Aim: To design and construct split-GFP and split-inteins SCN1A plasmids for subsequent SCN1A fragments complementation or trans-splicing assay.

Plasmids design:

The cytoplasmic loop (highlighted in yellow) connecting SCN1A DII to DIII were chosen as potential split site such that the resulting expression cassettes are small enough to be packaged into gene therapy vectors. The targeted split-GFP and split-inteins insertion sites (underscored) were designed to be flanked by extein amino acid combinations that allow efficient protein splicing.

>sp|P35498-2|SCN1A_HUMAN Isoform 2 of Sodium channel protein type 1 subunit alpha OS=Homo sapiens OX=9606 GN=SCN1A

MEQTVLVPPGPDSFNFFTRESLAAIERRIAEEKAKNPKPDKKDDDENGPKPNSDLEAGKNLPFIYGDIPPEMV
SEPLEDLDPYYINKKTFIVLNKGKAIFRFSATSALYILTPFNPLRKIAIKILVHSLFSMLIMCTILTNCVFMTMSNPP
DWTKNVEYTFTGIYTFESLIKIIARGFCLEDFTFLRDPWNWLDFTVITFAYVTEFVDLGNVSALRTFRVLRALKTI
SVIPGLKTIVGALIQSVKKLSDVMILTVFCLSVFALIGLQLFMGNLRNKCIQWPPTNASLEEHSIEKNITVNYNG
TLINETVFEFDWKSYIQDSRYHYFLEGFLDALLCGNSSDAGQCPEGYMCVKAGRNPNYGYTSFDTFSWAFLSL
FRLMTQDFWENLYQLTLRAAGKTYMIFFVLVIFLGSFYLINLILAVVAMAYEEQNQATLEEAEQKEAEFQQMI
EQLKKQQEAAQQAATATASEHSREPSAAGRLSDSSSEASKLSSKSAKERRNRRKKRKQKEQSGGEEKDEDEF
QKSESEDSIRRKGFRFSIEGNRLTYEKRYSSPHQSLLSIRGSLFSPRRNSRTSLFSFRGRAKDVGSENDFADDEHS
TFEDNESRRDSLFVPRRHGERRNSNLSQTSRSSRMLAVFPANGKMHSTVDCNGEVSLVGGPSVPTSPVGQL
LPEGTTTETEMRKRRSSSFHVSMDFLEDPSQRQRAMSIASILTNTVEELEESRQKCPPCWYKFSNIFLIWDCSP
YWLKVKHVVNLVVMDPFVDLAITICIVLNTLFMAMEHYPMTDHFNNVLTVGNLVFTGIFTAEMFLKIIAMDP
YYYFQEGWNIFDGFIVTLSLVELGLANVEGLSVLRSFRLLRVFKLAKSWPTLNMLIKIIGNSVGALGNLTLVLAII
VFIFAVVGMQLFGKSYKDCVCKIASDCQLPRWHMNDFFHSFLIVFRVLCGEWIETMWDCMEVAGQAMCL
TVFMMVMVIGNLVVLNLFLALLLSSFSADNLAATDDDNEMNNLQIAVDRMHKGVAYVKRKIYEFIQQSFIRK
QKILDEIKPLDDLNNKKDSCMSNHTAEIGKDLDYLKDVNGTTSGIGTGSSVEKYIIDESDYMSFINNPSLTVTVP
IAVGESDFENLNTEDFSSESDLEESKEKLNESSSSSEGSTVDIGAPVEEQPVVEPEETLEPEACFTEGCVQRFKC
CQINVEEGRGKQWWNLRRTCFRIVEHNWFETFIVFMILLSSGALAFEDIYIDQRKTIKTMLEYADKVFTYIFILE
MLLKWVAYGYQTYFTNAWCWLDFLIVDVSLVSLTANALGYSELGAIKSLRTLRALRPLRALSRFEGMRVVVN
ALLGAIPSIMNVLLVCLIFWLIFSIMGVNLFAGKFYHCINTTTGDRFDIEDVNNHTDCLKLIERNETARWKNVK
VNFDNVGFGYLSLLQVATFKGWMDIMYAAVDSRNVELQPKYEESLYMYLYFVIFIIFGSFFTLNLFIGVIIDNF
NQQKKKFGGQDIFMTEEQKKYYNAMKKLGSKKPQKPIPRPGNKFQGMVFDFVTRQVFDISIMILICLNMVT
MMVETDDQSEYVTTILSRINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLAELIEKYFVSPTLF
RVIRLARIGRILRLIKGAKGIRTLLFALMMSLPALFNIGLLLFLVMFIYAIFGMSNFAYVKREVGIDDMFNFETFG
NSMICLFQITTSAGWDGLLAPILNSKPPDCDPNKVNPGSSVKGDCGNPSVGIFFFVSYIIISFLVVVNMYIAVIL
ENFSVATEESAEPLSEDDFEMFYEVWEKFDPDATQFMEFEKLSQFAAALEPPLNLPQPNKLQLIAMDLPMVS
GDRIHCLDILFAFTKRVLGESGEMDALRIQMEERFMASNPSKVSYQPITTTLKRKQEEVSAVIIQRAYRRHLLK

RTVKQASFTYNKNKIKGGANLLIKEDMIIDRINENSITEKTDLTMSTSACPPSYDRVTKPIVEKHEQEGKDEKAK
GK*

Cloning pipeline:

Plasmids with superfolder-GFP(sfGFP) coding sequence inserted at each of the split sites (SCN1A-internal-sfGFP) will first be generated using Gibson assembly. These plasmids are then subcloned and split into two plasmids expressing split-GFP(SCN1A-$GFP_{(1-10)}$ and SCN1A-$GFP_{(11)}$). Split-intein sequences will subsequently be subcloned into these split-GFP constructs (SCN1A-N-$_{intein}$-$GFP_{(1-10)}$ and SCN1A-C-$_{intein}$-$GFP_{(11)}$) using Gibson assembly.

Note: Please see attached spreadsheet for primer sequences and cloning condition for constructing SCN1A-internal-sfGFP, SCN1A-$GFP_{(1-10)}$ and SCN1A-$GFP_{(11)}$ at position 1047. Plasmid maps and sequencing results for each construct are also attached.