Synthetic ribosome skip sequence derived from *Thosea asigna* virus

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TaV 2A protein sequence from mGRASP constructs

**Visually checking 2A skip in human cells**

CAGGS-mTagBFP2-T2A-iCre

CAG-loxP-3pA-loxP-
H2B-sfGFP-GlySerAla x9 or T2A-tdTomato

**No skip, GlySerAla x9**

No skip of tdTomato from H2B-sfGFP → nuclear localized tdTomato

**Skip, 2A**

Efficient skip of tdTomato from H2B-sfGFP → diffuse tdTomato

Calcium phosphate transfections of 293T transformed human embryonic kidney epithelial cells

Fluorescence from live cells

H2B-sfGFP only

tdTomato only

H2B-sfGFP-GSAx9-tdTomato

H2B-sfGFP-T2A-tdTomato
Quantifying 2A skip by fluorescence resonance energy transfer

293T cells (calcium phosphate transfections)

FRET from sfGFP (minus H2B) to tdTomato in DRAQ7- mTagBFP2+ cells (viability and transfection controls)

Control non-fluorescent plasmids

sfGFP GSAx9 tdTomato

sfGFP T2A tdTomato
Measuring the fluorescence intensities of first and second proteins

GFP or RFP Median Fluorescence Intensity in cells gated for GFP+ or RFP+

Constructs of sfGFP and tdTomato fused by GSAX9 or T2A sequences with different linkers

GGSGGRS EGRGSLTCDGVEENPG / P APGST M

Sample

293T cells (calcium phosphate transfections)
All T2A constructs skipped (see previous page)

TdTomato protein fluorescence of skipping constructs appear to be similar to the unskippable GSAX9 fusion, suggesting 1:1 ratio of skipped first and second proteins

Unfortunately sfGFP in GSAX9 is quenched due to FRET?
Additional calculations are necessary to demonstrate similar fluorescence of the first protein.
Visually checking 2A skip in mouse cells

Electroporations of mouse embryonic stem cells

CAG-loxP-3pA-loxP-H2B-sfGFP-T2A-tdTomato (-XE +APGST) + Bluescript2

CAG-loxP-3pA-loxP-H2B-sfGFP-T2A-tdTomato (-XE +APGST) + CAGGS-pac-T2A-iCre

CAG-loxP-3pA-loxP-H2B-sfGFP-T2A-tdTomato (-XE +APGST) + CAGGS-pac-T2A-iCre

Condensed chromosomes
A synthetic ribosome skip sequence that seems to work well in human and mouse cells

DNA sequence in the plasmid sequence files

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