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Supplemental Information

Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression

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The dCas9 Protein Peptide Sequence

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR
KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIA
QLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILL
SDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKP
ILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPL
ARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVK
YVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDK
DFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTIL
DFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRH
KPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL
DINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTK
AERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREI
NNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLA
NGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWD
PKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSL
FELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITG
LYETRIDLSQLGGD

The sgRNA Design

5’-
N20GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC
GAGUCGGUGCUUUUUU-3’

Different sgRNA designs: only the N20 matching region is shown

The mRFP-Targeting sgRNAs Used in Figure 2C

T1 5’-UGGUCCGCUGCCGUUCGCUU-3’

T2 5’-GCAGAAAAAAACCAUGGGUU-3’

T3 5’-AAAAAACCGGUUCAGCUGCC-3’

NT1 (also rfp in Fig. 4B) 5’-AACUUUCAGUUUAGCGGUCU-3’

NT2 5’-AGGACAGUUUCAGGUAGUCC -3’

NT3 5’-AACCGGUUUUUUAGCCAUGU -3’

The promoter-targeting sgRNAs used in Fig. 2D:

P1 5’-UUGACAGCUAGCUCAGUCCU-3’

P2 5’-CCCGGAAGAGAGUCAAUUCA-3’

P3 5’-CCCUGAAUUGACUCUCUUCC-3’

P4 5’-GAAUUCAUUAAAGAGGAGAA -3’

P5 5’-GAAUGGUGCAAAACCUUUCG -3’

Target Promoter Sequence

5’-
CGACACCATCGAATGGTGCAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTCAATTCAG
GGTGGTGAATTTGACAGCTAGCTCAGTCCTAGGTATAATAGATCTGAATTCATTAAAGAGGAGAAAGGTAC
C-3’

The mRFP-Targeting sgRNAs Used in Figure 5B

5’-AACUUUCAGUUUAGCGGUCU-3’

5’-UGGAACCGUACUGGAACUGC-3’

5’-GGUAGUCCGGGAUGUCAGCC-3’

5’-AGGACAGUUUCAGGUAGUCC-3’

5’-GUCUUGCAGGGAGGAGUCCU-3’

5’-GCAUAACCGGACCGUCGGAC-3’

5’-CUUUCAGAGCACCGUCUUCC-3’

5’-GAUGGUGUAGUCUUCGUUGU-3’

The sfGFP-Targeting sgRNA (gfp) Used in Figure 4B

5’- CAUCUAAUUCAACAAGAAUU -3’

The sfGFP-Targeting sgRNAs Used in Figure 5B

5’-CAUCUAAUUCAACAAGAAUU-3’

5’-AGUAGUGCAAAUAAAUUUAA-3’

5’-ACAAGUGUUGGCCACGGAAC-3’

5’-UUUCAUGUGAUCCGGAUAAC-3’

5’-CGUUCCUGUACAUAACCUUC-3’

5’-UAACUCGAUACGAUUAACAA-3’

5’-AUAAUGGUCUGCUAGUUGAA-3’

5’-AUGUGGUCACGCUUUUCGUU-3’

The Double-sgRNA Targeting Experiments in Figures 5F and S6

R1 5’-AACUUUCAGUUUAGCGGUCU-3’

R2 5’-UGGAACCGUACUGGAACUGC-3’

R3 5’-GAUGGUGUAGUCUUCGUUGU-3’

R4 5’-UUCCGGGUACAUACGUUCGG-3’

R5 5’-GGUAGUCCGGGAUGUCAGCC-3’

R6 5’-AGGACAGUUUCAGGUAGUCC-3’

R7 5’-UUGACAGCUAGCUCAGUCCU-3’

R8 5’-AACCGGUUUUUUAGCCAUGU-3’

R9 5’-AAAAAACCGGUUCAGCUGCC-3’

The lac Operon-Targeting sgRNAs Used in Figure 6B

lacZ 5’-UUGGGAAGGGCGAUCGGUGC-3’

lacI 5’-GCUGGCCUGGUUCACCACGC-3’

lacY 5’-GUAGCCAAAUCGGGAAAAAC-3’

lacA 5’-CGGUAAGCCUUCGCACAUAU-3’

crp 5’-ACAAGAACCAUUCGAGAGUC-3’

cya 5’-GUCAAGCAGCAGUAUAUGCU-3’

A site 5’-UGUGAGUUAGCUCACUCAUU-3’

O site 5’-AUGUUGUGUGGAAUUGUGAG-3’

P site 5’-CUUCCGGCUCGUAUGUUGUG-3’

The EGFP-Targeting sgRNAs Used in Figure 7

eT1 5’-GGGCGAGGAGCUGUUCACCG-3’

eT2 5’-GGCCACAAGUUCAGCGUGUC-3’

eNT1 5’-GCCCUUGCUCACCAUGGUUG-3’

eNT2 5’-GACCAGGAUGGGCACCACCC-3’

eNT3 5’-GGUGGUGCAGAUGAACUUCA-3’

eNT4 5’-GUGGUCACGAGGGUGGGCCA-3’

eNT5 5‘-GCACGGGGCCGUCGCCGAUG-3’