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Super Recombinator (SuRe), a CRISPR/Cas9-based platform for the rapid incorporation of multiple transgenes at the same genetic locus

Incorporation of genetic elements from foreign species to generate transgenic organisms in bacteria, fungi, plants, and animals for a variety of applications ranging from food crops, industrial enzymes, basic research, etc., has transformed our life. To achieve complex functions for academic research, agriculturial production, or industrial production, it is desirable to create transgenic organisms with multiple transgenic elements. Because of the underlying constraints imposed by genetic linkage and crossing-over, the incorporation of multiple transgenes is difficult, time-consuming, and largely limited to one transgene. Harnessing the recent advancement in CRISPR/Cas9 technology, researchers at Stanford created a novel system termed Super Recombinator (SuRe) that enables a significant reduction in turnover time for introducing N transgenes in the same locus from N-1 to $\log_2 N$ steps. Moreover, this strategy requires the selection of much fewer possible genotypes per step and does not involve embryo injection, thus further reducing labor, cost, and time. The success of the strategy has been demonstrated *in vivo*.

Stage of Development
In vivo demonstration of successful incorporation of multiple transgenes in animal brain.

Applications

- Introduction of multiple transgenes in synthetic biology
- Create transgenic structures much larger than vector capacity
- Simplify the recombination in multiploidy species

- Research tool development

Advantages

- Two recombination strategies are available: activation recombination strategy and coordination recombination strategy
- Faster turnover comparing traditional approach: The time complexity for recombining N genes is reduced from O(N) to O($\log_2$N)
- Much fewer requirements on genotype selection at each step
- High efficiency: The proportion of recombination product in progeny can reach 50%
- High capacity: It is capable of recombining transgenes up to 5 Mbp
- No need for embryo injection
- Flexible to target to specific transgenic vector or specific genomic locus.
- Can adapt to existing transgenic strains
- The method is generalizable for different species
- Software for optimizing recombination processes is available

Patents

- Published Application: WO2022082225
- Published Application: 20230323342
- Issued: 12,637,671 (USA)

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