Reagents:

Ligase reaction mix	Reservoir-1	A7
NEB Taq DNA ligase	Reservoir-1	A8
EDTA	Reservoir-1	A9

Steps:

1. Pick up tip.
2. Pre-wet tip with ligase reaction mix.
3. Transfer 25 µl ligase reaction mix from reservoir-1 A7 to column 3 of thermocycler plate, mix before 3 times, mix after 3 times.
4. Blow out into destination well.
5. Discard tip.
6. Pick up tip.
7. Pre-wet tip with NEB Taq DNA ligase.
8. Transfer 20 µl NEB Taq DNA ligase from reservoir-1 A8 to column 3 of thermocycler plate, mix after 3 times.
9. Transfer 90 µl reaction solution from column 3 of thermocycler plate to column 4 of thermocycler plate.
10. Blow out into destination well.
11. Discard tip.
12. Turn on thermocycler (51.7°C for 30 mins to ligate full-length cDNA, or 46.4°C for 30 mins to ligate cleaved cDNA).
13. Pick up tip.
14. Pre-wet tip with EDTA.
15. Transfer 30 µl EDTA from reservoir-1 A9 to column 4 of thermocycler plate, mix after 3 times.

16. Transfer 100 µl reaction solution from column 4 of thermocycler plate to column 3 of 96-well plate.
17. Blow out into destination well.
18. Discard tip.

Dynabeads™ cDNA purification step

Reagents:

Washed Dynabeads™ MyOne™ Streptavidin C1 magnetic beads in buffer	Reservoir-1	A10
Solution A (NaOH, NaCl)	Reservoir-1	A11
Solution B (NaCl)	Reservoir-1	A12
PCR buffer	Reservoir-2	A1

Steps:

1. Pick up tip.
2. Pre-wet tip with Dynabeads™ solution.
3. Transfer 50 µl Dynabeads™ solution from reservoir-1 A10 to column 3 of plate on magnetic block, mix before 3 times.
4. Blow out into destination well.
5. Discard tip.
6. Pick up tip.
7. Pre-wet tip with Solution A.
8. Transfer 150 µl Solution A from reservoir-1 A11 to column 3 of plate on magnetic block, mix after 3 times.
9. Blow out into destination well.
10. Engage magnetic module.
11. Leave for 2 mins.
12. Transfer 150 µl supernatant from column 3 of plate on magnetic block to reservoir-2 A12.
13. Discard tip.
14. Disengage magnetic module.
15. Repeat steps 6 to 14 one more time.
16. Pick up tip.
17. Pre-wet tip with Solution B.
18. Transfer 150 µl Solution B from reservoir-1 A12 to column 3 of plate on magnetic block, mix after 3 times.
19. Blow out into destination well.
20. Engage magnetic module.
21. Leave for 2 mins (or however long needed for beads to settle).
22. Transfer 150 µl supernatant from column 3 of plate on magnetic block to reservoir-2 A12.
23. Discard tip.
24. Disengage magnetic module.
25. Pick up tip.
26. Pre-wet tip with Solution B.
27. Transfer 150 µl Solution B from reservoir-1 A12 to column 3 of plate on magnetic block, mix after 10 times.

28. Transfer 100 µl reaction solution from column 3 of plate on magnetic block to column 3 of 96-well plate, mix after 3 times.
29. Blow out into destination well.
30. Incubate at room temperature for a total of 20 mins.
31. Mix reaction solution 10 times (at a slow speed) for column 3 of 96-well plate every 2 mins.
32. Blow out into destination well.
33. Repeat steps 31 and 32 until the 20 min incubation time is up.
34. Transfer 150 µl reaction solution from column 3 of 96-well plate to column 4 of plate on magnetic block, mix before 10 times.
35. Blow out into destination well.
36. Engage magnetic module.
37. Leave for 3 mins for Dynabeads™ to settle.
38. Transfer 130 µl supernatant from column 4 of plate on magnetic block to reservoir-2 A12.
39. Discard tip.
40. Pick up tip.
41. Pre-wet tip with Solution A.
42. Transfer 180 µl Solution A from reservoir-1 A11 to column 4 of plate on magnetic block.
43. Blow out into destination well.
44. Incubate at room temperature for 30 sec.
45. Transfer 180 µl supernatant from column 4 of plate on magnetic block to reservoir-2 A12.
46. Discard tip.
47. Repeat steps 40 to 46 for one more time.
48. Pick up tip.
49. Pre-wet tip with Solution A.
50. Transfer 180 µl Solution A from reservoir-1 A11 to column 4 of plate on magnetic block.
51. Blow out into destination well.
52. Incubate at room temperature for 30 sec.
53. Transfer 200 µl supernatant from column 4 of plate on magnetic block to reservoir-2 A12.
54. Discard tip.
55. Disengage magnetic module.
56. Pick up tip.
57. Pre-wet tip with PCR buffer.
58. Transfer 200 µl PCR buffer from reservoir-2 A1 to column 4 of plate on magnetic block, mix after 10 times.
59. Transfer 150 µl reaction solution from column 4 of plate on magnetic block to column 5 of thermocycler plate.
60. Blow out into destination well.
61. Discard tip.