E. coli Conji - One strain for all: Plasmid cloning and mobilization

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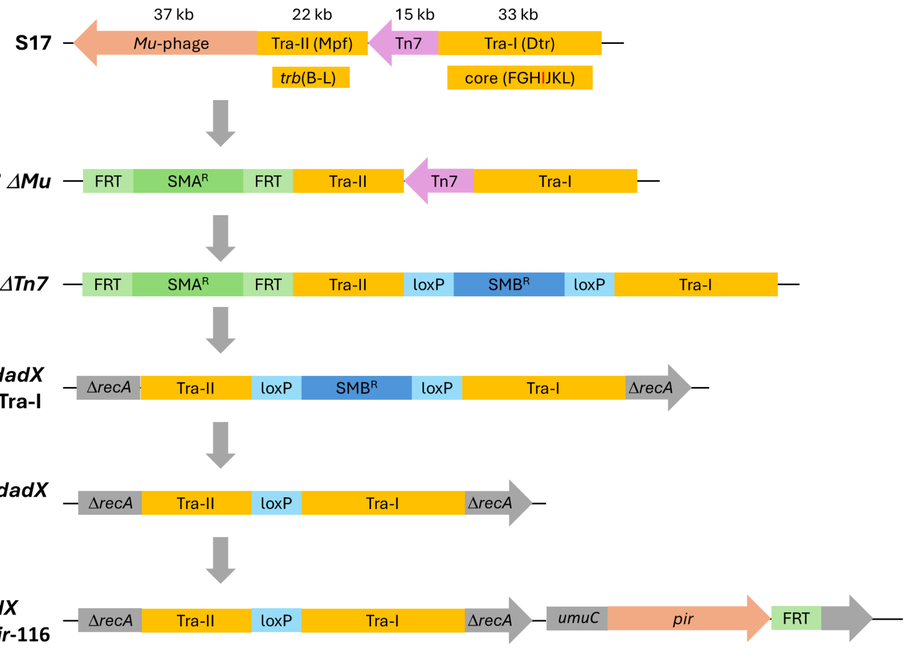
Conjugation is a critical method for the genetic manipulation of bacteria, enabling the transfer of plasmids across a broad range of bacteria and even other species. This makes it invaluable in strain engineering, allowing the introduction of novel traits and providing insights into genetic functions. For an easy and improved conjugation process Gen-H developed a new all-in-one strain, *E. coli* Conji, which is ideal for both cloning and plasmid mobilization, significantly improving the standard workflow.

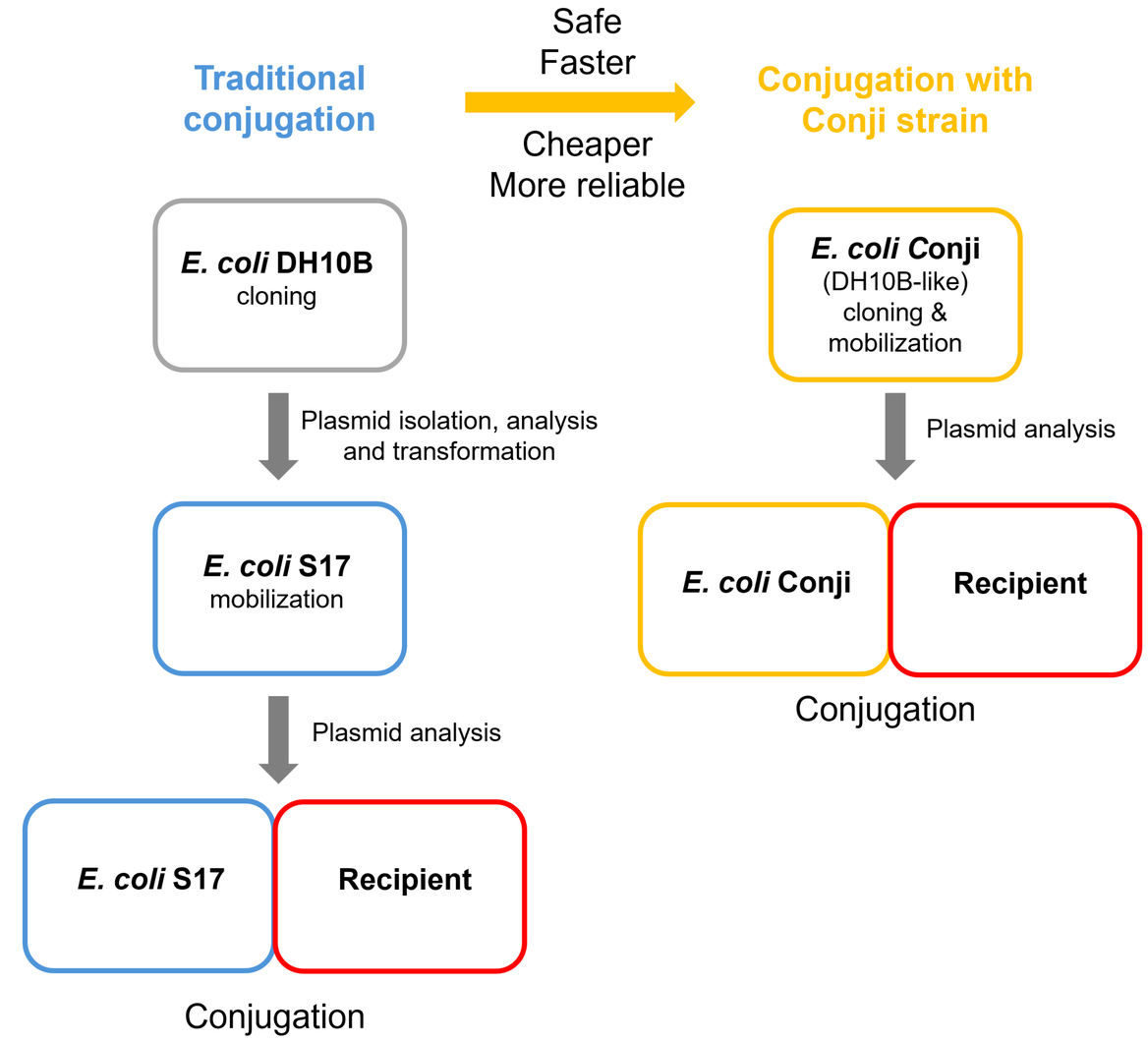
Problem: *E. coli* S17 is the most used donor strain for conjugation, although it has several important drawbacks. The strain carries the ~55 kb broad-host-range plasmid RP4 integrated into its chromosome, providing the Tra-I and Tra-II regions required for conjugative transfer. Cloning and DNA assembly are typically not carried out in *E. coli* S17 but in standard laboratory strains such as DH10B.

S17 often grows poorly and yields low amounts of plasmid DNA, complicating plasmid analysis. In addition, plasmids isolated from S17 frequently show undesired modifications, which hinder downstream analyses and pose a risk to project success. Therefore, S17 is only transformed with plasmids that are fully finalized for conjugation. The strain also carries a Mu prophage, which can enter the lytic cycle under stress, leading to plaque formation and posing a risk of Mu transfer to the recipient strain.

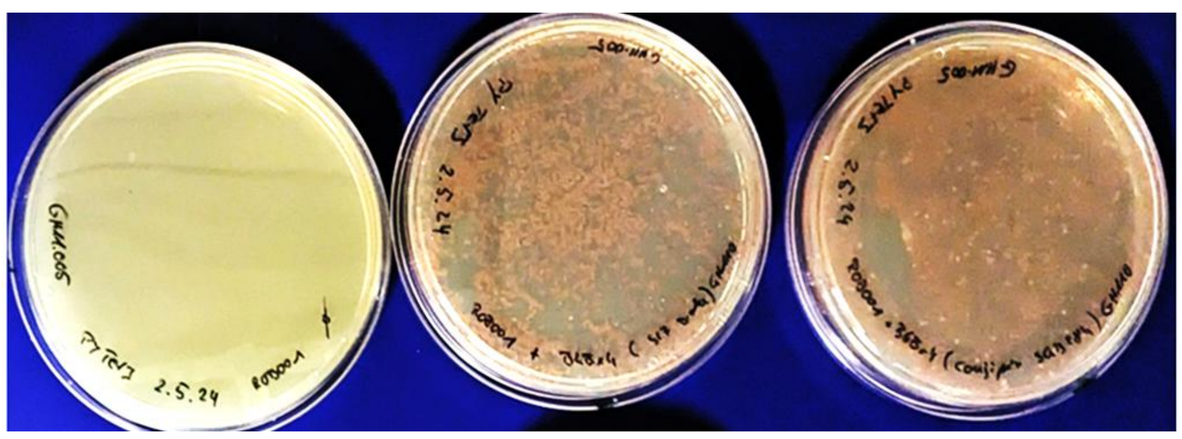


Conji generation: Deletion of Mu prophage and a Tn7 transposon from Tra apparatus in S17. Cleaned Tra-I and Tra-II regions were transferred into a DH10B background carrying an alanine auxotrophy (Δalr ΔdadX). The resulting strain, Conji, enables efficient plasmid conjugation while retaining the advantages of a typical cloning strain. In addition, the pir gene was integrated to support workflows requiring integrative R6K-based plasmids.

Conjugation workflow using S17 vs Conji



Comparison of conjugations of *Cereibacter sphaeroides* using traditional workflow with donor S17 and improved workflow with Conji using a standard plasmid encoding for a chromophore and a tetracycline resistance.



without donor **donor S17** **donor Conji**

Advantages of Conji

- **Time efficiency:** No need to re-transform plasmids into a separate donor strain.
- **High yield of plasmid DNA:** pDNA can be easily reisolated - unlike from the commonly used donor strain S17.
- **High reliability:** Stable plasmid maintenance with reliable sequence fidelity, which is superior to S17, where spontaneous mutations are frequently observed.
- **Improved safety:** Conji lacks the Mu prophage, eliminating the risk of stress-induced plaque formation.
- **High genetic flexibility:** DH10B-derived Conji is easy to manipulate, allowing further genomic modifications if needed.
- **Extended functionality Conji pir:** Supports genomic integration of large DNA constructs via pir-dependent suicide plasmids - an important tool for metabolic engineering.

Conjugation workflow using Gen-H´s *E. coli* Conji strain solves limitations of the established conjugation techniques and holds great potential for a broad applicability in strain engineering projects.