Primerize-2D: automated primer design for RNA multidimensional chemical mapping

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SUPPLEMENTAL MATERIAL

Supplementary Figure S1. Schematics for Primerize-2D enabled application of multidimensional chemical mapping methods.

(A). Mutate-and-map (M^2) provides rich base-pairing information by assessing which nucleotides are 'released' upon making single mutations at every other nucleotide.

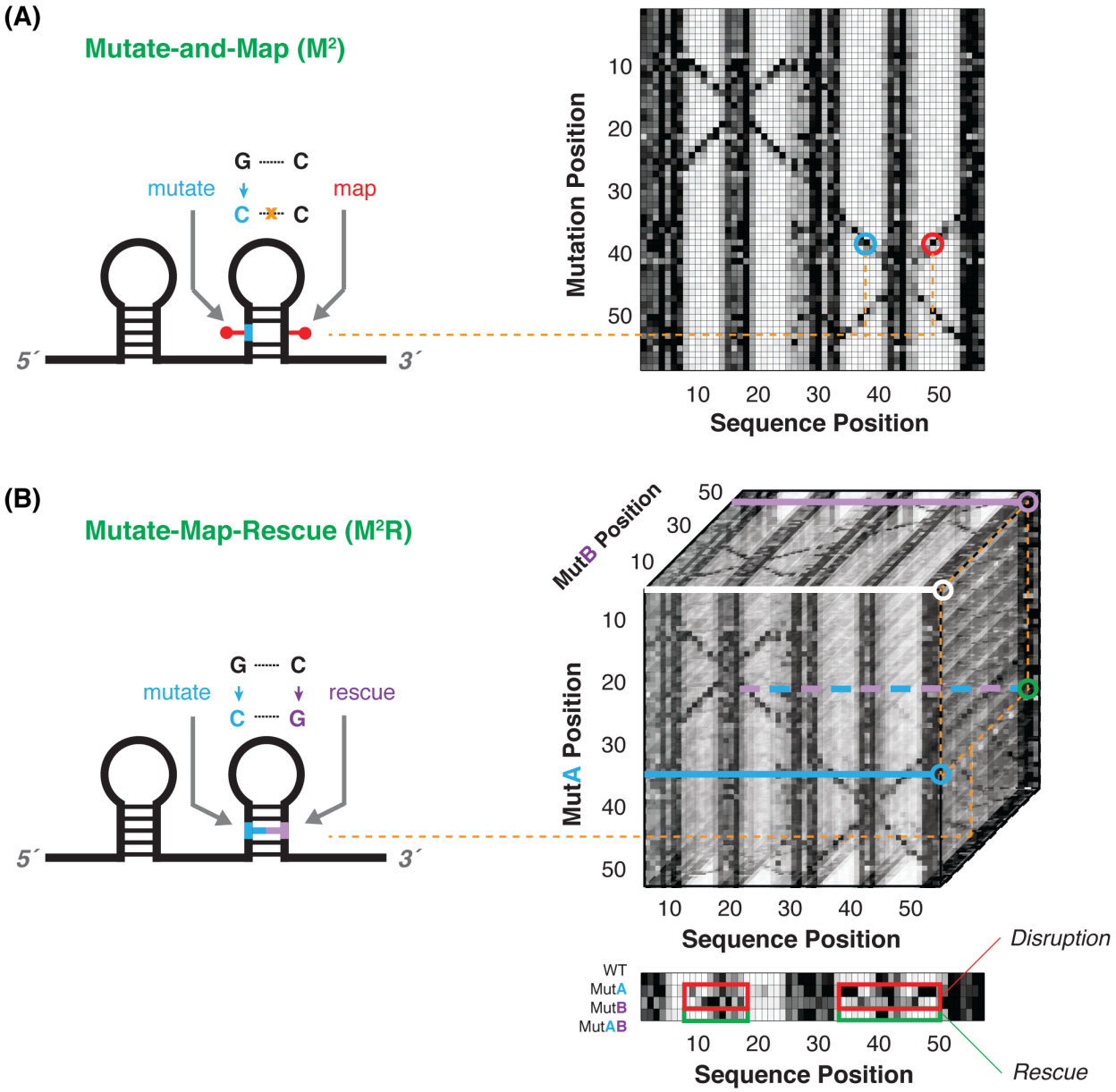
(B). Mutation-map-rescue (M^2R) validates or falsifies base pairs by detecting rescue of the RNA structure by predicted compensatory mutations.

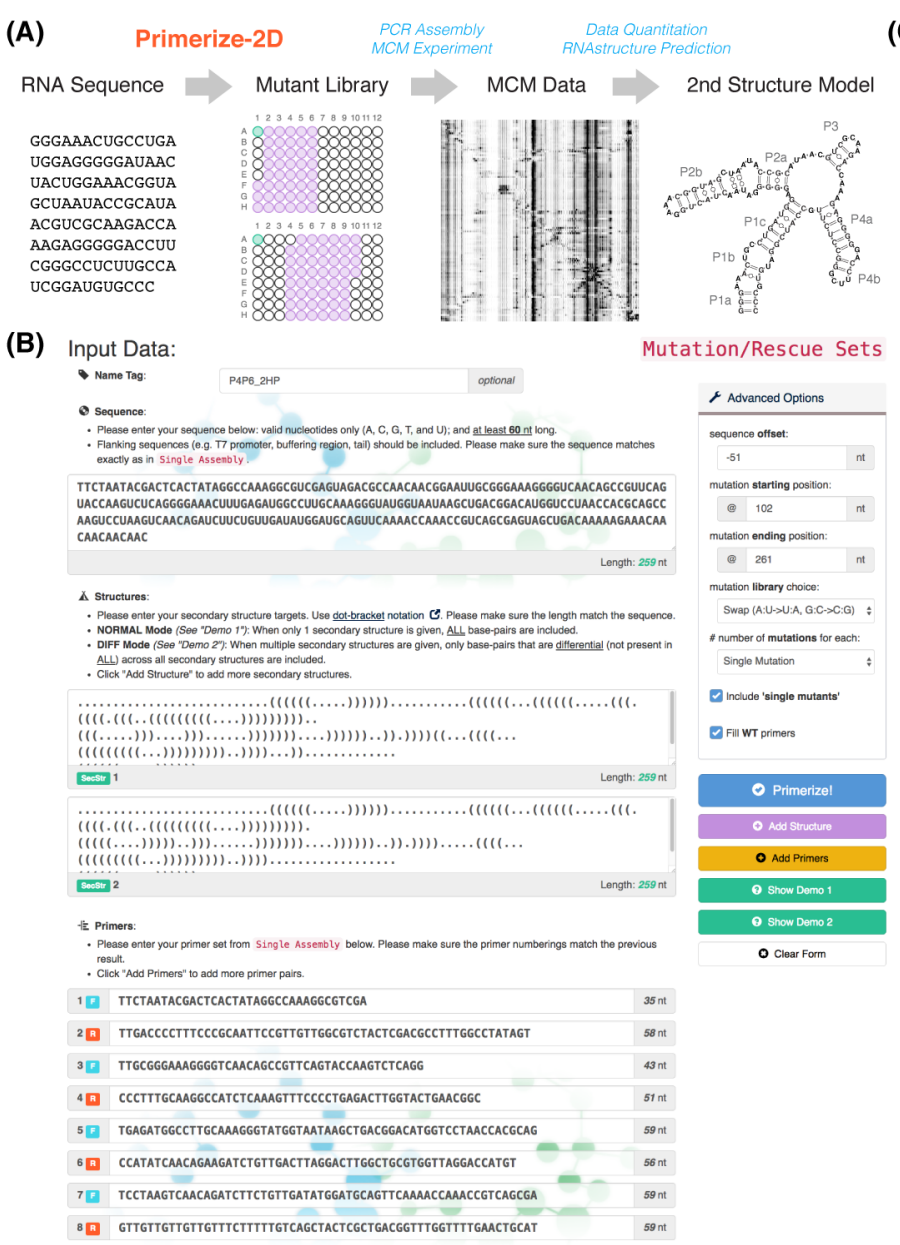
Supplementary Figure S2. Primerize-2D workflow and example input and output interface.

(A). Primerize-2D automates design of primer plates for mutate-and-map that involves synthesis of comprehensive single mutant library for multidimensional chemical mapping.

(B). Input interface of Primerize-2D server M^2R sets. Primerize takes a sense-strand DNA template sequence, and at least one secondary structure as input. Specific set of primers to use, as well as advanced options are available for customization.

(C). Output interface of Primerize-2D server M^2R sets. Primerize returns an illustration with sequence regions and targeted base pairs highlighted; graphic representation of construct primers in 96-well plate layout; and the general scheme of how primers assemble into the template. All results are assigned with a unique JOB_ID and are available for download.


(A)
Mutate-and-Map (M²)
G C
mutate
map
C C
5´
3´
Mutation Position
10
20
30
40
50
Sequence Position
(B)
Mutate-Map-Rescue (M²R)
G C
mutate
rescue
C G
MutB Position
MutA Position
Disruption
WT
MutA
MutB
MutAB
Rescue


(A)
Primerize-2D
PCR Assembly
MCM Experiment
Data Quantitation
RNAstructure Prediction
RNA Sequence
Mutant Library
MCM Data
2nd Structure Model
GGGAAACUGCCUGA
UGGAGGGGGAUAAC
UACUGGAAACGGUA
GCUAAUACCGCAUA
ACGUCGCAAGACCA
AAGAGGGGGACCUU
CGGGCCUCUUGCCA
UCGGAUGUGCCC
P3
P2a
P2b
P1c
P4a
P1b
P4b
P1a
(B)
Input Data:
Mutation/Rescue Sets
Name Tag:
P4P6_2HP
optional
Sequence:
Please enter your sequence below: valid nucleotides only (A, C, G, T, and U); and at least 60 nt long.
Flanking sequences (e.g. T7 promoter, buffering region, tail) should be included. Please make sure the sequence matches exactly as in Single Assembly.
TTCTAATACGACTCACTATAGGCCAAAGGCGUCGAGUAGACGCCAACAACGGAAUUGCGGGAAAGGGGUCAACAGCCGUUCAG
UACCAAGUCUCAGGGGAAACUUUGAGAUGGCCUUGCAAAGGGUAUGGUAAUAAGCUGACGGACAUGGUCCUAACCACGCAGCC
AAGUCCUAAGUCAACAGAUCUUCUGUUGAUAUGGAUGCAGUUCAAAACCAAACCGUCAGCGAGUAGCUGACAAAAAGAAACAA
CAACAACAAC
Length: 259 nt
Structures:
Please enter your secondary structure targets. Use dot-bracket notation. Please make sure the length match the sequence.
NORMAL Mode (See "Demo 1"): When only 1 secondary structure is given, ALL base-pairs are included.
DIFF Mode (See "Demo 2"): When multiple secondary structures are given, only base-pairs that are differential (not present in ALL) across all secondary structures are included.
Click "Add Structure" to add more secondary structures.
SecStr 1
Length: 259 nt
SecStr 2
Length: 259 nt
Primers:
Please enter your primer set from Single Assembly below. Please make sure the primer numberings match the previous result.
Click "Add Primers" to add more primer pairs.
1 F TTCTAATACGACTCACTATAGGCCAAAGGCGTCGA 35 nt
2 R TTGACCCCTTTCCCGCAATTCCGTTGTTGGCGTCTACTCGACGCCTTTGGCCTATAGT 58 nt
3 F TTGCGGGAAAGGGGTCAACAGCCGTTCAGTACCAAGTCTCAGG 43 nt
4 R CCCTTTGCAAGGCCATCTCAAAGTTTCCCCTGAGACTTGGTACTGAACGGC 51 nt
5 F TGAGATGGCCTTGCAAAGGGTATGGTAATAAGCTGACGGACATGGTCCTAACCACGCAG 59 nt
6 R CCATATCAACAGAAGATCTGTTGACTTAGGACTTGGCTGCGTGGTTAGGACCATGT 56 nt
7 F TCCTAAGTCAACAGATCTTCTGTTGATATGGATGCAGTTCAAAACCAAACCGTCAGCGA 59 nt
8 R GTTGTTGTTGTTGTTTCTTTTTGTCAGCTACTCGCTGACGGTTTGGTTTTGAACTGCAT 59 nt
Advanced Options
sequence offset:
-51
nt
mutation starting position:
@ 102
nt
mutation ending position:
@ 261
nt
mutation library choice:
Swap (A:U->U:A, G:C->C:G)
# number of mutations for each:
Single Mutation
Include 'single mutants'
Fill WT primers
Primerize!
Add Structure
Add Primers
Show Demo 1
Show Demo 2
Clear Form
(C)
Output Result:
JOB_ID: 1234567890fedcba
Save Result
INFO: DIFF Mode; (26).
52
102
TTCTAATACGACTCACTATAGGCCAAAGGCGTCGAGTAGACGCCAACAAC-GAATTGCGGGAAAGGGGTCAACAGCCGTTCAGTACCAAGTCTCAGGGGAAACTTTGAGATGGCCTTGCAAAGGGTAT
SUCCESS: All plates are ready to go. No editing is needed before placing the order.
Time elapsed: 1.2 s.
Plate Layout
Plate # 1 (26)
Primer 1 F (26)
Primer 2 R (26)
Primer 3 F (26)
Primer 4 R (26)
Primer 5 F (26)
Primer 6 R (26)
Primer 7 F (26)
Primer 8 R (26)
Assembly Scheme
What's next? Try our suggested experimental Protocol for PCR assembly.