

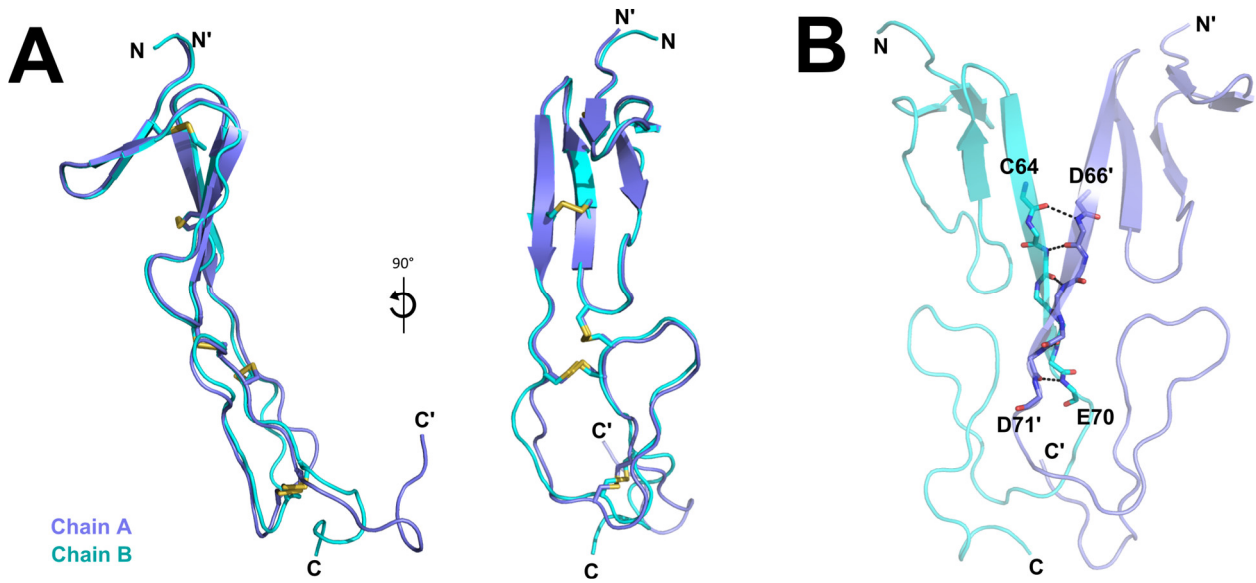
# Structural analyses of von Willebrand factor C domains of collagen 2A and CCN3 reveal an alternative mode of binding to bone morphogenetic protein-2

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## Supplemental Data

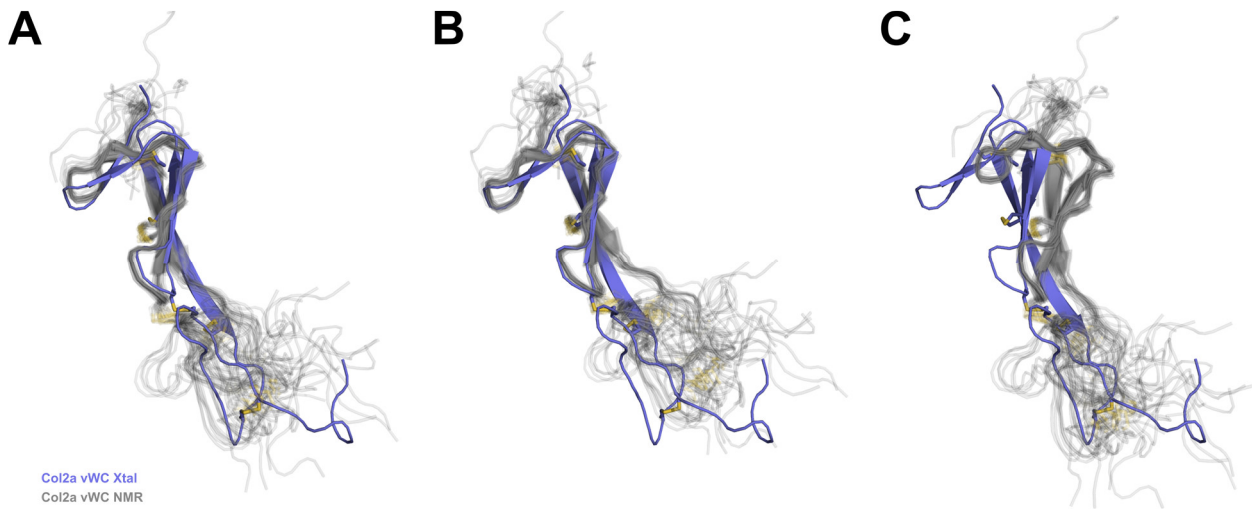
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FIGURE S1. Comparison of two molecules in the asymmetric unit of Col2a vWC.



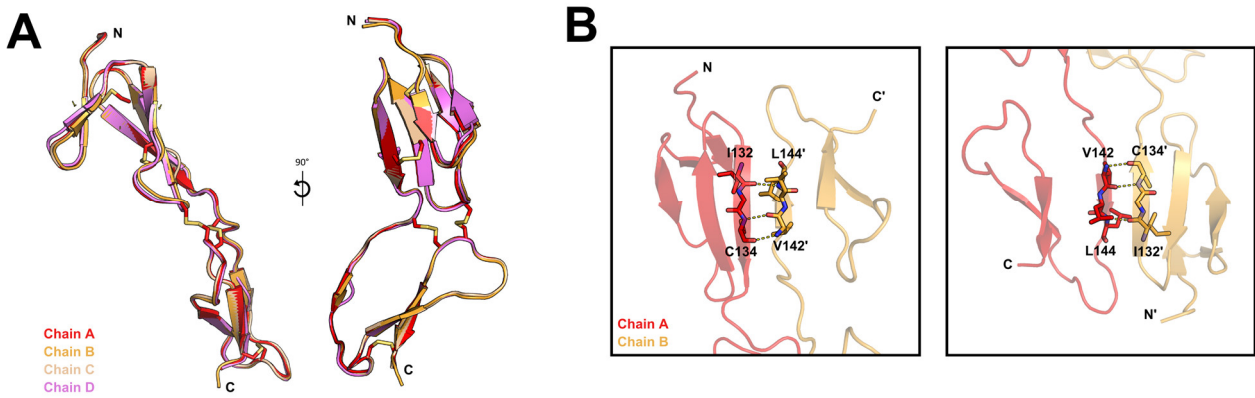
A, Overlay of the two chains in the asymmetric unit of Col2a vWC show very good agreement, with an average RMSD of 0.464 Å for 49 residues, excluding the first section of SD2 (residues 71-77) and the extreme C-terminus (residues 89-96). B, The arrangement of the two chains in the asymmetric unit is shown, with the intermolecular hydrogen bonds formed between  $\beta$ -strands in adjacent molecules as results from crystal packing highlighted.

FIGURE S2. Comparison of Col2a vWC crystal and NMR structures.



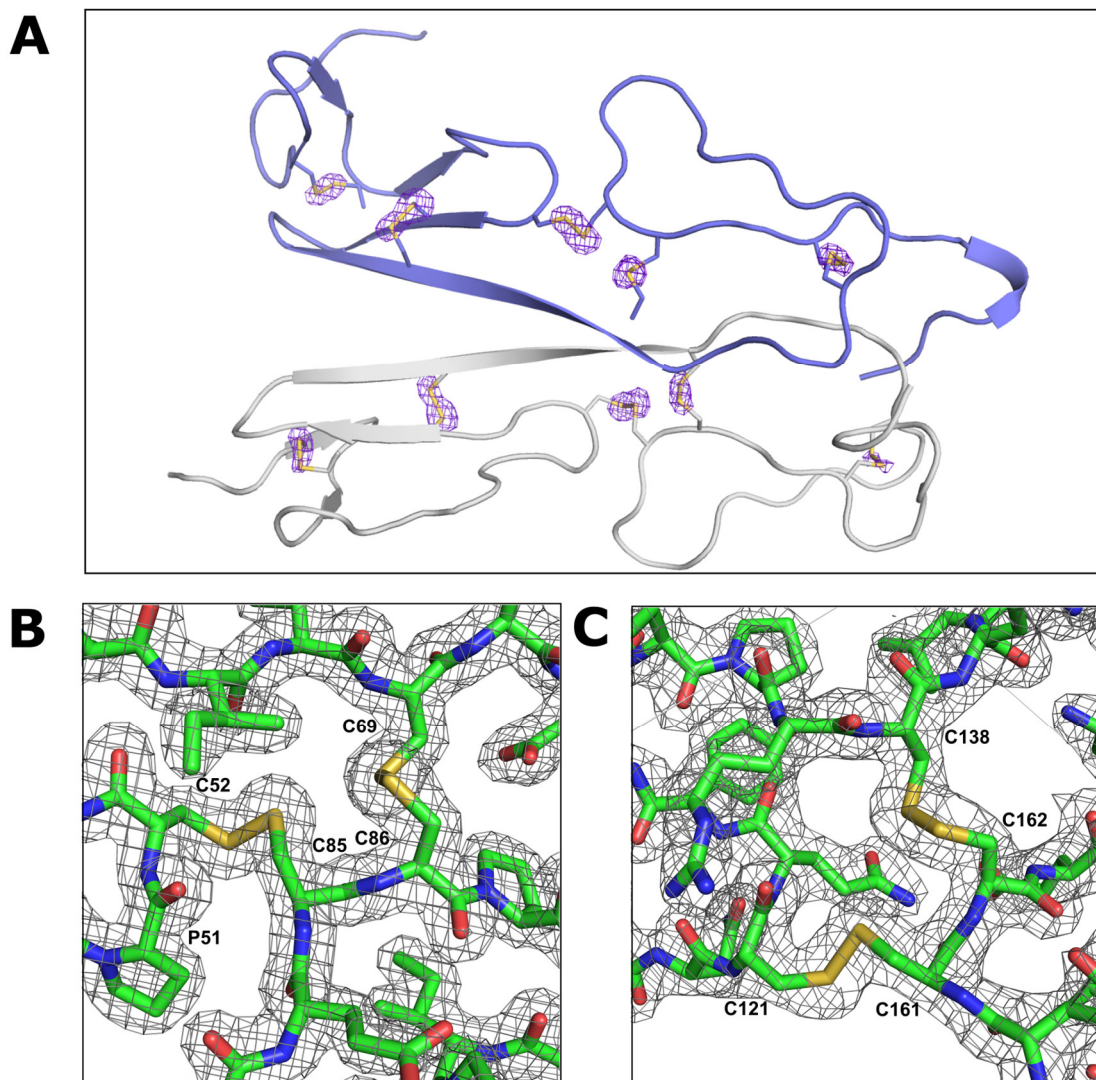
Overlays of our Col2a vWC crystal structure (*purple*) and the Col2a NMR ensemble (*grey*, PDB 1U5M), aligned according to the entire structure, SD1 (through I68), and SD2 (from C69) in *A*, *B*, and *C*, respectively.

FIGURE S3. Comparison of four chains in the asymmetric unit of CCN3 vWC.



*A*, Overlay of the four chains in the asymmetric unit of CCN3 vWC that are nearly identical, with RMSD of 0.336 Å, 0.121 Å, and 0.338 Å, between chains B and A, C and A, and D and A, respectively. *B*, The intermolecular hydrogen bonds formed between  $\beta$ -sheets in adjacent molecules as results from crystal packing are shown.

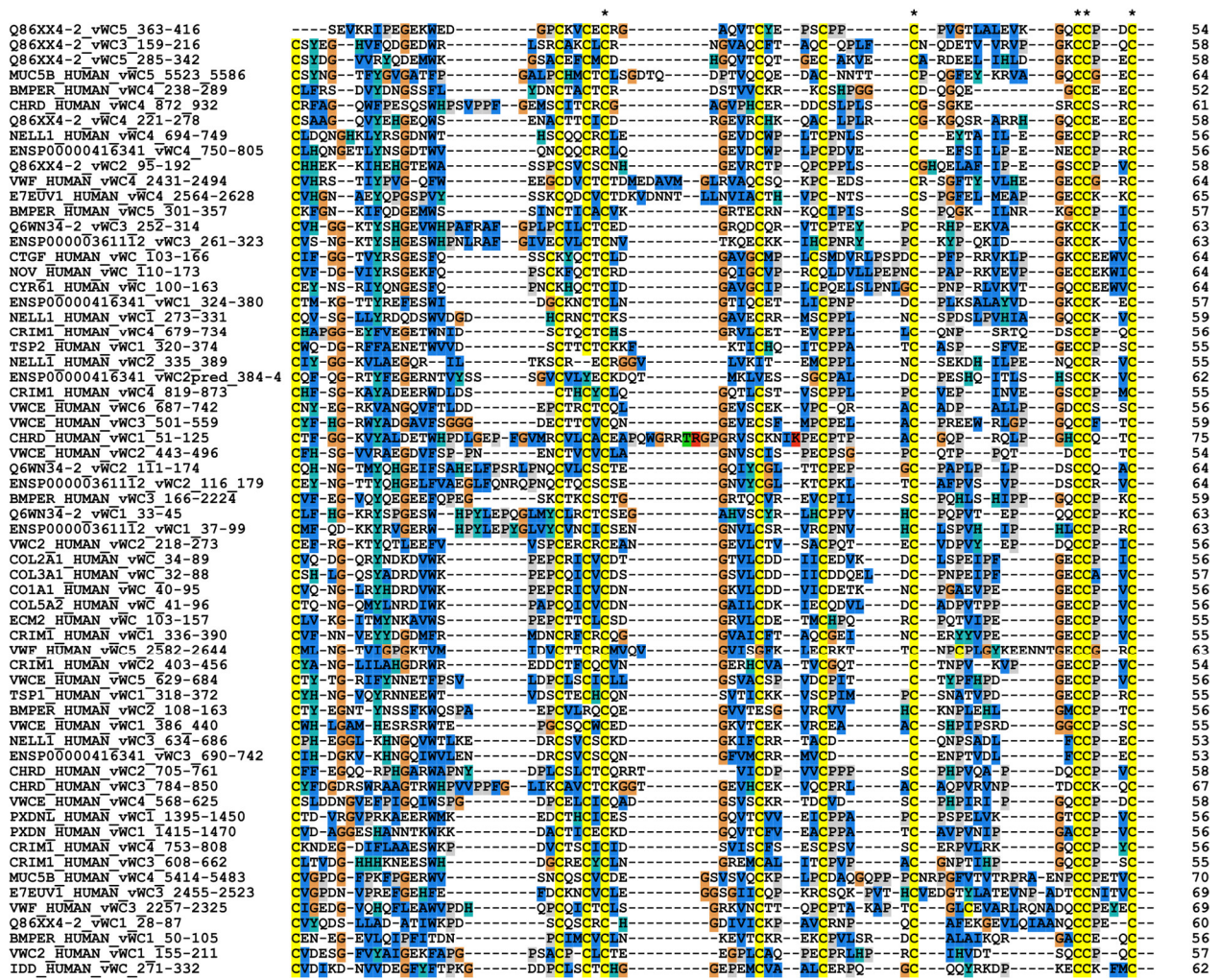
FIGURE S4. Electron density maps.



(A) Anomalous difference density for Col2a. Shown are ribbon diagrams of the two chains contained in the asymmetric unit (*purple* and *grey*). Side chains for cysteines forming disulfide bonds are shown as sticks, with sulphur atoms in *yellow*. A mesh of the anomalous difference density is contoured at  $4\sigma$ , showing clear density around the disulfide bonds, as calculated from the dataset collected at a wavelength of  $1.9\text{ \AA}$ . (B) Final 2Fo-Fc electron density map of the Col2a vWC domain, around the site of the two disulfide bonds connecting SD1 and SD2, contoured at  $1.0\sigma$ . All cysteines are labelled, as is proline 51 which is causing the shift of the subsequent disulfide bonds in Col2a vWC. (C) Final 2Fo-Fc electron density map of the CCN3 vWC, contoured at  $1.0\sigma$ , and centered at the interface between the subdomains SD1 and SD2.

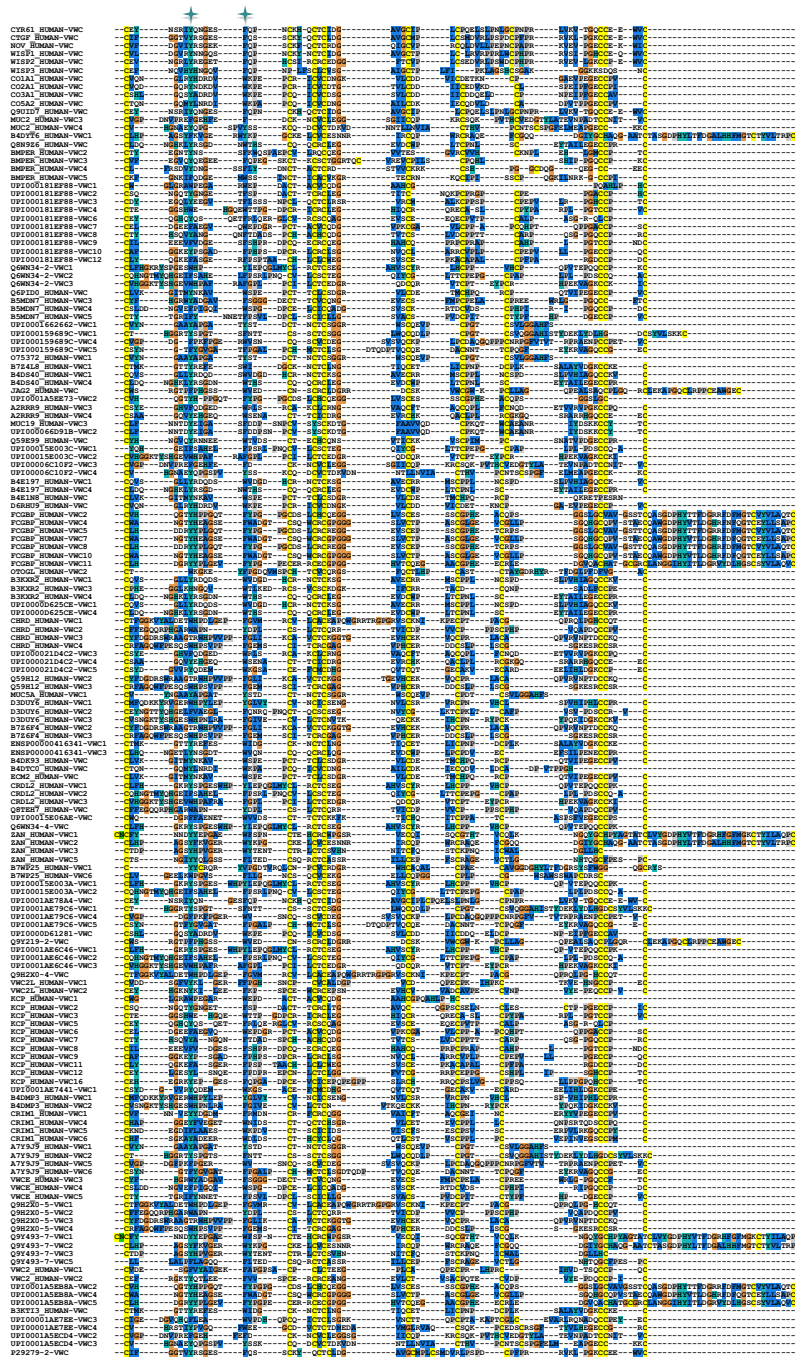


FIGURE S5. Sequence alignment of human vWC domains with extra proline before C<sub>2</sub>.



Sequences of human vWC domains with additional proline residues before the second cysteine were extracted from the SMART database (<http://smart.embl-heidelberg.de>) and aligned using Clustal X.

FIGURE S6. Sequence alignment of human vWC domains with conserved aromatic residues.



VWC sequences with conserved aromatic residues in the SD1 subdomain were extracted from the *SMART* database (<http://smart.embl-heidelberg.de>) and aligned using *Clustal X*. The positions of the aromatic residues in the sequences are marked with crosses at the top of the alignment.



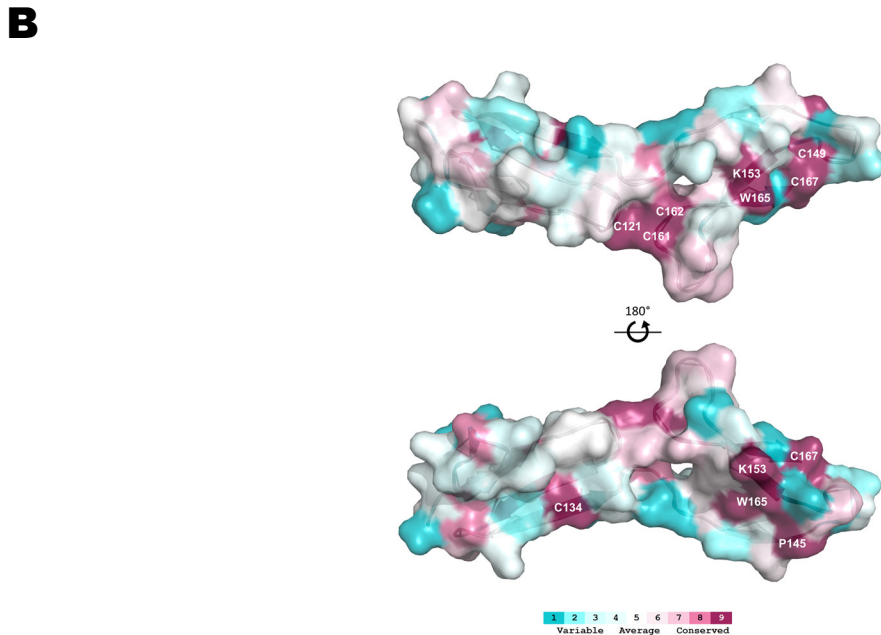
FIGURE S7. ConSurf analysis of Col2a vWC.



Conservation of residues in Col2a vWC across species was analysed using the ConSurf server (<http://consurf.tau.ac.il/>). These results projected onto the crystal structure of Col2a vWC is shown in Figure 5A.



FIGURE S8. ConSurf analysis of CCN3 vWC.



Conservation of residues in CCN3 vWC across species was analysed using the ConSurf server (<http://consurf.tau.ac.il/>), with sequence alignment shown in A, and projection of the conservation scores onto the crystal structure in B.

Supplemental Table 1

Protein	Primer	Sequence (5' → 3')	Restriction Site
<b>Col IIA vWC</b>	VWC_1	ATATAT <u>ACCATGG</u> CACAGGAAGCTGGATCCTGCGTTCAGGACGGTCAG	<i>NcoI</i>
	VWC_2	CGACAAAGACGTTTGGAAACCGGAACCGTGCCGTATCTGCGTTTGCGA	
	VWC_3	CCGTTCTGTGCGACGACATCATCTGCGAAGACGTTAAAGACTGCCTGT	
	VWC_4	ATCCCGTTTCGGTGAATGCTGCCCGATCTGCCCGACCGACCTGGCTACC	
	VWC_5	GTTTCCAAACGTCCTTGTGCGTTGTAACGCTGACCGTCCTGAACGC	
	VWC_6	GTCGTGCGACAGAACGGTACCGGTGTCGCAAACGCAGATACGG	
	VWC_7	GCATTCACCGAACGGGATTTCCGGGGACAGGCAGTCTTTAACGTC	
	VWC_8	TATATATA <u>AAGCTT</u> AACCGGAAGCGGTAGCCAGGTCGGTCG	<i>HindIII</i>
<b>Col IIA vWC mutants</b>	Col2aI30A_F	GCTTGGAACCGGAACCGTGCCGTGCCTGC	
	Col2aV22A_R	CGGCACGGTTCCGGTTTCCAAGCGTCTTTG	
	Col2aL39A_F	GCTTGGACACCGGTACCGTTGCGTGCG	
	Col2aV32A_R	CGCAACGGTACCGGTGTCGCAAGCGCAG	
	Col2aF58AI64A_F	CCCGGCCGGTGAATGCTGCCCGCCTGCCCG	
Col2aF58AI64A_R	CGGGCAGGCCGGGCAGCATTACCGGCCGGG		
<b>CCN3 vWC</b>	Nov5	TATAT <u>CCATGG</u> AGGGAGACAACCTGTGTGTTT	<i>NcoI</i>
	Nov62	TATATA <u>AAGCTT</u> AGTCAGAGAGTTCGACTCCTACG	<i>HindIII</i>