Supplementary Data

Investigating the sequence-dependent mechanical properties of DNA nicks for applications in twisted DNA nanostructure design

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Supplementary Methods

Supplementary Method S1. Mechanical rigidities and coupling coefficients of a base-pair (BP) step.

MD trajectories of BP steps were analyzed on the basis of a mechanical model with quasi-harmonic strain energy. Six BP step parameters describe bending (tilt and roll denoted by $\tau$ and $\rho$), torsional (twist denoted by $\omega$), shearing (shift and slide denoted by $D_x$ and $D_y$), and stretching (rise denoted by $D_z$) modes. The strain energy for the six deformable modes in BP steps was assumed to the following form

$$E(U) = \frac{1}{2} U^T K U = \frac{1}{2} (x - \langle x \rangle)^T K (x - \langle x \rangle)$$  \hspace{1cm} (1)$$

where $x$ represents the coordinate vector of BP step parameters as $x = [\tau \rho \omega D_x D_y D_z]^T$, whose average is denoted as the angle bracket, $U$ is displacement vector whose components indicate the displacement from average configuration, and $K$ is a positive definite stiffness matrix where the six diagonal and remaining symmetric off-diagonal terms represent mechanical stiffness (bending, torsion, shearing, and stretching) and coupling stiffness, respectively.

The probability distribution of BP step parameters can be approximated to an N-dimensional Gaussian function assuming moderate fluctuations under harmonic potential in contact with a heat bath of a certain temperature. The covariance matrix of the BP step parameters was used to induce the stiffness matrix of a BP step (1-3) as

$$K = k_B T F^{-1}$$  \hspace{1cm} (2)$$

where $k_B$ is the Boltzmann constant, $T$ is the absolute temperature used in MD simulation, and $F$ is the covariance matrix. Here, the covariance matrix was obtained using from the sampled MD trajectories of the BP step parameters (4) as

$$F_{ij} = \langle (x_i - \langle x_i \rangle)(x_j - \langle x_j \rangle) \rangle$$  \hspace{1cm} (3)$$

The strain energy can be rewritten as the sum of diagonal and off-diagonal components as

$$E(U) = \frac{1}{2} \sum_i K_{ii} U_i^2 + \sum_{i \neq j} K_{ij} U_i U_j$$  \hspace{1cm} (4)$$

where $i$ and $j$ represent the BP step parameters as $\tau$, $\rho$, $\omega$, $D_x$, $D_y$, and $D_z$. Diagonal terms contribute to mechanical rigidities corresponding to the six BP step parameters, and mechanical coupling coefficients are derived from off-diagonal terms between two BP step parameters. Mechanical rigidities of a BP step were then obtained by multiplying the diagonal stiffness components to the axial length of a BP step from linear elastic theory as
\begin{align}
B_τ &= K_{ττ}(Dz) \\
B_ρ &= K_{ρρ}(Dz) \\
C &= K_{ωω}(Dz) \\
Y_{Dx} &= K_{DxDx}(Dz) \\
Y_{Dy} &= K_{DyDy}(Dz) \\
S &= K_{DzDz}(Dz)
\end{align}

where \(B_τ\) and \(B_ρ\) represent bending rigidities corresponding to tilt and roll, \(C\) is torsional rigidity, \(Y_{Dx}\) and \(Y_{Dy}\) indicate shear rigidities corresponding to shift and slide, \(S\) is stretching rigidity, and \(\langle Dz \rangle\) is the average of rise, respectively. The detailed derivation of each mechanical rigidity above was explained in Supplementary Method S2. The equivalent isotropic bending or shearing rigidities (\(B\) or \(Y\)) were obtained using the harmonic mean of anisotropic bending (\(B_τ\) and \(B_ρ\)) or shearing rigidities (\(Y_{Dx}\) and \(Y_{Dy}\)), respectively (Supplementary Method S3) as

\begin{align}
B &= \frac{2B_τB_ρ}{B_τ+B_ρ} \\
Y &= \frac{2Y_{Dx}Y_{Dy}}{Y_{Dx}+Y_{Dy}}
\end{align}

Likewise, mechanical coupling coefficients of a BP step were calculated by multiplying the off-diagonal stiffness to the axial length of the BP step as

\[g_{ij} = K_{ij}(Dz)\]

where \(g_{ij}\) represents the mechanical coupling coefficients of two different BP step parameters. For example, \(g_{ωDz}\) represents the mechanical coupling coefficient of twist and rise, implying the twist-stretch coupling.
Supplementary Method S2. Derivation of mechanical rigidities of a base-pair (BP) step.

Mechanical rigidities were derived as assuming DNA structures of a homogeneous linear elastic material. The initial shape was assumed to have an area \((A)\) and length \((L)\) with axial z-direction. Then, the representative mechanical rigidities \((B_\tau, B_\rho, C, Y_{Dx}, Y_{Dy}, \text{and } S)\) were derived by elastic theory.

(A) **Bending rigidities.** Bending moment \((M_b)\) is obtained by integrating the axial stress \((\sigma_{zz})\) multiplied by the moment arm \((s)\) perpendicular to the direction of bending moment over the cross-section area \((A)\) as

\[
M_b = \int_A \sigma_{zz}sdA = EI \frac{\Delta \theta}{L} \tag{9}
\]

where axial stress \((\sigma_{zz})\), axial strain \((\varepsilon_{zz})\), and the second moment of area \((I)\) are defined as

\[
\sigma_{zz} = E\varepsilon_{zz} = E \frac{\Delta \theta}{L} \tag{10}
\]

\[
\varepsilon_{zz} = \frac{\Delta \theta}{L} \tag{11}
\]

\[
I = \int_A s^2dA \tag{12}
\]

Bending rigidity \((EI)\) is then evaluated as

\[
EI = \frac{M_b}{\Delta \theta} L = k_{\text{bending}} L \tag{13}
\]

where \(k_{\text{bending}}\) is the stiffness defined by the ratio of applied bending moment and corresponding bending deformation. Bending rigidities of DNA, therefore, can be calculated in terms of components of stiffness matrix and BP step parameters as

\[
B_\tau = (EI)_{\tau} = K_{\tau\tau}(Dz) \tag{14}
\]

\[
B_\rho = (EI)_{\rho} = K_{\rho\rho}(Dz) \tag{15}
\]

where \(B_\tau\) and \(B_\rho\) represent tilt and roll bending rigidities, and \(K_{\tau\tau}\) and \(K_{\rho\rho}\) indicate the diagonal tilt and roll components in the stiffness matrix, respectively.
(B) Torsional rigidity. Torsional moment ($M_z$) is obtained by integrating the shear stress ($\sigma_t$) multiplied by the moment arm ($r$) over the cross-section area ($A$) as

$$M_z = \int_A \sigma_t r dA = GJ \frac{\Delta \phi}{L} \quad (16)$$

where shear stress ($\sigma_t$), shear strain ($\gamma$), and the second polar moment of area ($J$) are defined as

$$\sigma_t = G\gamma = G \frac{r \Delta \phi}{L} \quad (17)$$

$$\gamma = \frac{r \Delta \phi}{L} \quad (18)$$

$$J = \int_A r^2 dA \quad (19)$$

Then, the torsional rigidity ($GJ$) is evaluated as

$$GJ = \frac{M_z}{\Delta \phi L} = k_{\text{torsion}} L \quad (20)$$

where $k_{\text{torsion}}$ is the stiffness defined by the ratio of applied torsional moment and corresponding torsional deformation. Therefore, we obtained the torsional rigidity of DNA corresponding to the twist parameter as

$$C = GJ = K_{\omega \omega} \langle Dz \rangle \quad (21)$$

where $C$ is generally referred to torsional rigidity of DNA, and $K_{\omega \omega}$ is the diagonal twist component in the stiffness matrix.

(C) Shearing rigidities. Shearing rigidity is derived from the shear modulus ($G$) defined by the ratio of shear stress ($\sigma_t$) to the shear strain ($\gamma$) as

$$G = \frac{\sigma_t}{\gamma} = \frac{F/A}{\Delta \delta / L} = \frac{F L}{A \Delta \delta} \quad (22)$$

where $F$ and $\Delta L$ are the applied shear force and corresponding shear displacement. The shearing rigidity ($GA$) is then obtained as

$$GA = \frac{F}{\Delta L} L = k_{\text{shearing}} L \quad (23)$$

where $k_{\text{shearing}}$ is the stiffness defined by the ratio of applied shear force and corresponding displacement. We then obtained the shearing rigidities of DNA as

$$Y_{Dx} = (GA)_{Dx} = K_{DxDx} \langle Dz \rangle \quad (24)$$

$$Y_{Dy} = (GA)_{Dy} = K_{DyDy} \langle Dz \rangle \quad (25)$$

where $Y_{Dx}$ and $Y_{Dy}$ denote shearing rigidities corresponding to the shift and slide, and $K_{DxDx}$ and $K_{DyDy}$ are the diagonal shift and slide components in the stiffness matrix, respectively.
(D) Stretching rigidity. Stretching rigidity is derived from Young’s modulus \((E)\) defined as the ratio of axial stress \(\sigma_{zz}\) to the axial strain \(\varepsilon_{zz}\) as

\[
E = \frac{\sigma_{zz}}{\varepsilon_{zz}} = \frac{F/A}{\Delta L/L} = \frac{FL}{AAL}
\]  

(26)

where \(F\) and \(\Delta L\) are the applied axial force and axial displacement. Stretching rigidity \((EA)\) is evaluated as

\[
EA = \frac{F}{\Delta L} = k_{\text{stretching}} L
\]

(27)

where \(k_{\text{stretching}}\) is the stiffness defined by the ratio of applied axial force and corresponding displacement. Therefore, the stretching rigidity of DNA is obtained corresponding to the rise mode as

\[
S = EA = K_{DzDz} Dz
\]

(28)

where \(S\) is generally referred to the axial rigidity of DNA, and \(K_{DzDz}\) is the diagonal component corresponding to the rise parameter in the stiffness matrix.
Supplementary Method S3. Derivation of an equivalent isotropic rigidity in bending and shearing.

Assuming a homogeneous linear elastic material, if a transverse load was applied to a beam, whose axis system coincides with the principal axes of bending, three-dimensional beam theory implies the following displacement field as

$$ u_z = y\theta_x(z) - x\theta_y(z) $$

(29)

where $z$ is the axial direction of the beam, $x$ and $y$ are the planar directions on the cross-section of the beam, and $\theta_x$ and $\theta_y$ are rigid body rotations of the cross-section respectively. The strain field can be evaluated by the differentiation of the displacement field as

$$ \varepsilon_{zz} = y\kappa_x(z) - x\kappa_y(z) $$

(30)

$$ \varepsilon_{xx} = \varepsilon_{yy} = \gamma_{xy} = \gamma_{yx} = \gamma_{zz} = 0 $$

(31)

where the curvatures are defined as

$$ \kappa_x = \frac{d\theta_x}{dz} $$

(32)

$$ \kappa_y = \frac{d\theta_y}{dz} $$

(33)

The axial stress is described by Hooke’s law as

$$ \sigma_{zz} = E\varepsilon_{zz} = E[y\kappa_x(z) - x\kappa_y(z)] $$

(34)

The strain energy ($\pi$) stored in the beam with anisotropic bending rigidities is evaluated as

$$ \pi = \int_A \int \frac{1}{2} \sigma_{zz} \varepsilon_{zz} dA dz = \int_z \frac{1}{2} (EI_y\kappa_x^2 + EI_x\kappa_y^2) dz $$

(35)

where $I_x$ and $I_y$ are the second moment of area with respect to x- and y-directions on the cross-section.

When an equivalent isotropic beam is assumed, the force equation under a bending moment is found as

$$ M_b = EI_y\kappa_x = EI_x\kappa_y = EI_{eqv}\kappa_{eqv} $$

(36)

If the strain energy stored in anisotropic and equivalent beams is the same, the following equation is satisfied

$$ \pi = \int_z \frac{1}{2} (EI_y\kappa_x^2 + EI_x\kappa_y^2) dz = \int_z \frac{1}{2} \left( EI_{eqv}\kappa_{eqv}^2 + EI_{eqv}\kappa_{eqv}^2 \right) dz $$

$$ = \int_z \frac{1}{2} \left( EI_{eqv}\kappa_{eqv}^2 + EI_{eqv}\kappa_{eqv}^2 \right) dz = \int_z \frac{1}{2} \left( \frac{2M_b^2}{EI_{eqv}} \right) dz $$

(37)

Equivalent bending rigidity ($B$ or $EI_{eqv}$) is then obtained by the harmonic mean of anisotropic bending rigidities as
\[ B = \frac{2B_1B_2}{B_1 + B_2} \]  

(38a)

or

\[ E I_{\text{eqv}} = \frac{2E I_x E I_y}{E I_x + E I_y} \]  

(38b)

Likewise, equivalent shearing rigidity \((Y \text{ or } G A_{\text{eqv}})\) is obtained as

\[ Y = \frac{2Y_{dx}Y_{dy}}{Y_{dx} + Y_{dy}} \]  

(39a)

or

\[ G A_{\text{eqv}} = \frac{2G A_x G A_y}{G A_x + G A_y} \]  

(39b)
Supplementary Method S4. Molecular dynamics simulation of twisting blocks of a six-helix-bundle (6HB) DNA origami structure.

We performed MD simulations of 6HB DNA origami structures with BP-insertion (Supplementary Figure S9). At each end of the simulated structure, 7-BP-long strands were added to eliminate the boundary effect. Since it was difficult to observe the effects of sequence control of nicked BP steps due to the limited system size, MD result of an arbitrary sequence was used to predict an approximately twist angle of 6HB structures. For the fast convergence of equilibrium states, the initial all-atom structures were developed after 100-ps-long the elastic network-guided simulation (5). All-atom explicit solvent simulations were then performed after energy minimization as previously described for DNA oligomers.

To smoothly convert the induced strain energy by BP-insertion into global torsion of structures, we applied weak harmonic constraints to Watson–Crick base-pairing from 0.5 kcal mol$^{-1}$ Å$^2$ to zero during 60-ns-long simulation as previous MD studies on DNA origami structures (5,6). Final 20-ns-long equilibrated trajectories were sampled to calculate twist angle (Supplementary Method S5).
Supplementary Method S5. Prediction of the twist angle of a six-helix-bundle (6HB) DNA origami structure.

The twist angle of a 6HB structure was calculated by the summation of local twist angles of twisting blocks between sequential hexagonal cross-sections locating at Holliday-junctions. To obtain the local twist angle between hexagonal cross-sections, we defined six vertexes locating at helices of the 6HB structure as the averaged point of local origins in four neighboring BPs next to the hexagonal cross-section. A hexagonal cross-section was determined to satisfy the smallest sum of the distance between each vertex and an arbitrary plane. Auxiliary vectors \( \vec{A}_1, \vec{A}_2, \vec{A}_3 \) were defined in hexagonal cross-sections: \( \vec{A}_1 \), \( \vec{A}_2 \), and \( \vec{A}_3 \) were defined as the vectors from vertex 1 to 4, 2 to 5, and 3 to 6, respectively. Subsequently, the triads in hexagonal cross-sections were determined: x-vector (\( \vec{x} \)) representing normalized axial direction was defined as a normal vector of each cross-section, y-vector (\( \vec{y} \)) in the hexagonal cross-sections was described as a normalized vector with projection of an averaged vector of auxiliary vectors on the cross-section, and the z-vector (\( \vec{z} \)) was calculated using the cross product of x- and y-vector. A rotation matrix (\( \mathbf{R} \)) between sequential triads was then obtained using the triads as

\[
\begin{bmatrix}
\vec{x}_{i+1} \\
\vec{y}_{i+1} \\
\vec{z}_{i+1}
\end{bmatrix} = \mathbf{R} \begin{bmatrix}
\vec{x}_i \\
\vec{y}_i \\
\vec{z}_i
\end{bmatrix}
\]

where \( i \) and \( i + 1 \) represent successive hexagonal cross-sections. The rotation vector (\( \vec{\omega} \)) equivalent to the rotation matrix was obtained as

\[
\vec{\omega} = [-W_{23}, W_{13}, -W_{12}]^T
\]

where \( W_{ij} \) is a component of the skew-symmetric matrix (\( \mathbf{W} \)) defined as \( \mathbf{W} = \frac{1}{2} (\mathbf{R} - \mathbf{R}^T) \). The local twist angle between sequential hexagonal cross-sections (\( \Omega \)) was finally calculated as

\[
\Omega = \sin^{-1}\left(\frac{\vec{\omega} \cdot \vec{x}_i}{||\vec{\omega}||} \right)
\]
We obtained the Gaussian mean and standard deviation of the twist angle in the unit twisting block from MD simulation (Supplementary Figure S9). If the twist angle of the unit twisting block ($\Phi_u$) follows Gaussian distribution ($N_u$) as

$$N_u = N_u(\Phi, \Phi_{u0}, \sigma_u) = \frac{1}{\sigma_u \sqrt{2\pi}} \exp\left(-\frac{(\Phi - \Phi_{u0})^2}{2\sigma_u^2}\right)$$ (43)

where $\Phi_{u0}$ and $\sigma_u$ are the Gaussian mean and standard deviation of the twist angle of the twisting block, we could calculate the twist angle ($\Phi$) of a DNA structure, in which same twisting blocks are connected in sequence and the number of twisting blocks is $m$, as the sum of the independent Gaussian distributions of the twisting blocks based on the probability theory as

$$N = N(\Phi, \Phi_0, \sigma) = mN_u = N(\Phi, m\Phi_{u0}, \sqrt{m}\sigma_u)$$ (44)

Since ten twisting blocks were employed in the entire structure, the approximate range of twist angle of the structure was estimated as the mean of $10\Phi_{u0}$ and the standard deviation of $\sqrt{10}\sigma_u$ using those of unit twisting blocks ($\Phi_{u0}$ and $\sigma_u$) by MD simulation.
**Supplementary Method S6. CanDo simulation based on finite element method.**

We performed CanDo simulation (7-9) for DNA origami structures used in experiments with the default setting (Supplementary Figure S10), where a double-stranded DNA was assumed as generic B-form DNA: geometry of diameter of 2.25 nm, axial rise of 0.34 nm per BP step, helicity of 10.5 BPs per turn, stretch rigidity (S) of 1100 pN, bending rigidity (B) of 230 pN nm$^2$, and torsional rigidity (C) of 460 pN nm$^2$. BP steps were modeled as two node linear elastic beam elements, and Holliday-junctions were modeled as rigid beam elements. To observe the effects of nicks on deformed shapes of DNA origami structures, we modeled nicks as same two node beam elements, but bending and torsional rigidities of nicked elements were modified by multiplying the mean B and C ratios of from experimental design as scale factors (Supplementary Table S15).
Supplementary Method S7. Relation of the trans ratio with the twist angle of 6HB DNA structures.

We followed the details of derivation in the previous study (10). Assuming that 6HB origami structures fluctuate in a harmonic potential, the Gaussian distribution of twist angle can be derived from the Boltzmann distribution as

$$N(\Phi, \Phi_0, \sigma) = N_0 \exp \left( -\frac{E}{k_B T} \right) = \frac{1}{\sigma \sqrt{2\pi}} \exp \left( -\frac{(\Phi - \Phi_0)^2}{2\sigma^2} \right)$$  \hspace{1cm} (45)

where $N_0$ is the normalization coefficient as $1/(\sigma \sqrt{2\pi})$, $E$ is torsional harmonic energy as $E = k_t(\Phi - \Phi_0)^2/2$, $\Phi$ is the twist angle of 6HB structure, $\Phi_0$ is the equilibrium twist angle, $\sigma$ is the standard deviation of twist angle as $k_t/(k_B T)$, and $k_B T$ is the product of the Boltzmann constant, $k_B$, and the absolute temperature, $T$. Here, the torsional stiffness of 6HB structure ($k_t$) has a relation with torsional persistence length ($L_p$) and the length of 6HB structure ($L$) as

$$k_t = \frac{C_{6HB}}{L} = \frac{L_p k_B T}{L}$$  \hspace{1cm} (46)

with

$$L_p = \frac{C_{6HB}}{k_B T}$$  \hspace{1cm} (47)

where the torsional persistence length of 6HB DNA structures ($L_p$) was reported as 530 nm in a previous study (11), and the length of our structure is approximately 300 nm in AFM images (Supplementary Figure S12). Using the equations above, the torsional harmonic energy yields the standard deviation of twist angle as

$$\sigma = \sqrt{\frac{k_B T}{k_t}} = \sqrt{\frac{L}{L_p}} = \sqrt{\frac{300}{530}} \approx 0.7524 \text{ [rad]}$$  \hspace{1cm} (48)

with

$$\frac{1}{2}k_t \sigma^2 = \frac{1}{2}k_B T$$  \hspace{1cm} (49)

The probability to deposit into trans monomer of the 6HB structures is obtained as

$$f(\Phi) = \frac{|\Phi - 2k\pi|}{\pi} \quad (2k-1)\pi \leq \Phi \leq (2k+1)\pi$$  \hspace{1cm} (50)

where $k$ is an integer as a parametric variable ($\cdots, -2, -1, 0, 1, 2, \cdots$). This probability to deposit into trans monomer is a kind of triangular function, which is plotted as
The trans ratio, \( TR(\Phi_0) \) in equilibrium is derived by the average probability of twist angle weighted in \( \Phi_0 \) for all \( \Phi \) domain as

\[
TR(\Phi_0) = \int_{-\infty}^{\infty} f(\Phi) N(\Phi, \Phi_0, \sigma) d\Phi = \sum_k \int_{(2k-1)n}^{(2k+1)n} \frac{1}{\alpha \sqrt{2\pi}} \exp \left( -\frac{(\Phi-\Phi_0)^2}{2\alpha^2} \right) d\Phi
\]

\[
= \frac{1}{\alpha \sqrt{2\pi}} \sum_k \left\{ \int_{(2k-1)n}^{(2k+1)n} \Phi \exp \left( -\frac{(\Phi-\Phi_0)^2}{2\alpha^2} \right) d\Phi - \int_{(2k-1)n}^{(2k+1)n} \Phi \exp \left( -\frac{(\Phi-\Phi_0)^2}{2\alpha^2} \right) d\Phi \right\}
\]

\[
= \frac{1}{\alpha \sqrt{2\pi}} \sum_k \left\{ \int_{(2k-1)n}^{(2k+1)n} \exp \left( -\frac{(\Phi-\Phi_0)^2}{2\alpha^2} \right) d\Phi + 2k\pi \int_{(2k-1)n}^{(2k+1)n} \exp \left( -\frac{(\Phi-\Phi_0)^2}{2\alpha^2} \right) d\Phi \right\}
\]

(51)

Using the Gaussian integrals,

\[
\int \Phi \psi(A + B\Phi) d\Phi = -\frac{1}{b^2} \left( \psi(a + b\Phi) + a\Psi(a + b\Phi) \right) + \text{Const.}
\]

(52)

\[
\int \Psi(A + B\Phi) d\Phi = \frac{1}{b} \Psi(a + b\Phi) + \text{Const.}
\]

(53)

where

\[
\psi(a + b\Phi) = \frac{1}{\sqrt{2\pi}} \exp \left( -\frac{1}{2} (a + b\Phi)^2 \right)
\]

(54)

\[
\Psi(a + b\Phi) = \int_{-\infty}^{\Phi} \psi(t) dt = \frac{1}{2} \left( 1 + \text{erf} \left( \frac{\Phi}{\sqrt{2}} \right) \right)
\]

(55)

\[
\text{erf} (\Phi) = \frac{1}{\sqrt{\pi}} \int_{-\infty}^{\Phi} \exp(-t^2) dt
\]

(56)

\[
a = -\frac{\Phi_0}{\sigma}
\]

(57)

\[
b = \frac{1}{\sigma}
\]

(58)

We then obtained the four integral terms in the equation (51) as

\[
\int_{(2k-1)n}^{(2k+1)n} \exp \left( -\frac{(\Phi-\Phi_0)^2}{2\alpha^2} \right) d\Phi = -\sigma^2 \left[ \exp \left( -\frac{(2k+1)n-\Phi_0)^2}{2\alpha^2} \right) - \exp \left( -\frac{(2kn-\Phi_0)^2}{2\alpha^2} \right) \right]
\]

\[
+ \frac{\sigma \Phi_0 \pi}{\sqrt{2}} \left[ \text{erf} \left( \frac{(2k+1)n-\Phi_0}{\alpha \sqrt{2}} \right) - \text{erf} \left( \frac{2kn-\Phi_0}{\alpha \sqrt{2}} \right) \right]
\]

(60a)

\[
\int_{(2k-1)n}^{(2k+1)n} \Phi \exp \left( -\frac{(\Phi-\Phi_0)^2}{2\alpha^2} \right) d\Phi = -\sigma^2 \left[ \exp \left( -\frac{(2k+1)n-\Phi_0)^2}{2\alpha^2} \right) - \exp \left( -\frac{(2kn-\Phi_0)^2}{2\alpha^2} \right) \right]
\]

\[
+ \frac{\sigma \Phi_0 \pi}{\sqrt{2}} \left[ \text{erf} \left( \frac{(2k+1)n-\Phi_0}{\alpha \sqrt{2}} \right) - \text{erf} \left( \frac{2kn-\Phi_0}{\alpha \sqrt{2}} \right) \right]
\]

(60b)

\[
\int_{(2k-1)n}^{(2k+1)n} \exp \left( -\frac{(\Phi-\Phi_0)^2}{2\alpha^2} \right) d\Phi = k\alpha \sqrt{2\pi} \left[ \text{erf} \left( \frac{(2k+1)n-\Phi_0}{\alpha \sqrt{2}} \right) - \text{erf} \left( \frac{2kn-\Phi_0}{\alpha \sqrt{2}} \right) \right]
\]

(60c)

\[
\int_{(2k-1)n}^{(2k+1)n} \Phi \exp \left( -\frac{(\Phi-\Phi_0)^2}{2\alpha^2} \right) d\Phi = k\alpha \sqrt{2\pi} \left[ \text{erf} \left( \frac{(2k+1)n-\Phi_0}{\alpha \sqrt{2}} \right) - \text{erf} \left( \frac{2kn-\Phi_0}{\alpha \sqrt{2}} \right) \right]
\]

(60d)

Hence, by substituting the equation (60a-d) into the equation (51), the relation of trans ratio with twist angle is derived as

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\[ \text{TR}(\Phi_0) = \sum_k \left\{ -\frac{\sigma}{\sqrt{2\pi}} \left[ \exp \left( -\frac{\left(2k\pi+\pi-\Phi_0\right)^2}{2\sigma^2} \right) - 2\exp \left( -\frac{(2k\pi-\Phi_0)^2}{2\sigma^2} \right) + \exp \left( -\frac{(2k\pi-\pi-\Phi_0)^2}{2\sigma^2} \right) \right] \right\} \] (61)

Here, the arguments in the k-summation rapidly approach to zero when \( \Phi_0 \) is out of the range of \([2k-1)\pi, (2k+1)\pi]\). This relation is illustrated in the range of \(0 \leq \Phi \leq 4\pi\) as

Here, since multiple solutions of twist angle existed for a specific trans ratio, we obtained the approximate domain of twist angle from MD results of twisting blocks (336 ± 11° for 1-BP-insertion and 550 ± 12° for 2-BP-insertion in Supplementary Figure S9) and CanDo results of entire 6HB structures (206-225° for 1-BP-insertion and 598-644° for 2-BP-insertion in Supplementary Figure S10). This suggests the twist angle of structures would be in the range of 180-360° for 1-BP-insertion and 540-720° 2-BP-insertion, respectively, as expressed above. We then present the numerical solution of twist angle for a representative trans ratio using the equation (61) as below.

| Numerical solution | Trans ratio |
|--------------------|-------------|
|                    | Trans ratio |
|                    | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 |
| **Twist angle [°]**|     |     |     |     |     |     |     |
| 1-BP-insertion (180-360°) | 346.8 | 311.6 | 289.7 | 270.0 | 250.3 | 228.4 | 193.2 |
| 2-BP-insertion (540-720°) | 706.8 | 671.6 | 649.7 | 630.0 | 610.3 | 588.4 | 553.2 |
Supplementary Notes

Supplementary Note S1. The sensitivity of mechanical properties in simulating environments and neighboring sequence variation.

In this study, we investigated the mechanical properties of nicked BP steps, suggesting that BP steps can have distinct C/B ratio. The twist angle of DNA structures can be, therefore, finely tuned continuously by choosing the sequences of a target C/B ratio based on the spectrum of C/B ratios established here. We employed CHARMM36 force field for MD simulation since it showed a good agreement with experiments in previous MD studies for DNA nanostructures. It remains open, however, whether our results would stay if we use other force fields, ionic conditions, and neighboring sequences.

To verify, we simulated all the BP steps for 100 ns employing the parmbsc1 force field (12), another widely used force field in DNA oligomer simulations, to obtain the mechanical properties and the C/B ratios. As in the previous results obtained using the CHARMM36 force field, the parmbsc1 force field provided the sequence-dependent properties for regular and nicked BP steps (Supplementary Figure S8 and Supplementary Tables S8-S13). It was also confirmed that nicks led to a significant reduction in torsional rigidity regardless of the force field used. On the whole, the geometry, mechanical rigidities, and coupling coefficients predicted by two force fields were comparable for both regular and nicked BP steps. While the parmbsc1 force field predicted relatively smaller torsional and shearing rigidities than the CHARMM36 as found in previous studies (12,13), the C/B ratios predicted by the parmbsc1 were not significantly deviated from those obtained using the CHARMM36 because the torsional rigidity decreases for both regular and nicked BP steps (C/B ratio = \( \frac{(C/B)_{\text{nicked}}}{(C/B)_{\text{regular}}} \)) (Supplementary Figure S8). Therefore, we could confirm that the trend of C/B ratio (and hence our design approach) is not sensitive to the choice of force field.

While limited, we increased the time duration from 100 to 300 ns for the GC/GnC step as a selected subset. We also performed additional simulations of 300 ns using parmbsc1 force field with 100 mM of NaCl (Supplementary Figure S5). It was apparent that RMSD values were maintained within 2-8 Å, suggesting that oligomers stayed near an equilibrium state. Results indicated that the mechanical properties of BP steps did not considerably alter compared to original simulation either the simulation time was extended or salt concentrations was changed from MgCl\(_2\) to NaCl concentration.

Furthermore, to investigate the effect of the next-nearest-neighbor sequence variation, another additional MD simulations performed for nicked GG/CnC and AA/TnT steps as a limited subgroup in
order to evaluate the effect of adjacent sequence variation of a BP step (Supplementary Figures S6 and S7). The dinucleotide GG/CnC step analyzed in this study is the same with the tetranucleotide AGGA/TCnCT step when considering neighboring sequences for original DNA oligomer (Supplementary Table S1). Likewise, the AA/TnT step was identical to with the hexanucleotide GAAAAAG/CTTnTTC step. We modified the sequence of the original oligomer for GG/CnC step from GACT₄-GAC-TcnCT-CAG-TCAG₄ to GACT₄-GAC-ACnCA-CAG-TCAG₄ to have the same dinucleotide GG/CnC step in the center but different tetranucleotide TGGT/ACnCA step. Likewise, the oligomer with AA/TnT step was also modified from GACT₄-GA-CTTnTTC-AG-TCAG₄ to GACT₄-GA-GTTnTTG-AG-TCAG₄ with different hexanucleotide GTAATG/CATTAC step. We performed 100-ns-long MD simulations with same protocol with the CHARMM36 force field and MgCl₂ condition. In both steps, RMSD values were maintained within 2-10 Å. The results indicate that the overall trend of the mechanical properties in sequence variation may be consistent with that of original steps, observing the less influence of sequence change as the farther from the central BP step. Nevertheless, since our MD simulation was limited in the nanosecond (ns) time-scale, it is promising that further investigations on mechanical properties of nicked BP steps remain under microsecond (µs) simulation and another testing environment.
Supplementary Note S2. Mechanical analysis of DNA bundle structures with BP-insertion.

We theoretically investigated mechanical parameters related to the twist angle of a DNA bundle structure with BP-insertion. A two-helix-bundles (2HB) structure was introduced as the smallest twisting structure by BP-insertion (A), where terminal ends were constraint by Holliday-junctions. It was assumed that two identical and straight helices (H1 and H2) have the radius of $r$, bending rigidity of $B$, and torsional rigidity of $C$. Also, there was nothing to induce strain energy since the end of two helices cross exactly at both ends.

When BPs are inserted but one of the Holliday-junctions is not constrained (B), the initial twist angle due to the helicity of DNA can be regarded as

$$\Phi_0 = \langle \omega \rangle N_{BP}$$

(62)

where $\langle \omega \rangle$ is the mean twist angle of each helix and the number of inserted BPs was denoted as $N_{BP}$. Also, each helix is elongated to a longitudinal length of $L$. 

![Diagram](image-url)
In equilibrium with the constraints at both ends of the helices (C), bending and torsional strain energy is induced at each helix to align the mismatch of connectivity and the center of twist. In addition, bending and torsional angle should satisfy a geometric constraint equation (g) as
\[ g = L\theta - r\Phi = 0 \] (63)
where θ and Φ represent the equilibrium bending and twist angle of a helix. Then, the induced strain energy in the helix can be described using the method of Lagrange multipliers as
\[ \pi = \int_0^L \frac{1}{2} M_b^2 \, dx + \int_0^L \frac{1}{2} M_z^2 \, dx + \lambda g \] (64)
where the bending (Mb) and torsional moment (Mz) were derived in Supplementary Method S2 as
\[ M_b = B \frac{\Delta \theta}{L} \] (65)
\[ M_z = C \frac{\Delta \Phi}{L} \] (66)
where the zero initial bending and initial twist angle by BP-insertion yield the angle deformation as
\[ \Delta \theta = \theta \] (67)
\[ \Delta \Phi = \Phi - \Phi_0 = \Phi - \langle \omega \rangle_{BP} \] (68)
Then, the strain energy is derived using the above equations as
\[ \pi(\theta, \Phi, \lambda) = \frac{1}{2L} B\theta^2 + \frac{1}{2L} C(\Phi - \langle \omega \rangle_{BP})^2 + \lambda(L\theta - r\Phi) \] (69)
The equilibrium configuration minimizes this strain energy, suggesting that the gradient of the strain energy is zero vector. We, therefore, obtain the system of equations as
\[ \frac{\partial \pi}{\partial \theta} = \frac{1}{L} B\theta + \lambda L = 0 \] (70)
\[ \frac{\partial \pi}{\partial \Phi} = \frac{1}{L} C(\Phi - \langle \omega \rangle_{BP}) - \lambda r = 0 \] (71)
\[ \frac{\partial \pi}{\partial \lambda} = L\theta - r\Phi = 0 \] (72)
From equations (70) and (72), the Lagrange multiplier, indicating the constraint force, was derived as
\[ \lambda = -\frac{1}{L^2} B\theta = \frac{1}{rL} C(\Phi - \langle \omega \rangle_{BP}) \] (73)
Here, the bending angle (θ) can be substituted to twist angle (Φ) with the constraint equation (72), providing a relation of twist angle with rigidities of each helix and the number of inserted BPs as
\[ \Phi = \langle \omega \rangle_{BP} \left(1 + \frac{\sigma^2/\mu^2}{C/B}\right)^{-1} \] (74)
This relation suggests that DNA bundle structures could be more twisted when BPs are more inserted or C/B is increased in helices. We found that nicks have significant effects on the reduction of torsional rigidity (C) rather than the reduction of bending rigidity (B). It is, therefore, speculated that the
twist angle of bundle structures could be controlled by adjusting the location of nicks to control C/B value. Here, we defined that C/B ratio of nicked BP steps normalized by corresponding regular BP steps as \( \frac{(C/B)_{\text{nicked}}}{(C/B)_{\text{regular}}} \) to quantify the effects of nicks from an ideal structure consisting of all regular BP steps. Utilizing the C/B ratio data from MD simulation, the twist angle of the structures with high C/B ratio could be deliberately designed greater than that with low C/B ratio, suggesting that the torsional energy stored by BP-insertion could be controlled by adjusting nicked BP steps. Future studies based on nonlinear analysis is promising to produce a more realistic theoretical prediction on the DNA structures since our approach is limited to only provide the insight of finding parameters to affect bundle structure due to the simple and linear assumption.
Supplementary Note S3. Comparison of the results of the reverse-phase cartridge (Bio-RP) and the polyacrylamide gel electrophoresis (PAGE) methods in the purification of staples.

We investigated whether the purification method used in the synthesis of oligonucleotides affects the trans ratio of assembled DNA origami structures. Here, 60 staples comprising ten twisting blocks of the flexible 1 design with 2-BP-insertion were purified using the polyacrylamide gel electrophoresis (PAGE) method, in which the manufacturer guarantees a higher purity of more than 95%. We obtained the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) data for representative ten staples (one staple per each twisting block) purified by both methods (Supplementary Table S19). According to the result of MALDI-TOF, purified staples using Bio-RP and PAGE showed almost identical mass spectrum with a single and clear peak, indicating only oligonucleotides with the desired length existed in the stock solution (Supplementary Figure S11). Noting that the maximum error of the MALDI-TOF spectrum is typically around 0.24%, we could confirm that both Bio-RP and PAGE methods were reliable to obtain the full-length and same sequence of staples. In addition, we constructed two versions of the flexible 1 design using the staples purified with Bio-RP and PAGE methods (Supplementary Figure S12). No distinguishable difference in the trans ratio was observed between these two versions of structures (Supplementary Table S16). The mean and standard deviation of the trans ratio by two methods were 0.7245 and 0.0609 for Bio-RP, and 0.7204 and 0.0272 for PAGE, respectively. Accordingly, we concluded that the trans ratio was hardly affected by the purification method for staples because both methods yield sufficiently high purity.
Supplementary Figures

Supplementary Figure S1. Root-mean-square deviation (RMSD) of DNA oligomers. Each RMSD value was calculated from the minimized structure omitting the four terminal BPs on each end.
Supplementary Figure S2. BP step parameters of the regular and nicked BP steps. Six figures represent overall BP step parameters: (A) tilt, (B) roll, (C) twist, (D) shift, (E) slide, and (F) rise. Blue represents the parameters of regular BP steps while red and green indicate the parameters of nicked BP steps.
Supplementary Figure S3. Comparison of the BP step parameters in stiffened BP steps by a nick.

GA/TnC, GC/GnC, and CG/CnG steps were stiffened by a nick for the stretching and bending rigidities with the decrease of roll ($\rho$) and shift ($Dx$).
Supplementary Figure S4. Mechanical rigidity distributions in DNA oligomers. Mechanical rigidities were calculated for total 33 BP steps except for the four terminal BPs on end in each oligomer: (A) tilt-bending, (B) roll-bending, (C) torsional, (D) shift-shearing, (E) slide-shearing, and (F) stretching rigidities. Noting that each oligomer has different sequences at the center at 16 to 18th BP steps, corresponding rigidities deviated, whereas a repeating tendency of rigidities occurs at the other BP steps due to the repetition of the sequence of AGTC from the center to terminal end. Black lines represent the mean and standard deviation of rigidity values of regular BP steps. Rigidities of 16-18th BP steps are illustrated as colored lines and marked with triangles for central nicked BP steps.
Supplementary Figure S4. (Continued)
Supplementary Figure S5. Effects of force field, simulation time, and salt condition on mechanical properties of nicked GC/GnC step. C36 and BSC1 represent the CHARMM36 and parmbsc1 force field, respectively. (A) RMSD trajectories. Each RMSD value was calculated from the minimized structure omitting the four terminal BPs on each end. (B, C) Mechanical rigidities and coupling coefficients. Each bar represents the overall value of the mechanical properties.
Supplementary Figure S6. Effects of the next-to-nearest-neighbor sequence on mechanical properties of nicked GG/CnC step. (A) RMSD trajectories. Each RMSD value was calculated from the minimized structure omitting the four terminal BPs on each end. (B, C) Mechanical rigidities coupling coefficients. Each bar represents the overall value of the mechanical properties.
Supplementary Figure S7. Effects of the second-adjacent sequence on the mechanical properties of the nicked AA/TnT step. (A) RMSD trajectories. Each RMSD value was calculated from the minimized structure omitting the four terminal BPs on each end. (B, C) Mechanical rigidities and coupling coefficients. Each bar represents the overall value of the mechanical properties.
**Supplementary Figure S8. Comparison of mechanical rigidities and C/B ratio in CHARMM36 and parmbsc1 force fields.** Equivalent bending and shearing rigidities were described (Supplementary Method S3). Sequence-dependent mechanical properties were listed in Supplementary Table S8-S13. The C/B ratios of CHARMM36 were sorted in ascending order and the corresponding values of parmbsc1 were indicated (Supplementary Table S14).
**Supplementary Figure S9. Twisting block design and MD results.** (A) caDNAno designs of twisting blocks with one or two BP-insertion. (B) Final configurations of twisting blocks in equilibrium by MD simulation. (C) RMSD and twist angle trajectories of twisting blocks. RMSD trajectory was calculated with respect to the minimized structure. Gaussian distribution of twist angles was obtained using the final 20-ns-long trajectory with mean and standard deviation as 33.6 ± 3.4° and 55.0 ± 3.7° for one and two BPs inserted blocks, respectively. The twist angles of 6HB structures with ten twisting blocks were then estimated as 336 ± 11° and 550 ± 12° for 1- and 2-BP-insertion (Supplementary Method S5).
Supplementary Figure S10. CanDo results of 6HB DNA origami structures with different helicities and nick rigidities. The details of CanDo simulations were explained in Supplementary Method S6.
Supplementary Figure S11. The results of MALDI-TOF using Bio-RP and PAGE methods. We obtained the MALDI-TOF results for representative ten staples (one staple per each twisting block). Red numbers in brackets indicate the ideal molecular weight of staple sequence (Supplementary Table S19).
Supplementary Figure S12. Atomic force microscope (AFM) images of twisted 6HB DNA origami structures. All scale bars represent 1 μm.
Supplementary Figure S12 (Continued).
Supplementary Figure S12 (Continued).
Supplementary Figure S13. Agarose gel electrophoresis result of twisted 6HB DNA origami structures. Ladder: 1kb DNA ladder (New England Biolabs N3232S). Scaffold: M13mp18 single-stranded scaffold DNA.
Supplementary Tables

**Supplementary Table S1. List of MD-simulated DNA oligomers.** Each oligomer is 42-BP-long DNA double helix and has the same sequence pattern, 5'-CTGA₅-MN-AGTC₅-3' / 5'-GACT₅-PQ-TCAG₅-3'. MN/PQ represents one of ten regular BP steps. Since MN/PQ or PQ/MN indicate identical one of ten BP steps, we selected notations by comparing M and P in order of A > G > T > C. For example, we chose AG/CT notation in AG/CT or CT/AG case. MnN/PQ or MN/PnQ represent one of sixteen nicked BP steps.

| Type | Base-pair (BP) step | Oligomer sequence (5’→3’) |
|------|---------------------|---------------------------|
| Regular | RR/YY | AA/TT | CTG₅₋TT-AGTC₅<br>GACT₅₋AA-TCAG₅ |
|       |       | AG/CT | CTG₅₋AG-AGTC₅<br>GACT₅₋CT-TCAG₅ |
|       |       | GA/TC | CTG₅₋TC-AGTC₅<br>GACT₅₋GA-TCAG₅ |
|       |       | GG/CC | CTG₅₋GG-AGTC₅<br>GACT₅₋CC-TCAG₅ |
|       | RY/RY | AC/CT | CTG₅₋AC-AGTC₅<br>GACT₅₋GT-TCAG₅ |
|       |       | AT/AT | CTG₅₋AT-AGTC₅<br>GACT₅₋AT-TCAG₅ |
|       |       | GC/GC | CTG₅₋GC-AGTC₅<br>GACT₅₋GC-TCAG₅ |
|       | YR/YR | TG/CA | CTG₅₋TG-AGTC₅<br>GACT₅₋CA-TCAG₅ |
|       |       | TA/TA | CTG₅₋TA-AGTC₅<br>GACT₅₋TA-TCAG₅ |
|       |       | CG/CG | CTG₅₋CG-AGTC₅<br>GACT₅₋CG-TCAG₅ |
| Nicked | RR/YnY | AA/TnT | CTG₅₋AA-AGTC₅<br>GACT₅₋TnT-TCAG₅ |
|       |       | AG/CnT | CTG₅₋AG-AGTC₅<br>GACT₅₋CnT-TCA₅ |
|       |       | GA/TnC | CTG₅₋GA-AGTC₅<br>GACT₅₋TnC-TCAG₅ |
|       |       | GG/CnC | CTG₅₋GG-AGTC₅<br>GACT₅₋CnC-TCAG₅ |
|       | RnR/YY | An/TT | CTG₅₋TT-AGTC₅<br>GACT₅₋AnA-TCAG₅ |
|       |       | AnG/CT | CTG₅₋CT-AGTC₅<br>GACT₅₋AnG-TCA₅ |
|       |       | GnA/TC | CTG₅₋TC-AGTC₅<br>GACT₅₋GnA-TCAG₅ |
|       |       | GnG/CC | CTG₅₋CC-AGTC₅<br>GACT₅₋GnG-TCAG₅ |
|       | RY/RnY | AC/GnT | CTG₅₋AC-AGTC₅<br>GACT₅₋GnT-TCAG₅ |
|       |       | AnC/GT | CTG₅₋GT-AGTC₅<br>GACT₅₋AnC-TCA₅ |
|       |       | AT/AnT | CTG₅₋AT-AGTC₅<br>GACT₅₋AnT-TCAG₅ |
|       |       | GC/GnC | CTG₅₋GC-AGTC₅<br>GACT₅₋GnC-TCAG₅ |
|       | YR/YnR | TG/CnA | CTG₅₋TG-AGTC₅<br>GACT₅₋CnA-TCAG₅ |
|       |       | TrnG/CA | CTG₅₋CA-AGTC₅<br>GACT₅₋TrnG-TCA₅ |
|       |       | TA/TrnA | CTG₅₋TA-AGTC₅<br>GACT₅₋TrnA-TCAG₅ |
|       |       | CG/CnG | CTG₅₋CG-AGTC₅<br>GACT₅₋CnG-TCAG₅ |
Supplementary Table S2. Sequence-dependent BP step parameters of regular BP steps. The mean and standard deviation are represented in the left and right column for each BP step parameter.

| Regular | τ [°] | ρ [°] | ω [°] | Dx [nm] | Dy [nm] | Dz [nm] |
|---------|-------|-------|-------|---------|---------|---------|
| AA/TT   | -3.62 | 4.34  | 6.17  | 5.52    | 36.40   | 3.99    | -0.03   | 0.05    | -0.01   | 0.04    | 0.34    | 0.03    |
| AG/CT   | -2.99 | 4.32  | 10.18 | 5.42    | 32.27   | 4.28    | -0.03   | 0.06    | -0.03   | 0.04    | 0.34    | 0.03    |
| GA/TC   | -1.11 | 5.16  | 3.70  | 5.64    | 39.02   | 4.25    | -0.01   | 0.05    | 0.01    | 0.05    | 0.34    | 0.03    |
| GG/CC   | 0.77  | 5.01  | 6.85  | 5.66    | 34.26   | 5.51    | -0.01   | 0.07    | -0.05   | 0.07    | 0.36    | 0.03    |
| AC/CT   | -0.50 | 4.78  | 5.03  | 5.86    | 32.10   | 5.09    | 0.04    | 0.06    | -0.04   | 0.05    | 0.32    | 0.04    |
| AT/AT   | -0.13 | 4.13  | 3.33  | 5.79    | 31.97   | 4.14    | 0.02    | 0.07    | -0.05   | 0.04    | 0.33    | 0.03    |
| GC/GC   | 0.01  | 4.86  | 2.09  | 5.55    | 38.32   | 4.86    | 0.01    | 0.06    | -0.04   | 0.04    | 0.34    | 0.03    |
| TG/CA   | 1.04  | 5.22  | 13.57 | 7.00    | 32.27   | 5.11    | 0.00    | 0.07    | -0.02   | 0.06    | 0.35    | 0.04    |
| TA/TA   | 1.92  | 5.24  | 8.93  | 8.11    | 35.71   | 5.31    | -0.02   | 0.07    | 0.01    | 0.07    | 0.35    | 0.04    |
| CG/CG   | -0.48 | 5.95  | 14.47 | 7.42    | 32.78   | 5.19    | -0.03   | 0.06    | 0.02    | 0.05    | 0.35    | 0.04    |
| Overall | -0.51 | 1.72  | 7.43  | 4.27    | 34.51   | 2.69    | -0.01   | 0.02    | -0.02   | 0.03    | 0.34    | 0.01    |
Supplementary Table S3. Sequence-dependent BP step parameters of nicked BP steps. The mean and standard deviation are represented in the left and right column for each BP step parameter.

| Nicked   | $\tau$ [°] | $\rho$ [°] | $\omega$ [°] | $D_x$ [nm] | $D_y$ [nm] | $D_z$ [nm] |
|----------|------------|------------|--------------|------------|------------|------------|
| AA/TnT   | -3.01      | 4.37       | 5.32         | 5.73       | 35.50      | 4.56       | -0.03 | 0.06 | -0.01 | 0.05 | 0.34 | 0.03 |
| AnA/TT   | -3.62      | 4.72       | 5.08         | 6.38       | 33.95      | 12.17      | -0.01 | 0.08 | -0.04 | 0.08 | 0.35 | 0.03 |
| AG/CnT   | -1.57      | 5.07       | 6.05         | 5.75       | 36.55      | 5.30       | -0.02 | 0.06 | 0.00  | 0.05 | 0.33 | 0.03 |
| AnG/CT   | -2.87      | 4.52       | 6.70         | 5.92       | 31.29      | 7.83       | 0.02  | 0.09 | -0.06 | 0.06 | 0.35 | 0.03 |
| GA/TnC   | -0.11      | 4.64       | 1.23         | 5.40       | 37.73      | 6.20       | -0.06 | 0.06 | 0.03  | 0.05 | 0.34 | 0.03 |
| GnA/TC   | -1.01      | 5.61       | 2.18         | 6.17       | 42.05      | 8.08       | -0.02 | 0.08 | 0.01  | 0.07 | 0.35 | 0.03 |
| GG/CnC   | -0.66      | 5.14       | 5.62         | 5.57       | 30.69      | 9.83       | -0.07 | 0.09 | -0.07 | 0.09 | 0.35 | 0.03 |
| GnG/CC   | 1.28       | 5.44       | 7.64         | 6.29       | 31.19      | 9.41       | 0.05  | 0.09 | -0.12 | 0.07 | 0.36 | 0.04 |
| AC/GnT   | -0.52      | 5.12       | 2.21         | 6.15       | 36.17      | 7.91       | 0.02  | 0.08 | -0.04 | 0.08 | 0.34 | 0.04 |
| AnC/GT   | -1.05      | 4.38       | 4.02         | 6.30       | 26.22      | 9.47       | 0.09  | 0.09 | -0.06 | 0.06 | 0.33 | 0.03 |
| AT/AnT   | 0.24       | 4.41       | 3.90         | 6.61       | 29.72      | 8.05       | -0.03 | 0.09 | -0.05 | 0.07 | 0.33 | 0.03 |
| GC/GnC   | -0.23      | 5.17       | 0.19         | 5.08       | 39.29      | 6.64       | -0.01 | 0.08 | -0.03 | 0.04 | 0.35 | 0.03 |
| TG/CnA   | 2.68       | 5.59       | 10.90        | 7.00       | 35.70      | 7.49       | 0.00  | 0.09 | -0.01 | 0.07 | 0.36 | 0.04 |
| TnG/CA   | -1.53      | 5.54       | 11.65        | 7.26       | 32.83      | 7.42       | 0.07  | 0.07 | -0.03 | 0.07 | 0.36 | 0.04 |
| TA/TnA   | 0.32       | 6.00       | 7.96         | 7.72       | 31.80      | 10.24      | -0.03 | 0.10 | -0.03 | 0.09 | 0.34 | 0.04 |
| CG/CnG   | -0.45      | 6.32       | 12.71        | 7.36       | 33.61      | 6.79       | -0.06 | 0.08 | 0.00  | 0.06 | 0.37 | 0.04 |
| Overall  | -0.76      | 1.59       | 5.83         | 3.68       | 34.02      | 3.95       | 0.00  | 0.05 | -0.03 | 0.04 | 0.35 | 0.01 |
Supplementary Table S4. Sequence-dependent mechanical rigidities of regular BP steps. The mean and standard deviation are represented in the left and right column for each rigidity. The standard deviation is calculated by multiplying the off-diagonal value of the stiffness matrix by the standard deviation of the rise.

| Regular | $B_x$ [pN nm$^2$] | $B_y$ [pN nm$^2$] | $C$ [pN nm$^2$] | $Y_{dx}$ [pN] | $Y_{dy}$ [pN] | $S$ [pN] |
|---------|------------------|------------------|----------------|-------------|-------------|---------|
| AA/TT   | 301.53           | 28.76            | 172.67         | 16.47       | 400.39      | 38.18   | 560.96 | 53.50 | 872.97 | 83.25 | 1920.88 | 183.18 |
| AG/CT   | 317.62           | 29.97            | 171.36         | 16.17       | 347.47      | 32.78   | 452.93 | 42.73 | 789.85 | 74.52 | 2087.34 | 196.93 |
| GA/TC   | 277.69           | 26.64            | 170.73         | 16.38       | 344.19      | 33.02   | 557.64 | 53.50 | 835.18 | 80.12 | 2361.15 | 226.52 |
| GG/CC   | 309.91           | 29.72            | 164.48         | 15.77       | 288.76      | 27.69   | 391.30 | 37.52 | 560.77 | 53.77 | 2145.27 | 206.70 |
| AC/GT   | 218.91           | 23.85            | 157.40         | 17.15       | 255.39      | 27.82   | 341.17 | 37.17 | 753.74 | 82.12 | 1711.07 | 186.42 |
| AT/AT   | 263.28           | 26.20            | 161.00         | 16.02       | 332.80      | 33.12   | 266.11 | 26.48 | 773.42 | 76.97 | 1475.58 | 146.84 |
| GC/GC   | 230.96           | 22.86            | 200.35         | 19.83       | 301.64      | 29.86   | 417.69 | 41.35 | 1340.78 | 132.73 | 2270.19 | 224.74 |
| TG/CA   | 189.65           | 20.22            | 132.03         | 14.08       | 281.44      | 30.01   | 353.84 | 37.73 | 478.38 | 51.01 | 1396.03 | 148.85 |
| TA/TA   | 174.92           | 17.75            | 123.80         | 12.56       | 309.72      | 31.42   | 333.37 | 33.82 | 380.62 | 38.62 | 1483.42 | 150.50 |
| CG/CG   | 173.43           | 19.80            | 129.45         | 14.78       | 276.46      | 31.57   | 502.78 | 57.41 | 595.34 | 67.98 | 1401.16 | 160.00 |
| **Overall** | **245.79**      | **55.83**        | **158.33**     | **23.71**   | **313.83**  | **42.90** | **417.78** | **99.62** | **738.11** | **267.10** | **1825.21** | **377.37** |
**Supplementary Table S5. Sequence-dependent mechanical rigidities of nicked BP steps.** The mean and standard deviation are represented in the left and right column for each rigidity. The standard deviation is calculated by multiplying the off-diagonal value of the stiffness matrix by the standard deviation of the rise.

| Nicked   | $B_\text{s}$ [pN nm$^2$] | $B_\text{p}$ [pN nm$^2$] | $C$ [pN nm$^2$] | $Y_{\text{dx}}$ [pN] | $Y_{\text{dy}}$ [pN] | $S$ [pN] |
|----------|------------------------|------------------------|-----------------|-------------------|-------------------|---------|
| AA/TnT   | 290.14                 | 26.04                  | 151.36          | 13.59             | 286.61            | 25.72   |
| AnA/TT   | 266.41                 | 26.66                  | 125.85          | 12.59             | 70.31             | 7.04    |
| AG/CnT   | 201.45                 | 18.40                  | 171.05          | 15.63             | 233.34            | 21.32   |
| AnG/CT   | 298.20                 | 29.83                  | 151.99          | 15.21             | 181.74            | 18.18   |
| GA/TnC   | 325.46                 | 28.28                  | 179.17          | 15.57             | 181.00            | 15.73   |
| GnA/TC   | 264.98                 | 26.14                  | 152.94          | 15.09             | 155.48            | 15.34   |
| GG/CnC   | 305.26                 | 29.09                  | 177.56          | 16.62             | 93.54             | 8.91    |
| GnG/CC   | 274.88                 | 30.13                  | 152.00          | 16.66             | 159.88            | 17.52   |
| AC/GnT   | 218.39                 | 23.12                  | 155.95          | 16.51             | 109.01            | 11.54   |
| AnG/CT   | 244.64                 | 24.90                  | 152.75          | 15.55             | 107.29            | 10.92   |
| AT/AnT   | 238.99                 | 25.07                  | 133.80          | 14.04             | 113.60            | 11.92   |
| GC/GnC   | 236.59                 | 20.83                  | 225.72          | 19.87             | 181.73            | 16.00   |
| TG/CnA   | 200.61                 | 20.22                  | 131.42          | 13.25             | 154.61            | 15.59   |
| TnG/CA   | 195.50                 | 20.62                  | 124.14          | 13.10             | 155.75            | 16.43   |
| TA/TnA   | 143.69                 | 17.80                  | 95.40           | 11.82             | 78.61             | 9.74    |
| CG/CnG   | 203.66                 | 21.56                  | 132.67          | 14.04             | 178.67            | 18.91   |
| **Overall** | 244.30                 | 48.87                  | 150.86          | 29.41             | 152.57            | 57.12   |
|           |                       |                       |                 |                   |                   |         |
|           | 350.04                 | 86.31                  | 526.44          | 222.42            | 1777.69          | 421.11  |


**Supplementary Table S6. Sequence-dependent mechanical coupling coefficients of regular BP steps.** The mean and standard deviation are represented in the left and right column for each coefficient. The standard deviation is calculated by multiplying the off-diagonal value of the stiffness matrix by the standard deviation of the rise.

| Regular | $g_{xy}$ [pN nm] | $g_{yx}$ [pN nm] | $g_{xz}$ [pN nm] | $g_{zx}$ [pN nm] | $g_{dy}$ [pN nm] | $g_{yd}$ [pN nm] | $g_{dz}$ [pN nm] | $g_{zd}$ [pN nm] |
|----------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| AA/TT    | -30.91           | -2.95            | -43.76           | -4.17            | -319.84          | -30.50           | -30.33           | -2.89            | -70.05           | -6.68            | -90.13           | -8.59            |
| AG/CT    | -83.18           | -7.85            | -77.92           | -7.35            | -344.10          | -32.46           | 15.82            | 1.49             | 9.08             | 0.86             | -102.98          | -9.72            |
| GA/TC    | -84.87           | -8.14            | -37.97           | -3.64            | -401.74          | -38.54           | -9.69            | -0.93            | -45.22           | -4.34            | -61.43           | -5.89            |
| GG/CC    | -135.35          | -12.98           | -54.14           | -5.19            | -392.32          | -37.62           | 26.71            | 2.56             | 14.63            | 1.40             | -10.54           | -1.01            |
| AC/GT    | -34.43           | -3.75            | -89.54           | -9.76            | -40.85           | 4.45             | -1.46            | -0.16            | 36.30            | 3.95             | 73.03            | 7.96             |
| AT/AT    | -10.37           | -1.03            | 3.11             | 0.31             | 4.51             | 0.45             | 11.12            | 1.11             | 26.75            | 2.66             | 83.95            | 8.35             |
| GC/GC    | -123.64          | -12.24           | -16.63           | -1.65            | -13.07           | -1.29            | 1.39             | 0.14             | 91.82            | 9.09             | 211.84           | 20.97            |
| TG/CA    | -41.36           | -4.41            | -20.37           | -2.17            | -55.92           | -5.96            | -18.19           | -1.94            | 23.68            | 2.52             | -141.52          | -15.09           |
| TA/TA    | -21.11           | -2.14            | 7.57             | 0.77             | -15.79           | -1.60            | 16.48            | 1.67             | 13.86            | 1.41             | -128.83          | -13.07           |
| CG/CG    | -138.13          | -15.77           | 24.41            | 2.79             | -5.57            | -0.06            | 11.90            | 1.36             | 71.97            | 8.22             | -180.78          | -20.64           |
| **Overall** | **-70.33**       | **-49.15**       | **-22.93**       | **-42.78**       | **-149.80**      | **187.63**       | **2.37**         | **17.62**        | **17.28**        | **47.91**        | **-34.74**       | **123.43**       |

| Regular | $g_{xby}$ [pN nm] | $g_{ybx}$ [pN nm] | $g_{xdz}$ [pN nm] | $g_{ydz}$ [pN nm] | $g_{dyx}$ [pN nm] | $g_{ydx}$ [pN nm] |
|----------|------------------|------------------|------------------|------------------|------------------|------------------|
| AA/TT    | 37.99            | 3.82             | -206.97          | -19.74           | -300.81          | -28.69           |
| AG/CT    | 108.65           | 10.25            | -114.53          | -10.81           | -310.71          | -29.31           |
| GA/TC    | 13.20            | 1.27             | -167.93          | -16.11           | -211.56          | -20.30           |
| GG/CC    | 78.30            | 7.51             | -227.37          | -21.80           | -310.65          | -29.79           |
| AC/GT    | 26.98            | 2.94             | -127.09          | -13.85           | -266.32          | -29.02           |
| AT/AT    | -5.57            | -0.65            | -118.97          | -11.84           | -164.19          | -16.34           |
| GC/GC    | 8.64             | 0.86             | -236.09          | -23.37           | -363.29          | -35.96           |
| TG/CA    | 36.09            | 3.85             | -90.49           | -9.65            | -274.23          | -29.24           |
| TA/TA    | 8.62             | 0.87             | -124.01          | -12.58           | -237.07          | -24.05           |
| CG/CG    | 25.41            | 2.90             | -73.78           | -8.43            | -335.03          | -38.26           |
| **Overall** | **33.73**       | **35.03**       | **-148.72**      | **57.47**        | **-277.39**      | **59.97**        |
Supplementary Table S7. Sequence-dependent mechanical coupling coefficients of nicked BP steps. The mean and standard deviation are represented in the left and right column for each coefficient.

The mean and standard deviation were calculated by multiplying the off-diagonal value of the stiffness matrix by the mean and standard deviation of the rise.

| Nicked    | $g_{12}$ [pN nm] | $g_{21}$ [pN nm] | $g_{13}$ [pN nm] | $g_{31}$ [pN nm] | $g_{11}$ [pN] | $g_{22}$ [pN] | $g_{33}$ [pN] |
|-----------|------------------|------------------|------------------|------------------|--------------|--------------|--------------|
| AA/TnT    | -27.83           | -2.50            | -66.30           | -5.95            | -310.03      | -27.83       | -32.85       | -2.95         | -7.03         | -0.63         | -59.67        | -5.36         |
| AnA/TT    | -27.26           | -2.73            | 38.30            | 3.83             | -373.65      | -23.78       | -33.31       | -3.33         | -0.75         | -0.08         | -43.74        | -4.38         |
| AG/CnT    | -71.08           | -6.49            | 23.52            | 2.15             | -447.10      | -13.44       | -21.85       | -2.00         | -4.34         | -0.40         | -107.53       | -9.82         |
| AnG/CT    | -59.02           | -5.91            | 12.91            | 1.29             | -286.55      | -28.67       | 1.33         | 0.13          | -5.52         | -0.55         | -125.54       | -12.56        |
| GA/TnC    | -120.75          | -10.49           | 12.57            | 1.09             | -452.28      | -39.30       | -7.43        | -0.65         | 28.29         | 2.46          | -5.81         | -0.50         |
| Gn/TCA    | -89.55           | -8.83            | 79.29            | 7.82             | -328.75      | -32.43       | 10.57        | 1.04          | 39.89         | 3.93          | -3.31         | -0.33         |
| GC/GnC    | -122.11          | -11.64           | 97.20            | 9.26             | -353.33      | -33.67       | 25.32        | 2.41          | 39.97         | 3.81          | -59.60        | -5.68         |
| Gn/CG     | -124.25          | -16.14           | 93.38            | 10.23            | -339.50      | -37.21       | -17.22       | -1.89         | 54.21         | 5.94          | -41.63        | -4.56         |
| Ac/GnT    | -60.26           | -6.38            | -73.25           | -7.75            | 48.44        | 5.13         | -15.20       | -1.61         | 82.57         | 8.74          | 78.52         | 8.31          |
| AnC/CT    | -31.40           | -3.20            | -6.71            | -0.68            | 5.82         | 0.59         | -14.71       | -1.50         | 28.14         | 2.86          | 0.11          | 0.01          |
| AT/AnT    | -18.47           | -1.94            | 14.36            | 1.51             | 70.88        | 7.44         | 20.77        | 2.18          | 8.39          | 0.88          | -26.42        | -2.77         |
| GC/GnC    | -138.14          | -12.16           | -19.00           | -1.67            | 46.14        | 3.67         | -11.14       | -0.98         | 168.19        | 14.81         | 214.44        | 18.88         |
| Tg/CnA    | -68.88           | -6.94            | -51.49           | -5.19            | -11.78       | -1.19        | -27.78       | -2.80         | 51.82         | 5.22          | -157.29       | -15.86        |
| Tn/GCA    | -98.71           | -10.41           | -4.98            | -0.52            | -105.70      | -11.15       | -16.73       | -1.76         | 36.08         | 3.81          | -135.67       | -14.31        |
| TA/TnA    | -16.64           | -2.06            | -48.42           | -6.00            | -78.89       | -9.77        | 4.29         | 0.53          | 22.01         | 2.73          | -78.58        | -9.73         |
| Cg/CnG    | -175.43          | -18.57           | 9.41             | 1.00             | 93.84        | 9.93         | -18.38       | -1.95         | 66.90         | 7.08          | -200.43       | -21.22        |

| Overall   | -79.55           | 50.05            | -5.23            | 52.82            | -149.43      | 180.24       | -9.64        | 17.69         | 38.05         | 43.80         | -47.01        | 98.36         |
## Supplementary Table S7 (Continued).

| Nicked | $g_{\text{u0z}_x}$ [pN nm] | $g_{\text{u0y}_x}$ [pN nm] | $g_{\text{u0z}_y}$ [pN nm] |
|--------|-----------------|-----------------|-----------------|
| AA/TnT | -3.00           | -0.27           | -143.15         |
| AnA/TT | 80.93           | 8.10            | -12.85          |
| AG/CnT | 3.54            | 0.32            | -147.34         |
| AnG/CT | 119.35          | 11.94           | -126.19         |
| GA/TnC | -92.72          | -8.06           | -124.40         |
| GnA/TC | 88.15           | 8.70            | -139.39         |
| GG/CnC | -56.45          | -5.38           | -84.93          |
| GnG/CC | 138.65          | 15.20           | -142.39         |
| AC/GnT | -53.18          | -5.63           | -63.98          |
| AnC/GT | 74.83           | 7.62            | -56.47          |
| AT/AnT | -65.71          | -6.89           | -18.30          |
| GC/GnC | -82.35          | -7.25           | -200.21         |
| TG/CnA | -50.58          | -5.10           | -103.10         |
| TnG/CA | 76.66           | 8.09            | -93.78          |
| TA/TnA | -67.53          | -8.36           | -27.52          |
| CG/CnG | -94.97          | -10.05          | -66.66          |
| **Overall** | 0.98        | 82.11           | -100.56         |

The table continues with similar data for other nicked pairs.
Supplementary Table S8. Sequence-dependent BP step parameters of regular BP steps using the parmbsc1 force field. The mean and standard deviation are represented in the left and right column for each BP step parameter.

| Regular | $\tau$ [°] | $\rho$ [°] | $\omega$ [°] | $D_x$ [nm] | $D_y$ [nm] | $D_z$ [nm] |
|---------|---------|---------|---------|----------|----------|----------|
| AA/TT   | -2.79   | 3.86    | -0.50   | 4.98     | 37.37    | 3.97     | -0.07    | 0.07     | -0.02    | 0.05     | 0.33     | 0.03     |
| AG/CT   | -1.95   | 4.44    | 2.26    | 5.52     | 33.00    | 5.76     | -0.02    | 0.09     | -0.05    | 0.07     | 0.33     | 0.03     |
| GA/TC   | -0.74   | 4.72    | 1.03    | 5.10     | 36.65    | 6.40     | -0.06    | 0.09     | -0.01    | 0.07     | 0.34     | 0.03     |
| GG/CC   | 2.04    | 4.85    | 3.24    | 6.31     | 35.38    | 5.31     | -0.01    | 0.09     | -0.06    | 0.08     | 0.35     | 0.03     |
| AC/CT   | -0.92   | 3.93    | -1.53   | 5.23     | 34.44    | 4.49     | 0.03     | 0.08     | -0.07    | 0.05     | 0.33     | 0.03     |
| AT/AT   | 0.58    | 3.64    | -1.28   | 4.54     | 32.75    | 3.52     | 0.00     | 0.06     | -0.09    | 0.04     | 0.33     | 0.03     |
| GC/GC   | 1.34    | 4.07    | 0.67    | 4.95     | 39.12    | 4.28     | 0.04     | 0.06     | -0.03    | 0.05     | 0.34     | 0.03     |
| TG/CA   | 2.61    | 5.34    | 6.40    | 6.81     | 32.19    | 6.03     | 0.08     | 0.10     | 0.00     | 0.05     | 0.32     | 0.03     |
| TA/TA   | 0.06    | 5.36    | 8.19    | 6.90     | 34.80    | 6.49     | -0.01    | 0.14     | 0.02     | 0.06     | 0.32     | 0.03     |
| CG/CG   | 3.33    | 5.14    | 10.95   | 6.39     | 33.75    | 6.21     | -0.08    | 0.08     | 0.02     | 0.05     | 0.33     | 0.03     |
| **Overall** | **0.36** | **2.00** | **2.94** | **4.25** | **34.95** | **2.22** | **-0.01** | **0.05** | **-0.03** | **0.04** | **0.33** | **0.01** |
Supplementary Table S9. Sequence-dependent BP step parameters of nicked BP steps using the parmbsc1 force field. The mean and standard deviation are represented in the left and right column for each BP step parameter.

| Nicked   | τ [°] | ρ [°] | ω [°] | Dx [nm] | Dy [nm] | Dz [nm] |
|----------|-------|-------|-------|---------|---------|---------|
| AA/TnT   | -3.52 | 4.19  | 2.29  | 6.40    | 35.87   | 6.07    | 0.00    | 0.06    | -0.04   | 0.06    | 0.32    | 0.03    |
| AnA/TT   | -1.49 | 4.54  | -0.21 | 5.01    | 32.83   | 7.74    | 0.00    | 0.06    | -0.08   | 0.06    | 0.33    | 0.03    |
| AG/CnT   | -3.00 | 5.16  | 4.01  | 6.32    | 29.35   | 8.08    | -0.01   | 0.07    | -0.08   | 0.08    | 0.32    | 0.03    |
| AnG/CT   | 0.65  | 5.27  | 2.22  | 6.18    | 28.44   | 12.03   | -0.02   | 0.13    | -0.14   | 0.07    | 0.33    | 0.03    |
| GA/TnC   | -0.93 | 4.75  | 1.01  | 6.29    | 33.16   | 10.16   | -0.03   | 0.08    | -0.03   | 0.07    | 0.32    | 0.03    |
| GnA/TC   | 2.04  | 5.95  | 2.55  | 6.67    | 36.24   | 15.73   | 0.03    | 0.14    | -0.08   | 0.09    | 0.33    | 0.03    |
| GG/CnC   | -2.57 | 4.92  | 8.10  | 6.36    | 30.06   | 9.27    | -0.11   | 0.10    | -0.03   | 0.08    | 0.31    | 0.03    |
| GnG/CC   | 1.81  | 5.57  | 4.95  | 6.00    | 32.36   | 8.76    | 0.03    | 0.09    | -0.14   | 0.07    | 0.35    | 0.03    |
| AC/GnT   | -2.89 | 4.54  | 0.14  | 5.56    | 36.34   | 6.92    | 0.09    | 0.07    | -0.09   | 0.06    | 0.33    | 0.03    |
| AnC/GT   | -0.26 | 4.17  | -0.31 | 4.98    | 27.51   | 6.75    | -0.06   | 0.07    | -0.08   | 0.07    | 0.32    | 0.03    |
| AT/AnT   | -0.62 | 4.02  | -0.48 | 4.80    | 30.16   | 5.64    | 0.00    | 0.07    | -0.09   | 0.05    | 0.32    | 0.03    |
| GC/GnC   | 0.00  | 4.40  | 0.09  | 5.09    | 39.41   | 7.00    | 0.01    | 0.07    | -0.05   | 0.06    | 0.34    | 0.03    |
| TG/CnA   | -0.65 | 5.30  | 10.43 | 6.51    | 31.51   | 8.35    | 0.05    | 0.09    | -0.03   | 0.07    | 0.33    | 0.03    |
| TnG/CA   | 1.16  | 5.44  | 12.19 | 6.35    | 26.31   | 9.58    | -0.08   | 0.09    | -0.08   | 0.06    | 0.32    | 0.04    |
| TA/TnA   | 2.66  | 7.99  | 8.63  | 8.54    | 32.73   | 17.63   | -0.05   | 0.16    | -0.01   | 0.10    | 0.33    | 0.04    |
| CG/CnG   | 0.15  | 4.81  | 10.46 | 5.36    | 30.15   | 7.56    | -0.03   | 0.07    | -0.02   | 0.06    | 0.32    | 0.03    |
| **Overall** | -0.47 | 1.88  | 4.13  | 4.42    | 32.03   | 3.58    | -0.01   | 0.05    | -0.07   | 0.04    | 0.33    | 0.01    |
Supplementary Table S10. Sequence-dependent mechanical rigidities of regular BP steps using the parmbsc1 force field. The mean and standard deviation are represented in the left and right column for each rigidity. The standard deviation is calculated by multiplying the off-diagonal value of the stiffness matrix by the standard deviation of the rise.

| Regular | $B_x$ [pN nm$^2$] | $B_y$ [pN nm$^2$] | $C$ [pN nm$^2$] | $Y_{dx}$ [pN] | $Y_{dy}$ [pN] | $S$ [pN] |
|---------|------------------|------------------|----------------|-------------|-------------|---------|
| AA/TT   | 354.13           | 28.01            | 189.04         | 14.95       | 347.38      | 27.48   |
|         | 1457.74          | 36.21            | 743.95         | 58.85       | 2398.20     | 189.70  |
| AG/CT   | 339.61           | 30.86            | 173.54         | 15.77       | 208.83      | 18.98   |
|         | 272.15           | 24.73            | 360.76         | 32.78       | 2247.31     | 204.20  |
| GA/TC   | 230.33           | 18.65            | 183.62         | 14.87       | 191.13      | 15.47   |
|         | 291.92           | 23.63            | 644.33         | 52.16       | 2125.85     | 172.10  |
| GG/CC   | 352.32           | 31.54            | 155.92         | 13.96       | 239.51      | 21.44   |
|         | 313.51           | 28.07            | 361.09         | 32.33       | 2407.37     | 215.52  |
| AC/CT   | 319.05           | 26.26            | 200.69         | 16.52       | 287.93      | 23.70   |
|         | 252.15           | 20.75            | 718.94         | 59.17       | 2554.25     | 210.24  |
| AT/AT   | 331.70           | 25.83            | 244.53         | 19.04       | 393.53      | 30.64   |
|         | 365.02           | 28.42            | 1012.94        | 78.87       | 2446.95     | 190.53  |
| GC/GC   | 323.66           | 25.22            | 224.49         | 17.49       | 294.53      | 22.95   |
|         | 447.32           | 34.86            | 660.64         | 51.48       | 2831.09     | 205.03  |
| TG/CA   | 205.69           | 18.84            | 130.02         | 11.91       | 198.38      | 18.17   |
|         | 180.30           | 16.51            | 639.19         | 58.53       | 1766.95     | 161.81  |
| TA/TA   | 187.88           | 17.24            | 130.70         | 11.99       | 232.12      | 21.29   |
|         | 91.57            | 8.40             | 509.50         | 46.74       | 1800.52     | 165.17  |
| CG/GG   | 238.97           | 21.47            | 130.46         | 11.72       | 177.32      | 15.93   |
|         | 317.64           | 28.53            | 718.86         | 64.58       | 1888.00     | 169.60  |
| Overall | 288.33           | 64.85            | 176.30         | 40.19       | 257.07      | 71.91   |
|         | 298.93           | 111.65           | 637.02         | 193.07      | 2226.65     | 316.17  |
Supplementary Table S11. Sequence-dependent mechanical rigidities of nicked BP steps using the parmbsc1 force field. The mean and standard deviation are represented in the left and right column for each rigidity. The standard deviation is calculated by multiplying the off-diagonal value of the stiffness matrix by the standard deviation of the rise.

| Regular | $B_\text{a}$ [pN nm$^2$] | $B_\text{p}$ [pN nm$^2$] | $C$ [pN nm$^2$] | $Y_{\text{dx}}$ [pN] | $Y_{\text{dy}}$ [pN] | $S$ [pN] |
|---------|----------------|----------------|----------------|----------------|----------------|--------|
| AA/TnT  | 32.67          | 29.18          | 112.16         | 10.14          | 171.76         | 15.53  | 445.96 | 40.33 | 420.60 | 38.03 | 2131.16 | 192.72 |
| AnA/TT  | 26.14          | 23.10          | 180.90         | 15.76          | 136.03         | 11.85  | 451.07 | 39.30 | 637.17 | 55.51 | 1989.50 | 173.32 |
| AG/CnT  | 225.68         | 22.50          | 120.85         | 12.05          | 101.69         | 10.14  | 228.24 | 22.64 | 278.70 | 27.79 | 2078.69 | 207.27 |
| AnG/CT  | 169.52         | 17.37          | 150.93         | 15.47          | 95.80          | 9.82   | 232.65 | 23.84 | 387.32 | 39.70 | 1296.95 | 132.93 |
| GA/TnC  | 286.92         | 25.83          | 147.30         | 13.26          | 94.51          | 8.51   | 292.48 | 26.33 | 380.45 | 34.25 | 2390.54 | 215.19 |
| GnA/TC  | 157.00         | 15.39          | 145.29         | 14.24          | 77.27          | 7.57   | 224.65 | 22.02 | 371.97 | 36.46 | 1507.45 | 147.77 |
| GG/CnC  | 288.84         | 30.74          | 126.59         | 13.47          | 68.24          | 7.26   | 188.83 | 20.10 | 245.40 | 26.12 | 2070.96 | 220.40 |
| GnG/CC  | 163.06         | 15.06          | 158.21         | 14.61          | 138.93         | 12.83  | 275.00 | 25.40 | 383.15 | 35.39 | 1565.90 | 144.64 |
| AC/GnT  | 242.32         | 21.58          | 171.80         | 15.30          | 137.10         | 12.21  | 344.28 | 30.66 | 546.96 | 48.71 | 2238.17 | 199.32 |
| AnG/GT  | 262.84         | 22.30          | 207.00         | 17.56          | 121.12         | 10.28  | 316.83 | 26.89 | 377.20 | 32.01 | 2349.27 | 199.35 |
| AT/AnT  | 274.58         | 22.49          | 205.69         | 16.85          | 158.86         | 13.01  | 337.50 | 27.64 | 659.28 | 53.99 | 2420.47 | 198.22 |
| GC/GnA  | 273.71         | 22.54          | 213.23         | 17.56          | 133.34         | 10.98  | 356.91 | 29.40 | 564.27 | 46.48 | 2468.90 | 203.36 |
| TG/CnA  | 198.08         | 19.68          | 132.30         | 13.14          | 92.87          | 9.23   | 216.09 | 21.47 | 343.66 | 34.14 | 1590.12 | 157.99 |
| TnG/CA  | 173.99         | 19.76          | 126.90         | 14.41          | 97.49          | 11.07  | 247.25 | 28.08 | 458.22 | 52.03 | 1339.69 | 152.13 |
| TA/TnA  | 120.94         | 13.25          | 104.35         | 11.43          | 81.66          | 8.94   | 260.88 | 28.57 | 324.59 | 35.55 | 1196.90 | 131.09 |
| CG/CnG  | 220.00         | 21.23          | 173.56         | 16.75          | 108.03         | 10.43  | 329.03 | 31.75 | 431.17 | 41.61 | 1814.63 | 175.13 |
| Overall | 227.83         | 58.43          | 154.82         | 34.47          | 113.42         | 30.02  | 300.35 | 76.01 | 425.63 | 119.98 | 1903.08 | 433.19 |
Supplementary Table S12. Sequence-dependent mechanical coupling coefficients of regular BP steps using the parmbsc1 force field. The mean and standard deviation are represented in the left and right column for each coefficient. The standard deviation is calculated by multiplying the off-diagonal value of the stiffness matrix by the standard deviation of the rise.

| Regular | $g_{1p}$ [pN nm$^{-2}$] | $g_{1w}$ [pN nm$^{-2}$] | $g_{2p}$ [pN nm$^{-2}$] | $g_{3px}$ [pN nm$^{-2}$] | $g_{3py}$ [pN] | $g_{3p2x}$ [pN] | $g_{3p2y}$ [pN] |
|---------|-----------------|-----------------|-----------------|-----------------|----------------|----------------|----------------|
| AA/TT   | 18.82           | 1.49            | 60.82           | 4.81            | -316.65        | -25.05         | -161.58        | -12.78          | 223.11          | 17.65          |
| AG/CT   | 26.49           | 2.59            | 52.31           | 4.75            | 47.68          | 4.33            | 47.96          | 4.36            | 170.93          | 15.53          | 270.92          | 24.62          |
| GA/TC   | 0.66            | 0.05            | -3.69           | -0.30           | 38.27          | 3.10            | -198.19        | -16.05          | -70.97          | -5.75          | 225.80          | 18.28          |
| GG/CC   | -21.07          | -1.69           | 7.73            | 0.69            | 37.57          | 3.36            | -9.02          | -0.81           | 161.96          | 14.50          | 404.06          | 36.17          |
| AC/CT   | 33.72           | 2.78            | 41.41           | 3.41            | 51.33          | 4.22            | -42.46         | -3.50           | 78.53           | 6.64           | 573.88          | 47.23          |
| AT/AT   | -1.43           | -0.11           | 2.00            | 0.16            | 59.20          | 4.61            | -71.95         | -5.60           | -6.78           | -0.53          | 471.14          | 36.88          |
| GC/GC   | 4.38            | 0.34            | 8.69            | 0.68            | 27.33          | 2.13            | -99.02         | -7.72           | 2.01            | 0.16           | 496.81          | 38.71          |
| TA/TA   | 6.82            | 0.62            | -29.11          | -2.67           | 58.59          | 5.37            | -25.52         | -2.34           | -27.31          | -2.50          | 42.34           | 3.88           |
| CG/GC   | -13.74          | -1.23           | -12.60          | -1.13           | 47.39          | 4.26            | -112.59        | -10.11          | -51.69          | -4.64          | 94.01           | 8.45           |
| Overall | 5.31            | 17.43           | 13.26           | 29.01           | 48.79          | 14.72           | -83.79         | 106.43          | 8.48            | 103.07         | 294.78          | 182.11         |

| Regular | $g_{1w}$ [pN nm] | $g_{1w}$ [pN nm] | $g_{1w}$ [pN nm] | $g_{2px}$ [pN nm] | $g_{2py}$ [pN nm] | $g_{p2x}$ [pN nm] | $g_{p2y}$ [pN nm] |
|---------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| AA/TT   | 24.56           | 1.94            | -6.28           | -0.50           | -329.44         | -26.06          | 29.59           | 2.34            | -21.04         | -1.66          | -6.48           | -0.51          |
| AG/CT   | -81.54          | 7.41            | 7.33            | 0.66            | -389.80         | 35.42           | 65.96           | 5.99            | -11.02         | -1.00          | -40.44          | -3.67          |
| GA/TC   | 34.49           | 2.79            | 20.60           | 1.67            | -181.36         | -14.68          | -20.43          | 1.65            | -32.65         | -2.64          | -15.82          | 1.28           |
| GG/CC   | -169.38         | -15.16          | -35.06          | -3.14           | -435.37         | -38.98          | 81.53           | 7.30            | 54.14          | 4.85           | 43.77           | 3.92           |
| AC/CT   | -41.87          | -3.45           | -10.97          | -0.90           | 62.29           | 5.13            | 22.85           | 1.88            | 89.89          | 7.32           | 194.57          | 16.01          |
| AT/AT   | 0.16            | 0.01            | 1.24            | 0.10            | -12.40          | -0.97           | 54.15           | 4.22            | 2.18           | 0.17           | 162.63          | 12.66          |
| GC/GC   | -129.99         | -10.13          | 0.88            | 0.07            | 43.79           | 3.41            | 10.36           | 0.81            | 99.89          | 7.78           | 229.88          | 17.91          |
| TA/TA   | -91.95          | -8.42           | 15.00           | 1.37            | 74.61           | -6.83           | -2.63           | -0.24           | 56.92          | 5.21           | -110.11         | -10.08         |
| CG/GC   | -140.86         | -12.55          | -13.12          | -1.18           | -59.56          | -5.35           | 36.48           | 3.28            | 27.93          | 2.51           | -82.54          | -7.41          |
| Overall | -65.34          | 70.42           | 2.88            | 15.82           | -140.51         | 182.99          | 29.52           | 31.23           | 29.71          | 45.54          | 26.15           | 127.40         |
Supplementary Table S13. Sequence-dependent mechanical coupling coefficients of nicked BP steps using the parmbsc1 force field. The mean and standard deviation are represented in the left and right column for each coefficient. The standard deviation is calculated by multiplying the off-diagonal value of the stiffness matrix by the standard deviation of the rise.

| Nicked | $g_{rx}$ [pN nm$^{-2}$] | $g_{ry}$ [pN nm$^{-2}$] | $g_{pz}$ [pN nm$^{-2}$] | $g_{Dxy}$ [pN] | $g_{Dyz}$ [pN] | $g_{Dzx}$ [pN] |
|--------|-----------------|-----------------|-----------------|-------------|-------------|-------------|
| AA/TnT | 5.89            | 0.53            | 29.36           | 2.66        | 30.89       | 2.79        |
| AnA/TT | 18.05           | 1.57            | 19.15           | 1.67        | 18.69       | 1.63        |
| AG/CnT | 12.99           | 1.21            | 36.73           | 3.66        | 26.50       | 2.64        |
| AnG/CT | 14.44           | 1.48            | -0.74           | 0.08        | 19.03       | 1.95        |
| GA/TnC | -17.05          | -1.53           | 27.96           | 2.52        | 38.85       | 3.50        |
| GnA/TC | 1.22            | 0.12            | -23.89          | -2.34       | 32.39       | 3.18        |
| GG/CnC | -40.90          | -4.35           | -4.98           | -0.53       | 15.45       | 1.64        |
| G/GnC  | -0.12           | -0.01           | -17.66          | -1.63       | 26.59       | 2.46        |
| AC/GnT | 16.63           | 1.48            | 20.76           | 1.85        | 9.21        | 0.82        |
| AnC/GT | 45.74           | 3.88            | 16.46           | 1.40        | 34.74       | 2.95        |
| AT/AnT | -16.78          | -1.37           | -16.90          | -1.38       | 18.97       | 1.55        |
| GC/GnC | -2.61           | -0.22           | 22.64           | 1.86        | 14.30       | 1.18        |
| TG/CnA | -14.33          | -1.42           | 6.14            | 0.61        | 31.75       | 3.15        |
| TnG/CA | 5.25            | 0.60            | -38.59          | -4.38       | 28.37       | 2.94        |
| TA/TnA | 7.04            | 0.77            | 0.99            | 0.11        | 35.33       | 3.87        |
| CG/CnG | -17.44          | -1.68           | 23.70           | 2.29        | 32.32       | 3.12        |

| Overall | 1.07         | 19.84          | 6.32            | 21.94       | 25.84       | 8.77        | -19.42 | 38.81 | 59.34 | 79.00 | 182.95 | 150.02 |

| Nicked | $g_{Dxy}$ [pN nm] | $g_{Dyz}$ [pN nm] | $g_{Dzx}$ [pN nm] |
|--------|-----------------|-----------------|-----------------|
| AA/TnT | -85.16          | -7.70           | -0.73           |
| AnA/TT | 2.51            | 0.22            | 91.58           |
| AG/CnT | -89.33          | -6.91           | -59.26          |
| AnG/CT | -12.45          | -1.28           | 50.52           |
| GA/TnC | -100.74         | -9.07           | -33.74          |
| GnA/TC | 20.02           | 1.96            | 87.91           |
| GC/GnC | -34.30          | -3.65           | -13.07          |
| AC/GnT | 34.36           | 3.17            | 37.57           |
| AT/AnT | -17.66          | -1.50           | 46.41           |
| GC/GnC | -96.96          | -7.99           | -69.11          |
| TG/CnA | -90.30          | -8.97           | -14.66          |
| TnG/CA | 51.51           | 5.85            | 73.73           |
| TA/TnA | -60.63          | -6.64           | -6.53           |
| CG/CnG | -93.89          | -9.06           | -25.91          |

| Overall | -37.71         | 50.56          | 1.82            |

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Supplementary Table S13 (Continued).

| Nicked    | $g_{\text{a} \text{a} \text{a}}$ [pN nm] | $g_{\text{a} \text{a} \text{b}}$ [pN nm] | $g_{\text{a} \text{b} \text{b}}$ [pN nm] |
|-----------|---------------------------------|---------------------------------|---------------------------------|
| AA/TnT    | 55.01                           | 4.97                            | -123.43                         |
| AnA/TT    | -79.42                          | -6.92                           | -135.48                         |
| AG/CnT    | -31.61                          | -3.15                           | -58.06                          |
| AnG/CT    | -108.22                         | -11.09                          | -44.00                          |
| GA/TnC    | -47.67                          | -4.29                           | -109.25                         |
| GnA/TC    | -86.77                          | -8.51                           | -64.68                          |
| GG/CnC    | 30.14                           | 3.21                            | -46.59                          |
| GnG/CC    | -115.67                         | -10.68                          | -93.47                          |
| AC/GnT    | -107.97                         | -9.62                           | -71.83                          |
| AnC/GT    | -42.03                          | -3.57                           | -51.60                          |
| AT/AnT    | -69.16                          | -5.66                           | -23.08                          |
| GC/GnC    | -92.13                          | -7.59                           | -52.87                          |
| TG/CnA    | -3.56                           | -0.35                           | -51.51                          |
| TnG/CA    | -75.32                          | -8.55                           | -34.38                          |
| TA/TnA    | -93.52                          | -10.24                          | -45.93                          |
| CG/CnG    | -42.25                          | -4.08                           | -23.51                          |
| Overall   | -56.88                          | 49.85                           | -64.35                          |

Note: The values represent the differences in energy barriers between the nicked and uncleaved states.
Supplementary Table S14. Rigidity ratio of nicked BP steps compared to regular BP steps.

Equivalent isotropic bending rigidity was used to calculate rigidity ratio values (Supplementary Method S3). The standard derivation (Std) of rigidity ratios in nicked BP steps compared to regular BP steps ($\sigma_{N/R}$) was approximated based on statistical theory as $\sigma_{N/R} = \frac{\alpha}{\beta} \sqrt{\sigma_\alpha^2 + \sigma_\beta^2}$ where $\alpha$ and $\beta$ represent the mean values of two independent properties, and $\sigma_\alpha$ and $\sigma_\beta$ indicate their standard deviations, respectively. For example, the standard deviation of $C$ ratio as $\frac{C_{\text{nicked}}}{C_{\text{regular}}}$ was calculated when the mean and standard deviation values of $C_{\text{nicked}}$ and $C_{\text{regular}}$ were substituted into $\alpha$, $\beta$, $\sigma_\alpha$, and $\sigma_\beta$. Likewise, the standard deviations of $B$ ratio or $C/B$ ratio were obtained. A figure below the table shows the rigidity ratio values for each BP steps in ascending order of the mean $C/B$ ratio.

| Rigidity ratio (nicked/regular) | C/B ratio | | | | C ratio | | | | B ratio | | |
|---------------------------------|-----------|---|---|---|---|---|---|---|---|---|---|
|                                 | Mean      | Std | Rank | Mean | Std | Rank | Mean | Std | Rank | Mean | Std | Rank |
| AA/TnT                          | 0.79      | 0.15 | 2   | 0.72 | 0.09 | 1    | 0.91 | 0.12 | 11  |
| AnA/TT                          | 0.23      | 0.04 | 16  | 0.18 | 0.02 | 16   | 0.78 | 0.11 | 16  |
| AG/CnT                          | 0.81      | 0.15 | 1   | 0.67 | 0.09 | 2    | 0.83 | 0.11 | 14  |
| AnG/CT                          | 0.58      | 0.11 | 5   | 0.52 | 0.07 | 9    | 0.90 | 0.12 | 12  |
| GA/TnC                          | 0.48      | 0.09 | 10  | 0.53 | 0.07 | 8    | 1.09 | 0.14 | 1   |
| GnA/TC                          | 0.49      | 0.10 | 9   | 0.45 | 0.06 | 10   | 0.92 | 0.13 | 9   |
| GG/CnC                          | 0.31      | 0.06 | 15  | 0.32 | 0.04 | 14   | 1.04 | 0.14 | 4   |
| GnG/CC                          | 0.61      | 0.13 | 3   | 0.55 | 0.08 | 5    | 0.91 | 0.13 | 10  |
| AC/GnT                          | 0.43      | 0.09 | 11  | 0.43 | 0.06 | 11   | 0.99 | 0.15 | 7   |
| AnC/CT                          | 0.41      | 0.09 | 12  | 0.42 | 0.06 | 12   | 1.03 | 0.15 | 5   |
| AT/AnT                          | 0.40      | 0.08 | 13  | 0.34 | 0.05 | 13   | 0.86 | 0.12 | 13  |
| GC/GnC                          | 0.56      | 0.10 | 7   | 0.60 | 0.08 | 4    | 1.08 | 0.14 | 3   |
| TG/CnA                          | 0.54      | 0.11 | 8   | 0.55 | 0.08 | 7    | 1.02 | 0.15 | 6   |
| TnG/CA                          | 0.57      | 0.12 | 6   | 0.55 | 0.08 | 6    | 0.98 | 0.15 | 8   |
| TA/TnA                          | 0.32      | 0.07 | 14  | 0.25 | 0.04 | 15   | 0.79 | 0.13 | 15  |
| CG/CnG                          | 0.60      | 0.13 | 4   | 0.65 | 0.10 | 3    | 1.08 | 0.17 | 2   |

A figure below the table shows the rigidity ratio values for each BP steps in ascending order of the mean $C/B$ ratio.
Supplementary Table S15. The number of nicked BP steps used in the experimental design and mean rigidity ratio. Mean rigidity ratio was used as a reference value to design DNA origami structures.

Mean rigidity ratio of each structure was calculated by dividing the summation of multiplying each rigidity ratio and the corresponding number of nicked BP steps by sixty as the total number of nicked BP steps.

A figure below the table shows the mean rigidity ratio values for each structure.

| The number of nicked BP steps | 1-BP-insertion | 2-BP-insertion | 1-BP-insertion | 2-BP-insertion |
|-------------------------------|----------------|----------------|----------------|----------------|
|                               | Stiff          | Flexible       | Moderate 1     | Moderate 2     | Flexible 1    | Flexible 2    |
| AA/TnT                        | 24             | 0              | 21             | 17             | 3             | 0              | 0              |
| AnA/Tt                        | 0              | 26             | 0              | 0              | 5             | 23            | 23             |
| AG/CnT                        | 12             | 0              | 6              | 10             | 0             | 0              | 0              |
| AnG/CT                        | 1              | 0              | 4              | 5              | 12            | 5             | 0              | 0              |
| GA/TnC                        | 0              | 0              | 0              | 0              | 16            | 4             | 2              | 1              |
| GnA/TC                        | 0              | 3              | 0              | 0              | 5             | 5             | 2              | 2              |
| GG/CnC                        | 2              | 0              | 1              | 0              | 1             | 6             | 11             | 15             |
| GtG/CC                        | 0              | 2              | 2              | 3              | 0             | 4             | 0              | 0              |
| AC/GnT                        | 0              | 1              | 0              | 0              | 11            | 1             | 1              | 2              |
| AnC/GT                        | 0              | 0              | 0              | 0              | 4             | 3             | 4              | 5              |
| AT/AnT                        | 1              | 5              | 1              | 1              | 2             | 2             | 4              | 1              |
| GC/GnC                        | 4              | 0              | 4              | 3              | 1             | 3             | 0              | 0              |
| TnG/CA                        | 1              | 0              | 2              | 2              | 2             | 3             | 0              | 0              |
| TA/TnA                        | 0              | 11             | 0              | 0              | 0             | 4             | 13             | 11             |
| CG/CnG                        | 11             | 1              | 13             | 13             | 0             | 3             | 0              | 0              |
| Sum                           | 60             | 60             | 60             | 60             | 60            | 60            | 60             | 60             |

| Mean rigidity ratio | 1-BP-insertion | 2-BP-insertion | 1-BP-insertion | 2-BP-insertion |
|---------------------|----------------|----------------|----------------|----------------|
|                     | Stiff          | Flexible       | Moderate 1     | Moderate 2     | Flexible 1    | Flexible 2    |
| C/B ratio           | 0.7062         | 0.2986         | 0.6737         | 0.6710         | 0.4915       | 0.4803       | 0.3062         | 0.3023         |
| C ratio             | 0.6580         | 0.2617         | 0.6352         | 0.6394         | 0.4889       | 0.4649       | 0.2722         | 0.2736         |
| B ratio             | 0.9431         | 0.8607         | 0.9536         | 0.9633         | 0.9979       | 0.9591       | 0.8706         | 0.8864         |

<figure>

A figure below the table shows the mean rigidity ratio values for each structure.

- C/B ratio
- C ratio
- B ratio

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Supplementary Table S16. Summary of AFM analysis. Trans ratio of DNA origami structures was calculated as the ratio of trans monomers for the total number of monomers. Trans ratio values of AFM images were used to calculate the standard deviation in brackets. Detailed values of AFM images were listed in Supplementary Table S11.

| The number of monomers | 1-BP-insertion | 2-BP-insertion |
|------------------------|----------------|----------------|
|                        | Stiff | Flexible | Stiff 1 | Stiff 2 | Moderate 1 | Moderate 2 | Flexible 1 | Flexible 2 | Flexible 1 (PAGE) |
| Trans                  | 760   | 860      | 722     | 681     | 714        | 895        | 818        | 788        | 523          |
| Cis                    | 1223  | 1308     | 631     | 490     | 489        | 498        | 311        | 317        | 203          |
| Trans + Cis            | 1983  | 2168     | 1353    | 1171    | 1203       | 1393       | 1129       | 1105       | 887          |

| Results                | 1-BP-insertion | 2-BP-insertion |
|------------------------|----------------|----------------|
|                        | Stiff | Flexible | Stiff 1 | Stiff 2 | Moderate 1 | Moderate 2 | Flexible 1 | Flexible 2 | Flexible 1 (PAGE) |
| Trans ratio (Std)      | 0.3833 (0.0767) | 0.3967 (0.0679) | 0.5336 (0.0519) | 0.5816 (0.0571) | 0.5936 (0.0239) | 0.6422 (0.0442) | 0.7245 (0.0609) | 0.7131 (0.0298) | 0.7204 (0.0272) |
| Twist angle [°] (Std)  | 293.3 (16.0) | 290.5 (13.8) | 624.0 (8.5) | 613.8 (11.4) | 611.6 (4.8) | 601.5 (9.6) | 582.1 (17.6) | 585.0 (9.5) | 582.2 (7.2) |
Supplementary Table S17. The number of monomers and trans-cis ratio from AFM images.

1-BP-insertion designs.

| Structure design | AFM image | The number of monomers | Trans ratio | Cis ratio |
|------------------|-----------|------------------------|-------------|-----------|
|                  |           | Trans  | Cis   | Cis + Trans |         |
| Stiff            | 1         | 22     | 32    | 54         | 0.4074  | 0.5926  |
|                  | 2         | 37     | 50    | 87         | 0.4253  | 0.5747  |
|                  | 3         | 52     | 59    | 111        | 0.4685  | 0.5315  |
|                  | 4         | 45     | 60    | 105        | 0.4286  | 0.5714  |
|                  | 5         | 33     | 50    | 83         | 0.3976  | 0.6024  |
|                  | 6         | 23     | 68    | 91         | 0.2527  | 0.7473  |
|                  | 7         | 20     | 53    | 73         | 0.2740  | 0.7260  |
|                  | 8         | 22     | 59    | 81         | 0.2716  | 0.7284  |
|                  | 9         | 25     | 60    | 85         | 0.2941  | 0.7059  |
|                  | 10        | 21     | 59    | 80         | 0.2625  | 0.7375  |
|                  | 11        | 26     | 58    | 84         | 0.3095  | 0.6905  |
|                  | 12        | 35     | 74    | 109        | 0.3211  | 0.6789  |
|                  | 13        | 48     | 65    | 113        | 0.4248  | 0.5752  |
|                  | 14        | 45     | 77    | 122        | 0.3889  | 0.6311  |
|                  | 15        | 52     | 55    | 107        | 0.4860  | 0.5140  |
|                  | 16        | 60     | 70    | 130        | 0.4615  | 0.5385  |
|                  | 17        | 35     | 66    | 101        | 0.3465  | 0.6535  |
|                  | 18        | 37     | 49    | 86         | 0.4302  | 0.5698  |
|                  | 19        | 34     | 47    | 81         | 0.4198  | 0.5802  |
|                  | 20        | 47     | 51    | 98         | 0.4796  | 0.5204  |
|                  | 21        | 41     | 61    | 102        | 0.4020  | 0.5980  |
|                  | Sum       | 760    | 1223  | 1983       | 0.3833  | 0.6167  |
| Flexible         |           |        |       |            | 0.0767  | 0.0767  |
|                  | 1         | 54     | 61    | 115        | 0.4696  | 0.5304  |
|                  | 2         | 47     | 64    | 111        | 0.4234  | 0.5766  |
|                  | 3         | 49     | 55    | 104        | 0.4712  | 0.5288  |
|                  | 4         | 42     | 51    | 93         | 0.4516  | 0.5484  |
|                  | 5         | 49     | 54    | 103        | 0.4757  | 0.5243  |
|                  | 6         | 56     | 53    | 109        | 0.5138  | 0.4862  |
|                  | 7         | 26     | 64    | 90         | 0.2889  | 0.7111  |
|                  | 8         | 29     | 66    | 95         | 0.3053  | 0.6947  |
|                  | 9         | 20     | 58    | 78         | 0.2564  | 0.7436  |
|                  | 10        | 36     | 67    | 103        | 0.3495  | 0.6505  |
|                  | 11        | 35     | 55    | 90         | 0.3889  | 0.6111  |
|                  | 12        | 45     | 80    | 125        | 0.3600  | 0.6400  |
|                  | 13        | 51     | 65    | 116        | 0.4397  | 0.5603  |
|                  | 14        | 36     | 68    | 104        | 0.3462  | 0.6538  |
|                  | 15        | 41     | 60    | 101        | 0.4059  | 0.5941  |
|                  | 16        | 44     | 60    | 104        | 0.4231  | 0.5769  |
|                  | 17        | 43     | 60    | 103        | 0.4175  | 0.5825  |
|                  | 18        | 33     | 74    | 107        | 0.3084  | 0.6916  |
|                  | 19        | 44     | 76    | 120        | 0.3667  | 0.6333  |
|                  | 20        | 38     | 57    | 95         | 0.4000  | 0.6000  |
|                  | 21        | 42     | 60    | 102        | 0.4118  | 0.5882  |
|                  | Sum       | 860    | 1308  | 2168       | 0.3967  | 0.6033  |

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Supplementary Table S17 (Continued).

2-BP-insertion designs.

| Structure designs | AFM image | The number of monomers | Trans | Cis | Cis + Trans | Trans ratio | Cis ratio |
|-------------------|-----------|-------------------------|-------|-----|-------------|-------------|-----------|
| Stiff 1           | 1         | 48                      | 54    | 102 | 0.4706      | 0.5294      |
|                   | 2         | 67                      | 49    | 116 | 0.5776      | 0.4224      |
|                   | 3         | 64                      | 57    | 121 | 0.5289      | 0.4711      |
|                   | 4         | 61                      | 47    | 108 | 0.5648      | 0.4352      |
|                   | 5         | 63                      | 65    | 128 | 0.4922      | 0.5078      |
|                   | 6         | 58                      | 62    | 120 | 0.4833      | 0.5167      |
|                   | 7         | 62                      | 34    | 96  | 0.6458      | 0.3542      |
|                   | 8         | 51                      | 52    | 103 | 0.4951      | 0.5049      |
|                   | 9         | 59                      | 56    | 115 | 0.5130      | 0.4870      |
|                   | 10        | 57                      | 50    | 107 | 0.5327      | 0.4673      |
|                   | 11        | 76                      | 52    | 128 | 0.5938      | 0.4063      |
|                   | 12        | 56                      | 53    | 109 | 0.5138      | 0.4862      |
|                   | Sum       | 722                     | 631   | 1353| Mean        | 0.5336      | 0.4664    |
|                   |           |                         |       |     | Std         | 0.0519      | 0.0519    |
|                   | Stiff 2   | 1                       | 62    | 55  | 117         | 0.5299      | 0.4701    |
|                   |           | 2                       | 66    | 57  | 123         | 0.5366      | 0.4634    |
|                   |           | 3                       | 56    | 47  | 103         | 0.5437      | 0.4563    |
|                   |           | 4                       | 60    | 45  | 105         | 0.5714      | 0.4286    |
|                   |           | 5                       | 76    | 32  | 108         | 0.7037      | 0.2963    |
|                   |           | 6                       | 58    | 43  | 101         | 0.5743      | 0.4257    |
|                   |           | 7                       | 70    | 42  | 112         | 0.6250      | 0.3750    |
|                   |           | 8                       | 70    | 37  | 107         | 0.6542      | 0.3458    |
|                   |           | 9                       | 56    | 41  | 97          | 0.5773      | 0.4227    |
|                   |           | 10                      | 57    | 45  | 102         | 0.5588      | 0.4412    |
|                   |           | 11                      | 50    | 46  | 96          | 0.5208      | 0.4792    |
|                   |           | Sum                     | 681   | 490 | Mean        | 0.5816      | 0.4184    |
|                   |           |                         |       |     | Std         | 0.0571      | 0.0571    |
|                   | Moderate 1| 1                       | 73    | 54  | 127         | 0.5748      | 0.4252    |
|                   |           | 2                       | 63    | 37  | 100         | 0.6300      | 0.3700    |
|                   |           | 3                       | 53    | 34  | 87          | 0.6092      | 0.3908    |
|                   |           | 4                       | 52    | 41  | 93          | 0.5591      | 0.4409    |
|                   |           | 5                       | 53    | 41  | 94          | 0.5638      | 0.4362    |
|                   |           | 6                       | 70    | 46  | 116         | 0.6034      | 0.3966    |
|                   |           | 7                       | 58    | 42  | 100         | 0.5800      | 0.4200    |
|                   |           | 8                       | 61    | 42  | 103         | 0.5922      | 0.4078    |
|                   |           | 9                       | 65    | 42  | 107         | 0.6075      | 0.3925    |
|                   |           | 10                      | 59    | 34  | 93          | 0.6344      | 0.3656    |
|                   |           | 11                      | 58    | 41  | 99          | 0.5859      | 0.4141    |
|                   |           | 12                      | 49    | 35  | 84          | 0.5833      | 0.4167    |
|                   |           | Sum                     | 714   | 489 | Mean        | 0.5935      | 0.4065    |
|                   |           |                         |       |     | Std         | 0.0239      | 0.0239    |
|                   | Moderate 2| 1                       | 102   | 45  | 147         | 0.6939      | 0.3061    |
|                   |           | 2                       | 85    | 41  | 126         | 0.6746      | 0.3254    |
|                   |           | 3                       | 67    | 38  | 105         | 0.6381      | 0.3619    |
|                   |           | 4                       | 67    | 51  | 118         | 0.5678      | 0.4322    |
|                   |           | 5                       | 69    | 39  | 108         | 0.6389      | 0.3611    |
|                   |           | 6                       | 65    | 47  | 112         | 0.5804      | 0.4196    |
|                   |           | 7                       | 74    | 43  | 117         | 0.6325      | 0.3675    |
|                   |           | 8                       | 72    | 31  | 103         | 0.6990      | 0.3010    |
|                   |           | 9                       | 71    | 34  | 105         | 0.6762      | 0.3238    |
|                   |           | 10                      | 78    | 53  | 131         | 0.5954      | 0.4046    |
|                   |           | 11                      | 79    | 37  | 116         | 0.6810      | 0.3190    |
|                   |           | 12                      | 66    | 39  | 105         | 0.6286      | 0.3714    |
|                   |           | Sum                     | 895   | 498 | Mean        | 0.6425      | 0.3575    |
|                   |           |                         |       |     | Std         | 0.0442      | 0.0442    |
## Supplementary Table S17 (Continued).

### 2-BP-insertion designs.

| Structure designs | AFM image | The number of monomers | Trans ratio | Cis ratio |
|-------------------|-----------|------------------------|-------------|----------|
|                   |           | Trans                  | Cis         | Trans + Cis |
| Flexible 1        | 1         | 104                    | 27          | 131       | 0.7939 | 0.2061 |
|                   | 2         | 68                     | 37          | 105       | 0.6476 | 0.3524 |
|                   | 3         | 76                     | 31          | 107       | 0.7103 | 0.2897 |
|                   | 4         | 64                     | 23          | 87        | 0.7356 | 0.2644 |
|                   | 5         | 75                     | 31          | 106       | 0.7075 | 0.2925 |
|                   | 6         | 87                     | 36          | 123       | 0.7073 | 0.2927 |
|                   | 7         | 81                     | 26          | 107       | 0.7570 | 0.2430 |
|                   | 8         | 72                     | 25          | 97        | 0.7423 | 0.2577 |
|                   | 9         | 75                     | 26          | 101       | 0.7426 | 0.2574 |
|                   | 10        | 77                     | 21          | 98        | 0.7857 | 0.2143 |
|                   | 11        | 39                     | 28          | 67        | 0.5821 | 0.4179 |
|                   |           | **Sum**                | **818**     | **311**   | **1129** | **Mean** | **0.7245** | **0.2755** |
|                   |           |                       |             |           |          | **Std**   | **0.0609** | **0.0609** |
| Flexible 2        | 1         | 61                     | 26          | 87        | 0.7011 | 0.2989 |
|                   | 2         | 54                     | 29          | 83        | 0.6506 | 0.3494 |
|                   | 3         | 67                     | 26          | 93        | 0.7204 | 0.2796 |
|                   | 4         | 67                     | 21          | 88        | 0.7614 | 0.2386 |
|                   | 5         | 79                     | 30          | 109       | 0.7248 | 0.2752 |
|                   | 6         | 59                     | 27          | 86        | 0.6880 | 0.3140 |
|                   | 7         | 62                     | 28          | 90        | 0.6889 | 0.3111 |
|                   | 8         | 76                     | 31          | 107       | 0.7103 | 0.2897 |
|                   | 9         | 65                     | 24          | 89        | 0.7303 | 0.2697 |
|                   | 10        | 78                     | 27          | 105       | 0.7429 | 0.2571 |
|                   | 11        | 62                     | 27          | 89        | 0.6966 | 0.3034 |
|                   | 12        | 58                     | 21          | 79        | 0.7342 | 0.2658 |
|                   |           | **Sum**                | **788**     | **317**   | **1105** | **Mean** | **0.7131** | **0.2869** |
|                   |           |                       |             |           |          | **Std**   | **0.0298** | **0.0298** |
| Flexible 1 (PAGE) | 1         | 70                     | 26          | 96        | 0.7292 | 0.2708 |
|                   | 2         | 74                     | 32          | 106       | 0.6981 | 0.3019 |
|                   | 3         | 77                     | 24          | 101       | 0.7624 | 0.2376 |
|                   | 4         | 69                     | 26          | 95        | 0.7263 | 0.2737 |
|                   | 5         | 71                     | 33          | 104       | 0.6827 | 0.3173 |
|                   | 6         | 83                     | 29          | 112       | 0.7411 | 0.2589 |
|                   | 7         | 79                     | 33          | 112       | 0.7054 | 0.2946 |
|                   |           | **Sum**                | **523**     | **203**   | **726**  | **Mean** | **0.7204** | **0.2796** |
|                   |           |                       |             |           |          | **Std**   | **0.0272** | **0.0272** |
Supplementary Table S18. Staple sequences used in 6HB DNA origami structures.

**Stiff design with 1-BP-insertion.**

| Index | Location | Staple sequence |
|-------|----------|-----------------|
| 1     | Twisting block 1 | TTGACCCCCAGCTGGAAAAGCTGAGTTAAATGGCTGGACAGAAAAC |
| 2     | Twisting block 1 | TTGCTAAAGGAGGAGGAGAGCATAGTTAAAGGCTGAGTTACAGTC |
| 3     | Twisting block 1 | TAAACCCGAGCCCCTTATAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 4     | Twisting block 1 | GGAACCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 5     | Twisting block 1 | GAAATCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 6     | Twisting block 1 | GCACACATACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 7     | Twisting block 2 | TTGACCCCCAGCTGGAAAAGCTGAGTTAAATGGCTGGACAGAAAAC |
| 8     | Twisting block 2 | TTGCTAAAGGAGGAGGAGGAGCATAGTTAAAGGCTGAGTTACAGTC |
| 9     | Twisting block 2 | TAAACCCGAGCCCCTTATAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 10    | Twisting block 2 | GGAACCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 11    | Twisting block 2 | GAAATCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 12    | Twisting block 2 | GCACACATACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 13    | Twisting block 3 | TTGACCCCCAGCTGGAAAAGCTGAGTTAAATGGCTGGACAGAAAAC |
| 14    | Twisting block 3 | TTGCTAAAGGAGGAGGAGGAGCATAGTTAAAGGCTGAGTTACAGTC |
| 15    | Twisting block 3 | TAAACCCGAGCCCCTTATAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 16    | Twisting block 3 | GGAACCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 17    | Twisting block 3 | GAAATCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 18    | Twisting block 3 | GCACACATACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 19    | Twisting block 4 | TTGACCCCCAGCTGGAAAAGCTGAGTTAAATGGCTGGACAGAAAAC |
| 20    | Twisting block 4 | TTGCTAAAGGAGGAGGAGGAGCATAGTTAAAGGCTGAGTTACAGTC |
| 21    | Twisting block 4 | TAAACCCGAGCCCCTTATAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 22    | Twisting block 4 | GGAACCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 23    | Twisting block 4 | GAAATCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 24    | Twisting block 4 | GCACACATACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 25    | Twisting block 5 | TTGACCCCCAGCTGGAAAAGCTGAGTTAAATGGCTGGACAGAAAAC |
| 26    | Twisting block 5 | TTGCTAAAGGAGGAGGAGGAGCATAGTTAAAGGCTGAGTTACAGTC |
| 27    | Twisting block 5 | TAAACCCGAGCCCCTTATAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 28    | Twisting block 5 | GGAACCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 29    | Twisting block 5 | GAAATCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 30    | Twisting block 5 | GCACACATACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 31    | Twisting block 6 | TTGACCCCCAGCTGGAAAAGCTGAGTTAAATGGCTGGACAGAAAAC |
| 32    | Twisting block 6 | TTGCTAAAGGAGGAGGAGGAGCATAGTTAAAGGCTGAGTTACAGTC |
| 33    | Twisting block 6 | TAAACCCGAGCCCCTTATAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 34    | Twisting block 6 | GGAACCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 35    | Twisting block 6 | GAAATCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 36    | Twisting block 6 | GCACACATACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 37    | Twisting block 7 | TTGACCCCCAGCTGGAAAAGCTGAGTTAAATGGCTGGACAGAAAAC |
| 38    | Twisting block 7 | TTGCTAAAGGAGGAGGAGGAGCATAGTTAAAGGCTGAGTTACAGTC |
| 39    | Twisting block 7 | TAAACCCGAGCCCCTTATAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 40    | Twisting block 7 | GGAACCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 41    | Twisting block 7 | GAAATCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 42    | Twisting block 7 | GCACACATACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 43    | Twisting block 8 | TTGACCCCCAGCTGGAAAAGCTGAGTTAAATGGCTGGACAGAAAAC |
| 44    | Twisting block 8 | TTGCTAAAGGAGGAGGAGGAGCATAGTTAAAGGCTGAGTTACAGTC |
| 45    | Twisting block 8 | TAAACCCGAGCCCCTTATAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 46    | Twisting block 8 | GGAACCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 47    | Twisting block 8 | GAAATCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 48    | Twisting block 8 | GCACACATACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 49    | Twisting block 9 | TTGACCCCCAGCTGGAAAAGCTGAGTTAAATGGCTGGACAGAAAAC |
| 50    | Twisting block 9 | TTGCTAAAGGAGGAGGAGGAGCATAGTTAAAGGCTGAGTTACAGTC |
| 51    | Twisting block 9 | TAAACCCGAGCCCCTTATAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 52    | Twisting block 9 | GGAACCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 53    | Twisting block 9 | GAAATCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 54    | Twisting block 9 | GCACACATACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 55    | Twisting block 10 | TTGACCCCCAGCTGGAAAAGCTGAGTTAAATGGCTGGACAGAAAAC |
| 56    | Twisting block 10 | TTGCTAAAGGAGGAGGAGGAGCATAGTTAAAGGCTGAGTTACAGTC |
| 57    | Twisting block 10 | TAAACCCGAGCCCCTTATAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 58    | Twisting block 10 | GGAACCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 59    | Twisting block 10 | GAAATCTACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 60    | Twisting block 10 | GCACACATACACACTAACTAGCTGAGTTAAAGGCTGAGTTACAGTC |
| 61    | Flag 1 | AAAAGGGGTAGACGTGAGTTAAAAGGCTGAGTTACAGTC |
### Supplementary Table S18 (Continued).

**Flexible design with 1-BP-insertion.**

| Index | Location | Staple sequence |
|-------|----------|-----------------|
| 1     | Twisting block 1 | CCCAGCTTGAATAAGCTTGAATGAAAACTTGAATTTGACGGACATA |
| 2     |   | AGCCCAAAGAGAAGAGGCAACGCGAGCAATATGGAAGG |
| 3     |   | TTTGCTAATGGAGGAGGATAGGTGTTATGACGAGG |
| 4     |   | ACCCTAACAAATCTACAAACTAAATAAGGTGACAG |
| 5     |   | ATTTTCTCAAGGATATAGCAGCAGTATGCAAGG |
| 6     |   | AGCCCGGAATAACCCCCGGATGTGAGCTTCTTGAATA |
| 7     | Twisting block 2 | ACTGACCATAAGGATGAAAAAGAGGCCAGATGGCCAGGACAG |
| 8     |   | AGATTTTCTTGAAAAAAGAAATACATCAACCTCAGAAAA |
| 9     |   | GTTTTGTGCTTGTGSGGACACACATTCAACATAATAGGCTTGGAC |
| 10    |   | AGCCGGGGAACCTCAGACATCAGCCACACATGGAGGAGAAGAGAC |
| 11    |   | CTTAATTTAGAGGCAAGAGGAGGACAGTATGAAAGG |
| 12    |   | AAACGGAATAGGTTGAGCAGTATGGCAGAATCAGG |
| 13    | Twisting block 3 | ACCGATGG TTCAGCACCAAGGACAGG |
| 14    |   | CAGGCAGTATAGGCGAGGAAGTGAAGACATTCTTTT |
| 15    |   | ACTAAGCTGGCTGAGAATAGCTTTGAGG |
| 16    |   | AACAGCTAATATGGAGTTGAGGACAG |
| 17    |   | GGCCGCTTAAATAGGCTGAGGTTGAGG |
| 18    |   | ATACAGCTTCTACATAAAACGAGATTGAGG |
| 19    |   | ACACCACGACCTTTAGGAGGTGAGGAGG |
| 20    |   | AGCGACCCAGACCTTTAGGAGGTGAGGAGG |
| 21    |   | ACAGAAGCTGGCATATAAGCCCAGCAGG |
| 22    |   | ACACTGCGGCAGGCTTGGAGGAGG |
| 23    |   | ACCTGACCGCAGCAGGCTTGGAGGAGG |
| 24    |   | ACCATGCCACCTTGGAGGAGG |
| 25    | Twisting block 4 | ATGTTGCTTCAGGACGAATAAGTGAAGAGG |
| 26    |   | AGTTAAGGAAAGGCGCTAGGAAAATAGG |
| 27    |   | CTCTTTTATGACGAGGACAGG |
| 28    |   | TCTTCAATGTTGACGAATAAGTGAAGAGG |
| 29    |   | ATCATTTAGGAGGAGGAGGAGG |
| 30    |   | CTCAAGAGCAGCAGCAGGAGGAGG |
| 31    |   | CTTAGAAGCTGGCAGCAGGAGG |
| 32    |   | CTTAGAAGCTGGCAGCAGGAGG |
| 33    |   | ACAGAAGCTGGCAGCAGGAGG |
| 34    |   | ACAGAAGCTGGCAGCAGGAGG |
| 35    |   | ATGACGAGAAGAGGAGGAGG |
| 36    |   | ATGACGAGAAGAGGAGGAGG |
| 37    | Twisting block 5 | ACCGAGAACACCGTCGTCAGACTGTAGCGCCTCTGAAGCTGAGACTC |
| 38    |   | AGTTTGCAGCGTCATGAAAAAGTTGAGG |
| 39    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 40    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 41    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 42    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 43    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 44    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 45    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 46    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 47    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 48    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 49    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 50    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 51    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 52    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 53    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 54    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 55    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 56    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 57    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 58    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 59    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 60    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |
| 61    |   | ATGGTTTAGAAATAACGAACTTTACTGAGGTGAGG |

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Flag 1: AAGGGGGAACGAGTGGATTTGGCACAGGAGGTCAGGAGGAGG

Flag 2: AAGGGTGAACGTGAGGTGAGGAAAGGAGGTCAGGAGGAGG
|   |   |
|---|---|
| 128 | CCATACCGAATCTTTAAAAATGACCAAGTAAATT |
| 129 | CCACGCTTTTCATCAACAACATGGTCACGGCTATAATTT |
| 130 | CCTCAAATATAATCAACATGGTAGACACGCAACGACAG |
| 131 | CCTGTTTTTCTACTTTCTTGGACTCTGAAATAACAG |
| 132 | CGCGCTTTCTGTTAAATCCATAAGCAGCTTAGATGATCAAAGAA |
| 133 | CGTCTTTACCAAGTATAGATGTTGCTGATCTATATTAGTT |
| 134 | GAAATACCGCAGGAAAAAGCTGTATATAACAGAAGAAGG |
| 135 | GACGAAGTGGAATCAAAGACGCCACAGCGCTTTGCA |
| 136 | GCTGAAGAACCTCCGGCTAGGGGATACGGGACCT |
| 137 | GCTAATAGAAATAAGTAGGAAATATTACGCTAAAAAT |
| 138 | GGGGACATCGGAAGGGCCACATTAAAGGAATTAATAGGA |
| 139 | GTATACCCAGAGCTGGCTTGCTAATGTAAATTAGCTG |
| 140 | GTTATCTTACGGAACAACTCAGTGTAATATACGTTA |
| 141 | TAGAACCTACCATAAAAAAAGGAAATAGGAAACCACGAGG |
| 142 | TATACCTCTGAAATAATGAGAGGACATTTGCAAT |
| 143 | TATATGCTCTCTCTGCTCTTCGGAGGCTTTACG |
| 144 | TATCCCATACTAGTGCTGATAAAAATAGGGAAAAAAACAGC |
| 145 | TGAATAACTACATCTTTAACGTTGCTATCCATTT |
| 146 | TGAATAACCTGAGTGTGTTGAAATACCTCTTTCATAGAGTC |
| 147 | TGCTATTAGCCAGACCCACAGCACGCCGCTCAACTGAA |
| 148 | TGAATAATCCACCTGCCGACACATCTAAGAAGCATGGCAG |
| 149 | TTATTTTGGATATCAGATCGAACATATTAAATTTGCAGTTTATA |
| 150 | TCGTAAAAGATTTGCAATTTAAAGGAATACCTTGAGAT |
| 151 | TTTACAAACATTGGAATACATGGCTAATATGACCTG |
| 152 | TTTCATAGTTATCAGTAAAGGCAAAATGACCAAGCAG |
| 153 | TTGCTACATCCGGAGTGAATACCAAAGTACCTGGTCAATACA |
| 154 | TTTCAGCTAATTGGGTTTTTTCCGAGCAGAC |
| 155 | TTTCATACAAATTTTTCACCAGTCACAAACTTTCAATAGG |
**Supplementary Table S18 (Continued).**

**Stiff 1 design with 2-BP-insertion.**

| Index | Location       | Staple sequence                                                                 |
|-------|----------------|---------------------------------------------------------------------------------|
| 1     | Twisting 1     | GCCAAAAAGGCTCCATCATTTTGACCCCCAGGCTTGATCAAGAGTT |
Flag 2

| DNA Sequence |
|--------------|
| AACGAGAAAAGCGGTATTGCTATATTTTATCTCACTACGGCTATG |
| AGGCCAATACGTCTAAATTATTTAAATTAATACAGGGTGATG |
| AGGTAAACCGGGGACCAAGGCTAAAGGAGAGGATTTTTCTG |
| AGGCAAAGCTCCACGCGGCTGTCAGGCTAGTTGAGTTG |
| AGGTTAAGGTTTGGCAGAGATAGCTTTCAGACAGGCTATG |
| ATTTAAGGCTGAGAACCGGTAAAGTGCTGGACAGAAGT |
| CAGAAAGCTCAGTTGCTGTTATACGACAGGCTACGAGATTG |
| CCGAATTTCGAAAGTCATCCCAAGACTTTTATTGAGTTG |
| CGGGTTGAGTAAACGTAAATTT |
| CGTAAAATTCATCATAATTCG |
| CTGACTAGCGCCGACAGTGGATATTTTATATTAC |
| CTGGAAGGTACCAAGCCGGAGACAGTCAGAACAAGAGTAAAA |
| CTGGTTTGTCTGGAAAAAGGTGGCATCACATTTGGTTATAGT |
| CTGTGTGAGTGTAAAGCCTCAACTGTTGGGATGTGCTGTTTC |
| GAAACAAAATTTTGCTTAGTAAATGAATTACCATCAGTCGAGGTG |
| GAACGAGAAAATTAATCTAGCTGATAAATAAAAGAAGTCATAA |
| GAGCTCACAATACATAGCAATTAACTGCTACCCCAACCGAGG |
| GATTAGCTGATCCGCTGAAATCCTCTATCAGTAATAGT |
| TATCCGCTCGAATTCGATATGAGCTGCATCGAGGATACC |
| TATTAAAGGAGAAAGAAACATGTAGCTACCCCAACCGAGG |
| TTCCAGGAGTGGCAGTACAACTCAGACAGACAAACAG |
| TTTCAGGAGTGAGCTAATCTGAGACACGGGAGGGAAGG |
| ACAAATCTCTTTGAGGTGCAGTATCAAGACAGGCAAGT |
| AGCAAGCTCAGTTGCTGTTATACGACAGGCTACGAGATTG |
| ATATTATTAGATAGTGACAAACCCAGTATTAAAGATAAATA |
| ATGTTAAGGTTTGGCAGAGATAGCTTTCAGACAGGCTATG |
| ATGTTTGTCTGGAAAAAGGTGGCATCACATTTGGTTATAGT |
| ATGTTGAGTGTAAAGCCTCAACTGTTGGGATGTGCTGTTTC |
| GTAGCTACGGCAAACCCCTCAAATGCTTATTAAGATCGCAAA |
| TTCCAGGAGTGAGCTAATCTGAGACACGGGAGGGAAGG |
| TTCCAGGAGTGAGCTAATCTGAGACACGGGAGGGAAGG |

Flag 2
| 128 | CCATACCGAATCTTTAAATAGAACAAATGCTCATGTAATTT |
| 129 | CCACGCTTTTCATCAACACTAGTTCCACCGCTCAATTCAAAT |
| 130 | CCTCAAAATAATAACAGTTGAAGATTAGAGCCGTCAAGAC |
| 131 | CCTGTCTTCTCATCTCTGTGACCCTCTGAAATAACGAG |
| 132 | CGCGCTCTGTGAAATCCATAGCAATGCTGATTCAAGGAA |
| 133 | CGACGCAAGAAATAAGGCAGGAGAGAGCACTTTGCA |
| 134 | CGTCTTTACCAAAAGGCTGTTTAAATAAAACAG |
| 135 | GACGAGCTGAGATCAAAGAAGCGAGAGAGCACTTTGCA |
| 136 | GCTGAGAACCTCCGCTTAAGGAAATACGGGCTT |
| 137 | GCTTAATATAAGTAGAATGAGAATGAGAAGGAGG |
| 138 | GGGACATGGAAAGGCAAAATTAAAGGTAAAG |
| 139 | GTAAACCCAGAAGCTGTAGAATCAAAGAG |
| 140 | GTGATCTTTGAGGAGCAACTAAGAACACTCGTGCAG |
| 141 | TAGAACCCTTACCATATAAAAAAGAATGAAAG |
| 142 | TATACCTTCTGATAATGAGAAGCAGATTGTTGAGT |
| 143 | TATATGTCCTTCTGTGCTGGAGAGGTTGTCAG |
| 144 | TATCCCATACGATCTGATATAAAATAAGGAAAAACAAAAG |
| 145 | TGAAACTACTACATTGTTGAAAAATAAGGGG |
| 146 | TGAAACTACTACATTGTTGAAAAATAAGGGG |
| 147 | TGCGCTCTGAGAAGCAGCAGACTCGTCAACTGAA |
| 148 | TTTAAAAAGGGCTTAGTGTGATGATATTGAAATAG |
| 149 | TTAATTTGATTACATGATGATGAAATAATACGCTGTGTTTATA |
| 150 | TTTTTAAAGGAGTACATGACGTAAATACGGAATAGCAGGATG |
| 151 | TTTCACAATGAGTAACATATTGAGAATGAGGATTACCTTGCAG |
| 152 | TTTTACATTACGTATAAAAGGCAATGACAGCAGG |
| 153 | TTGGTTGATACGGGAGTTGAATACGACGACGTCATCAGTCAATACAG |
| 154 | TTTTAGCAGTAATGAGGTTTTTTCTGAGGCAAGC |
| 155 | TTTTATACAAATTTTCACCAACTCAACAAACTTTCAGTAGG |
## Supplementary Table S18 (Continued).

**Stiff 2 design with 2-BP-insertion.**

| Index | Location          | Staple sequence                                                                 |
|-------|-------------------|---------------------------------------------------------------------------------|
| 1     | Twisting block 1  | GCCAAAAGGTCCTACATTTGAGCCGCCAGCTTTAGAAAGTGT                                       |
| 2     |                   | GAGAAATAGAAACACTGTTACATTGAGGGAACAATGGGAGGAAAGTGTT                                  |
| 3     |                   | ACTAAAGGGAGACACTCATTAGTTAGGTAAGGCGCCGCCATATACAA                                  |
| 4     |                   | GCGACGCTGGATTTATCGGGGAACCTTATAAACAGCGATCTCGGGAGGT                                 |
| 5     |                   | GTGGCCGATACCGAGCAGGAGAACTATGATGTAAGGGTGTGAGCAGGTATCAGGAAA                       |
| 6     |                   | TGGCTGCTTACACTACAGCGCCGATAGTGAAGGATTTGACGCTAGTATGAGC                             |
| 7     |                   | GTGACAGGAGCGCCACCAAGCATTTAACAGGAAATATTGCTAGGATG                                  |
| 8     |                   | TCACTCGTGAATTGAGAACTTACAGCGCCGACTTTGAGTTGAGGAAAGGGAAGGAA                        |
| 9     |                   | TCAACCTTGATTTATCAGGCTAGTATACAGGAAATATTGCTAGGATG                                  |
| 10    |                   | GTAACAGGAGGAAAGGGAAGGAAAATAACGAAAATGAGGAAAGGAAAGGAAAGGAA                       |
| 11    |                   | GAAAGGGGAGGACACTCATTAGTTAGGTAAGGCGCCGCCATATACAA                                  |
| 12    |                   | TAAAGACTCATGGCAGCAGCAGCACCAAGCAGACGGGTTAAGGGAAGGAAAGGAA                        |
| 13    |                   | GTTACGCTTGTTACAGCGCCGACCGTATTTGAGGATTTGAGGAAAGGAAAGGAA                        |
| 14    |                   | GCCACATCTCCACGTCACCACTACATTACAGCGGAGGAGGAAAGGAAAGGAA                           |
| 15    |                   | GCGACGCTGGATTTATCGGGGAACCTTATAAACAGCGATCTCGGGAGGT                                 |
| 16    |                   | TGGCTGCTTACACTACAGCGCCGATAGTGAAGGATTTGACGCTAGTATGAGC                             |
| 17    |                   | GTGACAGGAGCGCCACCAAGCATTTAACAGGAAATATTGCTAGGATG                                  |
| 18    |                   | TCACTCGTGAATTGAGAACTTACAGCGCCGACTTTGAGTTGAGGAAAGGGAAGGAA                        |
| 19    |                   | TCAACCTTGATTTATCAGGCTAGTATACAGGAAATATTGCTAGGATG                                  |
| 20    |                   | GTAACAGGAGGAAAGGGAAGGAAAATAACGAAAATGAGGAAAGGAAAGGAAAGGAA                       |
| 21    |                   | GAAAGGGGAGGACACTCATTAGTTAGGTAAGGCGCCGCCATATACAA                                  |
| 22    |                   | TAAAGACTCATGGCAGCAGCAGCACCAAGCAGACGGGTTAAGGGAAGGAAAGGAA                        |
| 23    |                   | GTTACGCTTGTTACAGCGCCGACCGTATTTGAGGATTTGAGGAAAGGAAAGGAA                        |
| 24    |                   | GCCACATCTCCACGTCACCACTACATTACAGCGGAGGAGGAAAGGAAAGGAA                           |
| 25    |                   | GCGACGCTGGATTTATCGGGGAACCTTATAAACAGCGATCTCGGGAGGT                                 |
| 26    |                   | TGGCTGCTTACACTACAGCGCCGATAGTGAAGGATTTGACGCTAGTATGAGC                             |
| 27    |                   | GTGACAGGAGCGCCACCAAGCATTTAACAGGAAATATTGCTAGGATG                                  |
| 28    |                   | TCACTCGTGAATTGAGAACTTACAGCGCCGACTTTGAGTTGAGGAAAGGGAAGGAA                        |
| 29    |                   | TCAACCTTGATTTATCAGGCTAGTATACAGGAAATATTGCTAGGATG                                  |
| 30    |                   | GTAACAGGAGGAAAGGGAAGGAAAATAACGAAAATGAGGAAAGGAAAGGAAAGGAA                       |
| 31    |                   | GAAAGGGGAGGACACTCATTAGTTAGGTAAGGCGCCGCCATATACAA                                  |
| 32    |                   | TAAAGACTCATGGCAGCAGCAGCACCAAGCAGACGGGTTAAGGGAAGGAAAGGAA                        |
| 33    |                   | GTTACGCTTGTTACAGCGCCGACCGTATTTGAGGATTTGAGGAAAGGAAAGGAA                        |
| 34    |                   | GCCACATCTCCACGTCACCACTACATTACAGCGGAGGAGGAAAGGAAAGGAA                           |
| 35    |                   | GCGACGCTGGATTTATCGGGGAACCTTATAAACAGCGATCTCGGGAGGT                                 |
| 36    |                   | TGGCTGCTTACACTACAGCGCCGATAGTGAAGGATTTGACGCTAGTATGAGC                             |
| 37    |                   | GTGACAGGAGCGCCACCAAGCATTTAACAGGAAATATTGCTAGGATG                                  |
| 38    |                   | TCACTCGTGAATTGAGAACTTACAGCGCCGACTTTGAGTTGAGGAAAGGGAAGGAA                        |
| 39    |                   | TCAACCTTGATTTATCAGGCTAGTATACAGGAAATATTGCTAGGATG                                  |
| 40    |                   | GTAACAGGAGGAAAGGGAAGGAAAATAACGAAAATGAGGAAAGGAAAGGAAAGGAA                       |
| 41    |                   | GAAAGGGGAGGACACTCATTAGTTAGGTAAGGCGCCGCCATATACAA                                  |
| 42    |                   | TAAAGACTCATGGCAGCAGCAGCACCAAGCAGACGGGTTAAGGGAAGGAAAGGAA                        |
| 43    |                   | GTTACGCTTGTTACAGCGCCGACCGTATTTGAGGATTTGAGGAAAGGAAAGGAA                        |
| 44    |                   | TCACTCGTGAATTGAGAACTTACAGCGCCGACTTTGAGTTGAGGAAAGGGAAGGAA                        |
| 45    |                   | TCAACCTTGATTTATCAGGCTAGTATACAGGAAATATTGCTAGGATG                                  |
| 46    |                   | GTAACAGGAGGAAAGGGAAGGAAAATAACGAAAATGAGGAAAGGAAAGGAAAGGAA                       |
| 47    |                   | GAAAGGGGAGGACACTCATTAGTTAGGTAAGGCGCCGCCATATACAA                                  |
| 48    |                   | TAAAGACTCATGGCAGCAGCAGCACCAAGCAGACGGGTTAAGGGAAGGAAAGGAA                        |
| 49    |                   | GTTACGCTTGTTACAGCGCCGACCGTATTTGAGGATTTGAGGAAAGGAAAGGAA                        |
| 50    |                   | GCCACATCTCCACGTCACCACTACATTACAGCGGAGGAGGAAAGGAAAGGAA                           |
| 51    |                   | TGGCTGCTTACACTACAGCGCCGATAGTGAAGGATTTGACGCTAGTATGAGC                             |
| 52    |                   | GTGACAGGAGCGCCACCAAGCATTTAACAGGAAATATTGCTAGGATG                                  |
| 53    |                   | TCACTCGTGAATTGAGAACTTACAGCGCCGACTTTGAGTTGAGGAAAGGGAAGGAA                        |
| 54    |                   | TCAACCTTGATTTATCAGGCTAGTATACAGGAAATATTGCTAGGATG                                  |
| 55    |                   | GTAACAGGAGGAAAGGGAAGGAAAATAACGAAAATGAGGAAAGGAAAGGAAAGGAA                       |
| 56    |                   | GAAAGGGGAGGACACTCATTAGTTAGGTAAGGCGCCGCCATATACAA                                  |
| 57    |                   | TAAAGACTCATGGCAGCAGCAGCACCAAGCAGACGGGTTAAGGGAAGGAAAGGAA                        |
| 58    |                   | GTTACGCTTGTTACAGCGCCGACCGTATTTGAGGATTTGAGGAAAGGAAAGGAA                        |
| 59    |                   | TCACTCGTGAATTGAGAACTTACAGCGCCGACTTTGAGTTGAGGAAAGGGAAGGAA                        |
| 60    | Flag 1            | AAAGGGGAGGACACTCATTAGTTAGGTAAGGCGCCGCCATATACAA                                  |

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| Line | Sequence |
|------|----------|
| 62   | AACGAGAAAGCGGTATTTGACCTATATTTATCTACGCTATTGACCATTAAGCATGAACAGGTTGTTT |
| 63   | AACGCAATCACGCTCAAAATTTAAATTAGCTGAGGAAATCTCACATGATATG |
| 64   | AGATAGGCAACGCGTGAACAAAGGCTGTTGTAAACGACGGTTAGG |
| 65   | AGTGCGGCTAACGCGGAAGCCACTACGGTGAACAGGACCAAGTAAAT |
| 66   | CAGAAACGCTGACACGAGGCTGACCTGACCATGATATG |
| 67   | CAAAACACAGCAGGAAAACAGGAGAAATCCGACGATATG |
| 68   | CCAGCTGCAGGTGTTGTGGGCGCATCGGATCGCACTTCGTG |
| 69   | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 70   | CGTGACGTATGTTATCCTGATGTGACGCTTATGAGGACCAAAAGCC |
| 71   | CGAAAGATTATCACTCTTTAATAATCAAGGAAGCTCATAAAAG |
| 72   | CTGACATCACGCAATAGCATACTGACGATATG |
| 73   | GAGCTGGCAACCGCTCCGGACAGGAAATCCGACGATATG |
| 74   | GCTTTTGATTTTCACTGGAAGAGGAAATCCGACGATATG |
| 75   | TATCCGCTGCAGGTGTTGTGGGCGCATCGGATCGCACTTCGTG |
| 76   | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 77   | CCAGCTGCAGGTGTTGTGGGCGCATCGGATCGCACTTCGTG |
| 78   | CGTGACGTATGTTATCCTGATGTGACGCTTATGAGGACCAAAAGCC |
| 79   | CGAAAGATTATCACTCTTTAATAATCAAGGAAGCTCATAAAAG |
| 80   | CTGACATCACGCAATAGCATACTGACGATATG |
| 81   | GAGCTGGCAACCGCTCCGGACAGGAAATCCGACGATATG |
| 82   | GCTTTTGATTTTCACTGGAAGAGGAAATCCGACGATATG |
| 83   | TATCCGCTGCAGGTGTTGTGGGCGCATCGGATCGCACTTCGTG |
| 84   | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 85   | CCAGCTGCAGGTGTTGTGGGCGCATCGGATCGCACTTCGTG |
| 86   | CTGACATCACGCAATAGCATACTGACGATATG |
| 87   | GAGCTGGCAACCGCTCCGGACAGGAAATCCGACGATATG |
| 88   | GCTTTTGATTTTCACTGGAAGAGGAAATCCGACGATATG |
| 89   | TATCCGCTGCAGGTGTTGTGGGCGCATCGGATCGCACTTCGTG |
| 90   | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 91   | CCAGCTGCAGGTGTTGTGGGCGCATCGGATCGCACTTCGTG |
| 92   | CTGACATCACGCAATAGCATACTGACGATATG |
| 93   | GAGCTGGCAACCGCTCCGGACAGGAAATCCGACGATATG |
| 94   | GCTTTTGATTTTCACTGGAAGAGGAAATCCGACGATATG |
| 95   | TATCCGCTGCAGGTGTTGTGGGCGCATCGGATCGCACTTCGTG |
| 96   | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 97   | CCAGCTGCAGGTGTTGTGGGCGCATCGGATCGCACTTCGTG |
| 98   | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 99   | CCAGCTGCAGGTGTTGTGGGCGCATCGGATCGCACTTCGTG |
| 100  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 101  | CCAGCTGCAGGTGTTGTGGGCGCATCGGATCGCACTTCGTG |
| 102  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 103  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 104  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 105  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 106  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 107  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 108  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 109  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 110  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 111  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 112  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 113  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 114  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 115  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 116  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 117  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 118  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 119  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 120  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 121  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 122  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 123  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 124  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 125  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 126  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
| 127  | CGGAAATTTTTTGACCAATAAAATCATCATAATGTGAGAGCC |
|   |   |
|---|---|
| 128 | CCATACCGGAAATCTTTAAATAGAAGCAGAATGCTCATGTAATTT |
| 129 | CCCAGCTTTTTCACAAAACATTTTTCACCGGCTCAATTTCAAT |
| 130 | CCTCAAAATAATCAACAGTGTGAATTAGAGCCGCTAGAAGAC |
| 131 | CCTGTTTTTCTTACTTCTTCTGACCTCTGAAATACGAG |
| 132 | CCGGCTTTCTGTGAAATCCATAGCGATAGCTTAGATTCAAAAGAA |
| 133 | CGTCTTTACCAGTAGTATAAGTCTCTGTATCATATAAGGT |
| 134 | GAATAACCGGACCAGAAAACCTGTAAAACAAAGAGAACAGG |
| 135 | GACGACGCTGAGAATCAAACGGAGAGACCAGCTTTTCGAAG |
| 136 | GCTGAGAACTCGGCCGTAGGGAATACGGCGTTTT |
| 137 | GCTTAAATAGAAATAAGTAGGAACTATTGCTAAAAAAT |
| 138 | GGGGATGCGAAAGGGCCATATTAGAAGTAAATGAATAAG |
| 139 | GTATACCCAGAGCTTGAGCTGAAATTGAAATATAGG |
| 140 | GTATCTTAGAGGCCCTAAACACTCTGATATTAAATAGT |
| 141 | TAAACAATACCATATAAAGAAATAGGAAACGAGAGGT |
| 142 | TATACCTCTGAAATAGAGGAGACATTTTCGAGTTTCAG |
| 143 | TATATGTCTTTTCTGCTGCGGAGGAGTTTCAG |
| 144 | TATCCCATACGTAGTTGATAAAATAAGGGAAAACAAACAGC |
| 145 | TGAATAACTCATTCTTTGTTCCGATTCTTCCAAATATA |
| 146 | TGAATAACCTGTAGTTGTTGAATACCTTTTTAAGAGTTCA |
| 147 | TGGCTTTATGCGAAACCAGACGAGACCGACTGCAACTGAA |
| 148 | TTAATTTAGCTTTAGATGCTATTACATAAAGCATGCGAGGAG |
| 149 | TTAATTTTAGCTTTAGATGCTATTACATAAAGCATGCGAGGAG |
| 150 | TTAATTTTAGCTTTAGATGCTATTACATAAAGCATGCGAGGAG |
| 151 | TTACAAACACACTTTGAGCAATACATGCAGAATACACACCAAT |
| 152 | TTACAGATTTAACGAATGAGCAGAATACACACCAAT |
| 153 | TTGCTTTACATCGGGATGTAATTACAGATCTCGTGCAATACA |
| 154 | TTATAGCGGTTAGGTTTTTTTCGAGGCAAGG |
| 155 | TTATACAAATTTTTCCACAGTCAACAAAACTTTTCAGTGGG |
Supplementary Table S18 (Continued).

Moderate 1 design with 2-BP-insertion.

| Index | Location | Staple sequence |
|-------|----------|-----------------|
| 1     | Twisting block 1 | CGTTTTCGACTAACGAGCATAGTAAAGAGATTTGAGCCAGCGCAGG |
| 2     |           | CGCCGACTTTGGAATTATCTCGGAACTAAAGAGGCTACTGGGAG |
| 3     |           | AAAAGCTTCCATACTTGCAATTCATTGGAGATAGTTGAGGGGAG |
| 4     |           | TGGAGATAGTAAAGAGGAGCTACTGGGAGATAGTTGAGGGGAG |
| 5     |           | CCTTTTCGACTAACGAGCATAGTAAAGAGATTTGAGCCAGCGCAGG |
| 6     |           | CCTTTTCGACTAACGAGCATAGTAAAGAGATTTGAGCCAGCGCAGG |
| 7     | Twisting block 2 | CGCTGTAAACCAGACGTAAACAACTTTCAACAGATTATATCGAAAT |
| 8     |           | CGCTGTAAACCAGACGTAAACAACTTTCAACAGATTATATCGAAAT |
| 9     |           | CGCTGTAAACCAGACGTAAACAACTTTCAACAGATTATATCGAAAT |
| 10    |           | CGCTGTAAACCAGACGTAAACAACTTTCAACAGATTATATCGAAAT |
| 11    |           | CCAGCCGACTAACGAGCATAGTAAAGAGATTTGAGCCAGCGCAGG |
| 12    |           | CCAGCCGACTAACGAGCATAGTAAAGAGATTTGAGCCAGCGCAGG |
| 13    | Twisting block 3 | GACGCTTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 14    |           | GACGCTTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 15    |           | GACGCTTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 16    |           | GACGCTTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 17    |           | GACGCTTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 18    |           | GACGCTTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 19    |           | GACGCTTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 20    |           | GACGCTTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 21    |           | GACGCTTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 22    |           | GACGCTTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 23    |           | GACGCTTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 24    |           | GACGCTTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 25    | Twisting block 5 | TAATCAGTGTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 26    |           | TAATCAGTGTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 27    |           | TAATCAGTGTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 28    |           | TAATCAGTGTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 29    |           | TAATCAGTGTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 30    |           | TAATCAGTGTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 31    | Twisting block 6 | CACGGTGTAAACCAGACGTAAACAACTTTCAACAGATTATATCGAAAT |
| 32    |           | CACGGTGTAAACCAGACGTAAACAACTTTCAACAGATTATATCGAAAT |
| 33    |           | CACGGTGTAAACCAGACGTAAACAACTTTCAACAGATTATATCGAAAT |
| 34    |           | CACGGTGTAAACCAGACGTAAACAACTTTCAACAGATTATATCGAAAT |
| 35    |           | CACGGTGTAAACCAGACGTAAACAACTTTCAACAGATTATATCGAAAT |
| 36    |           | CACGGTGTAAACCAGACGTAAACAACTTTCAACAGATTATATCGAAAT |
| 37    | Twisting block 7 | GGGATTTTAGAACCAGGCAGAGCCGCCACCCTCTACAGTGGAGATAGTTGAGGGGAG |
| 38    |           | GGGATTTTAGAACCAGGCAGAGCCGCCACCCTCTACAGTGGAGATAGTTGAGGGGAG |
| 39    |           | GGGATTTTAGAACCAGGCAGAGCCGCCACCCTCTACAGTGGAGATAGTTGAGGGGAG |
| 40    |           | GGGATTTTAGAACCAGGCAGAGCCGCCACCCTCTACAGTGGAGATAGTTGAGGGGAG |
| 41    |           | GGGATTTTAGAACCAGGCAGAGCCGCCACCCTCTACAGTGGAGATAGTTGAGGGGAG |
| 42    |           | GGGATTTTAGAACCAGGCAGAGCCGCCACCCTCTACAGTGGAGATAGTTGAGGGGAG |
| 43    |           | GGGATTTTAGAACCAGGCAGAGCCGCCACCCTCTACAGTGGAGATAGTTGAGGGGAG |
| 44    |           | GGGATTTTAGAACCAGGCAGAGCCGCCACCCTCTACAGTGGAGATAGTTGAGGGGAG |
| 45    |           | GGGATTTTAGAACCAGGCAGAGCCGCCACCCTCTACAGTGGAGATAGTTGAGGGGAG |
| 46    |           | GGGATTTTAGAACCAGGCAGAGCCGCCACCCTCTACAGTGGAGATAGTTGAGGGGAG |
| 47    |           | GGGATTTTAGAACCAGGCAGAGCCGCCACCCTCTACAGTGGAGATAGTTGAGGGGAG |
| 48    |           | GGGATTTTAGAACCAGGCAGAGCCGCCACCCTCTACAGTGGAGATAGTTGAGGGGAG |
| 49    | Twisting block 8 | TATACATGGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 50    |           | TATACATGGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 51    |           | TATACATGGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 52    |           | TATACATGGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 53    |           | TATACATGGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 54    |           | TATACATGGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 55    |           | TATACATGGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 56    |           | TATACATGGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 57    |           | TATACATGGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 58    |           | TATACATGGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 59    |           | TATACATGGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 60    |           | TATACATGGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |
| 61    | Flag 1    | GACGCTTGAATATGAGAGCTACCTTGGAGATAGTTGAGGGGAG |

Flag 1
---

AAACAAAATTTTGTCTTGAATAGTTAATACCATCAGTGCGAG
AAAGGGTGAAAGCTGAGTTTTGCCAGAGGGTCAGGAACGGTGT
62
AACGAGAAAACGGGTTTAGCTATATTTTATTCTACGTCATTG
63
AACGCCAATCAGCTCATAATTTTTATTTAATCAGCGGTAAT
64
AGATAGGCAACCGTGCAATAAAGCCTCAATTCCCATCAAAGC
65
AGCTGGCGGGTAACGCCAGGGCACGACGTTGTAAAACGACGGTTA
66
AGTAGCATAACCTGATTGCATCAAAAAGTAAACAGGGTGGTT
67
AGTTGAAAGGGGGAAGGGCGCCATTTTTCCGGTCAGGAATAA
68
ATATTCACTTCAAAAGTTTGACCATTAGAGGCAAGGGAGAGG
69
ATCGGTTTTTCATTGCAAACTCCAACAGGGGTAATAGTCCAC
70
ATGGCTTTTACCAGGGCCCACTACGTGATTGAACCCTCATAT
71
ATTAGCATAGATTTTATCGCGTTTTAATCGGAATCTAGCCCG
72
ATTTTAAGGGCGATACGACGATAAAAACGGTCATTATAATGC
73
CAGAAGCATGACCACAGGCGAGCCCTGAGAGAGTTGTGAGAC
74
CCAAAAACAGGATGGCAAACAAGAGAATCGAGTAACAATAGG
75
CCAGCTGCCAGGTTGTTGCGCATTAGCCTGGACTGCTCGTG
76
CCGAATTITTTGGCATAATACATACATACATTGGAAGCC
77
CCGTCACGTTGATTCTCCATGTGAGCGATGAAAAAAGCC
78
CCTGAGGAGCCCCAGTAAATAACGAGCTACACCACCG
79
CCTGATAACAATCATACATAACCTCCTGATATTGTATCATCG
80
CGAAAGATTTGAAATACCTCCTTATAAATCTAATGCCGAAAGA
81
CGGTTGAGTTACGTAATACCTCCTCCTCCTGAAATCTGAAAT
82
CGTTGAGTAAACGTAATTIT
83
CCTGAATTITCATACATACAGTTTCTGAGGCTGCTCCTACTG
84
CTGACAGACGGCGCCAGATGTAAGCAATTITTTATACCC
85
CTGGAAGGGCGGAAAGCGACGAGCTGCAAGAAAGAGGAGGAA
86
CTGGTTTCTCGGGAAAAAGGTGCGATACATTITTTATACCC
87
CTGTGAGCTGTAAATACCATACATACATTGGAAGCC
88
GAGCTCAGAATATTCTCCTCTTTGAAAATTGCAATAAAAGAGGCT
89
GAGCTCAGAATATTCTCCTCTTTGAAAATTGCAATAAAAGAGGCT
90
GACAGAGCTGCAATATTCTCCTCTTTGAAAATTGCAATAAAAGAGGCT
91
GCATTGCGCATTCTGCAATTCTGCAAGGCGGCCAGTGCCAGCTTT
92
GCATTCTGCCAGGAAACTCTGCCAGGCCGCGCTCTGGGGGTT
93
GCTTTTGTATITCTTTTAAATATGCAATGTAATAAGATCTCAAA
94
GGGGAACAACAGCAGGTCTTTGAAAGAGGAGGCAA
95
GGGCAACTACGGCAGCAATTTATGCAATGTAATAAGATCTCAAA
96
GGTACCTTCCAGAATATTCTCCTCTTTGAAAATTGCAATAAAAGAGGCT
97
GTGCTTTTGTATITCTTTTAAATATGCAATGTAATAAGATCTCAAA
98
GATATCCAAATTTACATACATACATTGGAAGCC
99
GATATCCAAATTTACATACATACATTGGAAGCC
100
GATATCCAAATTTACATACATACATTGGAAGCC
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111
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112
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113
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114
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115
GATATCCAAATTTACATACATACATTGGAAGCC
116
GATATCCAAATTTACATACATACATTGGAAGCC
117
GATATCCAAATTTACATACATACATTGGAAGCC
118
GATATCCAAATTTACATACATACATTGGAAGCC
119
GATATCCAAATTTACATACATACATTGGAAGCC
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GATATCCAAATTTACATACATACATTGGAAGCC
121
GATATCCAAATTTACATACATACATTGGAAGCC
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GATATCCAAATTTACATACATACATTGGAAGCC
123
GATATCCAAATTTACATACATACATTGGAAGCC
124
GATATCCAAATTTACATACATACATTGGAAGCC
125
GATATCCAAATTTACATACATACATTGGAAGCC
126
GATATCCAAATTTACATACATACATTGGAAGCC
127
GATATCCAAATTTACATACATACATTGGAAGCC

Flag 2

ACAATCTTTCATTTACGCTTGACGTAAACGCAATATTCTACGGTAC
112
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
113
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
114
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
115
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
116
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
117
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
118
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
119
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
120
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
121
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
122
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
123
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
124
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
125
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
126
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
127
AGCGAAAGTGAAATCTGCGGTTACTCAGTCTGAGCTTTTTTTTTTTT
| 128 | CCATACCGAATCTTATAATAGAACAAGACATGCTCATGTAATTT |
| 129 | CCACGCTTTTTCACCAACATGTTTCGCGCTTACATTCAAT |
| 130 | CCTCAAAATAATAACAGATTGAAGATTAGACGCGATCAGAC |
| 131 | CCTGTTTTTCTACTCTTTGCTGACCCCTCTGAAAATAACGGAG |
| 132 | CGCCGCTCTGTGAAAATCCAGATAGCTTATATGAATAAGAA |
| 133 | GACGACCTGAGATCAAAGACGACGAGAAGATTGCAATTTGCA |
| 134 | GCTGAGAACCTCCGGCTTAGGGAATAGCGCTTT |
| 135 | GCTTAAATACATAAGATAGGAAATACGATTTGCTAAATAAA |
| 136 | GGGACATGGCAAGGGGCAATATTAAAGAAGAAATATAAGCA |
| 137 | GTATATACCGAGCTTGGCTCAATCTGAATAATITGAGGTT |
| 138 | GTTATCGGAGGCATAAACACTCTGTATATGAAACGGT |
| 139 | TAAACATCCATAAATAAAGAAATATGAAAAGTTGAAACCCGAGG |
| 140 | TATACTTCGATAAAATAGGAGACAGATTGTAAGG |
| 141 | TATATGTCCTTCTGCTGGCGAGATGTTT |
| 142 | TATCCATTACATGTTGATAAAAATAAGGGAAAAACACAGC |
| 143 | TGAATAACCTGAGTGGTTGGAATATCCCTTTTAAGGCTA |
| 144 | TGCTATATGCCAAGCCACGCCACGCCTGANACGAG |
| 145 | TTAATAAACGCTGAGACGCTGACAAATGATAGCAGAAG |
| 146 | TTATTGATACGATAGCTCAATATAGAGATTGCTAGATTTA |
| 147 | TTCAAAACGATGACGAAATATGAAAGAAGTTATAGG |
| 148 | TTGTTTCTGTGCTGAGCCACTTAACTGAGGTAAGAG |
| 149 | TTGTACCAGTATGAGTCAAAAGGCAAAGGCTTCAATAG |
| 150 | TTTCAGCAGTATGAGTCAAAAGGCAAAGGCTTCAATAG |
| 151 | TTGGTATACGTCGGAGTCACTGAGAAGTTTACAGAGC |
| 152 | TTGTTATACGTCGGAGTCACTGAGAAGTTTACAGAGC |
| 153 | TTGTACCAGTATGAGTCAAAAGGCAAAGGCTTCAATAG |
| 154 | TTGGTATACGTCGGAGTCACTGAGAAGTTTACAGAGC |
| 155 | TTGGTATACGTCGGAGTCACTGAGAAGTTTACAGAGC |
## Supplementary Table S18 (Continued).

### Moderate 2 design with 2-BP-insertion.

| Index | Location | Staple sequence |
|-------|----------|-----------------|
| 1     | Twisting block 1 | AGAATAGAAAACACTGTTACTTAGCAGAATAGCCGAGTTA |
| 2     |           | CGTCCTTGAGCATACGAGGGCATAGTAAAGAATGTTGCGAAGCCGCC |
| 3     |           | GCCAAAAGCTCCATATCCTGGGCATTTGACCCCCAGCGTTTCAGAAGTTTTGT |
| 4     |           | CGCGAGCCTGGAATATCGGAACCCTAAAGGCGATCTACGGAGTG |
| 5     |           | AGTTGAGGGAACCCACAACTTAAACGATAAATACCTGTAAGCA |
| 6     |           | ATCCCACATACCCAGTCACCTAACACAGAAAGGATATTTC |
| 7     | Twisting block 2 | TTCCACAGGGGAAAAATACACCATATCACTAGGGCCAAAAAGAAC |
| 8     |           | AGAAGCCTGAGTGATGTTAAAAAGCGCTCAAACAGGTTAAGAGAGGC |
| 9     |           | AAGACCTTATCAGGCGGTTAGACAGCCACACGATTTAGTGATAATTT |
| 10    |           | GTATCGGCTGCATTTTCTATGCCGGCTCTGTTAAAATATCGCCG |
| 11    |           | GAACGATCTGAGTCTGCACTATATCCCTCAGAAGCTGCAAGAGGAGAAC |
| 12    |           | AGGGGCCGTCAGACCCGCGAAGAGAGACTACAGCGAAGAGGAGAAC |
| 13    | Twisting block 5 | CCACCCCTACTATGGTTGTGAATTACCTTATCAAATCAGCGAAAGAC |
| 14    |           | CAAAGAAGCTGGAAGCGGGAGCTAAACAGGGAGGGTTCCACCAC |
| 15    |           | CGGGTGCACCAATTGAGCAGGTAGACAGGGAAGGCCTTCTGAGGACTA |
| 16    |           | ATCTTACAAGTTTAAATAACAGTTTAGAGTATAATATTAGAGG |
| 17    |           | ACCATTAAAACAAATGAGTAACAGTGCCGATATAACATAAGAATTGA |
| 18    |           | GATATTCACCCATTAGGACAAAAGGGCGACATAACCCACCACTTGCAG |
| 19    | Twisting block 7 | GAATTAGATAACAGTGCCCTTTTCTTACGCGTACAGCCGGCCGGGTTT |
| 20    |           | CGTCAGTAGTAAAGACCTCATTTGATACGGAGCAAGGAGGGTCACAGCC |
| 21    |           | CTTAGCCGAAAAGCATTACATCGCAGCCGAGGATAGTGAGG |
| 22    |           | ATACGACGTGAGTTACCGTGAGTATTGCGAGGAGCTGTAAGAAGGAGG |
| 23    |           | CTTGCTGAGTTTTCTTATGAGGAGCTGTAAGAAGGAGG |
| 24    |           | GTTGTTATGAGAGCTGTAAGAAGGAGG |
| 25    |           | TAACGACGTGAGTTACCGTGAGTATTGCGAGGAGCTGTAAGAAGGAGG |
| 26    |           | GTTGTTATGAGAGCTGTAAGAAGGAGG |
| 27    | Flag 1    | AAAACAAAATTTTCTAGTAAATAGAATACCTGACACTGAGCAGG |

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## Supplementary Table S18 (Continued).

**Flexible 1 design with 2-BP-insertion.**

| Index | Location | Staple sequence |
|-------|----------|-----------------|
| 1     | Twisting block 1 | ACGCCAAAAACGCTCATTCTTTGACCCCCAGCTTTTACAAAGTGGTTTG |
| 2     |            | TCACCAGCTCTGAGGAAATTTAATCGCCAGAAGGCTGACCCAGAGGAGGA |
| 3     |            | ATACACTAAGGAAACACTCTGTTAGTGGCTAAGGAGGGCCGACATATA |
| 4     |            | ATAGAAAACACTCTGTTAGTGGCTAAGGAGGGCCGACATATAAGGAGG |
| 5     |            | TCCTTCTTACACTACCTCCGCTTTTAGTTATTAGTGGCTAAGGAGGG |
| 6     |            | CGTAAACCGAGGAGGAAATTTAATCGCCAGAAGGCTGACCCAGAGGAG |
| 7     | Twist block 2 | TTCCACAGGGGAAAAATACCACATTCAACTGAGGCGCCAAAAGA |
| 8     |            | AGAGCTTGACGACAGCCACTAAAGGAATTGCGAAAAGAGGAGACGGTCA |
| 9     |            | ATGCCACATCTCCACGTCACCAGTACAAACAGAAAGGAGATTCA |
| 10    |            | TCAGTTGAGGGGAAACCGCACCACACCTACAAACGGCAGATTACCTAGC |
| 11    |            | CGTTGAAATAGGAAACACTCATAGTTAGCGTAAGAGCCCCATACATA |
| 12    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 13    | Twist block 3 | ATTTGACATGTTTTTCTAGGTCGAGCTTAAAAATACCCGAGG |
| 14    |            | AAGACTTTATCAGCGGGATAGCAAGCCCAACAAGTGTACGTATTTAA |
| 15    |            | ATGCCTAACCACATAAGGAGGAAATTTAATACCCGAGG |
| 16    |            | CGTTGAAATAGGAAACACTCATAGTTAGCGTAAGAGCCCCATACATA |
| 17    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 18    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 19    | Twist block 4 | TTCCACAGGGGAAAAATACCACATTCAACTGAGGCGCCAAAAGA |
| 20    |            | AGAGCTTGACGACAGCCACTAAAGGAATTGCGAAAAGAGGAGACGGTCA |
| 21    |            | ATGCCACATCTCCACGTCACCAGTACAAACAGAAAGGAGATTCA |
| 22    |            | TCAGTTGAGGGGAAACCGCACCACACCTACAAACGGCAGATTACCTAGC |
| 23    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 24    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 25    | Twist block 5 | ATTTGACATGTTTTTCTAGGTCGAGCTTAAAAATACCCGAGG |
| 26    |            | AAGACTTTATCAGCGGGATAGCAAGCCCAACAAGTGTACGTATTTAA |
| 27    |            | ATGCCTAACCACATAAGGAGGAAATTTAATACCCGAGG |
| 28    |            | CGTTGAAATAGGAAACACTCATAGTTAGCGTAAGAGCCCCATACATA |
| 29    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 30    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 31    | Twist block 6 | TTCCACAGGGGAAAAATACCACATTCAACTGAGGCGCCAAAAGA |
| 32    |            | AGAGCTTGACGACAGCCACTAAAGGAATTGCGAAAAGAGGAGACGGTCA |
| 33    |            | ATGCCACATCTCCACGTCACCAGTACAAACAGAAAGGAGATTCA |
| 34    |            | CGTTGAAATAGGAAACACTCATAGTTAGCGTAAGAGCCCCATACATA |
| 35    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 36    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 37    | Twist block 7 | TTCCACAGGGGAAAAATACCACATTCAACTGAGGCGCCAAAAGA |
| 38    |            | AGAGCTTGACGACAGCCACTAAAGGAATTGCGAAAAGAGGAGACGGTCA |
| 39    |            | ATGCCACATCTCCACGTCACCAGTACAAACAGAAAGGAGATTCA |
| 40    |            | CGTTGAAATAGGAAACACTCATAGTTAGCGTAAGAGCCCCATACATA |
| 41    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 42    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 43    | Twist block 8 | TTCCACAGGGGAAAAATACCACATTCAACTGAGGCGCCAAAAGA |
| 44    |            | AGAGCTTGACGACAGCCACTAAAGGAATTGCGAAAAGAGGAGACGGTCA |
| 45    |            | ATGCCACATCTCCACGTCACCAGTACAAACAGAAAGGAGATTCA |
| 46    |            | CGTTGAAATAGGAAACACTCATAGTTAGCGTAAGAGCCCCATACATA |
| 47    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 48    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 49    | Twist block 9 | TTCCACAGGGGAAAAATACCACATTCAACTGAGGCGCCAAAAGA |
| 50    |            | AGAGCTTGACGACAGCCACTAAAGGAATTGCGAAAAGAGGAGACGGTCA |
| 51    |            | ATGCCACATCTCCACGTCACCAGTACAAACAGAAAGGAGATTCA |
| 52    |            | CGTTGAAATAGGAAACACTCATAGTTAGCGTAAGAGCCCCATACATA |
| 53    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 54    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 55    | Twist block 10 | TTCCACAGGGGAAAAATACCACATTCAACTGAGGCGCCAAAAGA |
| 56    |            | AGAGCTTGACGACAGCCACTAAAGGAATTGCGAAAAGAGGAGACGGTCA |
| 57    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 58    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 59    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 60    |            | ATCATAAAATGGGCGGCGGAAACGAGGTCACCAAGAGAAGGAAGGCA |
| 61    | Flag 1     | AAAAGGGTGACCTGTTGTTTTTCCAGAGGTCAGAGGAGGAGGAGGAGGA |
Flag 2

AACAAAATTTTGCTTAGTAAATGAATTACCATCAGTCGAGGTGC
AACGAGAAAAGCGGTTTAGCTATATTTTATTCTACGTCATTG
AACGCCAATCAGCTCAAATATTTAAATTTAATCAGCGGTAAT
AGATAGGCAACCGTGCAATAAAGCCTCAATTCCCATCAAAGC
AGCTGGCGGGTAACGCCAGGGCACGACGTTGTAAAACGACGGTTA
AGTAGCATAACCTGATTGCATCAAAAAGTAAACAGGGTGGTT
AGTTGAAAGGGGGAAGGGCGCCATTTTTCCGGTCAGGAATAA
ATATTCACTTCAAAAGTTTGACCATTAGAGGCAAGGGAGAGG
ATCGGTTTTTCATTGCAAACTCCAACAGGGGTAATAGTCCAC
ATGGCTTTTACCAGGGCCCACTACGTGATTGAACCCTCATAT
ATTAGCATAGATTTTATCGCGTTTTAATCGGAATCTAGCCCG
ATTTTTAAGGGCGATACGACGATAAAAACGGTCATTATAATGC
CAGAAGCATGACCACAGGCGAGCCCTGAGAGAGTTGTGAGAC
CCAAAAACAGGATGGCAAACAAGAGAATCGAGTAACAATAGG
CCAGCTGCCAGGGTGGTTTGGCGCATCGGATCGCACTTCGTG
CCGAAATTTTTTGACCAATAAATCATACATACATTGGAAGCC
CCGTCACGTTGATTCTCCATGTGAGCGATGAAAAAAGCC
CCTGAGAGCCCCGTAAATACCAAAAAATGAGGTCTAAATCAGG
CGTTCAGTTGATCTCTAGTGAGATCGGTTCTTTTATTACC
CTGGAAAGGTACCAAGCCGGAGACAGTCAGAACAAGAGTAAAA
CTGGTTTGTCTGGAAAAAGGTGGCATCACATTTGGTTATAGT
CTGTGTGAGTGTAAAGCCTCAACTGTTGGGATGTGCTGTTTC
GAACCAGACTGGATGTTGTTCCAGTTTGAATCACCAAGCTAA
GAACGAGAAATTAATCTAGCTGATAAATAAAAGAAGTCATAA
GAGCTCACAATACATACGACATTACCTGCTACCCAACCGACGC
GATCTGCTACATGCACTTTGTTTAAATATGCAATGTAATAAGATTCA
GGGAGAACAACATGTCCTTTTGATAAGACAAAATAAAAACCG
GGGCAACTTGCGTATTGGGCGCATTAATGAATCGGTGCCCGC
GGTAGCTGATCCGCCTGAAATCCTCTATCAGTAATAGT
GTACCTTCAAAAGACTCAGTCAATATCGCAGGTTACCCCAACGC
TTACCCTCAAAAGACTCAGTCAATATCGCAGGTTACCCCAACGC
TATCCGCTGAAATCTGCAATGTTACCTGACCCTGAGGAGGACC
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CTATCAATGCAATTTTACAAGGAGGATGTTTATTGGCC
TCTGGTGCGGCAAACGGCCATTCTCGATGGCGGGCTAGCAGCC
TGATATTGGTACGAGCATGTAGGTATAAACACCGGA
TGTTAGACGGGAACTCATATAACGCTGAGGATCAATAGT
TGACCGACAGCAAGGCGATTCCGCGTGAGTGAATGGTCCAG
TTTGTAACTAAAAAAACTATAGGTTGGAACAGGTTAATAGT
TTTCCAGGAGTGAACATTCACTACGGGCGAAGACTTTAATTTG
TTTACTGTATGGGTAACACGGGAGAGCTTAAATTTTAAAT
ACAAATCTTTTGGACGTTTCAGTAAATCAGAATACCCTGTCAC
AGCGAAACAGCAAAGAGGATGTTGGAAGAGGATTACACGCA
AGGGAACAGTATTCTGTAAATAGAGATAGAACAAAATGACG
AGCTGACCTTCAATCTGCTTTTATTATGCTTAAATATGAGG
AGTTAAGAGTTTTACAAATGACGCGGGATTACACGCA
AGTTGAAAGGGGGAAGGGCGCCATTTTTCCGGTCAGGAATAA
ATTAGATAGTATTGCTACAAACACCGTATTAAAGATATAAAA
ATTATGAACTATGGGAAAGAACGCGACGCGTTTCCTAAATAGG
CTAACAAATACCCGCTGAGCAAAGAAGAGGCGGCTAGCCTAAT
CATATTAAATACCCGCTGAGCAAAGAAGAGGCGGCTAGCCTAAT
CCATACCGAATCTTTAAATAGAACAAATGCTCATGTAATTT
CCCTCAAAATATAATCAACAGTGTGAATTAGAGCGCCTGAAGAC
CCTGTTTTTCTTACTTCTACTTCTAAGCTCCCTCTGAAATACGAG
CGGCGTTCTGTAAATCCATAGCGATAGCTTAGATTCAAAAGAA
GACGAGCGAGGTGAAATCAAGAAACGCGAGAGACCAGTTTTGCG
GCTGAGAACCTCCCAGTTGGAATACGGCGTTTT
GCTTAGACATCTGTAATAAGTAGGAATATTAGTTTTGATCAATTT
GGGACATCGCAAGACGCCATATTTAACAAGTAATTAATAGCA
GTATTAACCAGAGCTTGACGCTCAATCGTAAATTTATGCGTT
GTTATCTTAGGAGAATTTAAGAAGGTTACTTTTGAT
TATCCATTACTAGTGTGATAAAATAAGGGAAAACAACAGGC
TGAAATAACCTGAGTTTTGAATTTTCACTCTTTTTAAGAGCTCA
TGGCTATTAGCGAACCACCAGCAAGACGCGCTCAGACCAG
TTAAAGCGCTGAGAGCAGCAAATCTAAAGCATCGAGGAAG
TTAATTTTGTATTATACAGATGTACTTAAATATGTGATTTTATA
TTACAAAGGCTCAGTTAAGATGCGCAATATCTGCTTATCCAGAG
TTTACATAGTTATACGTAAGAAGCGCAAATATGACCAAGAGCG
TTTGTTACTGCAGTGTACCAAAGTCTGCTGTAACGTTCTCGAG
TTTACATCGAATTTTGTCTTTCGAGACGAC
TTTTATACAAATTTTCACCCAGTCAACAAATTTTCTACAGGA
| Index | Location    | Staple sequence                        |
|-------|-------------|----------------------------------------|
| 1     | Twisting block 1 | ATACACTAAAGGAAACTCATGTTAGCTTAGGACGCCCCCATACATA |
| 2     |              | CGGCACTTGGAATTATCGGAAACCTTAAAGGCGATCTACGGAGTGAAG |
| 3     |              | ACGGCAAAAGCCTCATATCTTTGGACCCCCGCGTTGAGATGTTTG |
| 4     |              | ATAGAAAAACCTGTTACATTTGAGGCCAAGATCGAGAGGTTT |
| 5     |              | TCGGCTTTGCACTAAGGAGCCATAGTAAAGGAAATTGTGCAGAACGGCG |
| 6     |              | CGTTAACCAGAGTTAACAACTTTTCACAGATATTATGAGATCGAGAATTCAT |
| 7     | Twisting block 2 | ATCATAAAATTAGGGCCGACCGAAAGGTCGAGAGACCTTTTTCTCAG |
| 8     |              | TTAGGGGAACCGGCAAAACCTTTAAACGATTAAACCTCTGAGATCACTC |
| 9     |              | ATGCACACTTCCACGCTACCGATCAACAAACAGAGGATGTCATAGTCAG |
| 10    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 11    |              | ACAGGGAAGAGGAGACCTTTAAAGGCAAAAAGAGGAGATGTCATAGTCAG |
| 12    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 13    | Twisting block 3 | AGCAAAGCAATGATTATAAACAGGCTGCAAACGGTTACAGAATTCAGATGA |
| 14    |              | ACGGCAAAAGCCTCATATCTTTGGACCCCCGCGTTGAGATGTTTG |
| 15    |              | ATAGAAAAACCTGTTACATTTGAGGCCAAGATCGAGAGGTTT |
| 16    |              | TCGGCTTTGCACTAAGGAGCCATAGTAAAGGAAATTGTGCAGAACGGCG |
| 17    |              | CGTTGAAATACGAAGGAACTGACCACTTTGGGTAGAAAAGGGA |
| 18    |              | AGAGCTTGACGACAGCCACTAAAGGAATTGCGAAAAGAGGAGACGGTCA |
| 19    |              | ATCATAAAATTAGGGCCGACCGAAAGGTCGAGAGACCTTTTTCTCAG |
| 20    | Twisting block 4 | ATGCACACTTCCACGCTACCGATCAACAAACAGAGGATGTCATAGTCAG |
| 21    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 22    |              | ACAGGGAAGAGGAGACCTTTAAAGGCAAAAAGAGGAGATGTCATAGTCAG |
| 23    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 24    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 25    | Twisting block 5 | AGCAAAGCAATGATTATAAACAGGCTGCAAACGGTTACAGAATTCAGATGA |
| 26    |              | ACGGCAAAAGCCTCATATCTTTGGACCCCCGCGTTGAGATGTTTG |
| 27    |              | ATAGAAAAACCTGTTACATTTGAGGCCAAGATCGAGAGGTTT |
| 28    |              | TCGGCTTTGCACTAAGGAGCCATAGTAAAGGAAATTGTGCAGAACGGCG |
| 29    |              | CGTTGAAATACGAAGGAACTGACCACTTTGGGTAGAAAAGGGA |
| 30    |              | AGAGCTTGACGACAGCCACTAAAGGAATTGCGAAAAGAGGAGACGGTCA |
| 31    |              | ATGCACACTTCCACGCTACCGATCAACAAACAGAGGATGTCATAGTCAG |
| 32    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 33    |              | ACAGGGAAGAGGAGACCTTTAAAGGCAAAAAGAGGAGATGTCATAGTCAG |
| 34    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 35    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 36    | Twisting block 6 | AGCAAAGCAATGATTATAAACAGGCTGCAAACGGTTACAGAATTCAGATGA |
| 37    |              | ACGGCAAAAGCCTCATATCTTTGGACCCCCGCGTTGAGATGTTTG |
| 38    |              | ATAGAAAAACCTGTTACATTTGAGGCCAAGATCGAGAGGTTT |
| 39    |              | TCGGCTTTGCACTAAGGAGCCATAGTAAAGGAAATTGTGCAGAACGGCG |
| 40    |              | AGAGCTTGACGACAGCCACTAAAGGAATTGCGAAAAGAGGAGACGGTCA |
| 41    |              | ATGCACACTTCCACGCTACCGATCAACAAACAGAGGATGTCATAGTCAG |
| 42    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 43    | Twisting block 7 | AGCAAAGCAATGATTATAAACAGGCTGCAAACGGTTACAGAATTCAGATGA |
| 44    |              | ACGGCAAAAGCCTCATATCTTTGGACCCCCGCGTTGAGATGTTTG |
| 45    |              | ATAGAAAAACCTGTTACATTTGAGGCCAAGATCGAGAGGTTT |
| 46    |              | TCGGCTTTGCACTAAGGAGCCATAGTAAAGGAAATTGTGCAGAACGGCG |
| 47    |              | AGAGCTTGACGACAGCCACTAAAGGAATTGCGAAAAGAGGAGACGGTCA |
| 48    |              | ATGCACACTTCCACGCTACCGATCAACAAACAGAGGATGTCATAGTCAG |
| 49    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 50    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 51    | Twisting block 8 | AGCAAAGCAATGATTATAAACAGGCTGCAAACGGTTACAGAATTCAGATGA |
| 52    |              | ACGGCAAAAGCCTCATATCTTTGGACCCCCGCGTTGAGATGTTTG |
| 53    |              | ATAGAAAAACCTGTTACATTTGAGGCCAAGATCGAGAGGTTT |
| 54    |              | TCGGCTTTGCACTAAGGAGCCATAGTAAAGGAAATTGTGCAGAACGGCG |
| 55    |              | AGAGCTTGACGACAGCCACTAAAGGAATTGCGAAAAGAGGAGACGGTCA |
| 56    |              | ATGCACACTTCCACGCTACCGATCAACAAACAGAGGATGTCATAGTCAG |
| 57    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 58    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 59    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 60    |              | CAGCAGAAAATACCACATTCAGTGGGGACCGGAAAAGAAGAGGTCAGGAG |
| 61    | Flag 1       | AAAAAAGTGAACGTGAGTTTTGCCAGAGGGTCAGGAACGGTGT |
AACAAAAATTGCTTAGTAAATGAAATACCATCAGTCGAGGTGC
AACGAGAAAAGCGGTTTAGCTATATTTTATTCTACGTCATTG
AACGCCAATCAGCTCAAATATTTAAATTTAATCAGCGGTAAT
AGATAGGCAACCGTGCAATAAAGCCTCAATTCCCATCAAAGC
AGCTGGCGGGTAACGCCAGGGCACGACGTTGTAAAACGACGGTTA
AGTAGCATAACCTGATTGCATCAAAAAGTAAACAGGGTGGTT
AGTTGAAAGGGGGAAGGGCGCCATTTTTCCGGTCAGGAATAA
ATATTCACTTCAAAAGTTTGACCATTAGAGGCAAGGGAGAGG
ATCGGTTTTTCATTGCAAACTCCAACAGGGGTAATAGTCCAC
ATGGCTTTTACCAGGGCCCACTACGTGATTGAACCCTCATAT
ATTAGCATAGATTTTATCGCGTTTTAATCGGAATCTAGCCCG
ATTTTAAGGGCGATACGACGATAAAAACGGTCATTATAATGC
CAGAAGCATGACCACAGGCGAGCCCTGAGAGAGTTGTGAGAC
CCAAAAACAGGATGGCAAACAAGAGAATCGAGTAACAATAGG
CCAGCTGCCAGGGTGTGTTGGCAGCTGGATGCTCCTCGTG
CCGTCAGGGTAAATCTCATACATACATTGGAAGCC
CCGTCACGTTGATTCTCCATGTGAGCGATGAAAAAAGCC
CCTGAGAGCCCCATCAAATATCACTCGATGAGTATTTTAGC
CGAAGATTGAGTATCCTGCTAACCAACCCGAAGAAGAGGCC
CGGTTGAGTAAACGTAAATTT
CGTAAAATTCATCATAATTCG
CTGACTAGGGCSCCAGGATGTAIAAAGCATTTTTATAC
CGTAAGGCTACAGCTCTACCTGCAAAGGAGCTGCAACTGTT
GCCATACCGTCTCACTGCAAAGGAGCTGCAACTGTT
GCTTTTGTGATTTTAAAATGCAATGTAATAAGATTCA
GGGAGAACAACATGTCCTTTTGATAAGACAAAATAAAAACCG
GGGCAACTTGCGTATTGGGCGCATTAATGAATCGGTGCCCGC
GGTAGCTGATCCGCCTGAAATCCTCTATCAGTAATAGT
GTACCTTCAAAAGAGACTCCAACGTCAATAGGTAACTTTTGC
GTAGCTACGGCAAACCCCTCAAATGCTTATTAAGATCGCAAA
TACCGCTCGAATCTGAACTGCTGAGCTCGCTCCTCGAGATTAC
TATTAAAAGGAAAGAAACATGTAAGCTACCCACTTATTTTGA
TCTCATATGGCATTTTCACCGAGAAGCTGATTTTIGGC
TCTGGTGCGCAAAGGCGGACCTTCTCGGACCGGCTACCGCT
TGATATTGAGTAGCGTCCAATACTGTCGAGCTATTCTGC
TGCTCAATTAACAGAGACTCTCAACAAAGGGTTTGATTTTGC
TGTAAGCTGCCCATGCAAGGAGCTCCATAGTTAAATACGTTA
TGTTAAGCCGGAACCTTACATAGTTAAATACGTTA
TTACCAGACCGAGACCGGCAGTCCCCTGCCGCTGAGTATTTTGC
TTGTTAATCAAAAAAACAATTAAGTGGGAAACAGGTTATAGG
TTTCCAGGAGTGAATCTCAGCGACAGGGTAAATTGCTA
TTTACTGTAAGGGGCTAACCGAGAAGCTTTATTTTGA
ACAATATCTTITTCAGCGTTCAGAAGCAATACCACTCGTCAC
AGCCAAACAGACAGACAGGGCGAAGCTGCAACTGTT
AGGGCGATTTCTGTAATAAGAGAATAAGAGGCAATATAAC
AGGCTGAATATGACTTCTCAGCTCGAGTCTGAGATTAT
ATACCAAAATCACCCACCTACGCCATTCAAGCAGTATTAACAC
ATATTTTGCGCATATCCAGATTTTGAACAGAAGCGGACC
ATCAATTATGAGAAAGATATTTACATACATTGCCGAGAAC
ATCATTGATTTTGGCGCAAAACCGAGACAGCTGCAACTGTT
AGTGAGTAAGTGGGAAACAGGTTAGACAGGTTA
ATTATTGATGTTGCGACAACCAGCTATTAAAGATAAAATA
CAAAAGCGTATTTCTAACAAGATACGTTAAGTCTGGA
CAATTACACAGGCGTGAGCAGAAAAAGAGGCGGCGCTCCTAAT
CATATTATACCGGTTAGAGCTGATGATAAAACCCCGA
| 128 | CCATACCGAATCTTTAAATAGAACAAATGCTCATGTAATTTTT |
| 129 | CCACGCTTTTTCATCAACACATGTTCCACCCGCTAAATTCAAT |
| 130 | CCTCAAAATATAATCAACAGTGTAGTAGGGGCTGGAACGAC |
| 131 | CCTGTTTTTCTACTTCTATCTGACTCCTGGAAATAACGGAG |
| 132 | GCCGCTTTCTGTGAAAATCCATAAGCTAGCTAGTATACAA |
| 133 | CGTGTTACCAAATGATAGATAAGCTGTTACCTGAAATAACGAG |
| 134 | GATGACCTTAGAGAAGGAAACAGCAGCACGTGGTTTTG |
| 135 | GCTGAGAACCTCCGGCTTAGGGAATACGGCGTTTTG |
| 136 | GCTTAATACATAATAGGTAGGGAAATACGGCGTTTTG |
| 137 | GTAATACATAATAGGTAGGGAAATACGGCGTTTTG |
| 138 | GTTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 139 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 140 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 141 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 142 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 143 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 144 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 145 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 146 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 147 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 148 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 149 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 150 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 151 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 152 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 153 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 154 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
| 155 | TTAAATTCCGTAGCTCGAAATAACGTTAGG |
Supplementary Table S19. MALDI-TOF results by Bio-RP and PAGE methods. For the comparison of Bio-RP and PAGE methods in oligonucleotides purification, representative ten staples were used to MALDI-TOF (Supplementary Figure S11). The error is the difference in molecular weight divided by the reference value.

| Index | Staple sequence | Molecular weight [Da] (Reference) |
|-------|-----------------|----------------------------------|
| 1     | TCGTCTTTTGACTAACGAGGCATAGTAAGAGAATTGTGCCAAGCGCA |
| 2     | TTCCACAGGGGAAAAATACCACATTCAACTGAGGCGCAAAGGA |
| 3     | ACACAGGAGCAGGAAACCATTATTACAAAAAGGAATACGTAG |
| 4     | CCTCGTAACCATCAGGACGTTGGGAAGGACCTTCTGAGGACTA |
| 5     | CGCCACCCTACTATGGTGTGAATTACCTTATCAAATCAGCCAAGA |
| 6     | ATTACTCCCTCGGATAAGTGCCGTCGAAGGCCGACGGAATACCCAA |
| 7     | CAGTCAGGAACAAACCGAGGAAACGCATTAGCAACATAGCCCCCTT |
| 8     | AGAGGTCTAGGCAACCTTATTTAAGAAGAAGTAGAAGCTGCTGCTT |
| 9     | CCCTGCTAGGAGAAGCAGAAACAAAGAAGATAACCGATCAGCAT |
| 10    | ACGGGGTCAGAACTCCTAATATCAGAGAGATTCAACCCGACCGATAGCACA |

| Index | Molecular weight [Da] (Bio-RP) | Molecular weight [Da] (PAGE) | Error [%] (Bio-RP) | Error [%] (PAGE) |
|-------|-------------------------------|-------------------------------|-------------------|-----------------|
| 1     | 14863.0                        | 14811.0                       | 0.15              | 0.20            |
| 2     | 13559.0                        | 13539.0                       | 0.10              | 0.04            |
| 3     | 13339.0                        | 13317.0                       | 0.08              | 0.08            |
| 4     | 13285.0                        | 13238.0                       | 0.24              | 0.11            |
| 5     | 14406.0                        | 14361.0                       | 0.11              | 0.20            |
| 6     | 14139.0                        | 14099.0                       | 0.18              | 0.10            |
| 7     | 14116.0                        | 14091.0                       | 0.12              | 0.06            |
| 8     | 13595.0                        | 13555.0                       | 0.23              | 0.07            |
| 9     | 14488.0                        | 14434.0                       | 0.14              | 0.23            |
| 10    | 15097.0                        | 15066.0                       | 0.13              | 0.07            |
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