HEK293T Landing Pad Recombination Protocol with Fugene (Based on a 24-well plate) V.1

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Version 1

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1 Works for me

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ABSTRACT

For using Fugene 6 to recombine Bxb1 attB plasmids into Bxb1 attP Landing Pads already integrated into HEK 293T cells.

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SAFETY WARNINGS

Engineering Controls: use of biosafety cabinet
PPE: lab coat and gloves

Day 0: Day of transfection 5m

1 Trypsinize and count the cells. 5m

2 Make 2 transfection mixtures per sample, 5m

Opti1 and **Opti2** (DNA volumes should be kept between 1.0 and 5.0 uL, if possible):

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2.1 **Opti1**: **23.8 µL Opti-MEM** + **1 µL Fugene6**

2.2 For the **Bxb1**-containing sample - **Opti2**: **23 µL Opti-MEM** + µL corresponding to **16 ng Bxb1 expression plasmid** + µL corresponding to **238 ng attB plasmid** ----**OR**----

2.3 For the **No-Bxb1** sample- **Opti2-noB**: **23 µL Opti-MEM** + µL corresponding to **254 ng attB plasmid**

3 Add **300 µL media** to each well.

(Be sure media contains doxycycline to a final concentration of **2 µg / mL** .)

4 Plate **120,000** cells per well within a 24-well plate.

5 Once at least 15 minutes have past since the mixtures were completed, add them dropwise throughout the well. Rock the plate back and forth to mix it around the well. 15m

Day 1: Adding more media to cells 5m

6 Add at least **500 µL media** to each well. 5m

Day 3+: Negative and/or positive selection 5m

7 If using a landing pad that encodes iCasp9, then add AP1903 to a final concentration of **10 nM** to induce apoptosis of un-recombined cells. Dying cells can be observed as soon as 2 hours after AP1903 addition, and can be removed by changing the media. 5m

If you want to estimate how many cells were recombined (this is recommended, especially for library -based experiments), make sure you leave some cells WITHOUT selection, so you can see what percent of the cells are reporter (eg. mCherry) positive. Then, you can multiply that percentage with the total number of cells you recombined (eg. 120,000 cells if only recombining a single well) to get the total number of recombined cells.

8 If using an attB vector encoding a positive selection marker, add the small molecule. For puromycin resistant recombination plasmids (this is most often the case), add puromycin to a final concentration of **1 µg / mL** . 5m

If performing both negative AND positive selection, we typically perform the negative selection first, and only perform the positive selection after the well has reached greater than 20% confluency.

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Day 7+: Read out the recombination 1h

9 Run the samples through the flow cytometer. Watch the cells on the days in between. They may need to be split at some point. It's probably easiest to simply plate all the cells into a 6-well plate when they need to be split. 1h

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