Supplemental Materials for:

Telomere proteins POT1, TRF1 and TRF2 augment long-patch base excision repair in vitro

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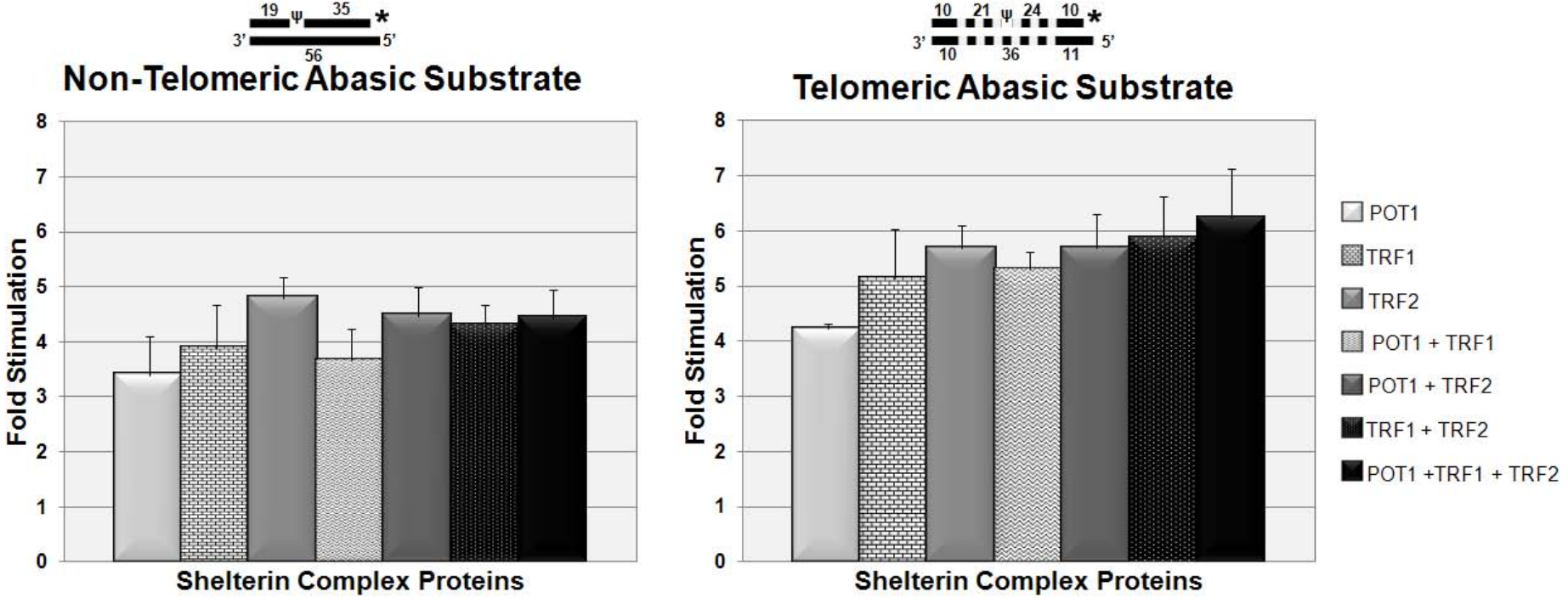
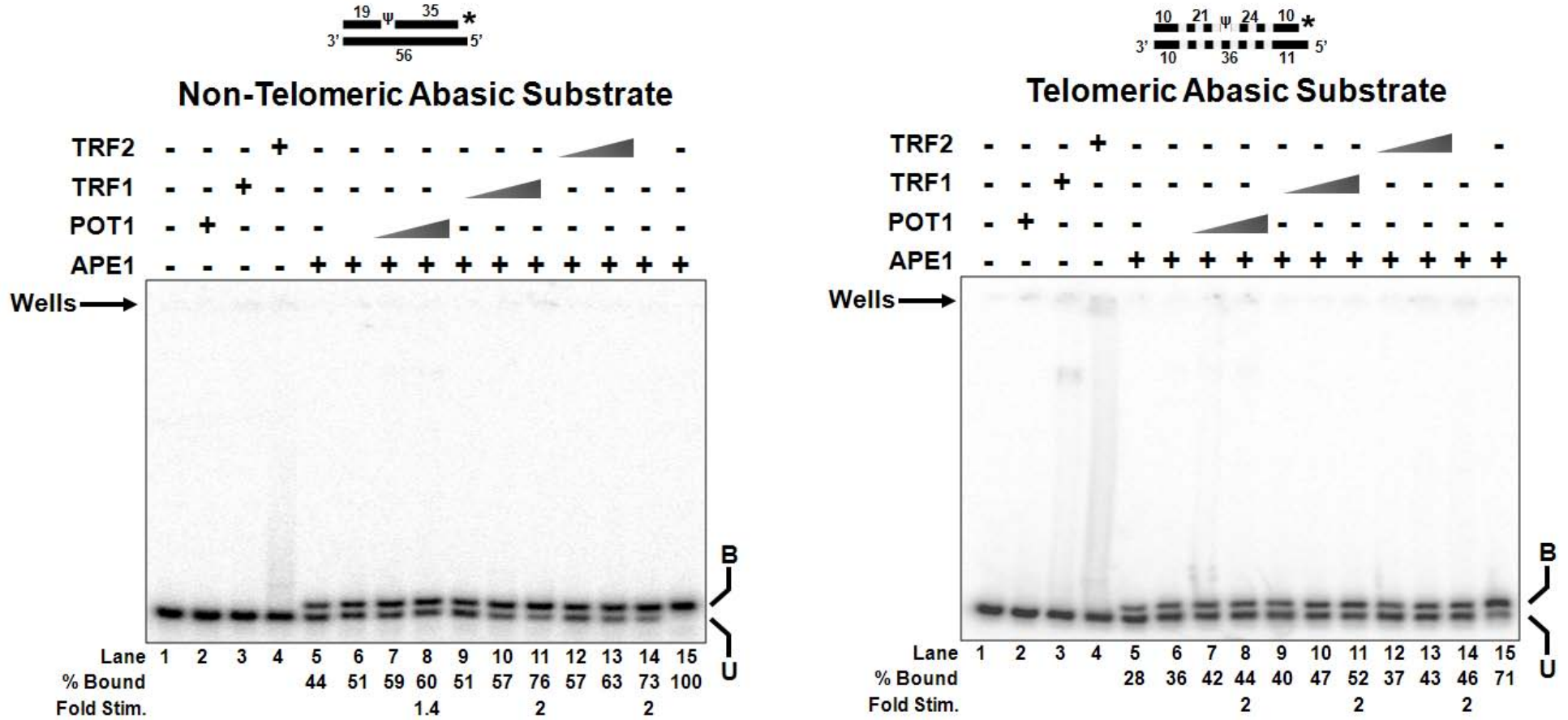
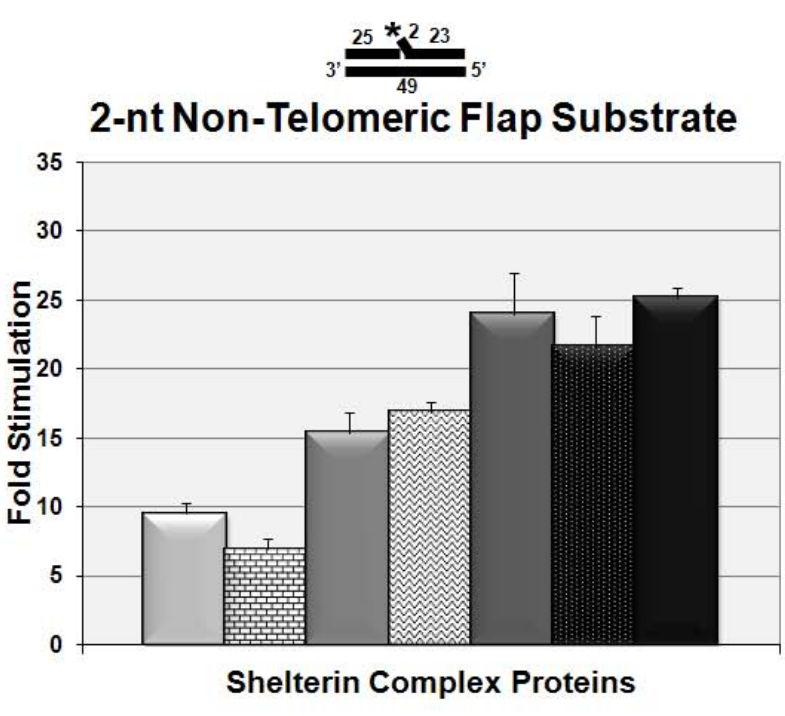
ABSTRACT

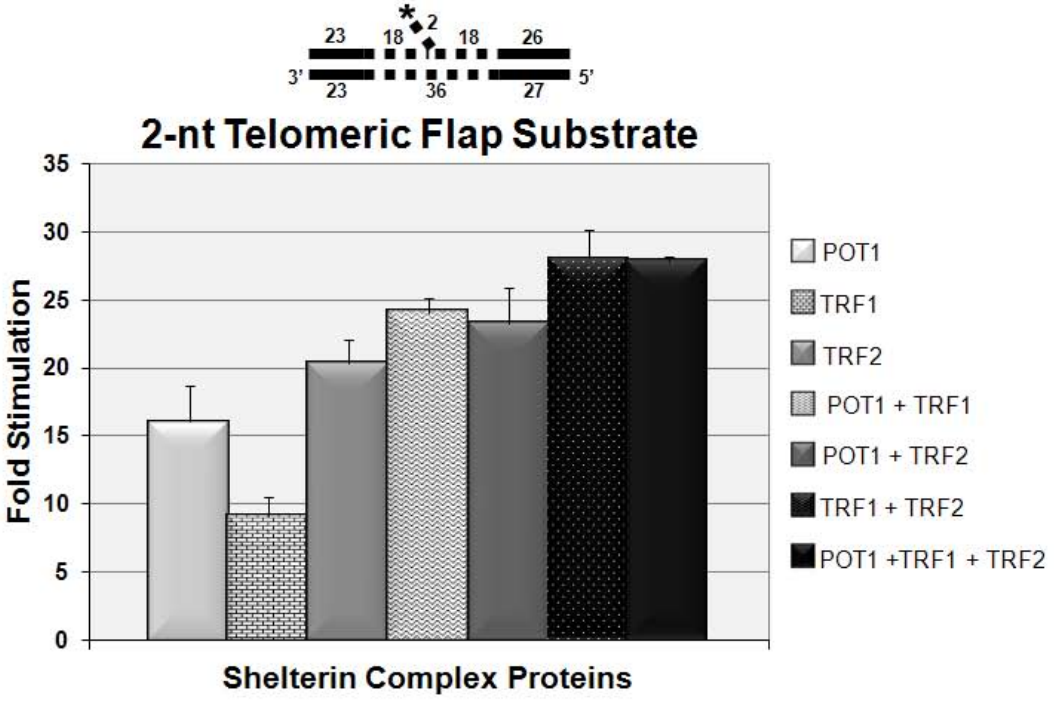
Human telomeres consist of multiple tandem hexameric repeats, each containing a guanine triplet. Guanosine-rich clusters are highly susceptible to oxidative base damage, necessitating base excision repair (BER). Previous demonstration of enhanced strand displacement synthesis by the BER component DNA polymerase β in the presence of telomere protein TRF2 suggests that telomeres employ long-patch (LP) BER. Earlier analyses in vitro showed that efficiency of BER reactions is reduced in the DNA-histone environment of chromatin. Evidence presented here indicates that BER is promoted at telomeres. We found that the three proteins that contact telomere DNA, POT1, TRF1 and TRF2, enhance the rate of individual steps of LP-BER and stimulate the complete reconstituted LP-BER pathway. Thought to protect telomere DNA from degradation, these proteins still apparently evolved to allow selective access of repair proteins.

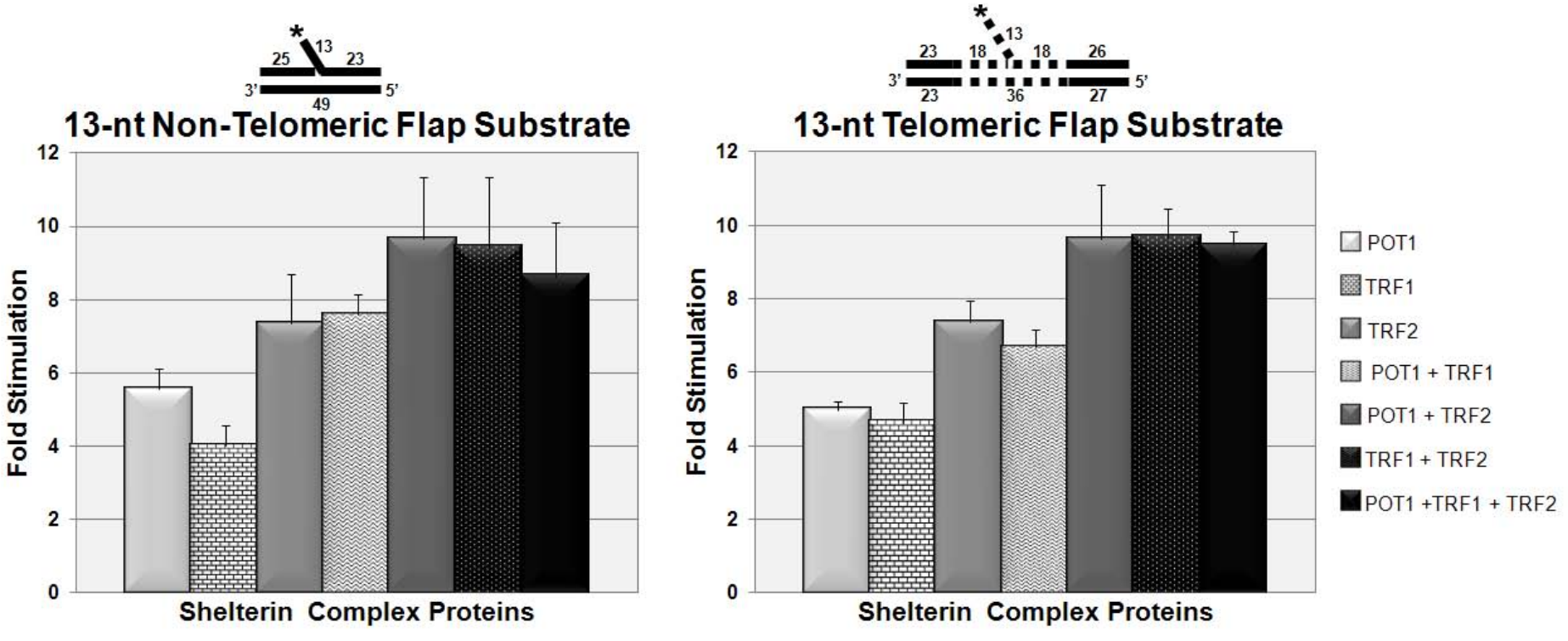
Supplemental Figures 1-5 followed by Supplemental Table 1.

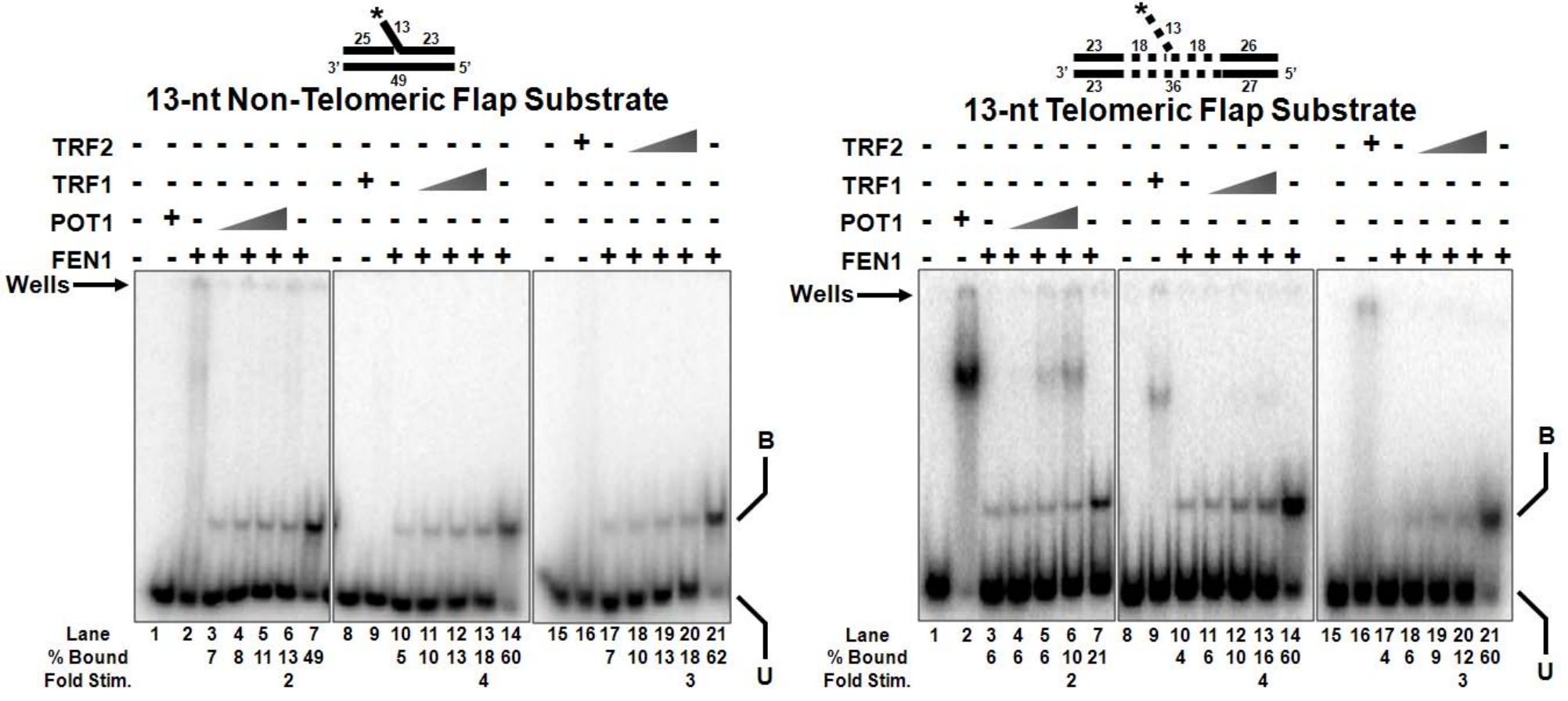

19 Ψ 35 *
3' 5'
56
Non-Telomeric Abasic Substrate
Fold Stimulation
0
1
2
3
4
5
6
7
8
Shelterin Complex Proteins
10 21 Ψ 24 10 *
3' 5'
10 36 11
Telomeric Abasic Substrate
Fold Stimulation
0
1
2
3
4
5
6
7
8
Shelterin Complex Proteins
POT1
TRF1
TRF2
POT1 + TRF1
POT1 + TRF2
TRF1 + TRF2
POT1 +TRF1 + TRF2

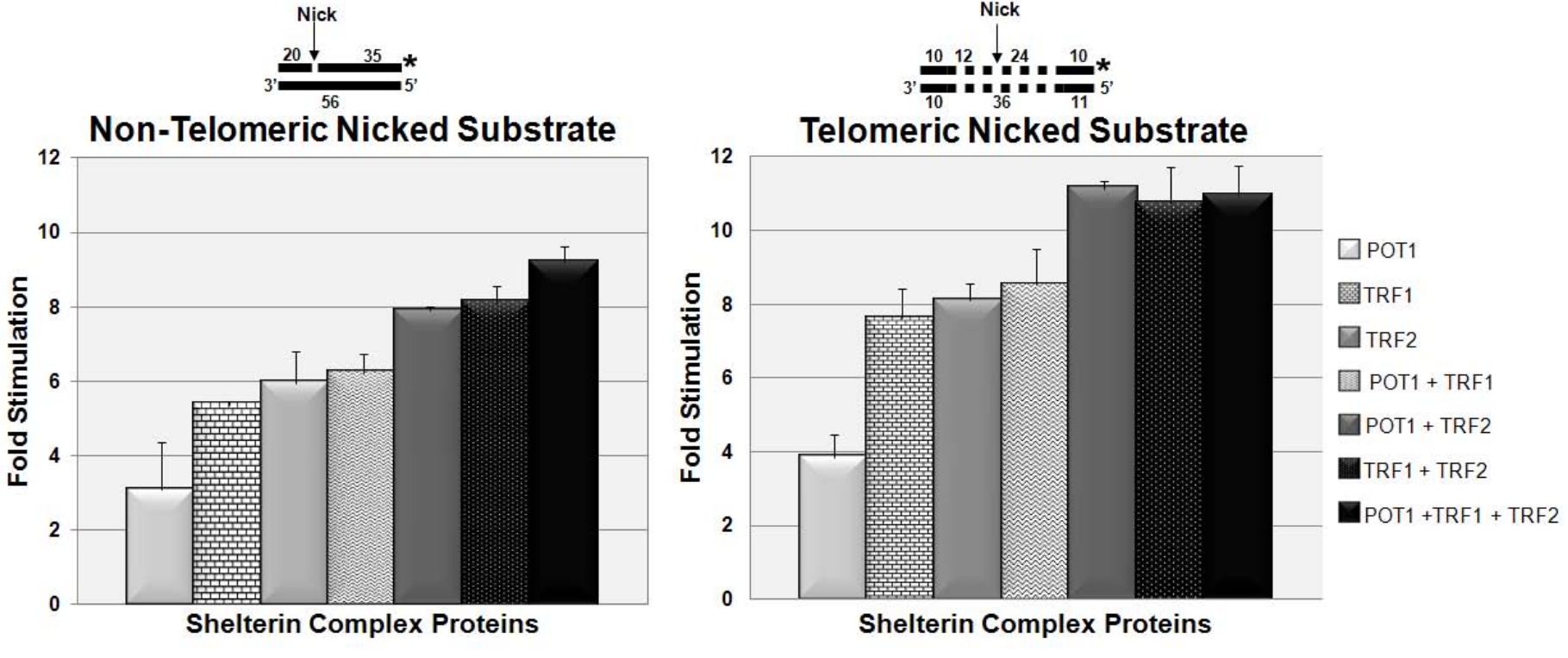

19 Ψ 35 *
3' 5'
56
Non-Telomeric Abasic Substrate
TRF2 - - - + - - - - - - - - - -
TRF1 - - + - - - - - - - - - - -
POT1 - + - - - - - - - - - - - -
APE1 - - - - + + + + + + + + + +
Wells
B
U
Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
% Bound 44 51 59 60 51 57 76 57 63 73 100
Fold Stim. 1.4 2 2
10 21 Ψ 24 10 *
3' 5'
10 36 11
Telomeric Abasic Substrate
TRF2 - - - + - - - - - - - - - -
TRF1 - - + - - - - - - - - - - -
POT1 - + - - - - - - - - - - - -
APE1 - - - - + + + + + + + + + +
Wells
B
U
Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
% Bound 28 36 42 44 40 47 52 37 43 46 71
Fold Stim. 2 2 2

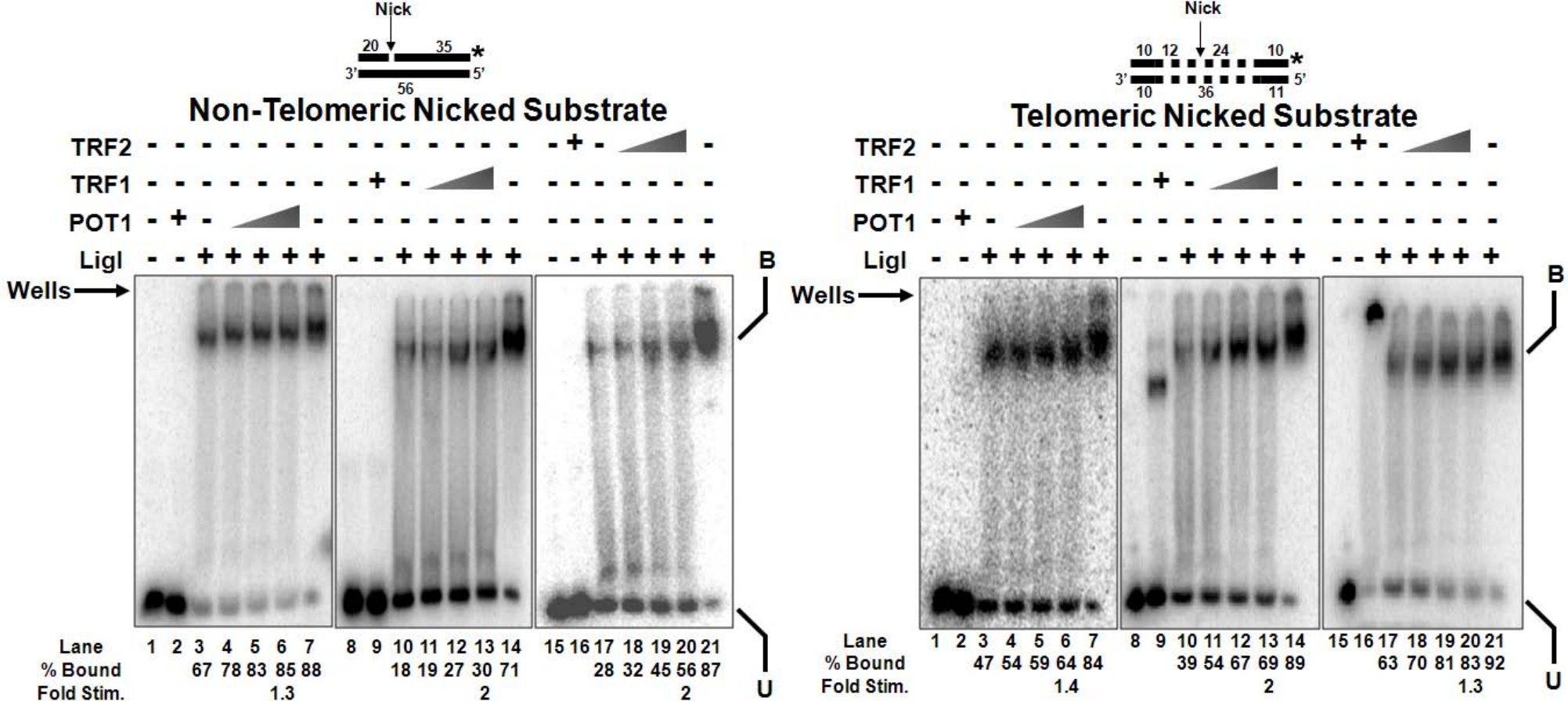

25 * 2 23
3' 5'
49
2-nt Non-Telomeric Flap Substrate
Fold Stimulation
0
5
10
15
20
25
30
35
Shelterin Complex Proteins


23 18 * 2 18 26
3' 5'
23 36 27
2-nt Telomeric Flap Substrate
Fold Stimulation
0
5
10
15
20
25
30
35
Shelterin Complex Proteins
POT1
TRF1
TRF2
POT1 + TRF1
POT1 + TRF2
TRF1 + TRF2
POT1 +TRF1 + TRF2


13-nt Non-Telomeric Flap Substrate
25
13
23
3'
5'
49
Fold Stimulation
0
2
4
6
8
10
12
Shelterin Complex Proteins
13-nt Telomeric Flap Substrate
23
18
13
18
26
3'
5'
23
36
27
Fold Stimulation
0
2
4
6
8
10
12
Shelterin Complex Proteins
POT1
TRF1
TRF2
POT1 + TRF1
POT1 + TRF2
TRF1 + TRF2
POT1 +TRF1 + TRF2


13-nt Non-Telomeric Flap Substrate
25
13
23
3'
5'
49
13-nt Telomeric Flap Substrate
23
18
13
18
26
3'
5'
23
36
27
TRF2
TRF1
POT1
FEN1
Wells
B
U
Lane
% Bound
Fold Stim.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
7 8 11 13 49 5 10 13 18 60 7 10 13 18 62
2 4 3
6 6 6 10 21 4 6 10 16 60 4 6 9 12 60 3
2 4


Nick
20
35
3'
5'
56
Non-Telomeric Nicked Substrate
Fold Stimulation
Shelterin Complex Proteins
Nick
10
12
24
10
3'
5'
10
36
11
Telomeric Nicked Substrate
Fold Stimulation
Shelterin Complex Proteins
POT1
TRF1
TRF2
POT1 + TRF1
POT1 + TRF2
TRF1 + TRF2
POT1 +TRF1 + TRF2


Nick
20
35
3'
5'
56
Non-Telomeric Nicked Substrate
TRF2
TRF1
POT1
LigI
Wells
B
U
Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
% Bound 67 78 83 85 88 18 19 27 30 71 28 32 45 56 87
Fold Stim. 1.3 2 2
Nick
10 12 24 10
3'
5'
10 36 11
Telomeric Nicked Substrate
TRF2
TRF1
POT1
LigI
Wells
B
U
Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
% Bound 47 54 59 64 84 39 54 67 69 89 63 70 81 83 92
Fold Stim. 1.4 2 1.3

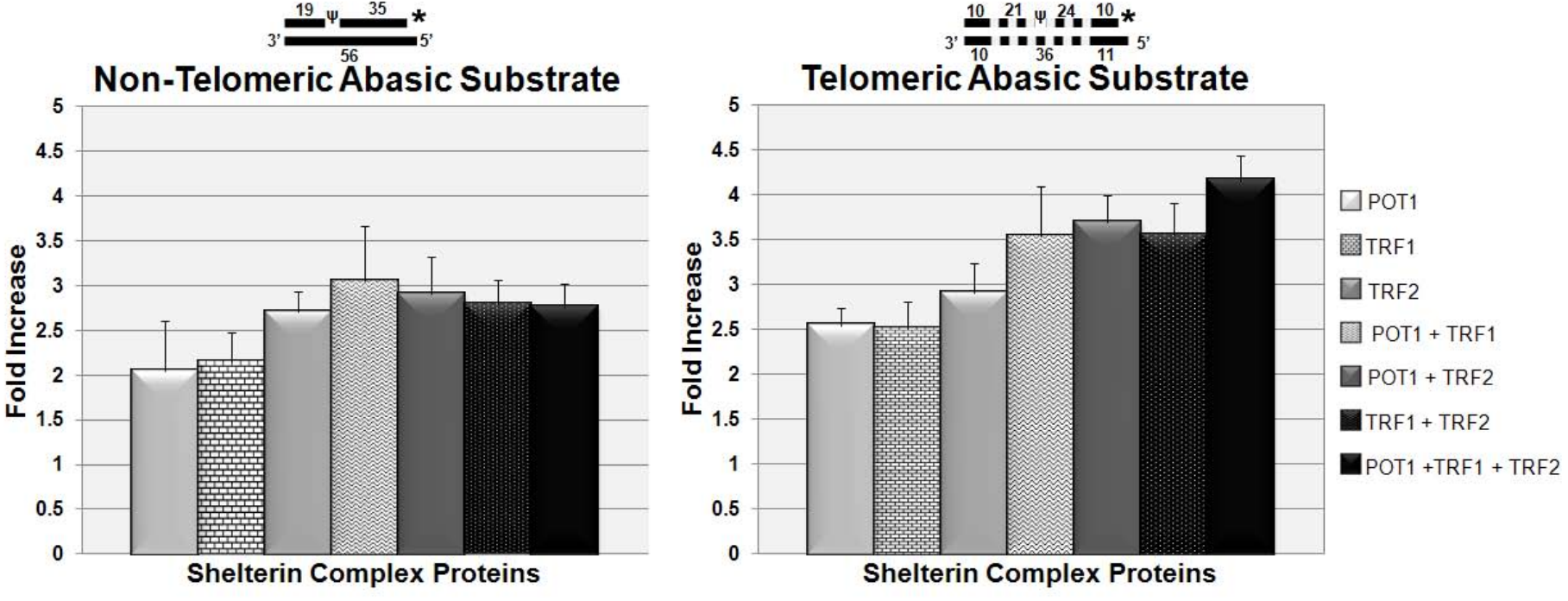

19 Ψ 35 *
3' 5'
56
Non-Telomeric Abasic Substrate
Fold Increase
Shelterin Complex Proteins
10 21 Ψ 24 10 *
3' 5'
10 36 11
Telomeric Abasic Substrate
Fold Increase
Shelterin Complex Proteins
POT1
TRF1
TRF2
POT1 + TRF1
POT1 + TRF2
TRF1 + TRF2
POT1 +TRF1 + TRF2

Table 1. Oligonucleotide Sequences

Ψ indicates a nucleotide containing a THF residue

Primer	Length(nts)	Sequence
Upstream		Listed 5' to 3'
U1	25	CGCCAGGGTTTTCCCAGTCACGACC
U2	20	CGACCGTGCCAGCCTAAAAC
U3	22	CGACCGTGCCTTAGGGTTAGGG
U4	41	CTAGAACTGAGTGCCAGATAGCATTAGGGTTAGGGTTAGGG
Downstream		Listed 5' to 3'
D1	25	GTCGTTTTACAACGACGTGACTGGG
D2	36	ATTCACTGGCCGTCGTTTTACAACGACGTGACTGGG
D3	46	GGTTAGGGTTAGGGTTAGGGTCCGATCGCCAGTCGCGTGCCTAGCG
D4	57	GGGTTAGGGAGGGTTAGGGTTAGGGTTAGGGTCCGATCGCCAGTCGCGTGCCTAGCG
D5	35	ACTTGCCCGTGCCCACCATCCCGACGCCACCTCCTG
D6	54	CGACCGTGCCAGCCTAAAAΨACTTGCCCGTGCCACCATCCCGACGCCACCTCCTG
D7	34	TTAGGGTTAGGGTTAGGGTTAGGGCCACCTCCTG
D8	55	CGACCGTGCCTTAGGGTTAGGGTTAGGGTTAΨGGTTAGGGTTAGGGCCACCTCCTG
Template		Listed 3' to 5'
T1	49	GCGGTCCCAAAAGGGTCAGTGCTGGGCAAAATGTTGCTGCACTGACCCG
T2	57	GCTGGCACGGAATCCCAATCCCAATCCCAATCCCAATCCCAATCCCGGTGGAGGACG
T3	56	GCTGGCACGGTCGGATTTTGTGAACGGGCACGGTGGTAGGGCTGCGGTGGAGGACG
T4	86	GATCTTGACTCACGGTCTATCGTAATCCCAATCCCAATCCCAATCCCAATCCCAATCCCAGGCTAGCGGTCAGCGCACGGATCGCG