**Supplementary File 4. *N. gonorrhoeae* strains used in this study**

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| --- | --- | --- | --- |
| Strain name | Description | Source | Strain number |
| GCGS0457 | CROS clinical isolate; recipient strain for transformations | GISP, CDC |  |
| GCGS1013 | CRORS clinical isolate | GISP, CDC |  |
| GCGS1014 | CRORS clinical isolate | GISP, CDC |  |
| GCGS1095 | CRORS clinical isolate | GISP, CDC |  |
| GCPH44 | CRORS clinical isolate | ([De Silva et al., 2016](#_ENREF_11)) |  |
| 28BL | CROS laboratory strain | Gift of S. Johnson |  |
| SP300-SP311 | 12 independent CRORS transformants: GCGS0457 + gDNA from GCGS1014 | This study |  |
| SP312-SP314 | 3 independent CRORS transformants:GCGS0457 + gDNA from GCGS1095 | This study |  |
| GCGS0457 RpoDE98K | Point mutation introduced on PCR product | This study | SP316 |
| GCGS0457 RpoBR201H | Point mutation introduced on PCR product | This study | SP319 |
| GCGS0457 RpoDΔ92-95 | Deletion introduced on PCR product | This study | SP323 |
| GCGS0092 | CROS clinical isolate | GISP, CDC |  |
| GCGS0092 RpoBR201H | CRORS transformant; point mutation introduced on PCR product | This study | SP349 |
| GCGS0275 | CROS clinical isolate | GISP, CDC |  |
| GCGS0275 RpoBR201H | CRORS transformant; point mutation introduced on PCR product | This study | SP354 |
| GCGS0465 | CROS clinical isolate | GISP, CDC |  |
| GCGS0465 RpoBR201H | CRORS transformant; point mutation introduced on PCR product | This study | SP358 |
| GCGS0336 | CROS clinical isolate | GISP, CDC |  |
| GCGS0336 RpoBR201H | CRORS transformant; point mutation introduced on PCR product | This study | SP340 |
| GCGS0524 | CROS clinical isolate | GISP, CDC |  |
| GCGS0524 RpoBR201H | CRORS transformant; point mutation introduced on PCR product | This study | SP368 |
| GCGS0364 | CROS clinical isolate; develops spontaneous CRORS via *rpoB* mutation *in vitro* | GISP, CDC |  |
| GCGS0364 RpoBG158V | Point mutation introduced on PCR product | This study | SP377 |
| GCGS0364 RpoBP157L | Point mutation introduced on PCR product | This study | SP375 |