Direct Single-Molecule Observation of Mode and Geometry of RecA-Mediated Homology Search.

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Supporting Information

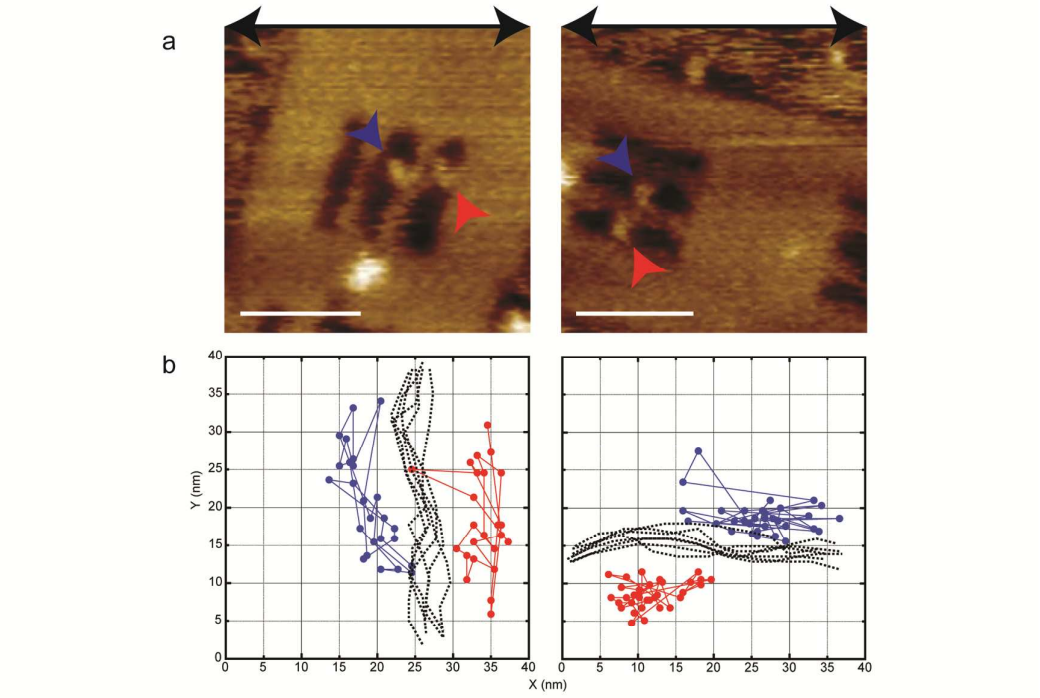

Figure S1. The observed motions of RecA nucleoprotein filaments are independent of the HS-AFM scan angle within the DNA origami frames. a) HS-AFM images of the same DNA origami frame orientated with the central DNA strands perpendicular (left) and parallel (right) to the fastscan axis of the scanning probe (indicated by the black arrow), respectively. b) X–Y plots of the positions of the centre of mass of the two nucleoprotein filaments relative to the origami at different time points (red and blue arrows in the AFM image, and red and blue points in the X–Y plot, respectively). The dotted lines indicate the position of the dsDNA at the same time points. No significant difference in nucleoprotein motion is observed. Scale bar = 40 nm. Z scale = 6 nm.

Quantification of observed nucleoprotein filament motions:
Where nucleoprotein filaments are observed to be associated with dsDNA within the DNA frames, their positions relative to the frame structure can be tracked. The position of the centre of mass of the nucleoprotein filament is measured along the length of the associated DNA strand from one end. The termini furthest from the polarity marker is used as zero. All measurements were conducted using the ImageJ software (see methods).

All distances were measured in nm (using appropriate pixel to nm scaling). The measurements in nm were converted to bp, assuming a standard B form helical rise of 3.4 nm per 10 bp. The positions of the nucleoprotein filament were plotted for a given image sequence, from which the motions of the nucleoprotein filaments were extracted. From this, two types of observed motions were identified, hops and slides, along with stalls.

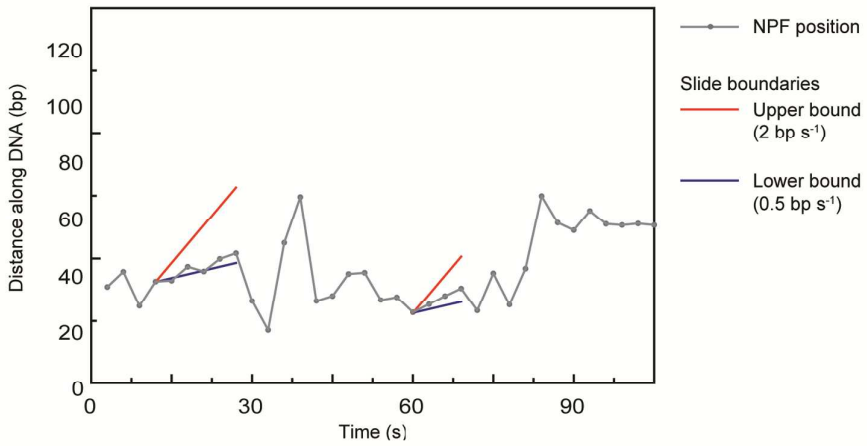

Figure S2. Definition of "slide" motion boundaries. A representative motion trace with two instances of sliding motions highlighted. The upper (red) and lower (green) bounds used to define the sliding motions are provided.

Sliding motions are defined by three or more consecutive points of unidirectional motion whose velocity falls between an upper bound of 2 bp s^{-1} and a lower bound of 0.5 bp s^{-1}. An example of this can be seen in figure S2 (the same plot as figure 2), where two representative slides are defined and the upper (red) and lower (blue) bounds are indicated.

Hopping motions are defined as large distance moves, often associated with a change in direction and where the rate exceeds that of the upper bound (2 bp s^{-1}).

Stalls are defined as any consecutive points where the rate falls below the lower bound of 0.5 bp s^{-1}. In practice, these are found to be distinctive from the designated lower bound, typically with observed rates of 0.1 – 0.2 bp s^{-1}.

Several representative motion plots are given in figure S3 where examples of sliding motions are highlighted in line with the described boundary conditions.

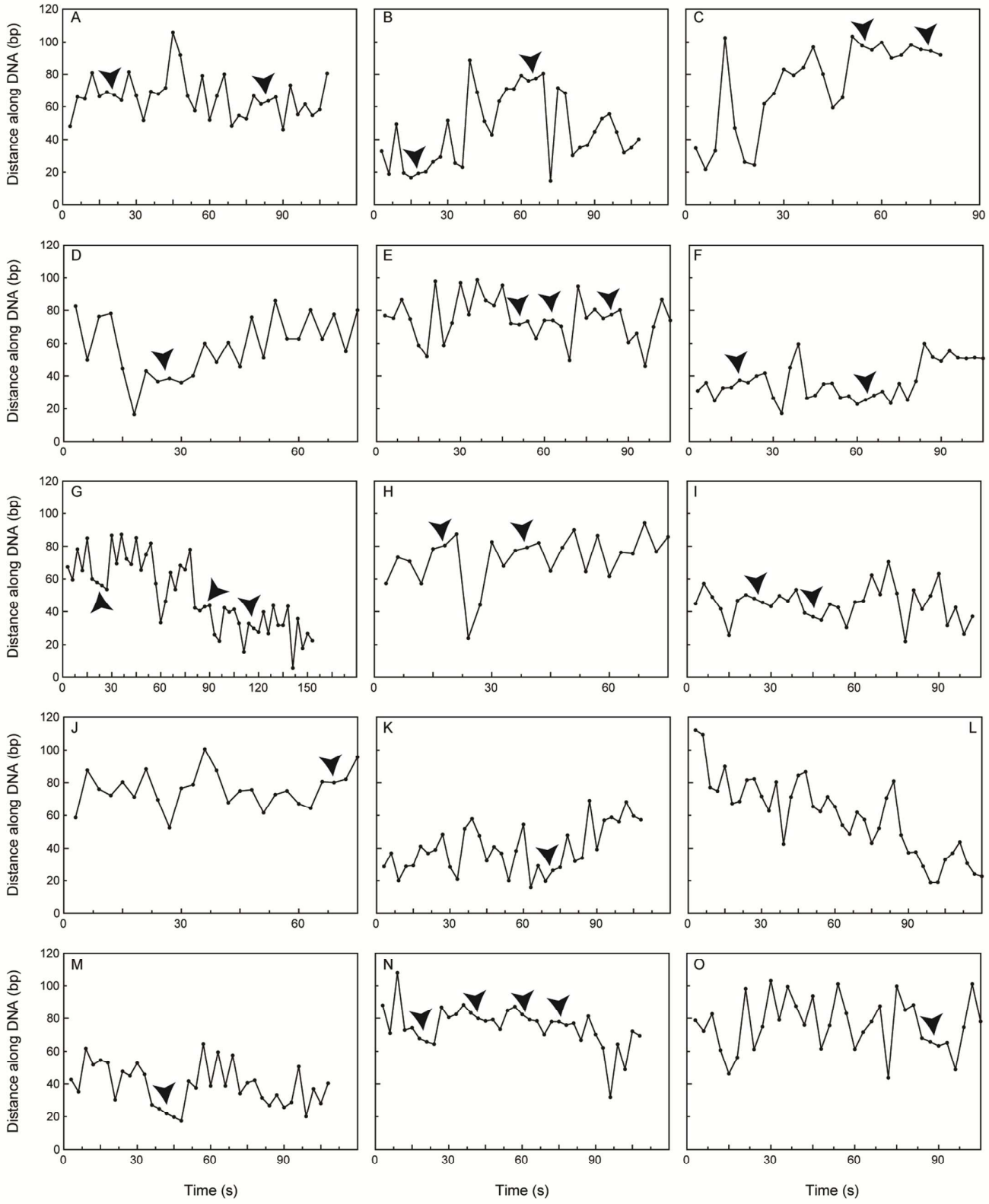

Figure S3. (A – O) Representative motion traces of nucleoprotein filaments moving along a dsDNA molecule. The filaments centre of mass is plotted as distance of the filament from the end of the dsDNA strand closest to the polarity marker vs time. Occurrences of facilitated diffusion, ``slides'' along the DNA are indicated (black arrows).

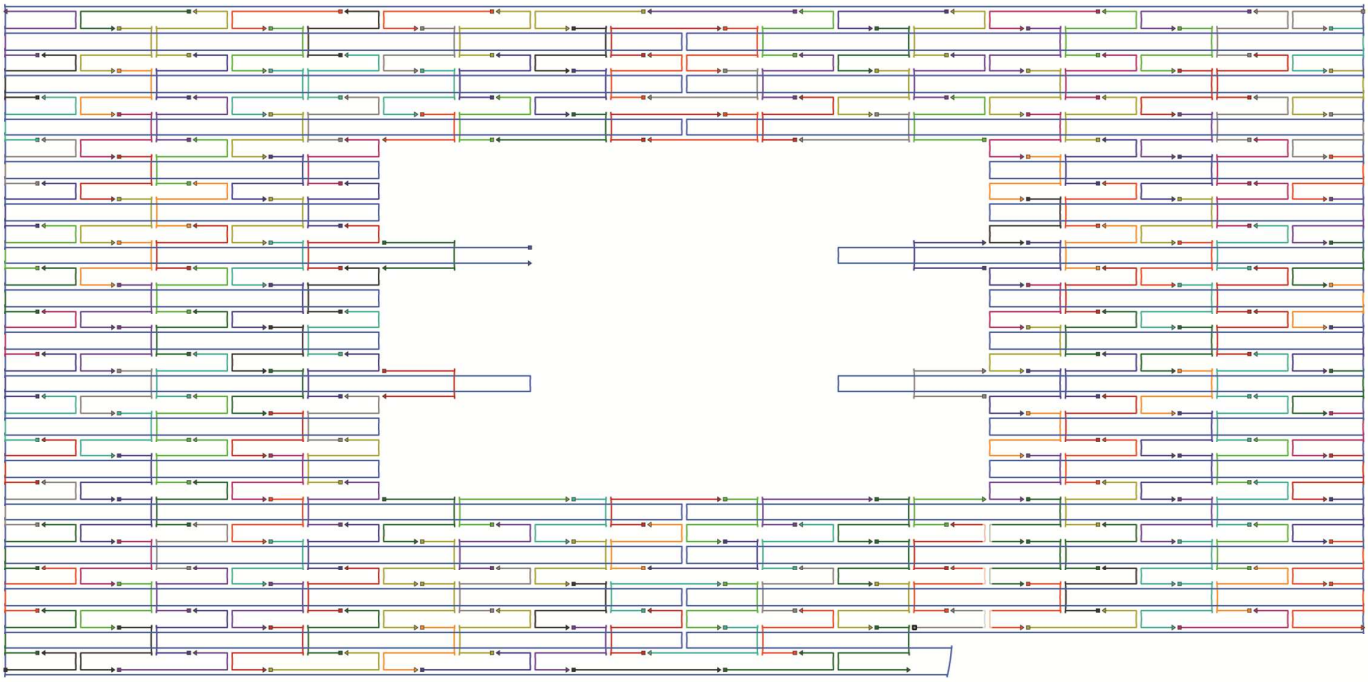

Figure S4. Schematic diagram depicting the scaffold and staple routing of the DNA frame. The M13mp18 ssDNA scaffold which runs around the entire structure (blue line) is held in place by 220 oligonucleotide staples (various colours) to form the desired frame structure.

Descriptions of Movies:

Movie S1: RecA nucleoprotein filament searching for homology within a DNA origami frame. Observations of the motion of nucleoprotein filaments undertaking a search for sequence homology are depicted. These motions are shown to be independent of the AFM scan angle. Example observations of the distinctive modes, facilitated "sliding" along the dsDNA and "hopping" – where the nucleoprotein filament detaches from the dsDNA and re-engages at a distal sequence – are presented.

Movie S2: Homology searching on non-contiguous DNA strands. A nucleoprotein filament is shown to search along one dsDNA strand before switching to a different strand within the DNA frame. The centre of mass of the nucleoprotein filament is presented as a vector plot with respect to the positions of the dsDNA molecules (black and grey lines) over time (Z) for clarity.

Movie S3: A stable synaptic joint formed at the region of homology. A nucleoprotein filament is shown stably bound at the region of homology, moving in tandem with the dsDNA substrate. The centre of mass of the nucleoprotein filament is presented as a vector plot with respect to the position of the dsDNA molecule (grey line) over time (Z) for clarity.

Central strand Sequences:

The sequences of the oligonucleotides utilised to form the central dsDNA strands and the nucleoprotein filament oligonucleotide are given below. The Region of homology is highlighted in bold on the reaction dsDNA strand.

Control dsDNA Strand
Bottom:
GACGGGAGAATTAACTCTCAAGACGATAGTTACTAGATAAGGAATTCTGG
TCGGGCTGAAGAAAGGATCGCAGTGCTTTCGTGCACACAGTTTAAATATG
CAACTA
Top:
CTGTAGCTCAACATGTCTGTGTGCACGAAAGCACTGCGATCCTTTCTTCAG
CCCGACCAGAATTCCTTATCTAGTAACTATCGTCTTGAGGAACACCCTGAA
CAAA

Reaction dsDNA Strand
Bottom:
CGACAATAAACAACATAGTGAGGAGCAACGCGCACGGATCCATGGTAGG
AATTCAACAACAATGAATATTTGGAACACTCTAGAGTCTCCAGCAAACAA
GAGAATC
Top:
TTGCCTGAGAGTCTGGGGAGACTCTAGAGTGTTCCAAATA**TTCATTGTTG**
TTGAATTCCTACCATGGATCCGTGCGCGTTGCTCCTCACTGTTCAGCTAA
TGCAGA

Nucleoprotein Filament Oligo
TTCATTGTTGTTGAATTCCTACCATGGATC

DNA frame design and sequences:

Oligo name	**Oligo sequence (5' to 3')**
DF2S 0[111] 1[95]	AATAATAATTTTTTCACGTTGAAAAGGGAGTT
DF2S 0[143] 1[127]	GAGAATAGAAAGGAACAACTAAAGACCCTCAG
DF2S 0[175] 0[144]	TGCTAAACAACTTTCAACAGTTTCAGCGGAGT
DF2S 0[207] 1[191]	ACGTTAGTAAATGAATTTTCTGTATACCGCCA
DF2S 0[239] 1[223]	CGTAACGATCTAAAGTTTTGTCGTCCGCCACC
DF2S 0[271] 1[255]	TGTAGCATTCCACAGACAGCCCTCAGGGATAG
DF2S 0[295] 1[287]	GTCACCAGTACAAACTCGTAACAC
DF2S 0[47] 1[31]	TCGGTTTATCAGCTTGCTTTCGAGTGCGCCGA
DF2S 0[79] 1[63]	AAAAAAGGCTCCAAAAGGAGCCTTCATAACCG
DF2S 1[128] 3[127]	CAGCGAAAAGAGGCTTTGAGGACTAGGCGCAG
DF2S 1[160] 3[159]	TATCACCGTATAAGTATAGCCCGGGCCAGAAT
DF2S 1[192] 3[191]	CCCTCAGAACCAGGCGGATAAGTGCGTTCCAG
DF2S 1[224] 3[223]	CTCAGAGCGAAGGATTAGGATTAGGATACAGG
DF2S 1[256] 3[255]	CAAGCCCATGAAAGTATTAAGAGGGGGGTCAG
DF2S 1[288] 3[287]	TGAGTTTCCTGCCTATTTCGGAACAAACAGTT

DF2S 1[32] 3[31]	CAATGACAGAGGCAAAAGAATACATACCAAGC
DF2S 1[64] 3[63]	ATATATTCTGCCACTACGAAGGCATGTATCAT
DF2S 1[96] 3[95]	AAAGGCCGGGAAGTTTCCATTAAACGCGACCT
DF2S 10[15] 8[16]	GAAGCAAACAGAAAACGAGAATGAAATGCTTT
DF2S 10[239] 8[240]	GAAGGAAAAAGAACTGGCATGATTTTATTTTG
DF2S 10[271] 8[272]	AAAAGTAACAGTATGTTAGCAAACTAAAAGAA
DF2S 10[47] 8[48]	TTTTAATTAGGTCTTTACCCTGACAATCGTCA
DF2S 10[79] 8[80]	AAGAGGAAAAAGCGGATTGCATCAATGTTTAG
DF2S 11[224] 13[223]	ATCAGAGAAGAGAATAACATAAAAATCCTGAA
DF2S 11[256] 13[255]	GCCCAATAAACGTCAAAAATGAAATTCCAGAG
DF2S 11[288] 13[287]	GCAATAGCTCCCAATCCAAATAAGACAGCCAT
DF2S 11[32] 13[31]	TACCTTTATTTAGTTTGACCATTAAGCTATAT
DF2S 11[64] 13[63]	TTTTTGCGACAGTTGATTCCCAATAGGTGGCA
DF2S 11[88] 12[88]	ATTGCTGAATATAATGAAGTACGGTGTCTGGA
DF2S 12[15] 10[16]	TCGCAAATCTCCAACAGGTCAGGACCAGACCG
DF2S 12[215] 11[215]	ACAGGGAAGCGCATTAGTCAGAGGGTAATTGA
DF2S 12[239] 10[240]	CTTTACAGGATAACCCACAAGAATAGTTACCA
DF2S 12[271] 10[272]	TTTTGTTTATAAGAGCAAGAAACATTTTTAAG
DF2S 12[47] 10[48]	CGAGTAGAATTGCTCCTTTTGATAATATCGCG
DF2S 12[79] 10[80]	TCCATATAGATGGCTTAGAGCTTAAAAAGATT
DF2S 13[224] 15[223]	TCTTACCAACCCAGCTACAATTTTCGTAGGAA
DF2S 13[256] 15[255]	CCTAATTTAAGCCTTAAATCAAGAAAATCAGA
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DF2S 13[32] 15[31]	TTTCATTTAGCAATAAAGCCTCAGTTATGACC
DF2S 13[64] 15[63]	TCAATTCTCATACAGGCAAGGCAACTTTATTT
DF2S 14[15] 12[16]	GCTAAATCGGTCAATAACCTGTTTGATACATT
DF2S 14[239] 12[240]	TATTTTGCACGCTAACGAGCGTCTATAGCAGC
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DF2S 15[224] 17[223]	TCATTACCCCGTTTTTATTTTCATACAATAGA
DF2S 15[256] 17[255]	TATAGAAGACCAAGTACCGCACTCATCCCATC
DF2S 15[288] 17[287]	GAGGCGTTTCTTTCCTTATCATTCAATCAATA
DF2S 15[32] 17[31]	CTGTAATAATTCAAAAGGGTGAGAGATATTCA
DF2S 15[64] 17[63]	CAACGCAAAAATGCAATGCCTGAGCCGGAGAG
DF2S 16[15] 14[16]	AGACAGTCGGTTGTACCAAAAACAAGCATAAA
DF2S 16[239] 14[240]	CAAGCAAGGCGCCCAATAGCAAGCTTAGTTGC
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DF2S 16[47] 14[48]	AGGTAAAGCTTTTGCGGGAGAAGCAGAATTAG
DF2S 16[79] 14[80]	TATATTTTGGATAAAAATTTTTAGTTAACATC
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DF2S 4[239] 2[240]	CACCAGAGGGTAATAAGTTTTAACCTGAGACT
DF2S 4[271] 2[272]	GCCACCACGTAACAGTGCCCGTATCTATTATT

DF2S 4[47] 2[48]	CCAAATCAAAGTACAACGGAGATTCCAACCTA
DF2S 4[79] 2[80]	AGTAATCTAAATTGTGTCGAAATCCGGGTAAA
DF2S 5[128] 6[112]	GGACGTTGACGGAACAACATTATTACAGGTAG
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DF2S 5[224] 7[223]	GTTTGCCAGTAGCACCATTACCATGGTTTACC
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DF2S 5[64] 7[63]	TTTCAACTTAATGCAGATACATAAAAAGAAGT
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DF2S 6[15] 4[16]	CATAACCCCTTGCCCTGACGAGAACATTCAGT
DF2S 6[175] 4[176]	AGCACCGTGCGTCAGACTGTAGCGTGATATTC
DF2S 6[215] 4[208]	TAGCAAGGCCGGAAACTTCGGTCATAGCCCCCCAGGAGGT
DF2S 6[239] 4[240]	AATCACCATCTTTTCATAATCAAAAGAACCAC
DF2S 6[271] 4[272]	GACTTGAGCCACCACCGGAACCGCCCCTCAGA
DF2S 6[47] 4[48]	GGAATTACTAAATTGGGCTTGAGATTCATTAC
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DF2S 7[224] 9[223]	AGCGCCAAAATAGAAAATTCATATATAACGGA
DF2S 7[256] 9[255]	ATTGAGGGACACCACGGAATAAGTAAGACTCC
DF2S 7[288] 9[287]	TTATTCATTAAAGGTGGCAACATAGTAGAAAA
DF2S 7[32] 9[31]	CCAAAATACATTGAATCCCCCTCACCATAAAT
DF2S 7[64] 9[63]	TTTGCCAGGCGTCCAATACTGCGGTATTATAG
DF2S 8[15] 6[16]	AAACAGTTTCGTTTACCAGACGACAACACTAT
DF2S 8[239] 6[240]	TCACAATCAGACAAAAGGGCGACAGCCAGCAA
DF2S 8[271] 6[272]	ACGCAAAGAGGGAAGGTAAATATTCCGTCACC
DF2S 8[47] 6[48]	TAAATATTGCGAGAGGCTTTTGCACGCCAAAA
DF2S 8[79] 6[80]	ACTGGATAAGGGGGTAATAGTAAAGAATACCA
DF2S 9[224] 11[223]	ATACCCAACCGAGGAAACGCAATAGCGCTAAT
DF2S 9[256] 11[255]	TTATTACGGCAGATAGCCGAACAATGAGTTAA
DF2S 9[288] 11[287]	TACATACATATCTTACCGAAGCCCATGAAATA
DF2S 9[32] 11[31]	CAAAAATCCGAGCTTCAAAGCGAATTAGAGAG
DF2S 9[64] 11[63]	TCAGAAGCGCCCGAAAGACTTCAAAGAGGTCA