Genetic manipulations of nonmodel gut microbes

Wen-Bing Jin[1,2]* and Chun-Jun Guo[1,2,3,4,5]*

[1]Jill Roberts Institute for Research in Inflammatory Bowel Disease, Weill Cornell Medicine, Cornell University, New York, NY 10021, USA
[2]Friedman Center for Nutrition and Inflammation, Weill Cornell Medicine, Cornell University, New York, NY 10021, USA

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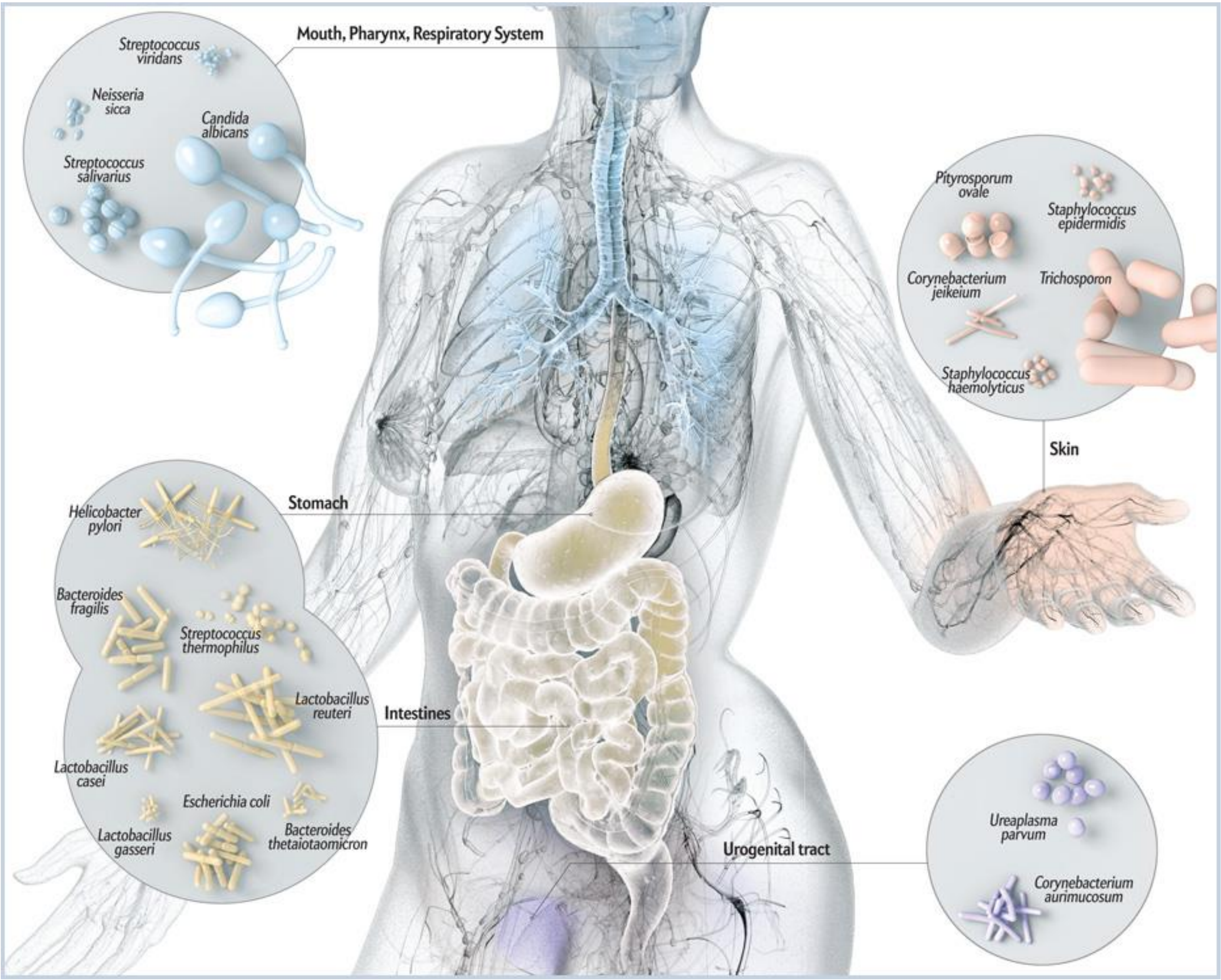
The human microbiota



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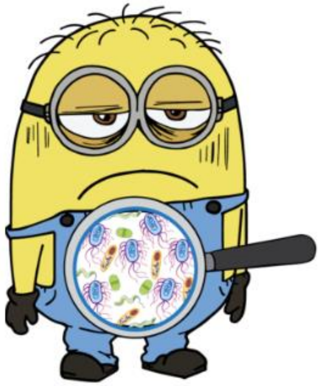
Gut microbiota genes-Metabolites-Host biology

- Invisible organ/virtual organ
- Minor fraction of our total mass (~2 kg)
- 3.3 million genes outweigh human genes by ~150 times



❖ IBD, colorectal cancer

❖ Obesity, type 2 diabetes, metabolic liver disease, cardio-metabolic disease

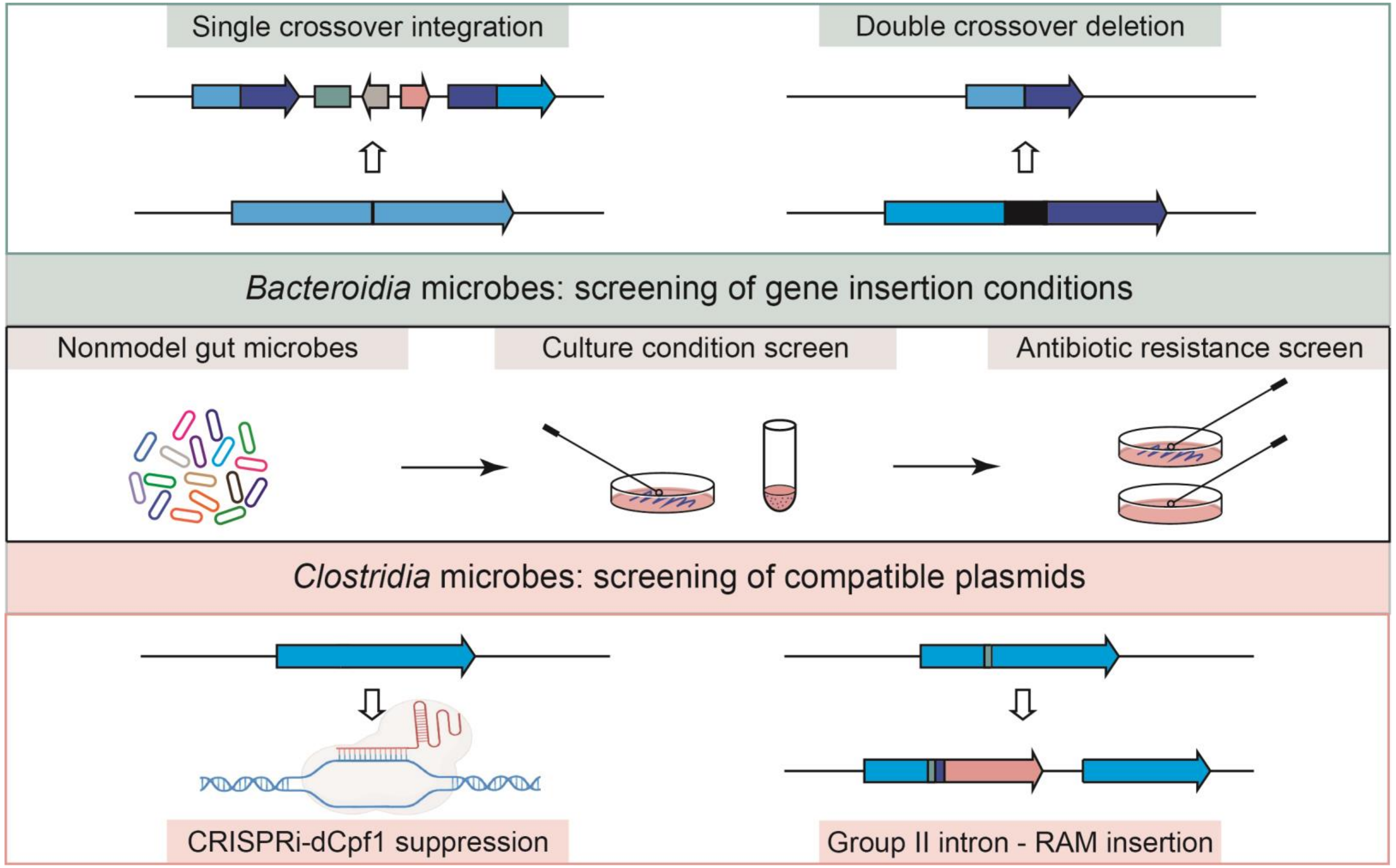
❖ Gut-brain axis

Gut microbiota genes → **Metabolites** → **Host biology**

Nature. 2010; 464: 59. *Science*. 2021; 374: 1087. *Nature*. 2022; 606:754. *Science.* 2017; 356: 2770. *ACS Cent Sci.* 2021; 7: 20. Dodd Lab website.

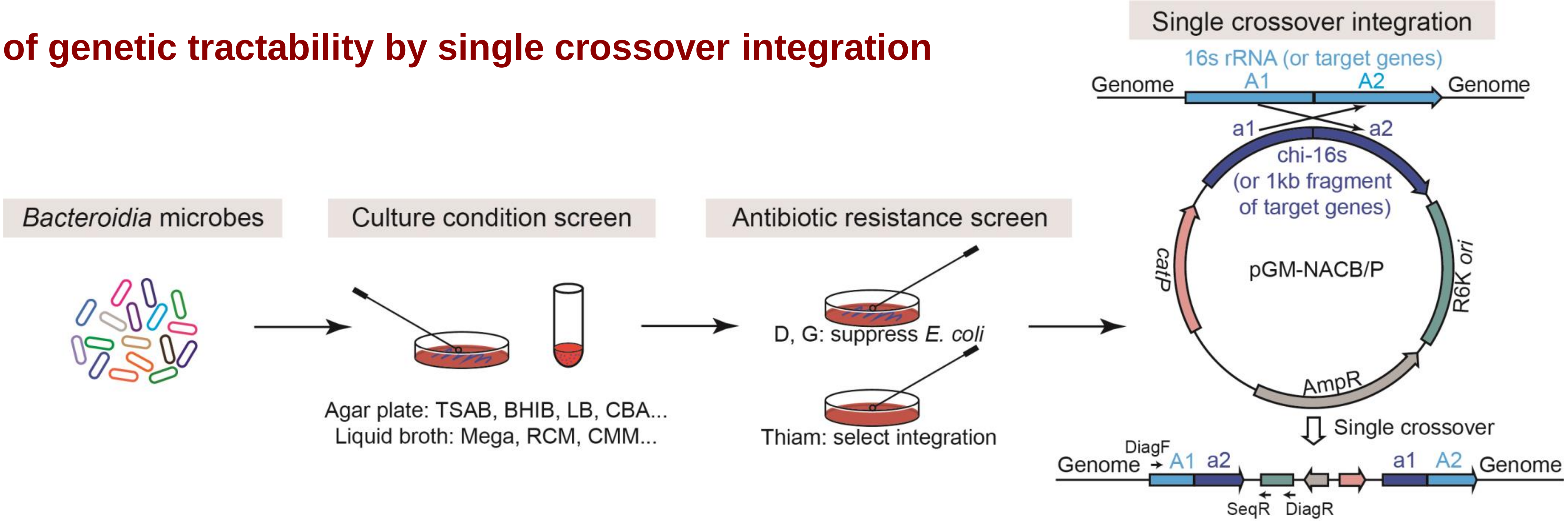
Highlights

- ✓ **A step-by-step genetic strategy that facilitates the study of functional genes encoded by gut commensals**
- ✓ **Establish genetic tools in nonmodel gut *Bacteroidia* and *Clostridia* without prior knowledge of genomic sequence**
- ✓ **A high-throughput genetics screening and manipulating platform for human gut microbes**

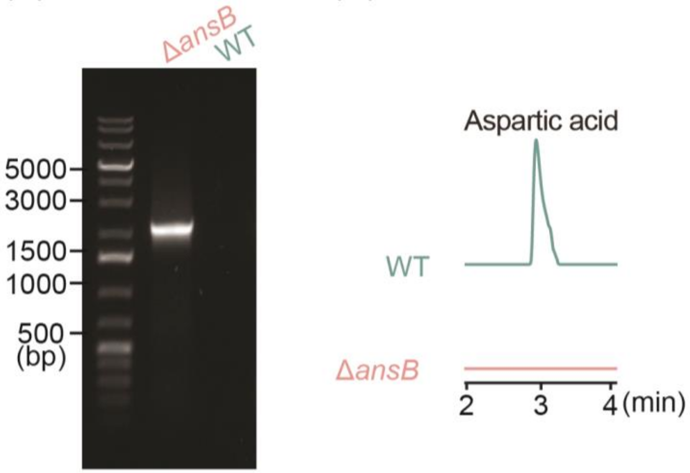


Genetic tools for nonmodel *Bacteroidia* microbes

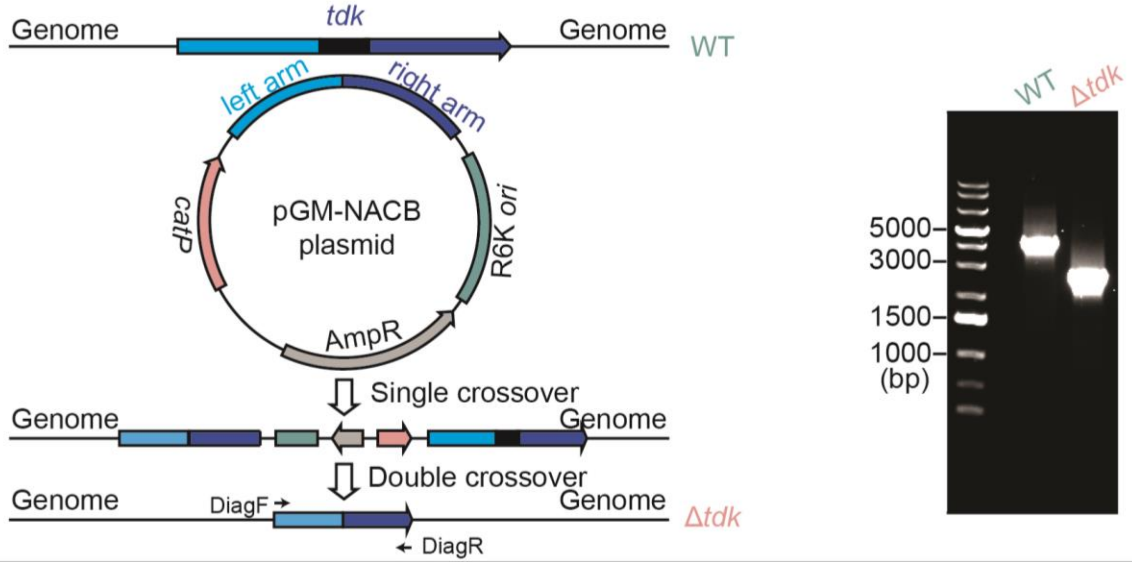
- **Screen of genetic tractability by single crossover integration**



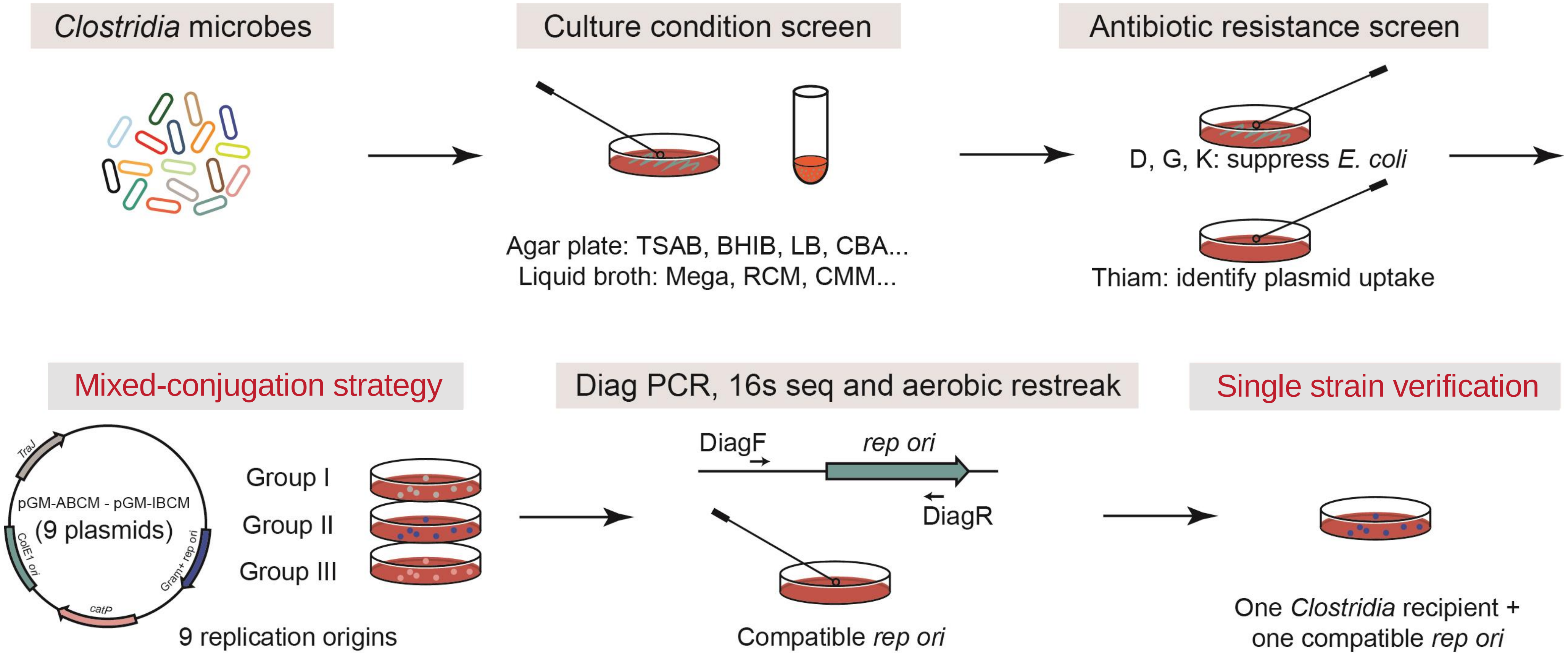
- **Knockout via single crossover integration**



- **Knockout via double crossover deletion**



Gene transfer method for nonmodel *Clostridia* microbes



Clostridia microbes

Culture condition screen

Agar plate: TSAB, BHIB, LB, CBA...
Liquid broth: Mega, RCM, CMM...

Antibiotic resistance screen

D, G, K: suppress *E. coli*

Thiam: identify plasmid uptake

Mixed-conjugation strategy

9 replication origins

Diag PCR, 16s seq and aerobic restreak

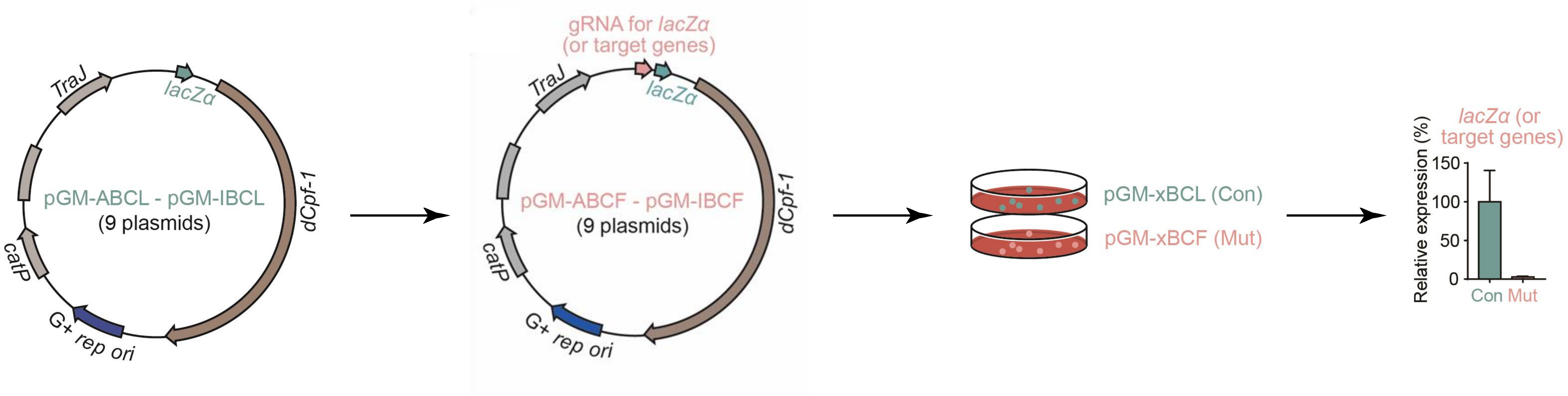
Compatible *rep ori*

Single strain verification

One *Clostridia* recipient +
one compatible *rep ori*

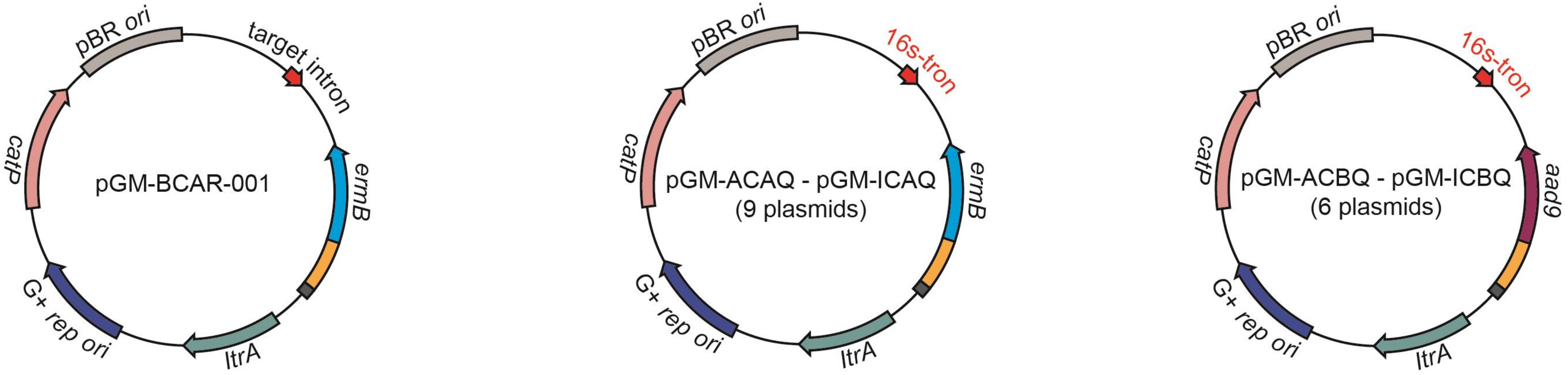
CRISPRi-dCpf1 system for *Clostridia* microbes

- **CRISPRi-dCpf1 system combined with report gene *lacZα***



Group II intron system for *Clostridia* microbes

- **Group II intron system-mediated gene insertion**



Summary

- In this protocol, we introduced an efficient and generalizable approach to establish genetic tools in nonmodel gut microbes.

- For *Bacteroidia* microbes, genetic tractability was screened using single crossover integration, and gene knockout could be performed via single crossover insertion and double crossover deletion developed based on this.

- For *Clostridia* microbes, plasmids with compatible replication origin were firstly identified, then CRISPRi-dCpf1-mediated suppression and Group II intron-induced insertion genetic systems were developed.

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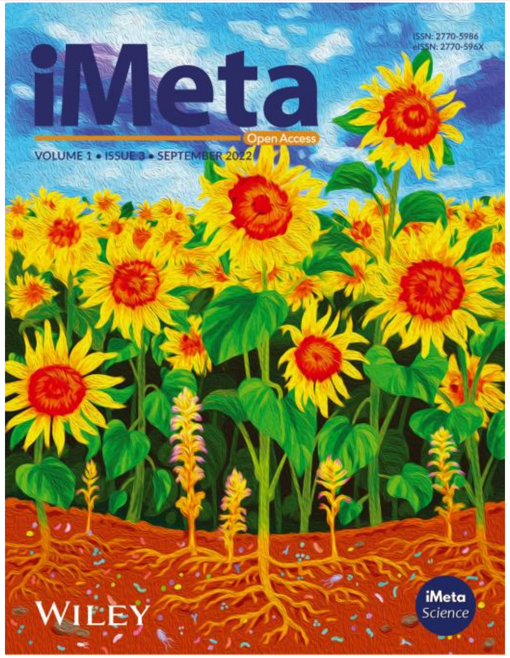

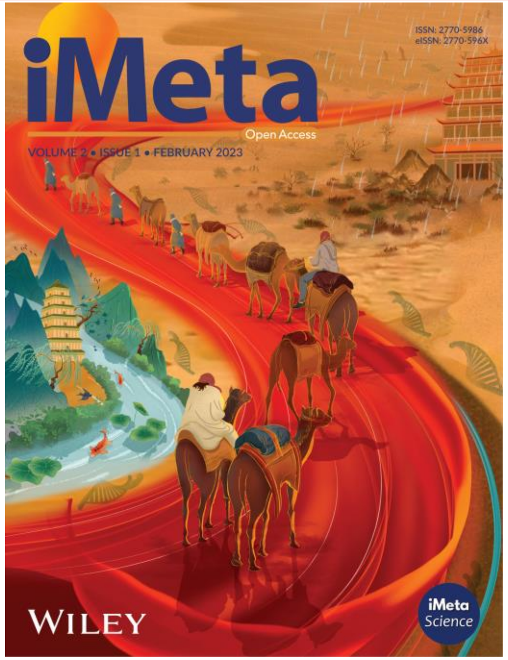

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