

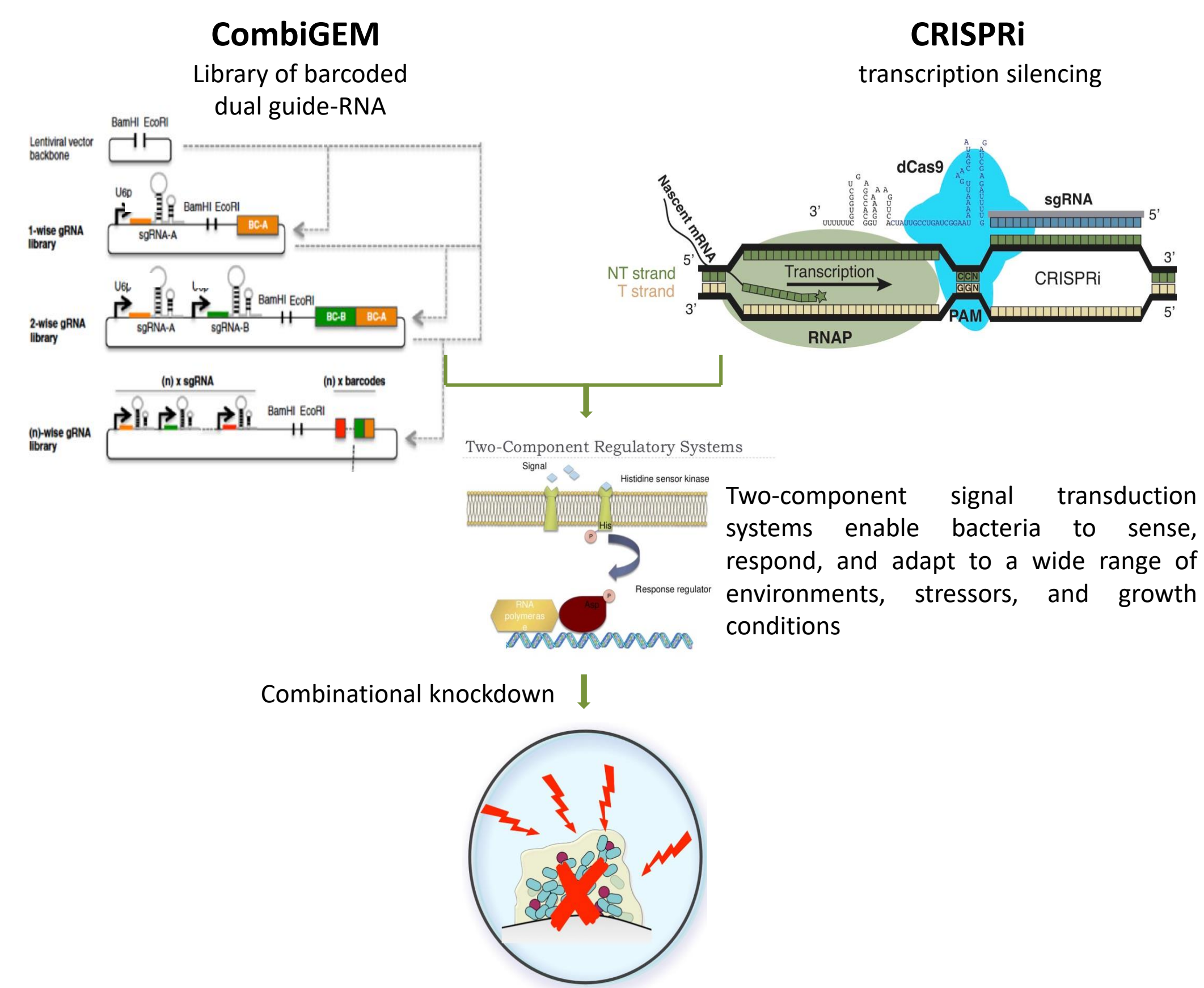
IMPACT ON AMR

In the era of antimicrobial resistance, bacterial biofilms serve as incubators for dissemination of resistant traits, at the same time shielding bacteria from antibiotics. Minimal inhibitory concentrations of antibiotics for bacteria within biofilms are typically much higher than for planktonic cells, making biofilm-associated infections not only much harder to clear, but contributing to a tendency to relapse. Therefore, **multifaceted strategies** should be deployed to eradicate biofilm-associated infections.

Enterococcal infections are often biofilm-associated, hard to treat, and may become life-threatening. Despite the importance of biofilms to Enterococcal infection, analysis of biofilm factors has been mono-factorial and largely in vitro.

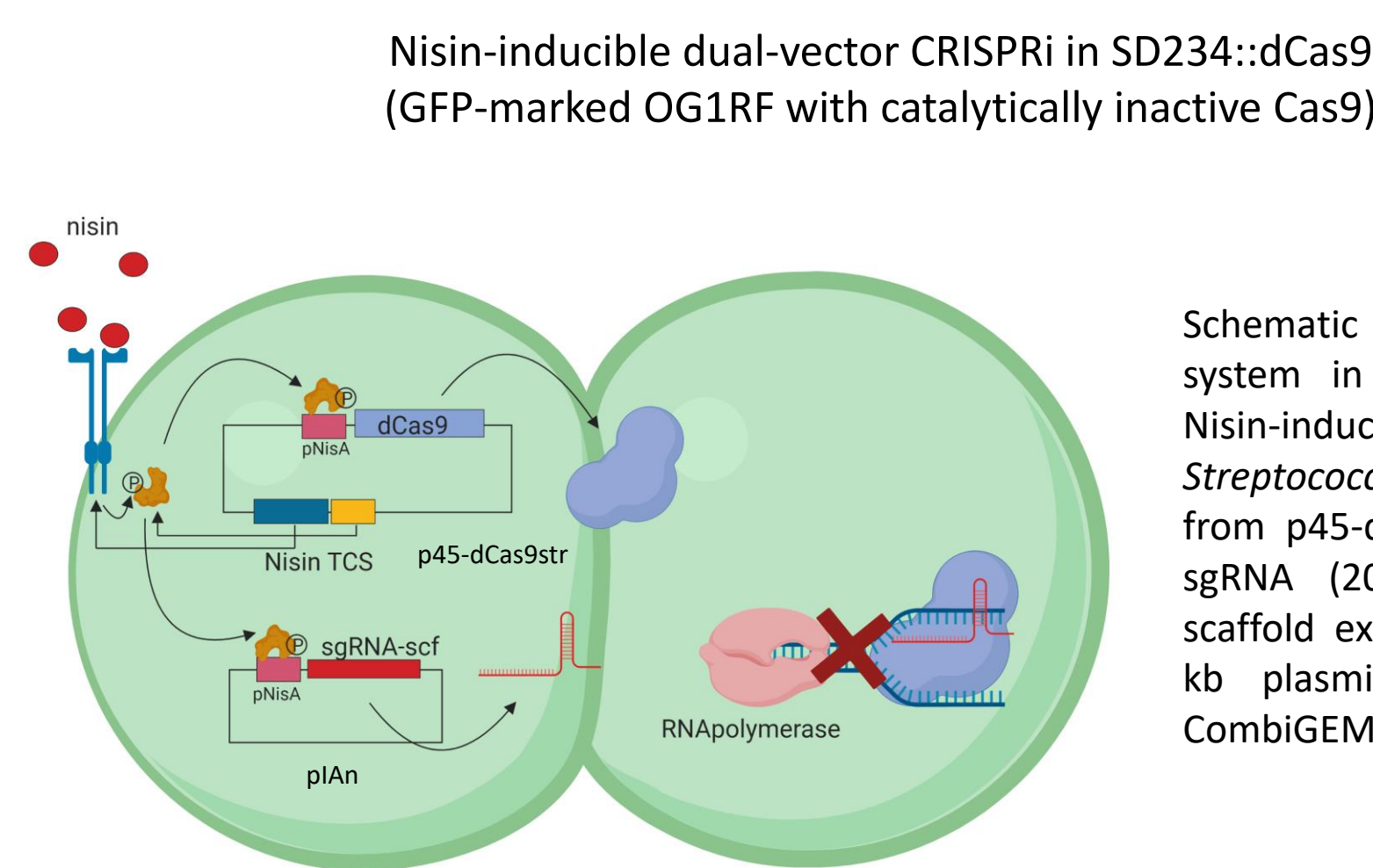
OUR APPROACH

CombiGEM-CRISPRi to characterize TCS involved in biofilm formation



Utilize combinatorial genetic en mass (combiGEM)-based DNA assembly with CRISPR interference technology to identify two-component system(s) (TCS) from *Enterococcus faecalis* important for biofilm formation.

Test model



Schematic diagram of CRISPRi system in *Enterococcus faecalis*. Nisin-inducible dCas9 (from *Streptococcus pyogenes*) expressed from p45-dCas9str; nisin-inducible sgRNA (20 nt) linked to Cas9 scaffold expressed from pIAn (3.2 kb plasmid modifiable through CombiGEM).

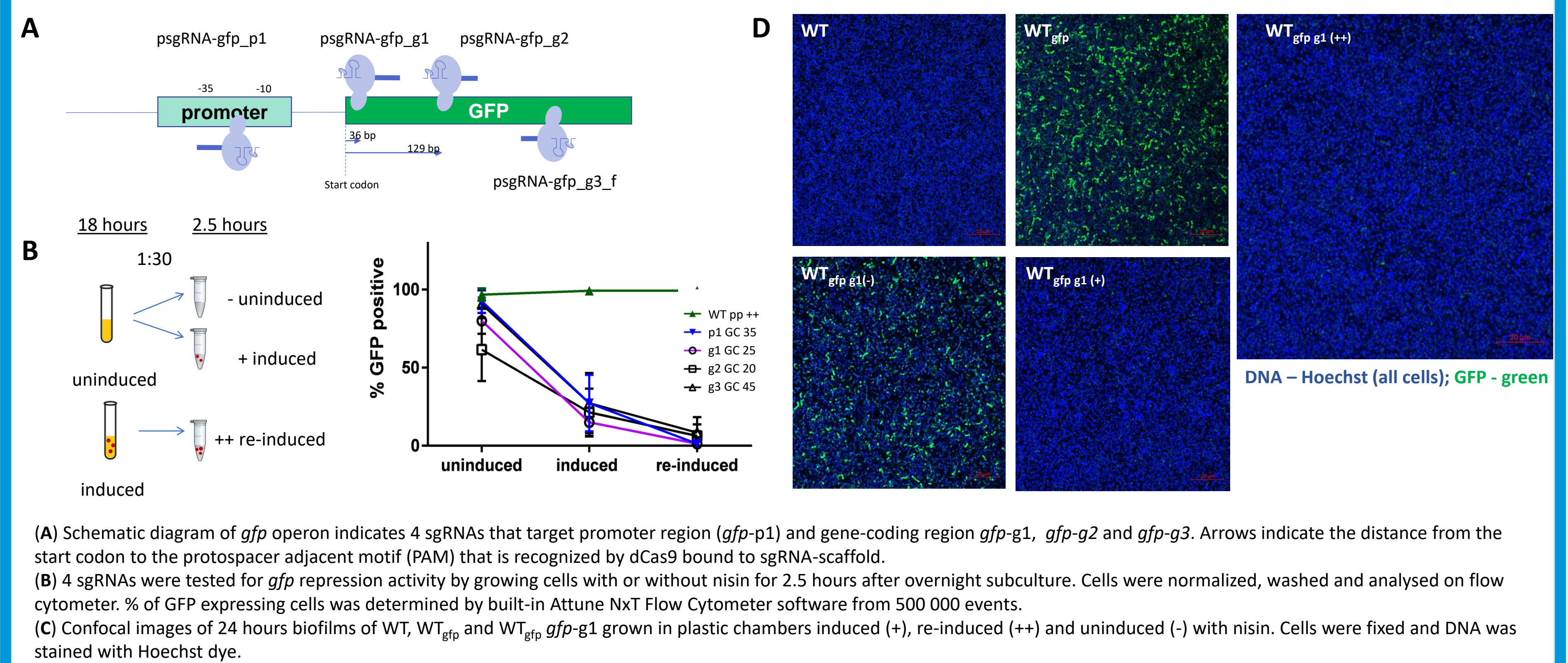
RESULTS

CRISPRi in *Enterococcus faecalis*:

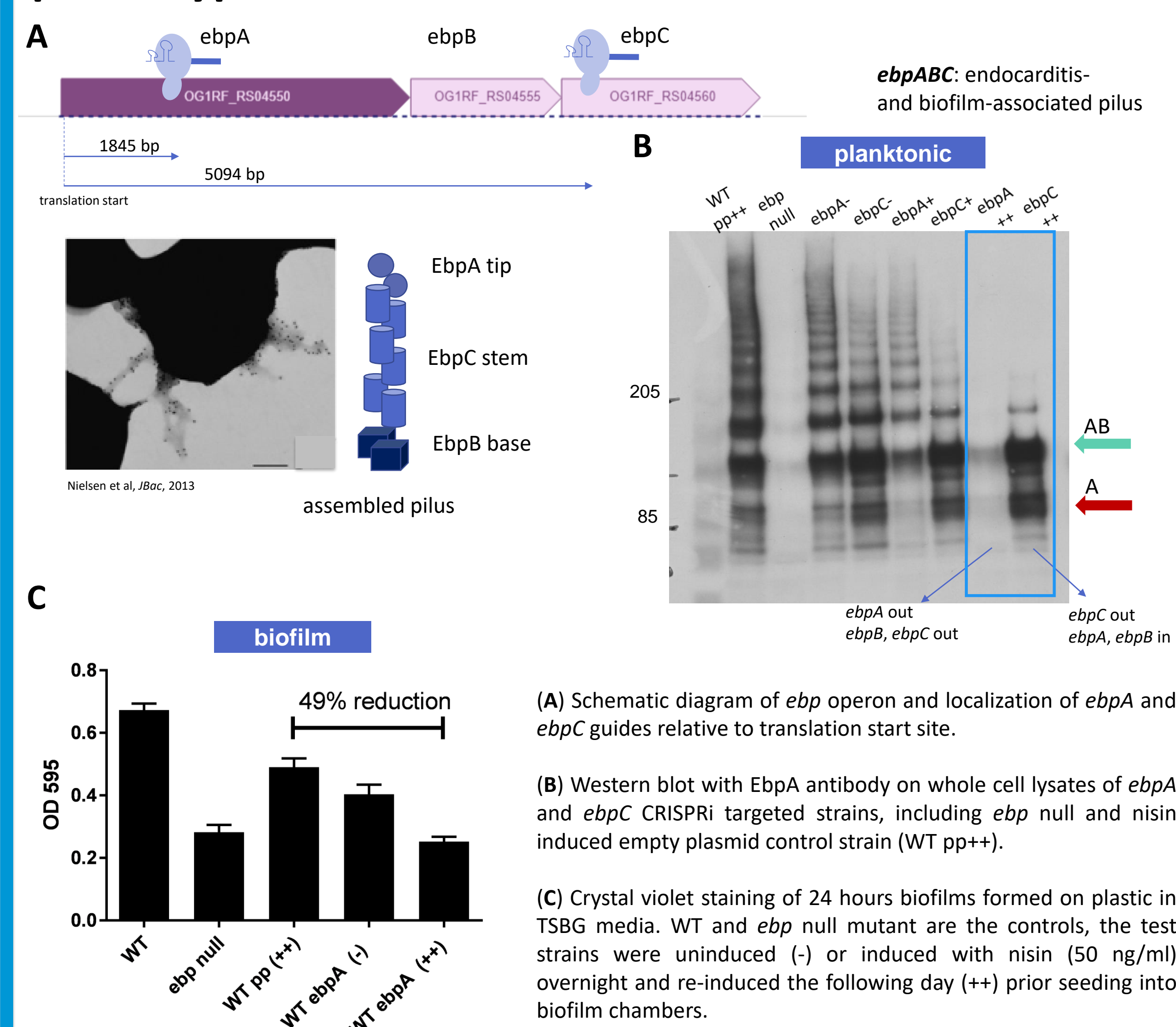
- Most efficient on presensitized cultures
- Efficient in:
 - distal targeting
 - whole-operon silencing
 - template/non-template strand targeting
- Mimics gene knockout phenotypes in planktonic and biofilm assays
- Can be multiplexed

KEY FINDINGS

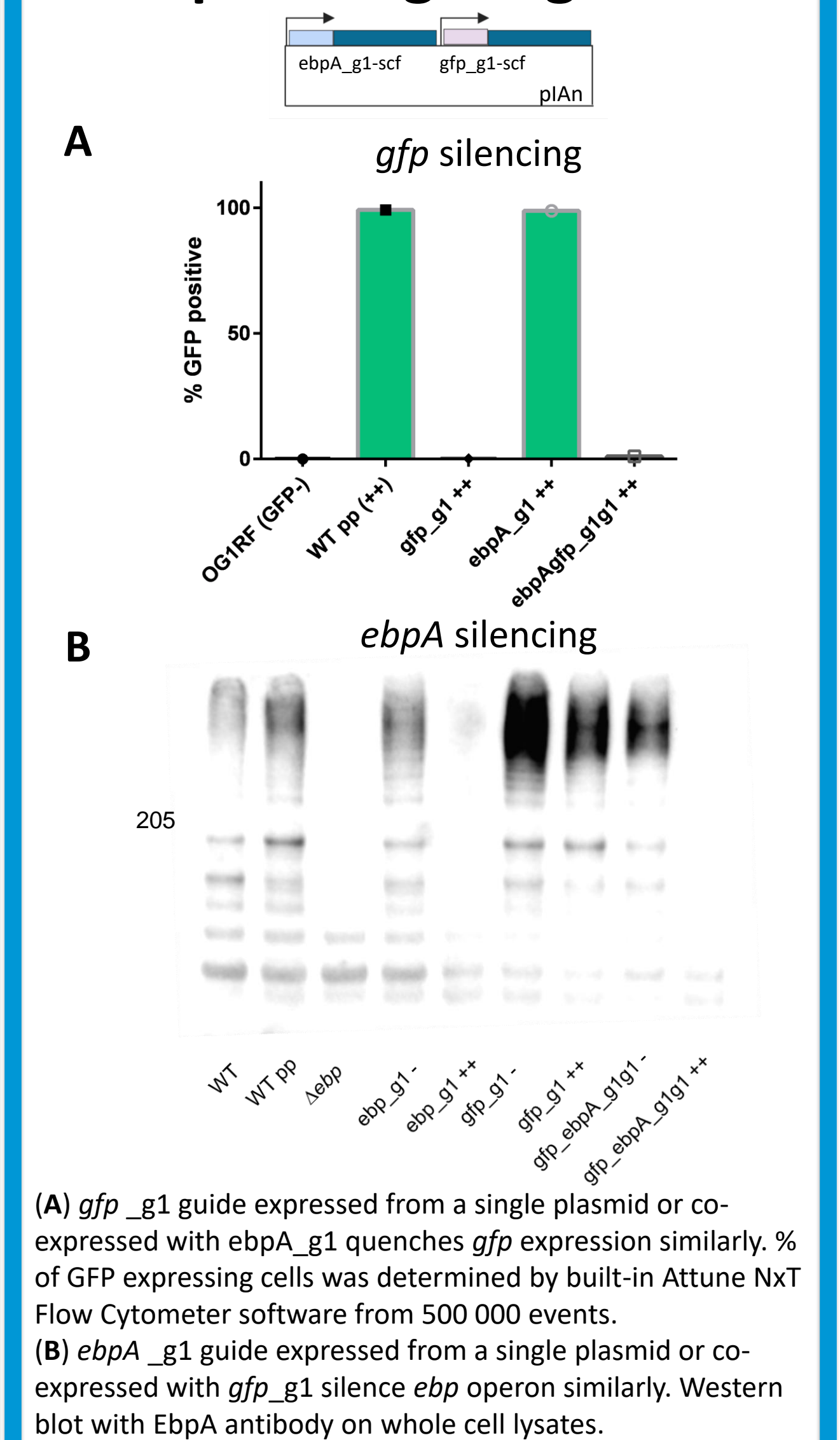
Efficient and inducible CRISPRi in *Enterococcus faecalis*



CRISPRi recapitulates biofilm-related knockout phenotype



Multiplex targeting



FUTURE VISION

- Combinatorial library design and screen for TCS genes involved in biofilm formation
- Validation of top hits in relative biofilm-associated infection models
- Novel drug-combination discovery: small molecule inhibitors screening, drug screening
- Effective combinatorial therapies
- Collaborative effort for drug delivery to biofilms

Platform for rapid identification of genetic combinations responsible for biofilm formation, infection and immune suppression that may serve as potent antimicrobial targets.