Synergistic Activation Mediators

ZhangLab

CRISPR/Cas9 Synergistic Activation Mediator (SAM) pooled human library

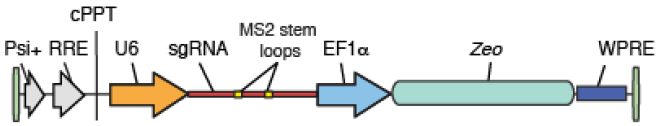
CRISPR/Cas9 Synergistic Activation Mediator (SAM) is an engineered protein complex for the transcriptional activation of endogenous genes. It consists of three components:

1. A nucleolytically inactive Cas9-VP64 fusion,
2. An sgRNA incorporating two MS2 RNA aptamers at the tetraloop and stem-loop 2
3. The MS2-P65-HSF1 activation helper protein.

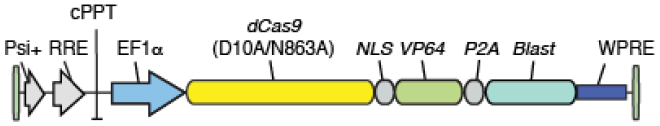
SAM can be combined with a human genome-wide library to activate all known coding isoforms form the RefSeq database (23,430 isoforms).

Here we describe how to amplify the human SAM library to have sufficient quantity to produce lentivirus, while maintaining full library representation.

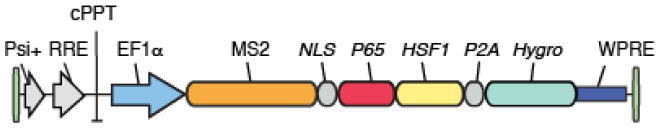
Library description: The SAM library consists of 3 unique sgRNAs targeting each human RefSeq coding isoform in the proximal promoter (> 90% of sgRNAs are targeted to the first 200bp upstream of the TSS of their target). The total library size is 70,290 guides. For SAM gain-of-function screening, this sgRNA library has to be combined with two additional SAM constructs – dCas9-VP64 and MS2-P65-HSF1. **Cells have to be transduced with all three SAM components**. To enable co-transduction and selection, all three lentiviral vectors have unique resistance markers as shown below:



guide RNA lentiviral expression vector



dCas9-VP64 lentiviral expression vector



MS2-p65-HSF1 lentiviral expression vector

Representation: The SAM library is a pool of many different vectors mixed together. The library contains 70,290 unique sgRNAs. To ensure no loss of representation, it is important to amplify the library using the protocol given on the next page. **The SAM library should not be transformed using chemically competent cells or amplified in liquid cultures. Please read over the entire protocol before starting library amplification.**

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Citation: Please reference the following publications for the use of this material.

Konermann S*, Brigham MD*, Trevino AE, Joung J, Abudayyeh OO, Barcena C, Hsu PD, Habib N, Gootenberg JS, Nishimasu H, Nureki O & Zhang F. Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. *Nature,* doi:10.1038/nature14136 (201,)

SAM library pooled electroporation, plating, determination of transformation efficiency and maxi prep

1. The SAM library is distributed by Addgene at a concentration of 50 ng/uL

2. Electroporate the library
    a. Add 1 uL of 50 ng/uL SAM library to 25 uL of electrocompetent cells with an efficiency of ≥10^9 cfu/ug on ice. We have had good success with Lucigen E. cloni 10G Elite.
    b. Electroporate using the manufacturer's suggested parameters/protocol.
    c. Recover in 975 uL recovery media (i.e. media provided with cells) and transfer to a loosely capped tube with an additional 1 mL of recovery media.
    d. Repeat for a total of 8 electroporations and rotate at 250 rpm for 1 hour at 37 C

3. Plate a dilution to calculate transformation efficiency
    *Note the library plasmids have **ampicillin resistance** – prepare all plates accordingly.*
    a. Pool all 16 mL of electroporated cells. Mix well.
    b. Remove 20 uL and add to 1 mL of recovery media, mix well, and plate 100uL onto a pre-warmed 10cm petri dish (ampicillin). This is a 10,000-fold dilution of the full transformation and will enable you to estimate transformation efficiency to ensure that full library representation is preserved.

4. Plate the transformations
    Follow Step a) if your lab has 24.5 cm^2 bioassay plates for large-scale bacteria culture; otherwise follow Step b), which substitutes 20 standard (10 cm round) petri dishes.
    a. Plate 2mL of transformation on each of 8 pre-warmed 24.5 cm^2 bioassay plates (ampicillin) using a spreader. Spread the liquid culture until it is largely absorbed into the agar and won't drip when turned upside down. At the same time, **make sure the liquid culture does not completely dry out as this will lead to poor survival!**
    b. Alternatively, spread 200 uL of transformation mix per petri onto 80 pre-warmed petri dishes (ampicillin).

5. Grow all plates inverted overnight at 37 C. We recommend growing for 12-14h.

6. Calculate transformation efficiency
    a. Count the number of colonies on the dilution plate.
    b. Multiply this number of colonies by 80,000 for the total number of colonies on all plates.
    c. Proceed if the total number of colonies is at least 7 x 10^7. This efficiency is equivalent to 100X colonies per sgRNA in the library.

7. Harvest colonies
    a. Pipette 10 mL of LB onto each 24.5 cm^2 bioassay plate (or, 500 uL per 10 cm petri dish)
    b. Scrape the colonies off with a cell spreader/scraper.
    c. Pipette off the liquid plus scraped colonies into a tube and repeat the procedure a second time on the same plate with additional 5-10 mL. *Note: Weigh this tube prior to adding any liquid to it.*

8. Weigh the bacterial pellet to determine the proper number of maxiprep columns to use
    a. Spin down all liquid to pellet the bacteria and then discard the supernatant.
    b. Weigh the bacterial pellet and subtract the weight of the tube

9. Maxi-prep for downstream virus production and future amplification
    a. Each column of a Maxi-prep can handle approximately 0.45 g of bacterial pellet.
    b. Process the entire bacterial pellet on a sufficient number of maxiprep columns in parallel.
    c. Pool the resulting DNA and quantify

We recommend using an Endo-free maxiprep kit such as the Qiagen EndoFree Plasmid Maxi Kit (#12362) or the Macherey-Nagel NucleoBond® Xtra Maxi EF (#740426.50).