"E. coli conji pir"- One strain for all: plasmid cloning and mobilization

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Background:

Conjugation is a critical method for the genetic manipulation of bacteria, allowing the transfer of plasmids between a broad range of bacteria and beyond. This makes it invaluable in strain engineering, enabling the introduction of novel traits and providing insights into genetic functions (Waksman 2019). The *E. coli* S17 donor strain has become the standard for conjugation, despite several notable drawbacks. One major issue is the presence of the *Mu* prophage, which can lead to unwanted plaque formation (Ferrieres et al., 2010). Moreover, the S17 strain is not genetically optimized for plasmid propagation and analysis. Especially large or complex plasmids are assembled and analysed in standard cloning strains like *E. coli* DH10B and subsequently transfered to S17. Thus, these limitations represent a significant bottleneck for the genetic manipulation of all organisms depending on conjugation for DNA transfer.

Approach:

We applied various recombineering techniques to introduce the genes necessary for the conjugation (in total 55 kbp) in the genome of an *E. coli* DH10B-derivative to allow plasmid conjugation with the advantagous DH10B strain. Additionally, we introduced the *pir*-gene in the genome to support also strain engineering strategies using integrative R6K-plasmids.

Results:

"E. coli conji *pir*" is a genetically engineered derivative of *E. coli* DH10B, designed as an ideal strain for both cloning and plasmid mobilization. Additionally, the integrated *pir*-gene supports strain engineering strategies using *pir*-dependent suicide plasmids, a widely appreciated technique for metabolic engineering. We selected *Cereibacter sphaeroides* as an example of a recipient organism to demonstrate successful plasmid conjugation with our versatile "*E. coli* conji *pir*" strain. The conjugation efficiencies we achieved were at least comparable to those of the traditional donor strain S17.

Conclusion:

Our engineered "*E. coli* conji *pir*" is a versatile donor strain that significantly improves the conjugation workflow by various means (Figure 1). Time reduction: No plasmids retransformation into a donor strain is required as the DH10B derivative serves as donor strain itself. Reliability: Plasmids can be conveniently reisolated and analyzed in good amounts and quality unlike the most common donor strain S17. Robustness: The genetic DH10B-background is genetically optimized to propagate large plasmids which are of high value for metabolic engineering projects, outcompeting the capacities of S17. Safety: The DH10B derivative "*E. coli* conji *pir*" does not contain the *Mu* prophage and thus limits the risk of plaque formation in cultures in contrast to the traditional donor strain S17. Flexibility: The DH10B-derivative is genetically amenable, allowing further genetic modifications, if desired. "*E. coli* conji *pir*" allows even the genomic integration of large DNA constructs with *pir*-dependent suicide plasmids- an appreciated technique for metabolic engineering. Thus, our new conjugation workflow using "*E. coli* conji *pir*" strain solves the limitations of the established conjugation techniques and importantly holds great potential for a broad applicability in strain engineering projects.

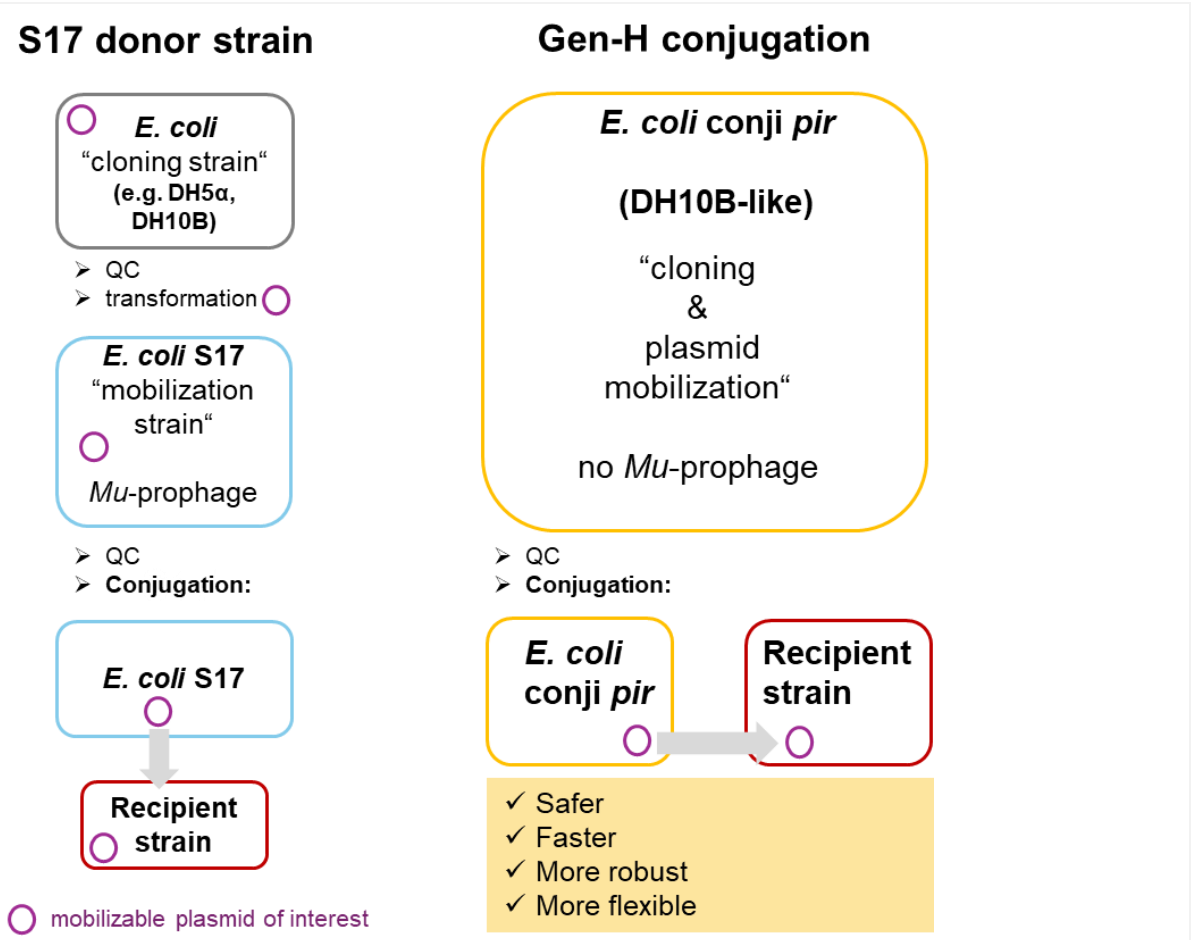

Figure 1: Schematic presentation of the traditional conjugation workflow with the donor strain S17 in comparison with the new platform strain "*E. coli* conji *pir*" for plasmid cloning and mobilization (developed by Gen-H Genetic Engineering Heidelberg GmbH).

References

1) Ferrières, L., Hémery, G., Nham, T., Guérout, A. M., Mazel, D., Beloin, C., & Ghigo, J. M. (2010). Silent mischief: bacteriophage Mu insertions contaminate products of Escherichia coli random mutagenesis performed using suicidal transposon delivery plasmids mobilized by broad-host-range RP4 conjugative machinery. *Journal of Bacteriology*, *192*(24), 6418–6427.
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