**SPARC README**

Written by Helen Yang, Ph.D. (Wilson Lab) July 24, 2019.

Updated by Jesse Isaacman-Beck, Ph.D. (Clandinin Lab) September 3, 2019.

*How do SPARC and SPARC.2 work?*

 The SPARC construct is a UAS-Effector construct that uses PhiC31 recombinase to probabilistically remove a STOP cassette to enable expression of the effector in a subset of the cells in a GAL4 line. PhiC31 catalyzes irreversible recombination between one attP and one attB site; SPARC contains two attP sites and one attB sites, so there are two possible, mutually exclusive reactions between the attB site and one of the two attP sites. One of these reactions removes the STOP cassette, allowing UAS-mediated expression of the effector (rxn 1). The other reaction removes the spacer and preserves the STOP cassette, thereby resulting in no expression of the effector, even in GAL4+ cells (rxn 2). When the two attP sites are identical, these reactions happen at approximately equal probability, thereby producing ~45% sparsening. Truncating the attP site from its full length (attP60) to shorter sequences (attP38 and attP34) decreases the efficiency of the PhiC31-mediated recombination. When attP38 or attP34 are paired with full length attP60 in the same construct, the two possible reactions do not occur at the same probability; the reaction between the attB and the full length attP60 is more likely. The construct with attP38 results in ~15% of GAL4+ cells expressing the effector, and the construct with attP34 results in ~5% of GAL4+ cells expressing the effector. We note that while we have seen Gal4 driver-dependent variation in these percentages, we routinely see that the 3 SPARC modules express at 3 precise levels. Therefore, we have named these 3 modules SPARC-D, SPARC-I, and SPARC-S (D = “Dense”, I = “Intermediate”, and S = “Sparse”)

Jesse Isaacman-Beck

 With some SPARC-effector pairs (LexAp65, mCD8::GFP), we have seen leaky expression of effector in the absence of PhiC31. We therefore generated a new version - SPARC2 – that incorporates ribozyme sequences into the cassette. We have found that these sequences decrease leaky expression up to ~10,000-fold and they ensure that the transgenes express in the appropriate proportions of neurons.

*SPARC transgene genomic location:*

Because the SPARC and SPARC2 modules depend on PhiC31 recombinase and PhiC31’s recognition sequences attP and attB, donor vectors cannot be inserted into traditional attP-based landing sites. Instead, we use CRISPR-HDR to insert SPARC and SPARC.2 Donor constructs into the genome; we targeted the region around the attP40 landing site (2L:5,108,448..5,108,448), a site widely used for transgenesis. To date, all SPARC and SPARC.2 transgenes have been inserted in this location as validated by the PCR described below:

**Insertion site validation**:

To validate the insertion site of SPARC transgenes, we PCR amplified genomic DNA with primer pairs in which one primer recognizes genomic DNA beyond the homology arm and the other recognizes sequence within the transgene. For every SPARC or SPARC2 transgenic fly, we ensure amplification of PCR product from both the 5’ and 3’ sides of the transgene. The primer pairs are as follows:

|  |  |  |
| --- | --- | --- |
| Primer Name | Sequence | Product Size (bp) |
| attP40-HAR-Verify-F1 | CTGGACATCACCTCCCACAAC |  |
| attP40-HAR-Verify-R1 | ACATCGATCTCGAATGGATTCTCGG | ~1500bp |
| attP40-HAL-Verify-F3 | GAGCGGAAAGCAATGTTTATGCGA |  |
| attP40-HAL-Verify-R2 | CGGCCGCGCTGGTGGTTGTTT | ~1100bp |

Note 1: The attP40-HAR-Verify-F1primer recognizes sequence in the floxed 3xP3-DsRed sequence. Therefore, one must validate the insertion site using DNA from SPARC transgenic flies that still retain the floxed 3xP3-DsRed sequence.

Note: HAL primers have some background amplification in control flies, try a higher annealing temperature to get rid of this.

*Using SPARC*

* A minimum of three transgenes is required: the GAL4 driver, the SPARC-effector, and the source of PhiC31.
* When building flies, **DO NOT** make a stable stock combining the PhiC31 transgene with the SPARC-effector. Any expression of PhiC31 in the germline can result in recombination between the attP and attB sites of the SPARC transgene that will be passed on to the next generation, and your stock will have flies where the SPARC construct no longer results in sparse expression of the effector.
* The PhiC31 transgenes are: nSyb-PhiC31, tubP-PhiC31, and UAS-PhiC31. The choice of PhiC31 source will affect the pattern of sparsening. nSyb-PhiC31 expresses PhiC31 in neurons pretty late in development (likely post-mitotically) such that expression of the SPARC effector in each neuron in the GAL4 line is independent. tub-PhiC31 expresses relatively early, resulting in clonally related populations having the same expression of the SPARC effector. The timing of UAS-PhiC31 expression, and therefore the independence of expression among clonally related cells, depends on the GAL4 line.

Here are the transgenic lines that we have sent to you:

SPARC and SPARC2 Flies sent to BDSC: Inserted with CRISPR-HDR into a region near the *attP40* locus

1. *+; 20XUAS-SPARC-D-GCaMP6f; +*
2. *+; 20XUAS-SPARC-I-GCaMP6f; +*
3. *+; 20XUAS-SPARC-S-GCaMP6f; +*
4. *+; 20XUAS-SPARC-D-jGCaMP7f; +*
5. *+; 20XUAS-SPARC-I-jGCaMP7f; +*
6. *+; 20XUAS-SPARC-S-jGCaMP7f; +*
7. *+; 20XUAS-SPARC2-D-LexA; +*
8. *+; 20XUAS-SPARC2-I-LexA; +*
9. *+; 20XUAS -SPARC2-S-LexA; +*
10. *+; 20XUAS-SPARC2-D-Syn21-CsChrimson::tdTomato-3.1; +*
11. *+; 20XUAS-SPARC2-I-Syn21-CsChrimson::tdTomato-3.1; +*
12. *+; 20XUAS-SPARC2-S-Syn21-CsChrimson::tdTomato-3.1; +*
13. *+; 20XUAS-SPARC2-D-mCD8::GFP; +*
14. *+; 20XUAS-SPARC2-I-mCD8::GFP; +*
15. *+; 20XUAS-SPARC2-S-mCD8::GFP; +*

PhiC31 transgenic strains:

1. y,w; *P{nSyb-IVS-PhiC31}suHw{attP5}; +*
2. y,w; *P{20XUAS-IVS-PhiC31} suHw{attP5}; +*
3. y,w; *P{Tub-IVS-PhiC31} suHw{attP5}; +*
4. y,w, *P{nSyb-IVS-PhiC31}attP18; +*
5. y,w, *P{20XUAS-IVS-PhiC31}attP18; +*
6. +, *P{nSyb-IVS-PhiC31}attP18; s/cyo; pr/TM6B (Good for split Gal4s; because of balancers, less healthy)*
7. +, *P{20XUAS-IVS-PhiC31}attP18; s/cyo; pr/TM6B (Good for split Gal4s; because of balancers, less healthy)*