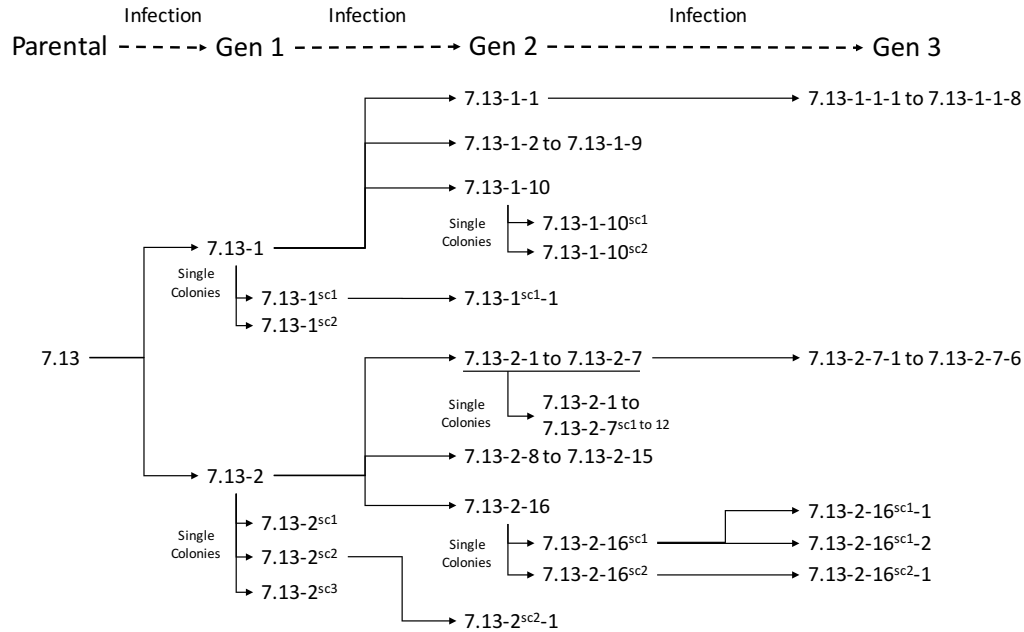
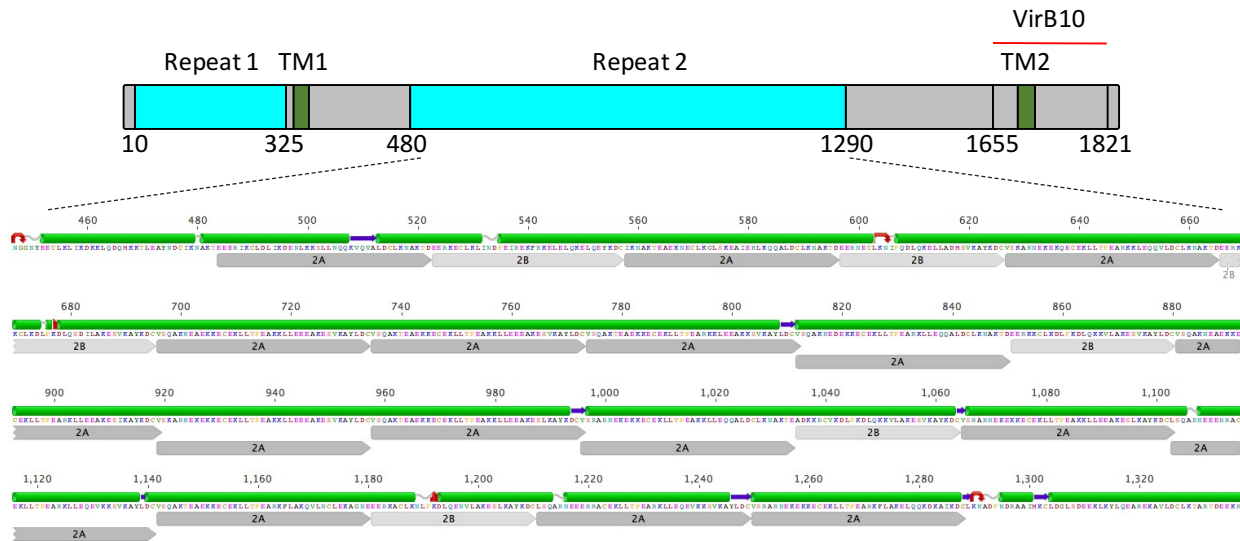


## Supplementary Figure S1



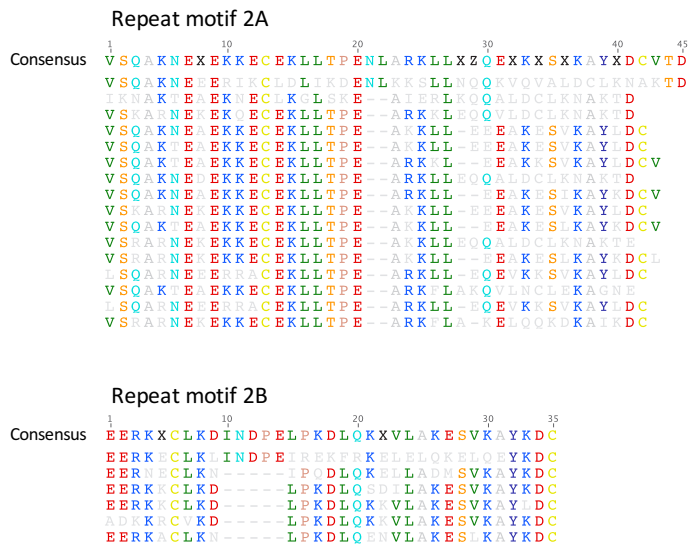
Overall experimental flow diagram of sequential *in vivo* infections showing the original parental 7.13 strain, the 7.13 derivative *H. pylori* strains by generation and passages (Gen) in Mongolian gerbils.

## Supplementary Figure S2



Conserved domain model of *cagY* from *H. pylori* strain 7.13 highlighting the repeat domains, transmembrane domains (TM1 and TM2) and identity with VirB10 (orange line). Bottom; details of repeat domain 2 showing the predicted secondary structure (green: alpha helix; purple: beta-sheets, and red: beta turns) and repeat motifs 2A and 2B (gray). Predicted secondary structures were calculated using the Garnier tool from EMBOSS.

## Supplementary Figure S3

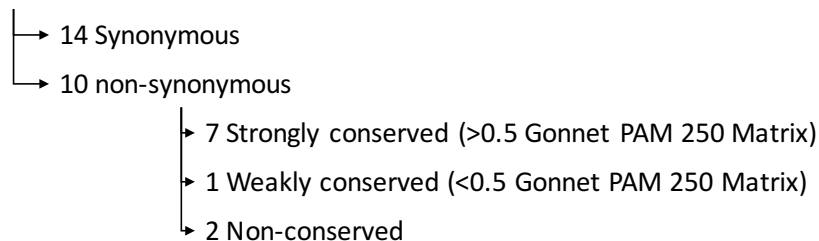


Consensus amino acid sequences of motif 2A and motif 2B from the CagY repeat domain 2 of *H. pylori* strain 7.13. Each individual motif sequence (Motif 2A, n=16; Motif 2B, n=6) was compared, and a consensus motif sequence was determined for Motif 2A and 2B. Alignment of motifs were done using Geneious software version 6.0.6.

## Supplementary Figure S4

J99

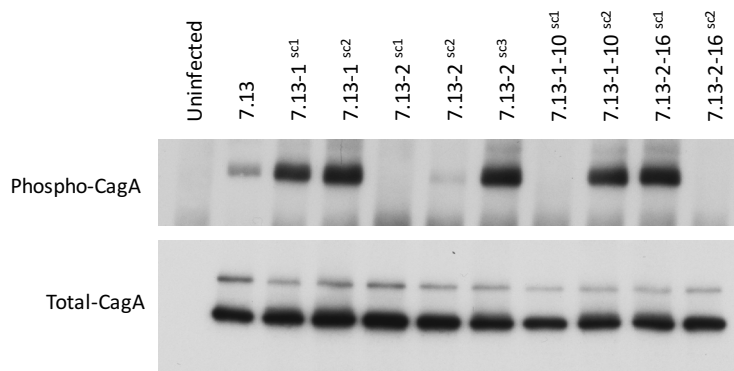
24 SNPs



All non-synonymous SNPs are located in the CagY repeat domain 2 and predict changes in secondary protein structure

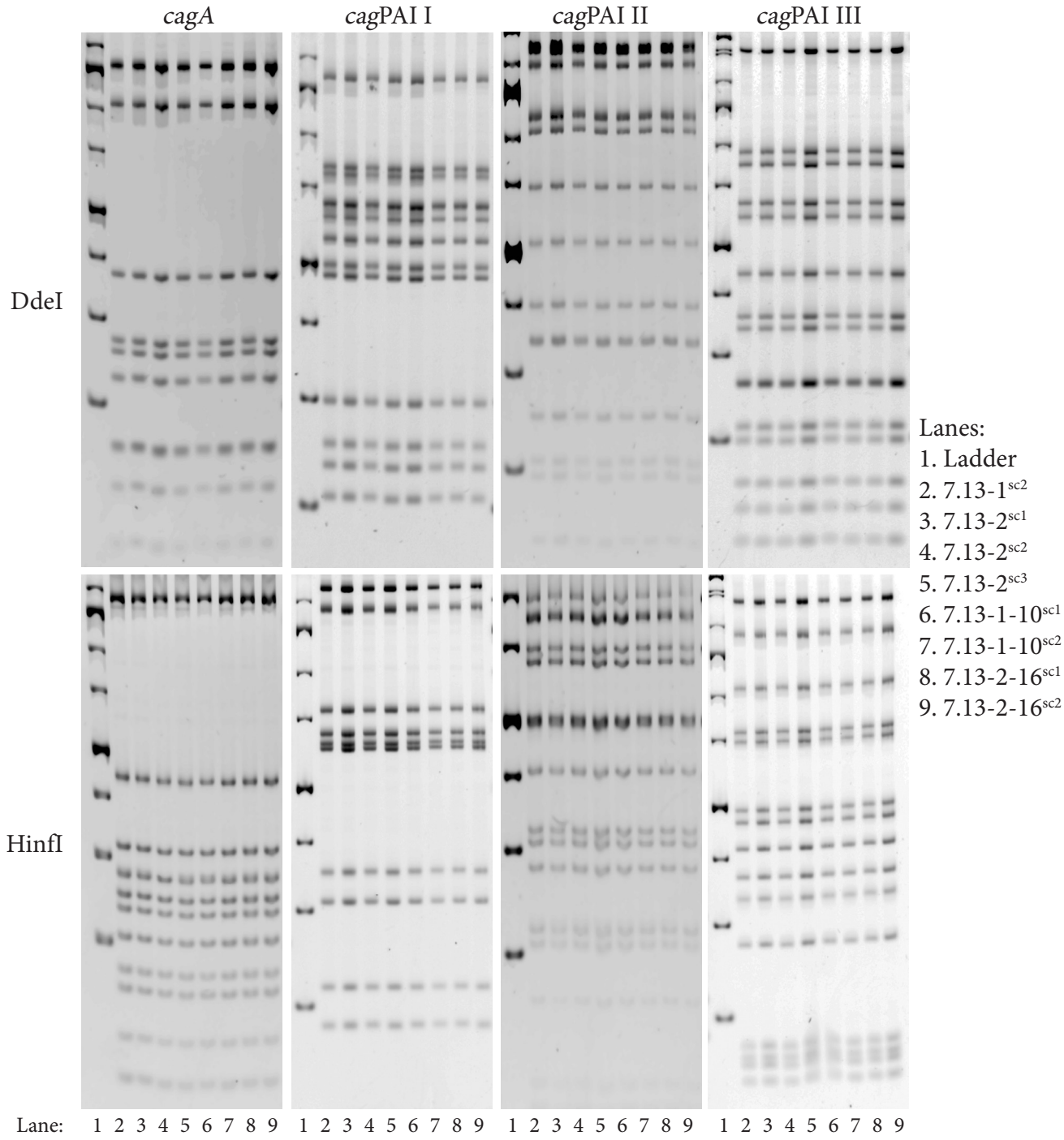
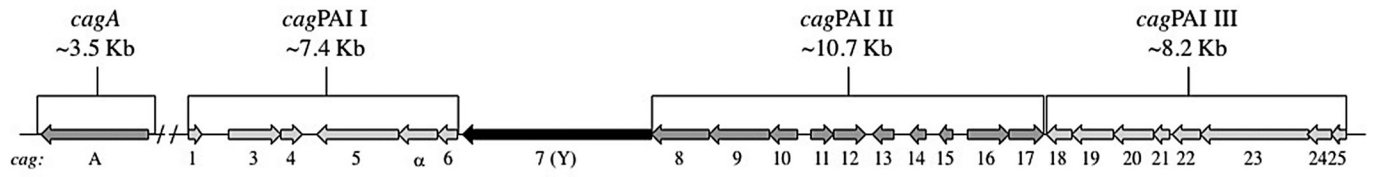
Flow diagram of single nucleotide polymorphisms found in *cagY* sequences of the *H. pylori* archival and recent J99 strains.

## Supplementary Figure S5



Western blot analysis for phosphorylated and total CagA using AGS cells co-cultured with *H. pylori* single colony strains carrying different rearrangements in *cagY*

Supplementary Figure S6



RFLP of PCR amplicons generated from *cagA* and the *cagPAI*, which was segregated into three separate components excluding *cagY*, from eight different single colony isolates of *H. pylori* that contain different *cagY* rearrangements. Top: schematic representation of the *cagPAI* and the different PCR amplicons. Bottom: RFLP gels of PCR amplicons digested with DdeI and HinfI



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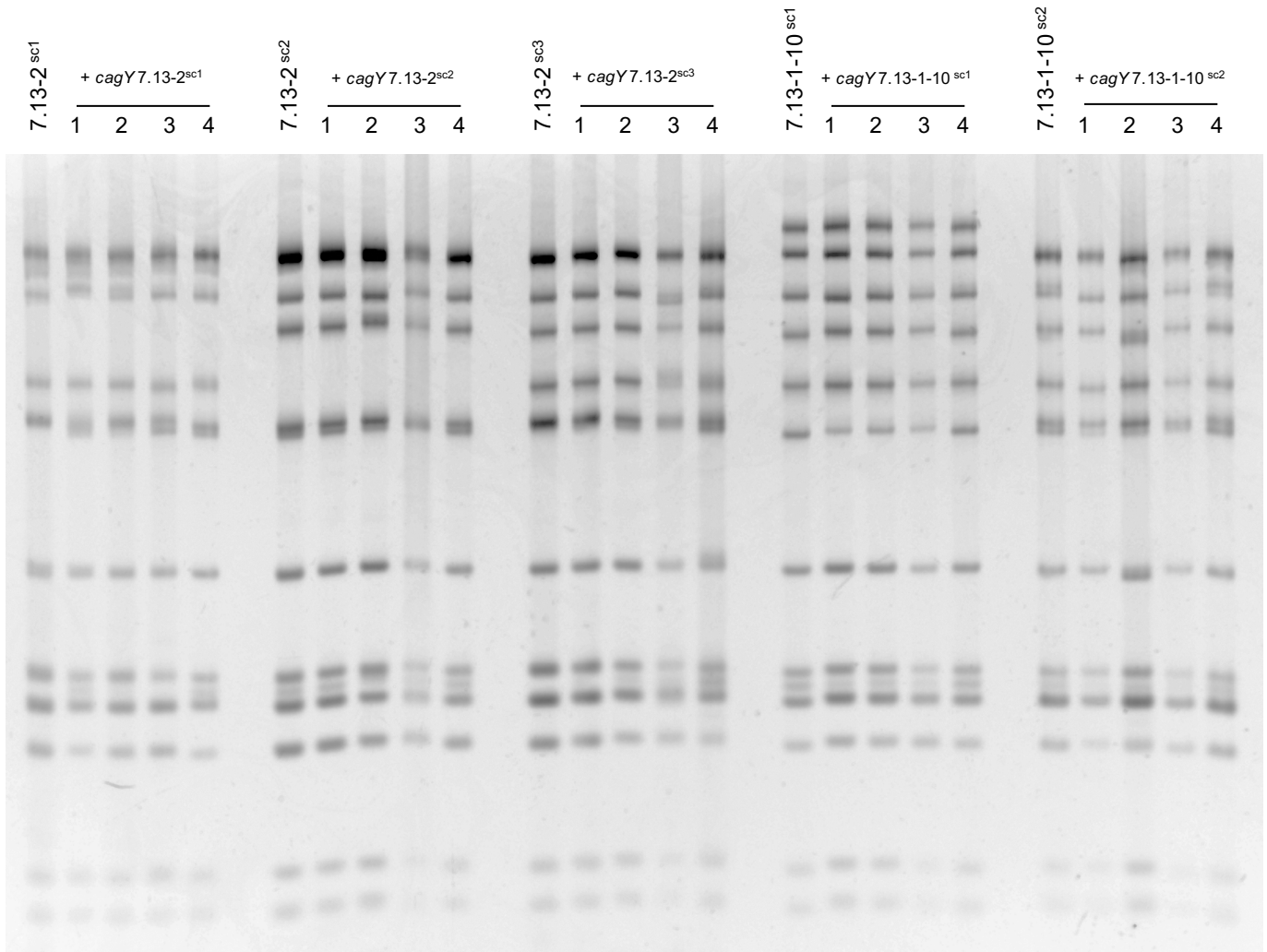
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Supplementary Figure S8



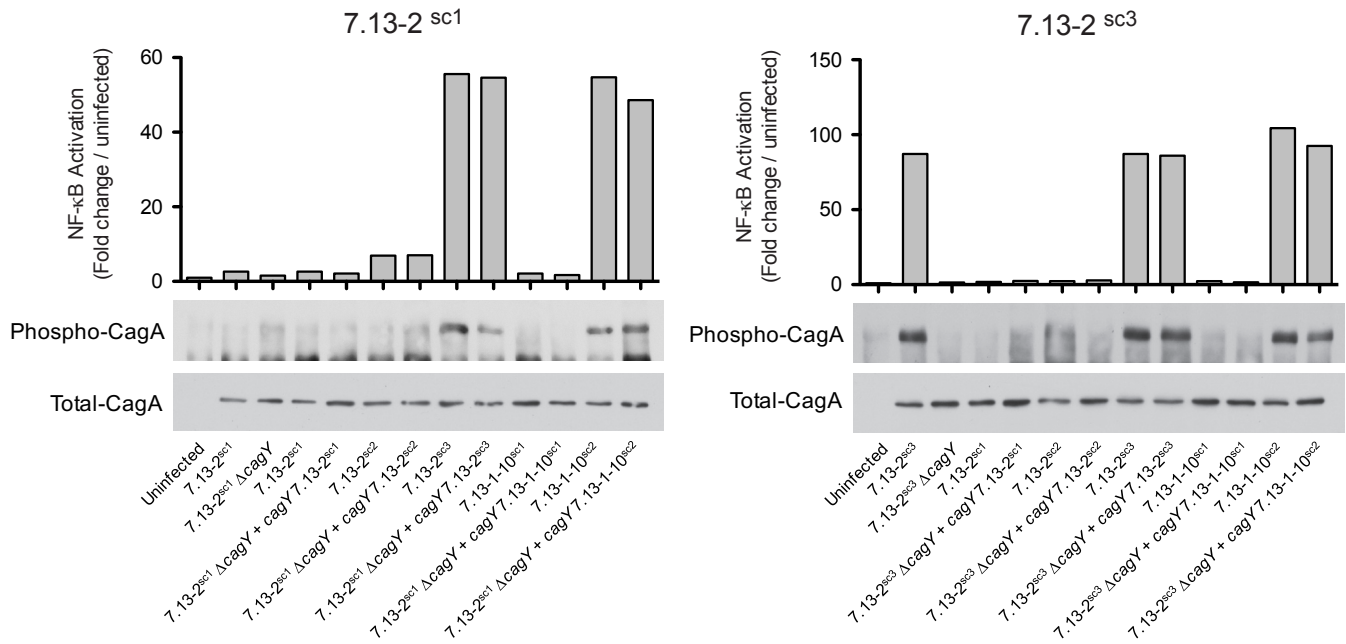
Lanes:

1. *Hp* 7.13-1<sup>sc2</sup>  $\Delta$ *cagY*
2. *Hp* 7.13-2<sup>sc1</sup>  $\Delta$ *cagY*
3. *Hp* 7.13-2<sup>sc2</sup>  $\Delta$ *cagY*
4. *Hp* 7.13-2<sup>sc3</sup>  $\Delta$ *cagY*

*cagY* RFLP of *H. pylori* recipient strains 7.13-1<sup>sc2</sup>, 7.13-2<sup>sc1</sup>, 7.13-2<sup>sc2</sup>, and 7.13-2<sup>sc3</sup> complemented with *cagY* from donor strains 7.13-2<sup>sc1</sup>, 7.13-2<sup>sc2</sup>, 7.13-2<sup>sc3</sup>, 7.13-1-10<sup>sc1</sup>, and 7.13-1-10<sup>sc2</sup>

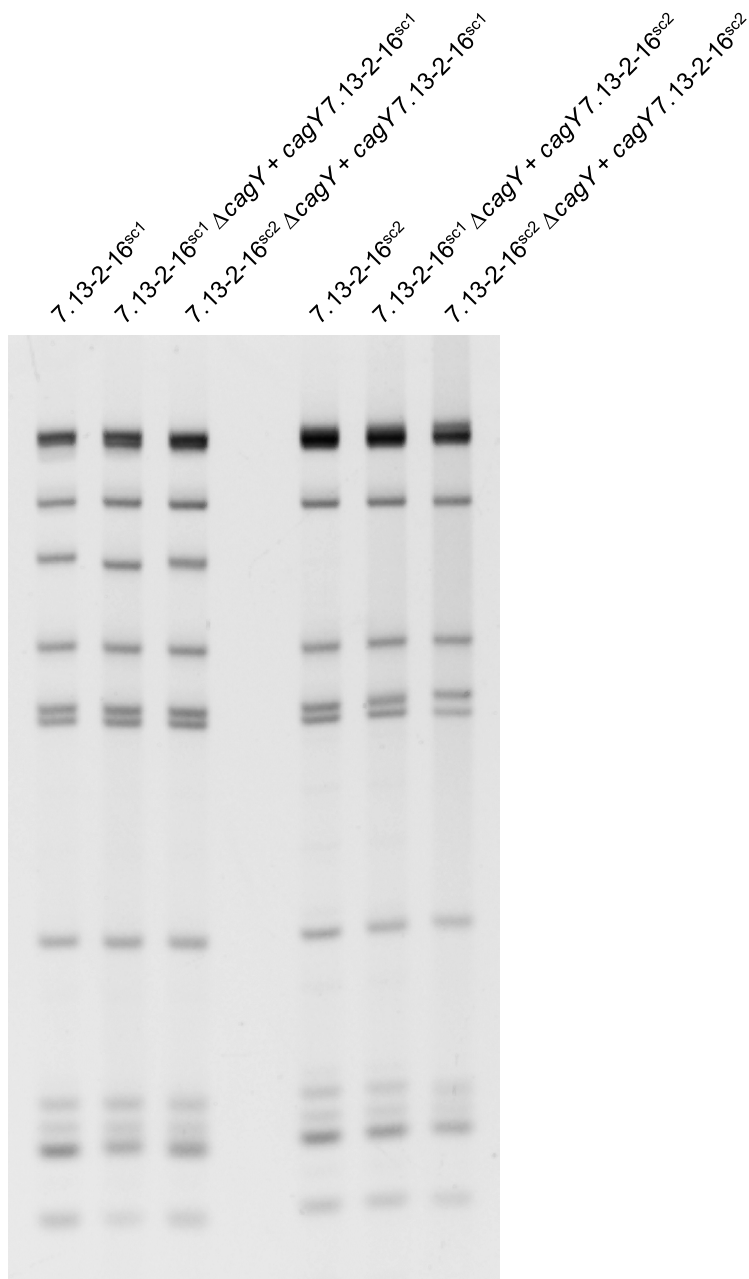


Supplementary Figure S9



NF-κB activation and CagA translocation using *H. pylori* wild type strains 7.13-2<sup>sc1</sup> and 7.13-2<sup>sc3</sup> complemented with *cagY* from strains 7.13-2<sup>sc1</sup>, 7.13-2<sup>sc2</sup>, 7.13-2<sup>sc3</sup>, 7.13-1-10<sup>sc1</sup>, and 7.13-1-10<sup>sc2</sup>

Supplementary Figure S10



*cagY* RFLP of *H. pylori* recipient strains 7.13-2-16<sup>sc1</sup>, and 7.13-2-16<sup>sc2</sup> complemented with either endogenous *cagY* or with *cagY* from the sibling *H. pylori* strain