S1 Supporting Materials and Methods.

To create pKT1-sar5-F and pKT1-sar5D-F, the backbone of pKT1 was amplified using primers:

pKT1_vector_F (Vector.FOR):
AATGTAACTGTATTCCAGCGATGACGAAATTCT
pKT1_vector_R (Vector.REV):
ATGTTCATGACTCCATTATATTGTACATGTGCA

To create pKT1-sar5-F, the FLAG-tagged sar5 was amplified from GBS strain using primers:

pKT1_sar5F_F:
CAATAATAATGGAGTCATGAACATATGTTTAGTCAATATAATTTTGAAAAAGGTT
TAAATTTTCCAT
pKT1_sar5F_R - (FLAG-tag in red):
ATCGCTGAATACAGTTACATTTAATTTTTCCAT
CGTCGTTTTAAAAACTGCTGCTG

To create pKT1-sar5D-F, the FLAG-tagged sar5D was amplified using primers:

pKT1_sar5D_F:
CAATAATAATGGAGTCATGAACATATGTTTAGTCAATATAATTTTGAAAAAGGTT
TAAATTTTCCAT
pKT1_sar5D_R - (FLAG-tag in red):
ATCGCTGAATACAGTTACATTTAATTTTTCCAT
CGTCGTTTTAAAAACTGCTGCTG

The PCR amplified products were adjoined by Gibson assembly.
Cloning strategy of pKT1-sar5-F:

**GIBSON ASSEMBLY**

Choose 1 .. 7189

Choose 1 .. 3009

**Fragment 1**

7189 bp

**Fragment 2**

3009 bp

**PCR**

Amplify 2407 .. 753 using:

- pKT1_vector_F
- pKT1_vector_R

**NCTC 9828 sar5**

2941 bp

10,153 bp
Cloning strategy of pKT1-sar5-F:

**pKT1_sar5D_F**
9622 bp

**GIBSON ASSEMBLY**
Choose 1 .. 7189

**Fragment 1**
7189 bp
- trfA
- orfV
- kan
- Xyls
- Pm
- orfT
- rnbT1T2

**PCR**
Amplify 2407 .. 753 using:
- pKT1_vector_F
- pKT1_vector_R

**pKT1**
8842 bp

Choose 1 .. 2478

**Fragment 2**
2478 bp
- Sar5

**PCR**
Amplify 1258 .. 3664 using:
- pKT1_sar5D-F_F
- pKT1-sar5-F_R

**93-33 sar5D**
4762 bp