**Assessing the activities of T2A-creER$^{T2}$ variants in a cell-based assay**

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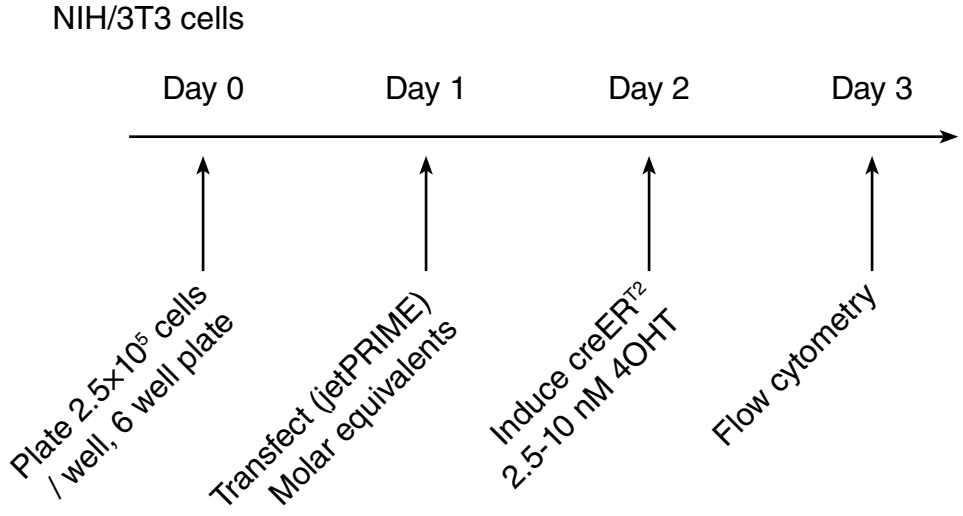
Assay design:




Constructs transfected:


+

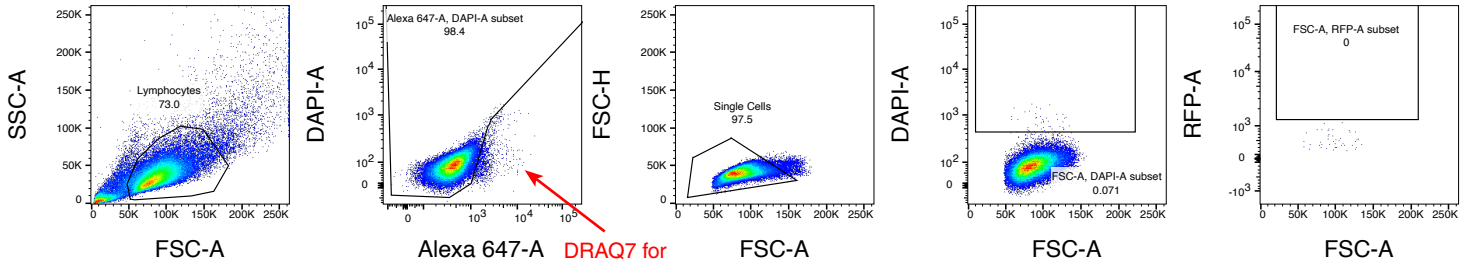
pCAG-loxP-3pA-loxP-tdTomato-WPRE-bpA

+

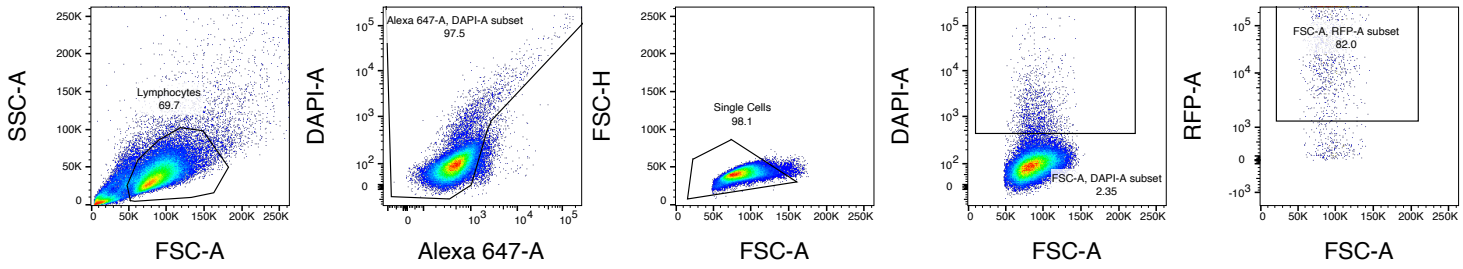
pBluescript II SK- carrier

## Flow cytometry gating examples:

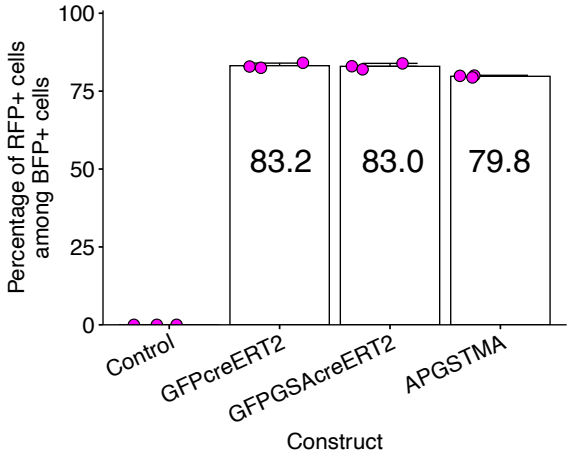
Control

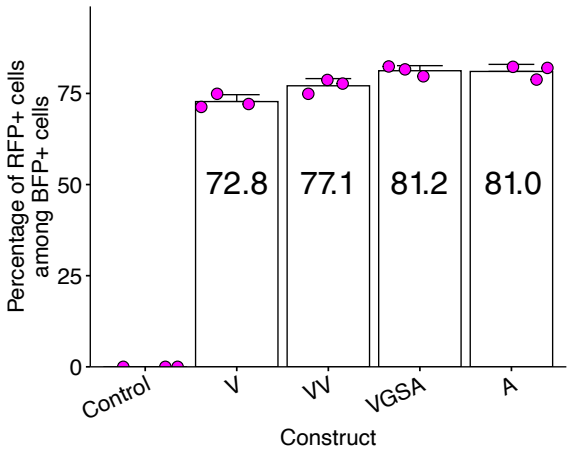



A




## Result summary:

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        Pairwise comparisons using t tests with pooled SD

data:  all_data$Percentage and all_data$Construct

                A       APGSTMA Control GFPcreERT2 GFPGSAcreERT2 V       VGSA
APGSTMA         0.82310 -       -       -          -             -       -
Control         < 2e-16 < 2e-16 -       -          -             -       -
GFPcreERT2      0.58470 0.08274 < 2e-16 -          -             -       -
GFPGSAcreERT2 0.70925 0.10939 < 2e-16 1.00000    -             -       -
V               2.6e-05 0.00018 < 2e-16 1.5e-06    1.8e-06       -       -
VGSA            1.00000 0.82310 < 2e-16 0.70925    0.70925       2.0e-05 -
VV              0.03289 0.26133 < 2e-16 0.00085    0.00113       0.01796 0.02437

P value adjustment method: holm
```

N-terminal amino acids of T2A-skipped creER$^{T2}$ seem to modulate recombination efficiency, presumably due to differential stabilities of the proteins

See Kats et al., *Molecular Cell*, 70, 488-501 (2018),
Reinhardt et al., *Stem Cell Reports*, 14, 1-8 (2020)

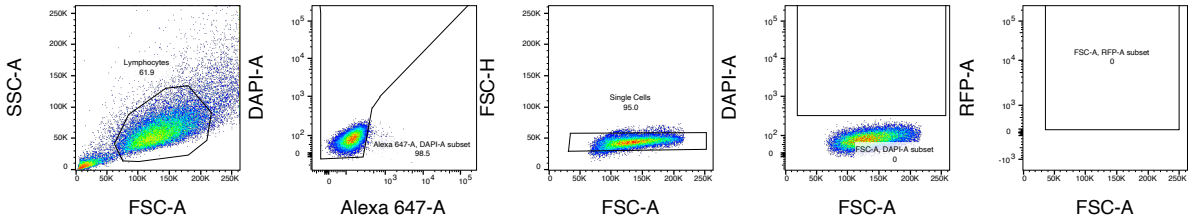
# Additional variants

## pCAGGS-mTagBFP2-GGSGGRS EGRGSLLTCGDVEENPG / P x NLL-iCreER$^{T2}$

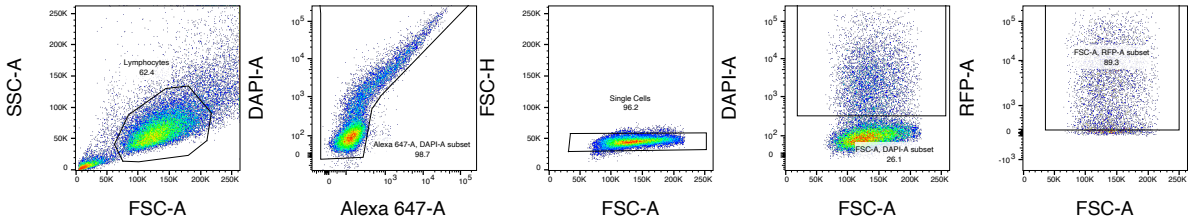
## x = AA, AAA, AAAA, AAGSA, AGSA, AGSAS, APGSTMA, and V

## As well as GFPcreER$^{T2}$

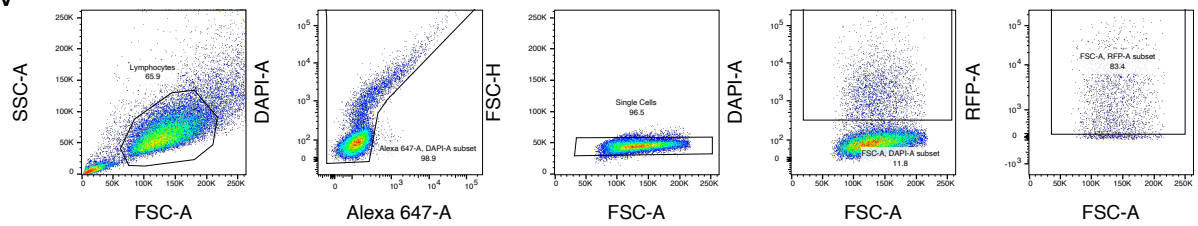
Control

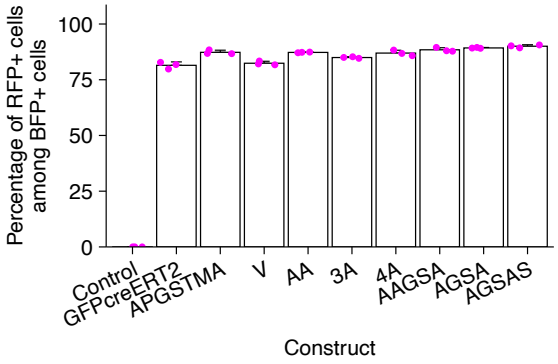



AGSAS




V




| | Construct | Mean_Percentage | SD_Percentage | Median_Percentage |
|---|---|---|---|---|
| 1 | 3A | 84.96667 | 0.3511885 | 85.0 |
| 2 | 4A | 86.96667 | 1.2583057 | 86.8 |
| 3 | AA | 87.26667 | 0.1527525 | 87.3 |
| 4 | AAGSA | 88.43333 | 0.9291573 | 88.0 |
| 5 | AGSA | 89.26667 | 0.2081666 | 89.2 |
| 6 | AGSAS | 90.03333 | 0.6658328 | 90.2 |
| 7 | APGSTMA | 87.30000 | 0.9539392 | 86.8 |
| 8 | Control | 0.00000 | 0.0000000 | 0.0 |
| 9 | GFPcreERT2 | 81.46667 | 1.5275252 | 81.8 |
| 10 | V | 82.40000 | 0.8888194 | 82.1 |

```
        Pairwise comparisons using t tests with pooled SD

data:  all_data$Percentage and all_data$Construct

           3A      4A      AA      AAGSA   AGSA    AGSAS   APGSTMA Control GFPcreERT2
4A         0.1140  -       -       -       -       -       -       -       -
AA         0.0489  1.0000  -       -       -       -       -       -       -
AAGSA      0.0013  0.4122  0.8460  -       -       -       -       -       -
AGSA       9.8e-05 0.0489  0.1140  1.0000  -       -       -       -       -
AGSAS      1.1e-05 0.0049  0.0128  0.3081  1.0000  -       -       -       -
APGSTMA    0.0467  1.0000  1.0000  0.8460  0.1140  0.0136  -       -       -
Control    < 2e-16 < 2e-16 < 2e-16 < 2e-16 < 2e-16 < 2e-16 < 2e-16 -       -
GFPcreERT2 0.0013  3.3e-06 1.5e-06 8.6e-08 1.3e-08 2.6e-09 1.4e-06 < 2e-16 -
V          0.0226  4.5e-05 1.9e-05 8.6e-07 1.1e-07 1.9e-08 1.8e-05 < 2e-16 1.0000

P value adjustment method: holm
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From three separate experiments, V is low, AGSAS is high, others in between

The numbers are somewhat different, variabilities in transient transfections

Statistically significant but subtle differences, will the subtle differences be useful in vivo?

The characterization is still work in progress

Welcome to use the plasmids

Online March 2020
Revised January 2021

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