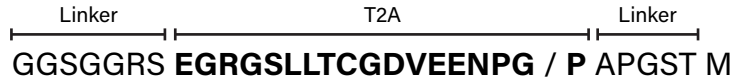


**Synthetic ribosome skip sequence derived from *Thosea asigna* virus**

Documentation for Addgene deposit

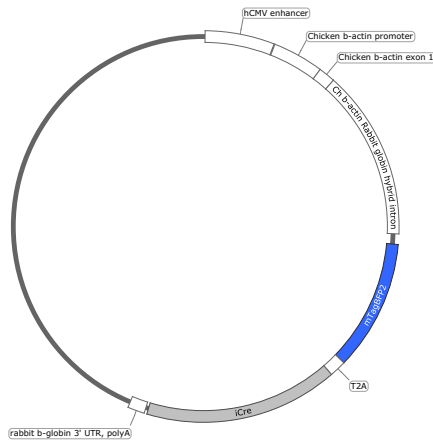
Hyung-song Nam and Mario R. Capecchi

TaV 2A protein sequence from mGRASP constructs  
Kim et al., *Nature Methods*, 9, 96–102 (2012)

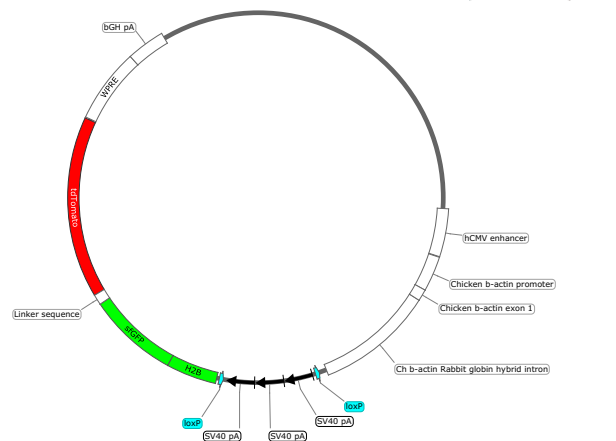


*Visually checking 2A skip in human cells*

CAGGS-mTagBFP2-T2A-iCre

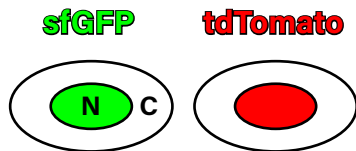


CAG-loxP-3pA-loxP-  
H2B-sfGFP-GlySerAla x9 or T2A-tdTomato



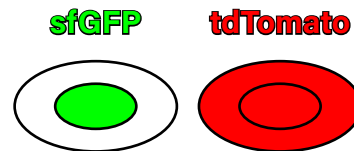
**No skip, GlySerAla x9**

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



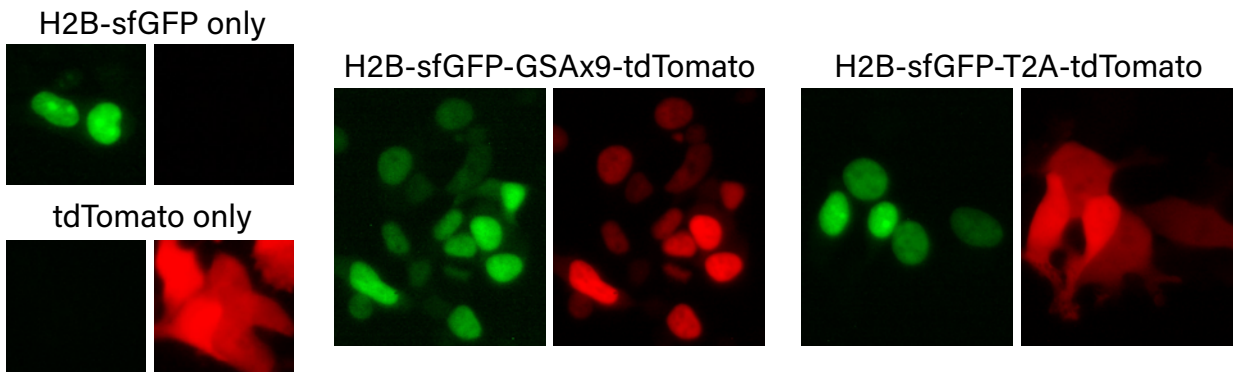
**Skip, 2A**

Efficient skip of tdTomato from  
H2B-sfGFP → diffuse tdTomato



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

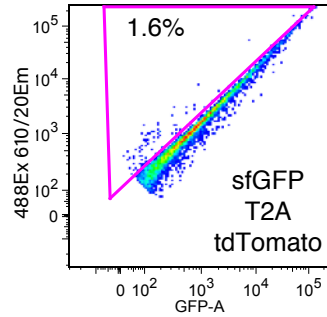
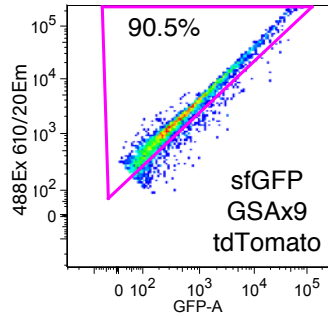
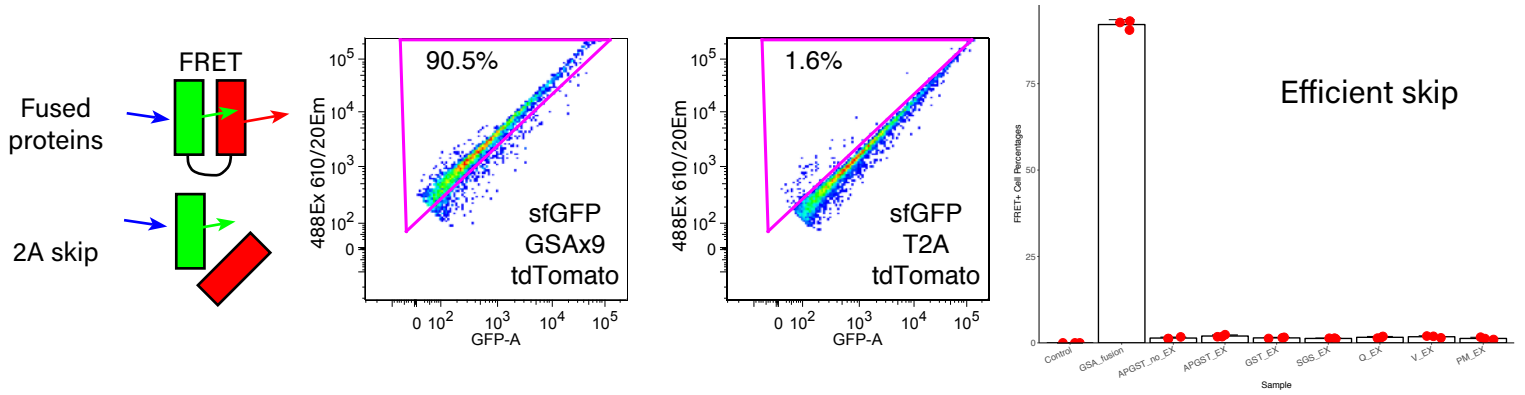
Fluorescence from live cells



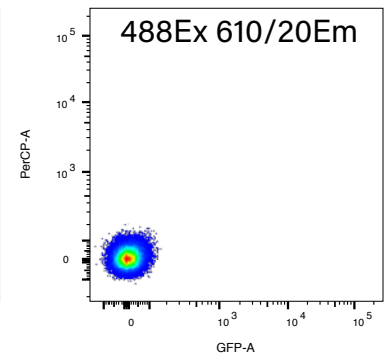
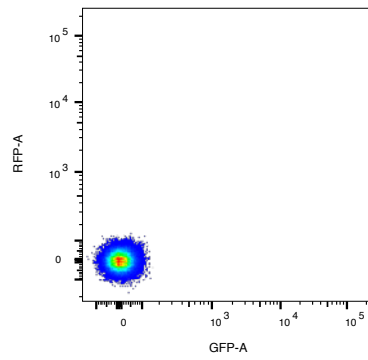
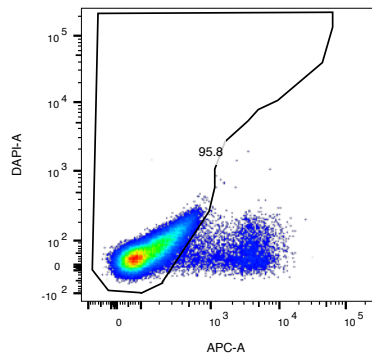
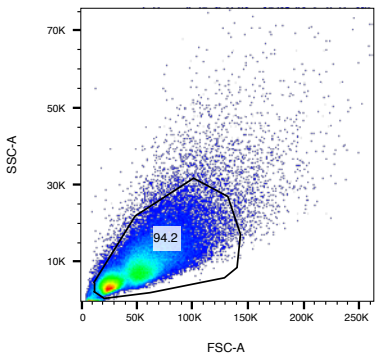
# Quantifying 2A skip by fluorescence resonance energy transfer

293T cells (calcium phosphate transfections)

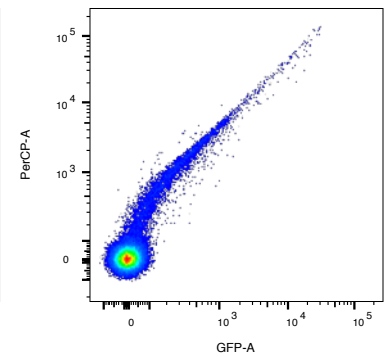
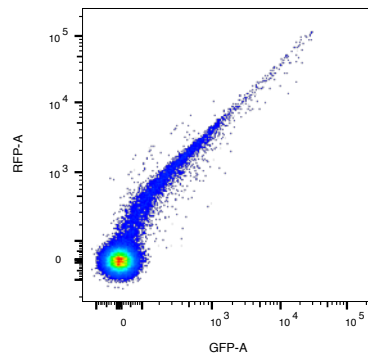
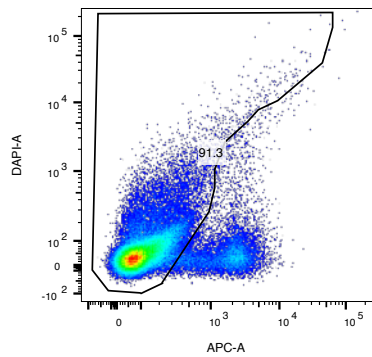
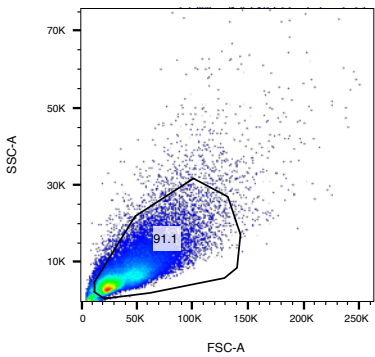
FRET from sfGFP (minus H2B) to tdTomato  
in DRAQ7- mTagBFP2+ cells (viability and transfection controls)



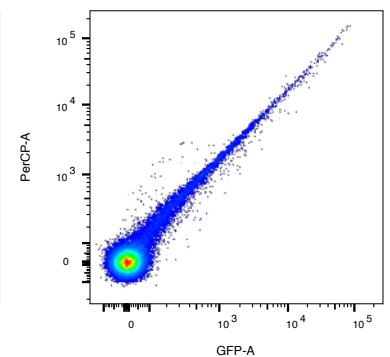
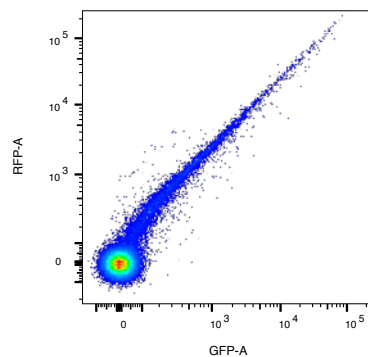
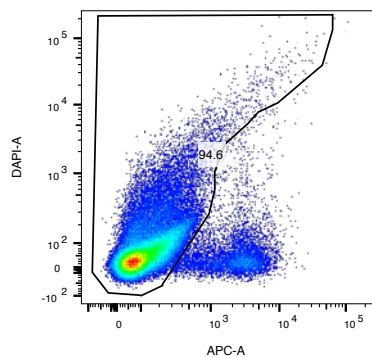
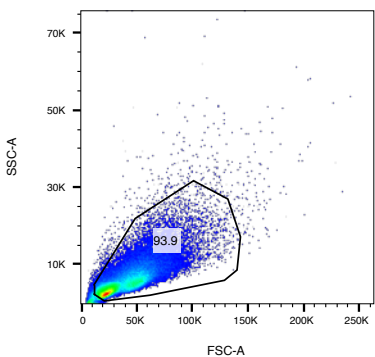
## Control non-fluorescent plasmids



## sfGFP GSAX9 tdTomato



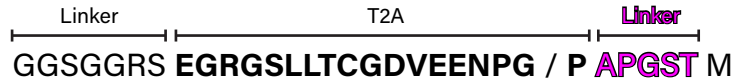
## sfGFP T2A tdTomato



Measuring the fluorescence intensities of first and second proteins

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

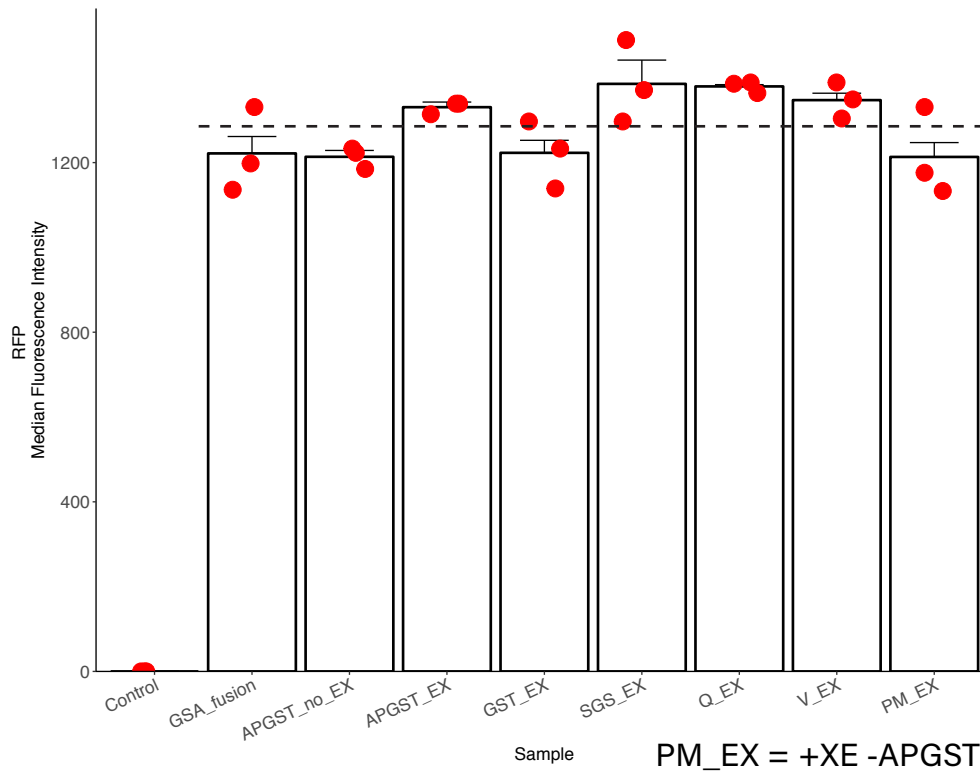
Constructs of sfGFP and tdTomato fused by GSx9 or T2A sequences with different linkers



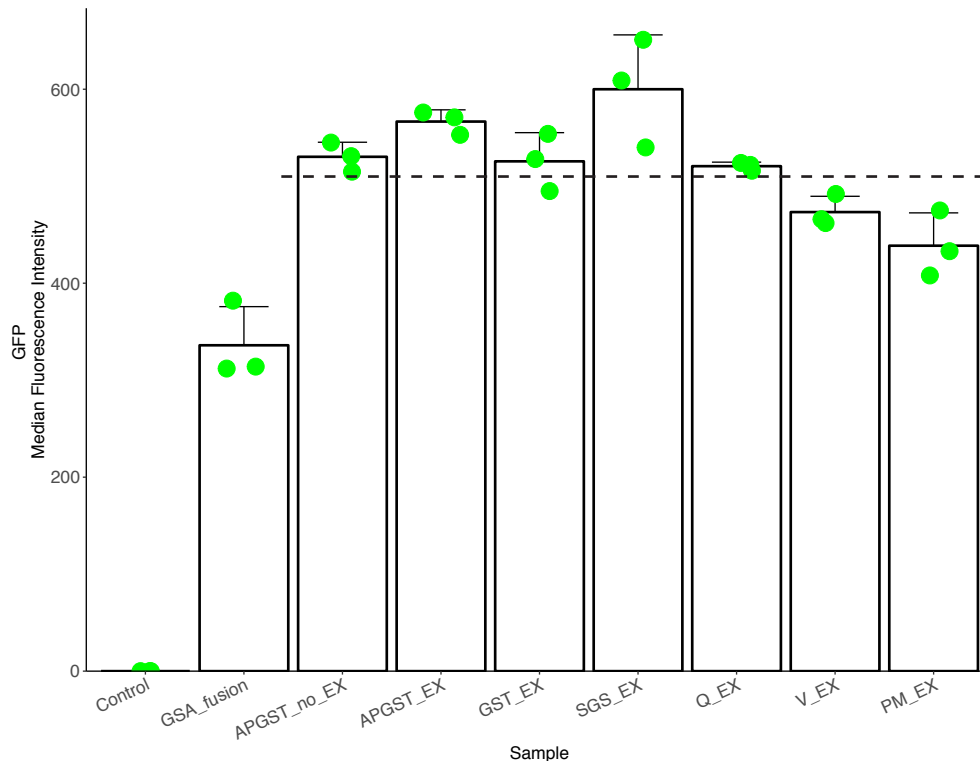
+/- XmaI and EcoRI sites (XE)

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 GSx9 fusion, suggesting 1:1 ratio of skipped first and second proteins

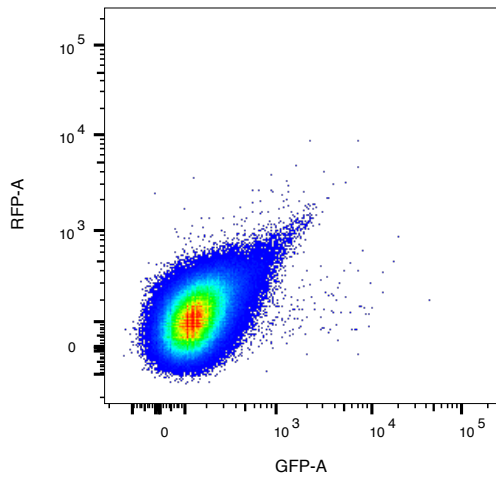


Unfortunately sfGFP in GSx9 is quenched due to FRET? Additional calculations are necessary to demonstrate similar fluorescence of the first protein

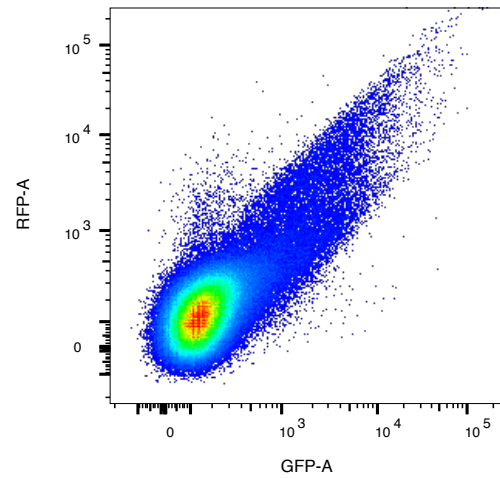
*Visually checking 2A skip in mouse cells*

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

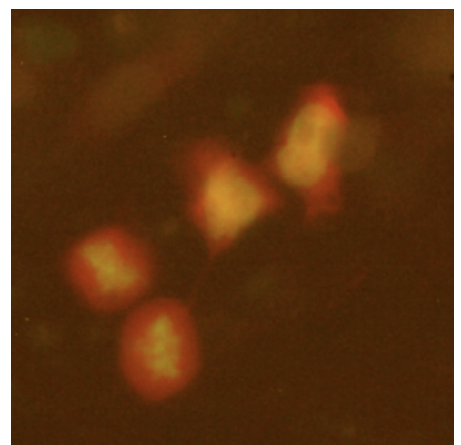
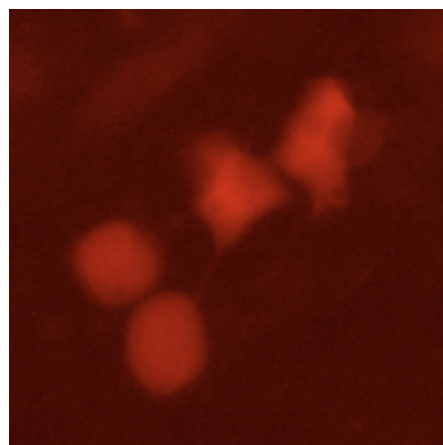
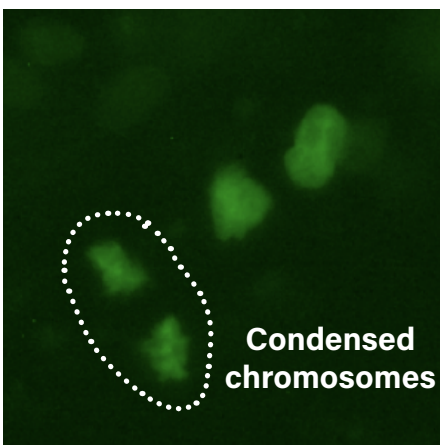
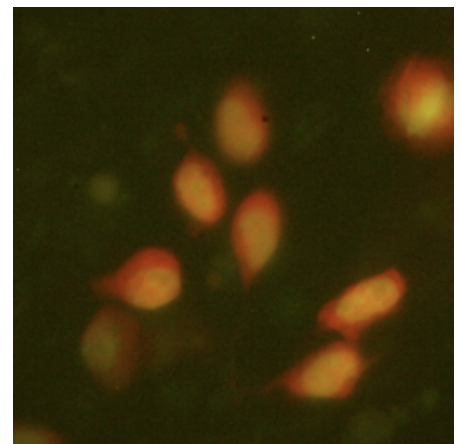
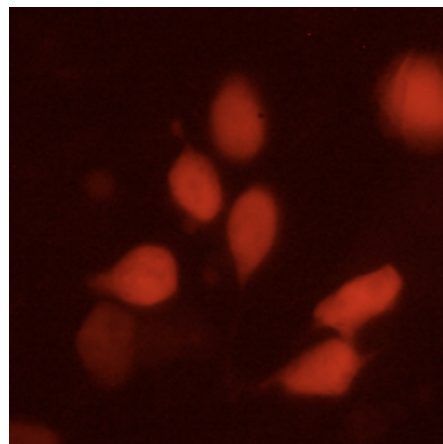
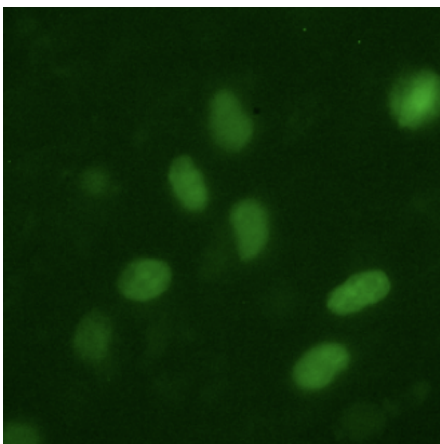


CAG-loxP-3pA-loxP-H2B-sfGFP-T2A-tdTomato (-XE +APGST) + CAGGS-pac-T2A-iCre

**GFP**

**RFP**

**Merge**



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

Linker T2A Linker  
GGSGGRS **EGRGSLT**CGDVEENPG / P APGST M

DNA sequence in the plasmid sequence files

Online November 2018  
Department of Human Genetics, University of Utah School of  
Medicine

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Flow Cytometry Core Facility