Title: Drug Repositioning for Novel Genome Engineering:
Technology With High Therapeutic Efficacy and Low Side Effects

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Drug repositioning—the repurposing of existing drugs for new applications—provides high safety but is still limited in gene therapy. As gene therapy research grows globally, safe genome engineering technologies are increasingly needed. Cre recombinase, used in the Cre–loxP system to remove specific genes, lacks temporal control which can result in harmful gene recombination. Although trimethoprim (TMP), which was originally used as an antibiotic, was cleverly repurposed to enable the ON/OFF regulation of Cre activity, its low DNA recombination efficiency has hindered its clinical applicability. This study reports an improved strategy that significantly reduces OFF-state activity while achieving high ON-state activity of Cre at low TMP concentrations, resulting in substantially increased DNA recombination efficiency compared with conventional methods. First, Cre was temporally inactivated by artificial splitting and degradation, destroying its structure. Second, a dimerization-promoting protein was introduced into fragments of the split Cre. Third, a novel design for the degradation domain was developed and incorporated. Various designs were tested to select the optimal design. The novel technology achieved a 94.4% reduction in Cre activity in the OFF-state compared with the conventional method, equivalent to cells without Cre, indicating low side effects. In the ON-state, it achieved high DNA recombination activity, equivalent to wild-type Cre, highlighting its high therapeutic efficacy. Overall, the ON/OFF change ratio improved to 2045% at only 1/650 TMP concentration compared with the maximum plasma concentration in clinical use. This novel chemogenetic genome engineering technology offers efficiency and safety with the potential to save many lives.