



FIG. S1. Overexpression and purification of TraC_{N292} protein. The TraC_{N292} protein was overproduced by IPTG induction of BL21(DE3) cells harboring pET22bN292TraC, and was purified as described in Materials and Methods. Protein fractions were electrophoresed in a 12.5% SDS-polyacrylamide gel and stained with Coomassie brilliant blue. Lane 1, molecular-mass standards; lane 2, total cell fraction; lane 3, crude cell extract; and lane 4, purified fraction after affinity resin treatment. The locations of the molecular mass markers (in kilodaltons) are indicated at the left.

1 Motif 1

NAH7 MFNVTSIKGSNQYAAAGY^YTA-ADDY^YAKESPG-EWQGI GAELLGLAGPIDQKELAKLFDGKLPNGEVMPKTFDKETGNR
pWW0 .F..L..D...F^SN-...A...E.A...R...R.A...T.
R388 .LSHMVLRQDIGR..S[.].EDG...DG^SAS...K...E...S.EV.S.RFRE.LA.NIGE.HRIMRSATRQDSKE

Motif 2 Motif 3

NAH7 RMGL^DLTFSAPKSVSMQAL^IAGDKDVVA^AHRAVTKAMEHVEKLAQARRKEHGKSMLE^RTGNLIIGKFR^HELSRAKDPQL
pWW0M.....S.E.R..T.L.VN..T...S...V.....M.....M
R388 .I.....L...V...AEI^IK...ARTL.QA.AR...Q.IQ..TRI.T...V.....T..ER....

NAH7 ^HTHAVVMNAT^RREDGKWRAIHND^IFKIQP^IDAMYKELAKELRELGYEIRVLDKDG^NFELAHISR^DQIEAFSSRAKVI
pWW0MT..S.....LR...Y...V.....I..Q...A.....NQ.....S...
R388IL.M.K.S...Q...L^K.E.V.RTRYL^G.V.NA...H..Q^K..Q^L..Y^G....D...D.Q...G..K.TE^Q.

292

NAH7 EEALAKDGKTRSNATALEKQIIAMAT^RPRKDERDRHLVKEYVVTKARDL^GIEFGGRSQLDNQ^EYGRRES^ESHAEHN^LPE
pWW0 .D.....AD..P.....E...D..A..H...R..AP.NGG-P..YS..Q
R388 A.WY.AR.LDPNSV^SLEQ..AAK^VLS.AK.TSV..EALRAE.QAT[.]KE...D.SR.EWSGREK^G.SEKQA----.SFMP

NAH7 GITAGQAVVQYAINH^LTEREQVVGESDLR^TAAALRRAVGLASPSEVD^EEIKRLV^KQGT^LIESPPT^YR--MATGK-DGAALS
pWW0 ..P.....N...V.....T.DQ.N..R.....--.PN.DL.SP^V..
R388 SDE.AKRA.R.....Q^S.MD.RE.VDT.MKH...A.RLEDI^QK.LL.QTET.Y..REA.R..PGG^Q..PT.EPGKT

473

NAH7 PAGWRALLKEQK^GWSEKEAQYV^KMAINRGS^LVEAEKRY^TTQ^RALKREKAILAIERSGRG^VAPLLSKEQVAKALES^TL
pWW0H.Q.L.....R..DK..K...EP.....K.....T...T..MT.....G...
R388 R.E.V.E.AA-.MKQGA.RER.DN..KT.G.PI.P...T.E...R.Q...D...A...VIAA.AARER.A.TN.

NAH7 SAGQYQAVEVIVSTSN^RFVGIQGDAGT^GKTYSDRAV^KLIESVNNAMTERST^TDAVFRVVALAPYGN^VTALKNEGLDA
pWW0 .P..F.....N.....D...A..ATNNPQDIT^GY.....
R388 NQ..RE.A.L...AA..V..V..F.....SHML.T.KQM..G-----EGYH.R...A..S..K..RELN^VE.

NAH7 HTLASFFHTKDK^LDER^TIVVLDEAGVVGARQMEQLM^RIEQSGARLVQLGDT^KQTEAIEAGK^PFAQLQNGM^QTARIKE
pWW0N.G.AK...I.....R..E.....I.....S.....
R388 N....LRA...NI.S..VL.I.....PT.L...TLKLA.KA..V.LM...A..K...R..D...AA...HMR.

670

NAH7 IQRQKNQELKIAVQHAADGN^PGK^SLEHVNHVEELRE^PGQRHQAI^VRDYMSL^TPQERKEV^LIAGTNKDRK^QINAMTRES^L
pWW0DP...R.....E.Q.T...LK...TASD.....A...DQ.....E..T.A.QA.
R388P.....EL..AGKASS...RIKD.T.IKNHHE.RA.VAEA.IA.K.D..DRT...S...EA.RE..QIV..G.

NAH7 GLVGNKELP^TLN^RVDTTQAERRYAPS^YKGMIVQPEK^DYIRAGLSRGELY^TVDQALPG^NVL-VVKDKNG^NRVEFN^RPKL
pWW0 .F.K...FE.....S.D.....Q...I...K...V...V.....RS...F...QA
R388 .TA.K.I.FD.V.....H^SKN.QV.HVI...R..AKT..Q...R.VETG...R.T.IGEHD.Q.IQ.S.MTH

806

NAH7 TKLSVYNLEKPEF^SVGDLV^RITRNDQKLDLTNGDRM^VVGNGANGVIELASLKEKDG^TPERVVALP^TNRPLHLEHAYSAT^V
pWW0K...LA...I..N.Q.P.....SIEG..VQ.....VN.Q...T.S...K.....
R388 .I...QP.RA.LA..TI...KH...A...K..AVEDRK^VTVTDG.-----N.E..DK..VD..AT..

914

NAH7 HSAQGLTND^RVMISINTKSL^TTSQNLWYVAISRARHEARI^YTDSIAGLPAAIANRYDK^TTALS^L-----Q^QARE
pWW0R.....L.....V.A...K.....K.....E..
R388 .S...S...L.DAHAE.R..RKDVY.....F...VF.NDRGK.....RENI.SA.HD.ARD^RGGRSAAAER^Q..

NAH7 RQRNESIKP^RTVLDGKELERK^QRSALDGAGL^GKSGV
pWW0 ..RD..Q...S..RA...TG...PSS.NARI
R388 Q..ERERN^RQ.QQPAHDRQ^KAA.E.ER.MEA.R---

FIG. S2. Comparison of relaxases from NAH7, pWW0, and R388. These proteins have the relaxase, AAA, and UvrD-like helicase domains, which correspond to the amino-acid positions from 15 to 292, from 473 to 670, and from 806 to 914, respectively, in the NAH7 relaxase. Motifs 1 to 3 are conserved in the relaxase domain of MOB_F-family relaxases (1). The Y18, Y26, D85, H150, H161, and H163 residues in the R388 relaxase have been shown to be important for conjugation (2), and they are depicted in red. dot: amino-acid residue identical to that of NAH7; and hyphen, no amino-acid residue.

REFERENCES

1. **Garcillan-Barcia MP, Francia MV, de la Cruz F.** 2009. The diversity of conjugative relaxases and its application in plasmid classification. *FEMS Microbiol Rev* **33**:657-687.
2. **Guasch A, Lucas M, Moncalian G, Cabezas M, Perez-Luque R, Gomis-Ruth FX, de la Cruz F, Coll M.** 2003. Recognition and processing of the origin of transfer DNA by conjugative relaxase TrwC. *Nat Struct Biol* **10**:1002-1010.