

PROTOCOL 3S2

Supplementary protocol for assembly of gRNA arrays with new promoters

To construct gRNA arrays with new promoters (not included in this vector set), **PROTOCOLS 3A, 3B, 3C** and **3S1** can be used with following modifications:

Additional primers needed to include a new promoter in the assembly of **Csy4** arrays:

XXX (forward primer)

TGCTCTTCGCGCXXXXXXXXXXXXXXXXXXXX – replace the green Xs with a primer binding to the 5' end of the new promoter

YYY (reverse primer)

TCGTCTCXXXXXXXXXXCTGCCTATACGGCAGTGAACXXXXXXXXXXXXXXXXXXXX – replace red Xs with first 12 bases – reverse complement, of the first gRNA spacer being assembled. Replace the green Xs with a primer (reverse) binding to the 3' end of the new promoter

Additional primers needed to include a new promoter in the assembly of **tRNA** arrays:

XXX (forward primer)

TGCTCTTCGCGCXXXXXXXXXXXXXXXXXXXX – replace the green Xs with a primer binding to the 5' end of the new promoter

ZZZ (reverse primer)

TCGTCTCCAGGXXXXXXXXXXXXXXXXXXXX – replace the green Xs with a primer (reverse) binding to the 3' end of the new promoter

TRNA-FWD

TCGTCTCCCTGCAGGAACAAAGCACCA

Additional primers needed to include a new promoter in the assembly of **ribozyme** arrays:

XXX (forward primer)

TGCTCTTCGCGCXXXXXXXXXXXXXXXXXXXX – replace the green Xs with a primer binding to the 5' end of the new promoter

RRR (reverse primer)

TCGTCTCXXXXXXXXXXTCCGGTGACAAAAGCXXXXXXXXXXXXXXXXXXXX – replace red Xs with first 12 bases – reverse complement, of the first gRNA spacer being assembled. Replace the green Xs with a primer (reverse) binding to the 3' end of the new promoter

Note 1: Make sure that the new promoter does not contain AarI, BsaI, Esp3I and SapI sites as necessary.

In **PROTOCOLS 3A** and **3S1** (Csy4 arrays), primers XXX and YYY replace the original primers in PCR reaction #1 (step 2). In **PROTOCOL 3B** (tRNA arrays), an additional PCR reaction is performed (and its product included in the assembly), using primers XXX and ZZZ. Primer TRNA-FWD replaces the promoter specific primer in reaction #1. In **PROTOCOL 3C** (ribozyme arrays), primers XXX and RRR replace the original primers in PCR reaction #1 (step 2). A template containing the new promoter sequence is used in reactions with primers XXX and YYY, XXX and YYY, XXX and RRR.

Further steps are identical with **PROTOCOLS 3A, 3B, 3C** and **3S1**.

Note 2: Forward primer specific to the new promoter must be used for colony PCR and sequencing.