How to prevent spontaneous recombination from inactivating the selection system for ubiquitylation [*Nature methods* 13 (11), 945-952 | *Olga Levin-Kravets, Gali Prag and co-workers*].

Background: The original paper describes an *E. coli*-based genetic selection system for ubiquitylation. In this system, two compatible plasmids express the ubiquitylation apparatus where ubiquitin and its target are tethered to fragments of a split antibiotic resistance protein (DHFR). In both plasmids expression takes place from the bacteriophage-λ left promoter, P_L. This promoter was sub-cloned from the pZ family vectors originally constructed by Lutz and Bujard[1]. Two operator sequences of the Tn*10* tetracycline resistance operon[2] (*tet*O) are located between the –10 (Pribnow box) and the –35 sequence elements and upstream to the –35 (**Fig. 1**). To achieve constitutive expression, we used *E. coli* strains that do not express the Tet repressor (*Tet*R).

Results and discussion: We recently discovered that endogenous spontaneous recombination events remove fragments from the promoter. These deletions typically contain the –35 sequence, and their removal inactivates the promoter and eliminates the expression of crucial components of the ubiquitylation cascade (**Fig. 1**). It is possible that during the co-evolution of *E. coli* with λ, the bacteria developed a defense recombination mechanism that removes or mutates elements of P_L promoter. Regardless of the reason, this phenomenon has been observed previously (personal communication with Prof. Shoshy Altuvia from the Hebrew University, Jerusalem). We assume that expression directed by P_L due to the absence of the repressor, as well as the presence of homologous sequences, make this construct prone to recombination.

It is worth clarifying that we did not identify any errors in the published data. However, we found that long use of these plasmids may lead to such recombination event which inactivate the system and growth arrest.

Conclusions: We strongly recommend to maintain, manipulate and purify these P_L containing plasmids only from bacteria strains that express *Tet*R (such as DH5αZ1 or MG1655 Z1)[3]. Ubiquitylation dependent growth experiments could be performed followed DNA plasmids transformation to strains that lack *Tet*R in a temporarily manner and plasmid should not be recovered from these strains.

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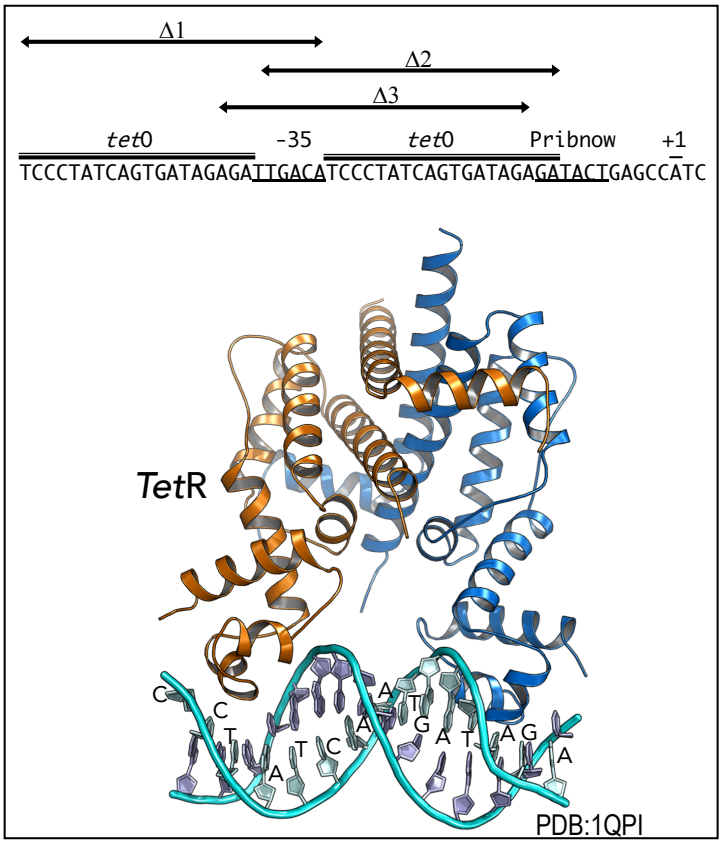

Figure 1. DNA sequence of the $P_{LtetO-1}$ promoter and *Tet*R binding sites. The sequence of the $P_{LtetO-1}$ promoter and key elements are shown. Representative deletions (Δ1-Δ3) that we found are shown as double arrowhead above the sequence. Below the crystal structure of the *Tet*R *complex with tetO*^{4} demonstrating the binding and the potential protective activity of the repressor on the DNA.

References and Notes

1. Lutz R. and Bujard H. (1997) *Nucleic Acids Res.* **25**, 1203–1210.
2. Wissmann A., Meier I., Wray L.V., Jr Geissendörfer M. and Hillen W. (1986) *Nucleic Acids Res.*, **14**, 4253–4265.
3. These strains can be now obtained from *Addgene* and/or directly from the Prag laboratory at TAU.
4. Orth P., Schnappinger D., Hillen W., Saenger W. and Hinrichs W. (2000) *Nat. Struct. Biol.* **7**, 215-219.