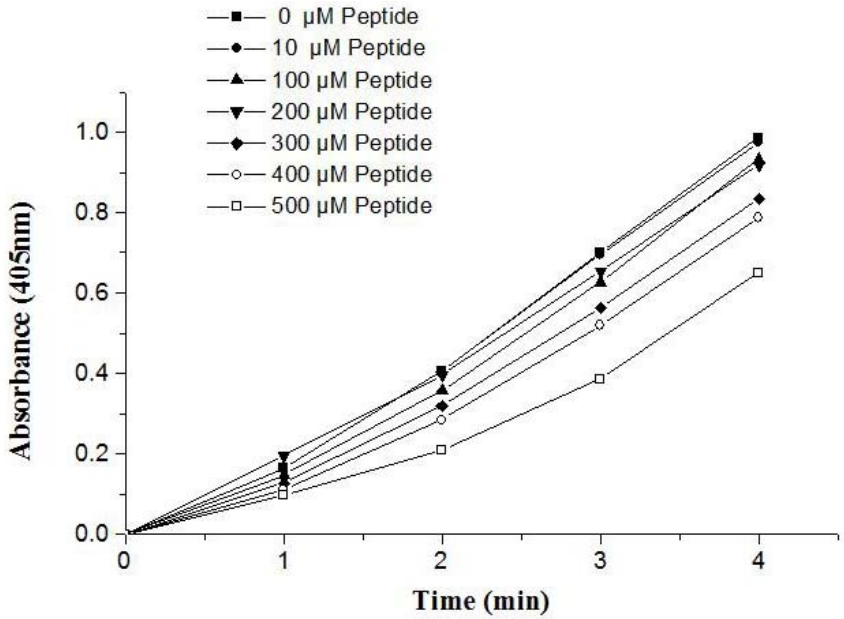
Supplementary Material

A)



B)

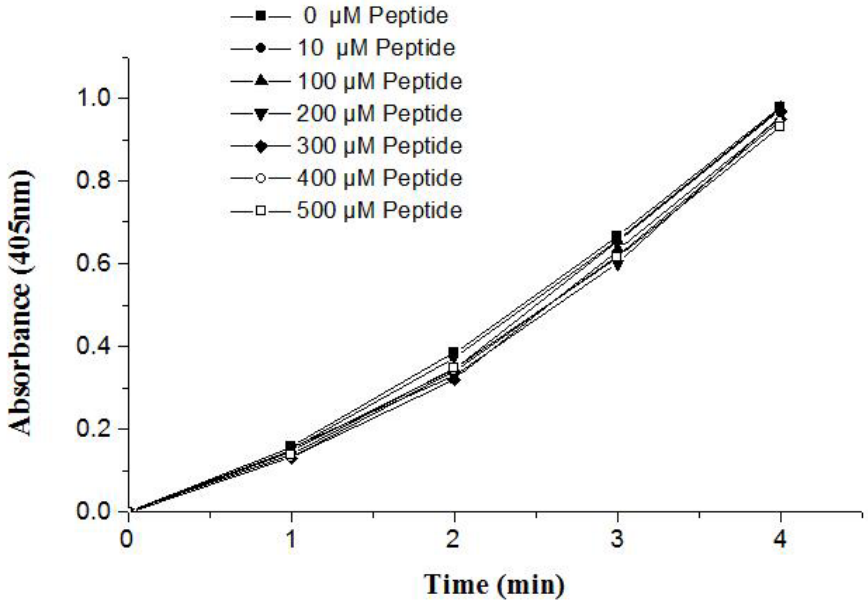
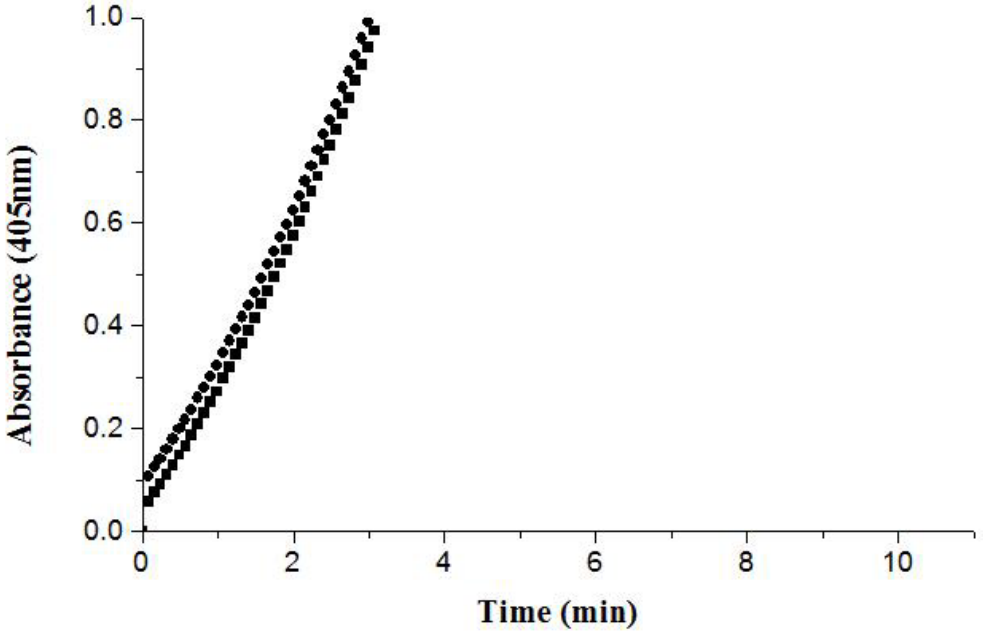


Figure S1. Time course of HPG activation assays in presence of various concentrations of the 170 loop peptide mimotope. A) Aliquots of preformed SK.HPN complex (5 nM) were added to HPG (0.05 µM) in assay buffer containing various concentrations of the 170-loop sequence cyclic peptides (0-500µM). B) Aliquots of preformed SK.HPN complex (5 nM) were added to HPG (0.05 µM) in assay buffer containing various concentrations of the K180A mutated 170-loop sequence cyclic peptides (0-500µM) and the activator activities were measured spectrophotometrically at 405 nm. The specific activity at each concentration of inhibitory peptide was calculated by obtaining the slopes of the activation progress curves as change in absorbance/t^2.

A)



B)

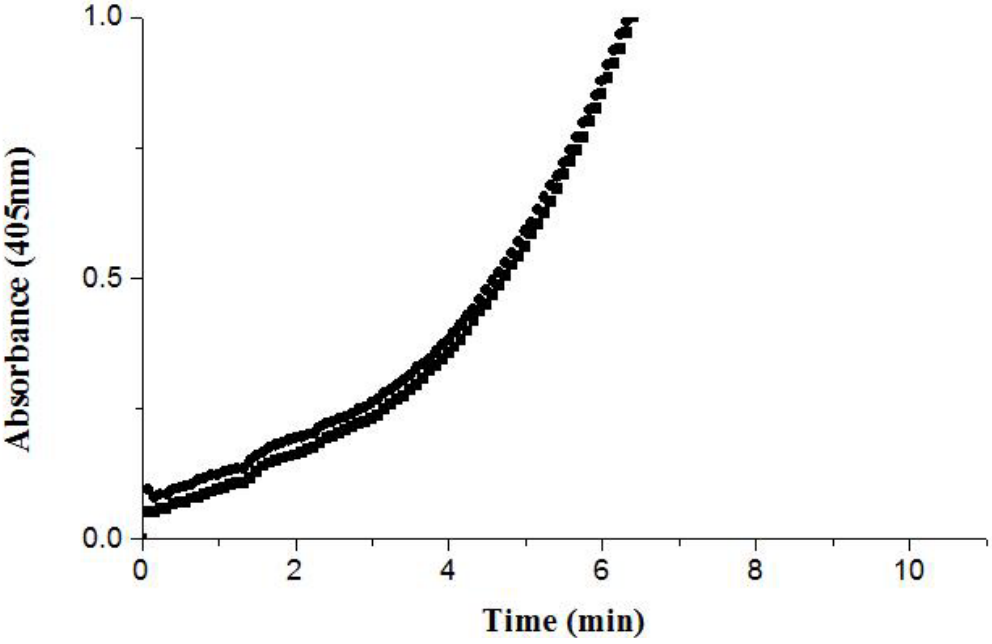
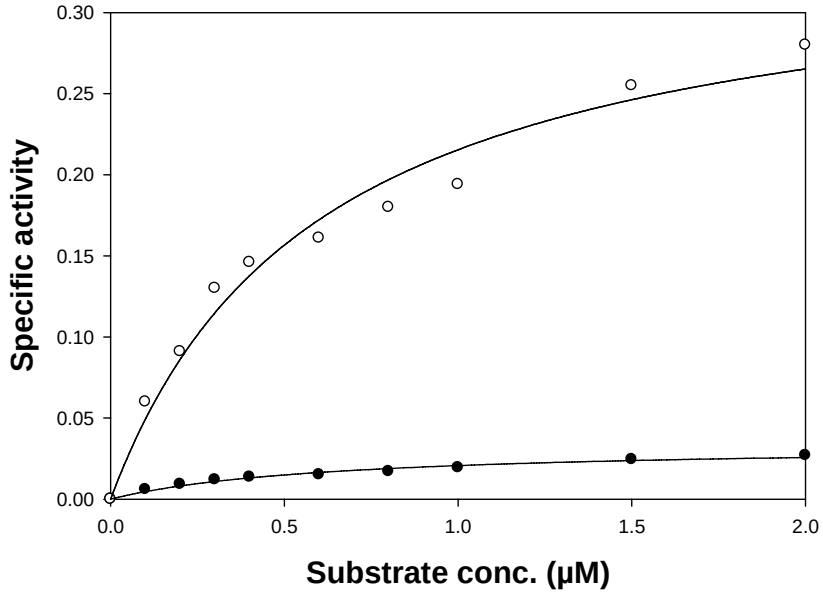


Figure S2. Time course of the amidolytic activity generation in HPG by wild type SK and 170 loop mutant. Equimolar complexes of wtSK/SK mutants and HPG were made, and transferred into cuvette containing 0.5 mM Chromozym®PL in 50 mM Tris-Cl, pH 7.4 and the generation of amidolytic activity was monitored at 405 nm. Assays were performed either at 37ºC with 10 nM of SK.HPG complex (A) or at 4ºC with 100 nM of SK.HPG complex (B). The amidolytic activity levels attained as a function of time by wild type SK (solid circle), K180A (solid square), are shown

A)



B)

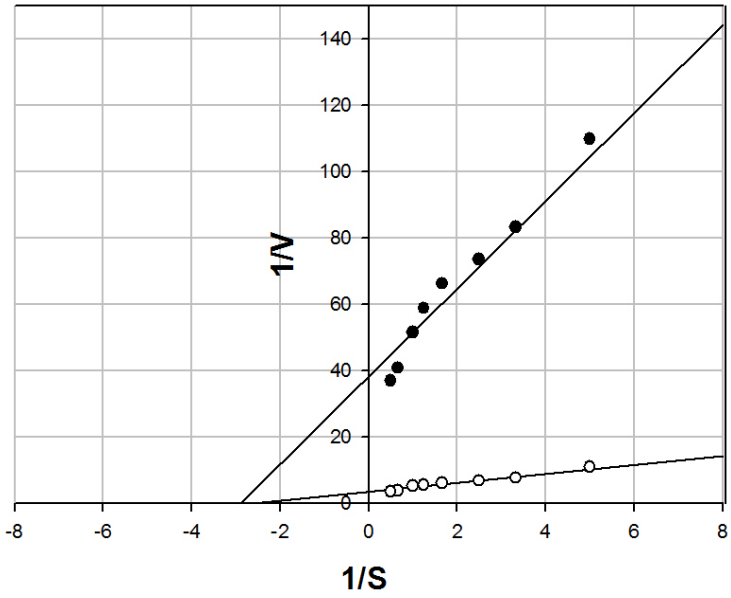
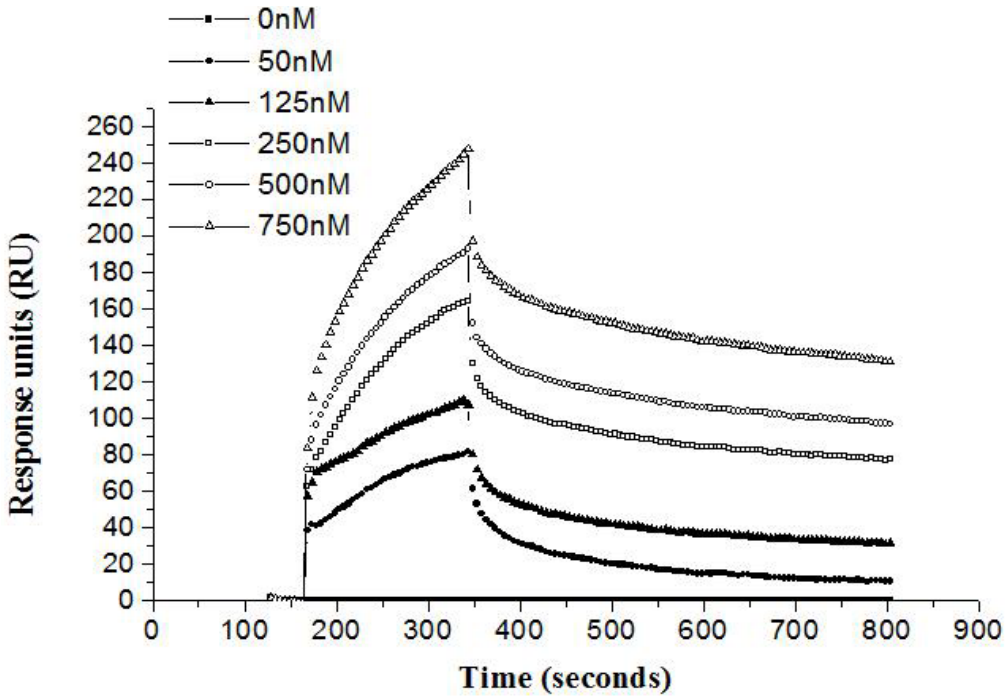


Figure S3. Michaelis Menten and Lineweaver-Burk plots for determination of the steady state kinetic constants. Representative data (same for both panels) are given on HPG activation by the preformed complex of SK/170loop mutant (K180A) with plasmin. Panel A: HPG at concentrations ranging from 0.1μM to 2.0μM was activated by SK.HPN complex (open circles) and K180A.HPN (solid circles) and plotted as Michaelis Menten plot. Panel B: HPG at concentrations ranging from 0.1μM to 2.0μM was activated by wtSK.HPN complex (solid circles) and K180A.HPN (open circles) plotted as Lineweaver-Burk plots.. Details can be seen in the Experimental Procedures section

A)



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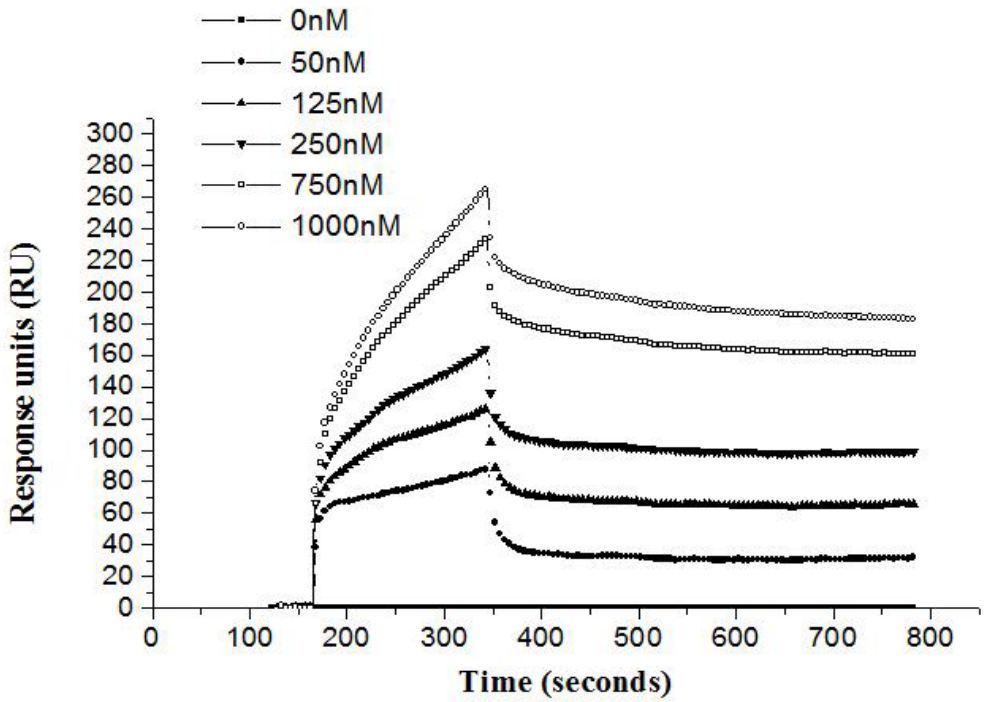
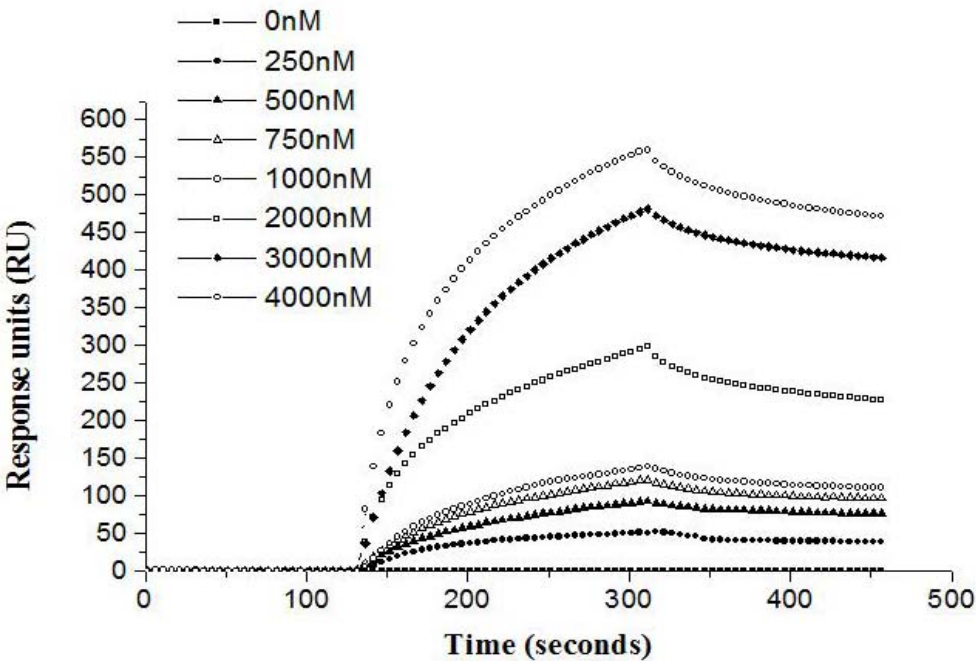


Figure S4. Representative sensorgrams of association and dissociation curves for HPG binding to preformed Binary complex of A) HPG: SK and (B) HPG: K180A as studied by SPR

The interaction of substrate HPG with wtSK/ loop mutants: HPG, (binary complex) was determined by global fitting to a 1:1 binding model, using the BIAcore 3000 evaluation software as determined under "Experimental Procedures". A stable binary complex between wtSK/ SK loop mutants and HPG immobilized on to the SA chip was made, and the binding of varying concentration of substrate HPG (0.05-1µM) was monitored.

A)



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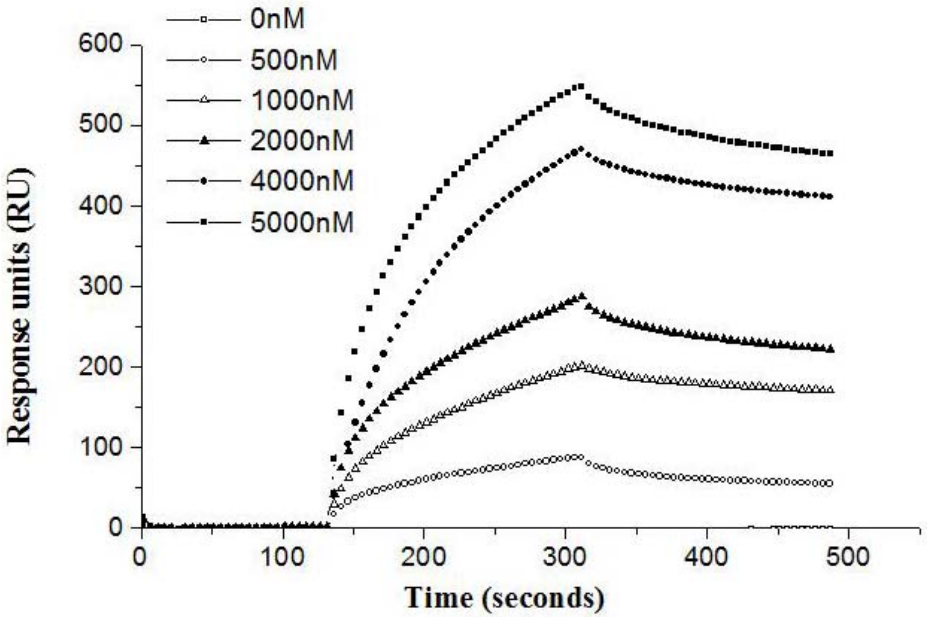

Figure S5. Representative sensorgrams of association and dissociation curves for microplasminogen binding to preformed binary complex of (A) HPG: SK and (B) HPG: K180A as studied by SPR. The interaction of substrate µPG with wtSK/ loop mutants: HPG,(binary complex) was determined by global fitting to a 1:1 binding model, using the BIAcore 3000 evaluation software as described under "Experimental Procedures". A stable binary complex between wtSK/ SK loop mutants and HPG immobilized on to the SA chip was made, and the binding of varying concentration of substrate µPG (1-6µM) was monitored.

Table SI. Sequence of PCR primers used for construction of SK mutants

The mutant primers shown in this table were used for the construction of substitution mutants

170 loop mutant	Oligonucleotide sequence
P171A Upstream primer	5’ TTC ACT CCC TTA AAT GCA GAT GAC GAC TTC AGA 3’
P171A Downstream primer	5’ TCT GAA GTC GTC ATC TGC ATT TAA GGG AGT GAA 3’
D172A Upstream primer	5’CCC TTA AAC CCT GCC GAC GAT TTC AGA CCA GGT 3’
D172A Downstream primer	5’ ACC TGG TCT GAA ATC GTC GGC AGG GTT TAA GGG 3’
D173A Upstream primer	5’ CCC TTA AAC CCT GAT GCG GAC TTC AGA CCA GGT 3’
D173A Downstream primer	5’ ACC TGG TCT GAA GTC CGC ATC AGG GTT TAA GGG 3’
D174A Upstream primer	5’ TTA AAC CCA GAT GAC GCG TTC AGA CCA GGT CTC 3’
D174A Downstream primer	5’ GAG ACC TGG TCT GAA CGC GTC ATC TGG GTT TAA 3’
R176A Upstream primer	5’ GAT GAC GAT TTC GCC CCA GGT CTC AAA GAT TAT 3’
R176A Downstream primer	5’ ATA ATC TTT GAG ACC TGG GGC GAA ATC GTC ATC 3’
P177A Upstream primer	5’ CTG AAC CCC GAT GAC GAT TTT AGA GCC GGC CTC AAA GAT ACT AAG 3’
P177A Downstream primer	5’ CTT AGT ATC TTT GAG GCC GGC TCT AAA ATC GTC ATC GGG GTT CAG 3’
K180A Upstream primer	5’ GAT GAC GAT TTC AGA CCA GGT CTC GCC GAT ACT 3’
K180A Downstream primer	5’ CAA TAG CTT AGT ATC GGC GAG ACC TGG TCT GAA 3’
K 180 R Upstream primer	5’ GAT GAC GAT TTC AGA CCA GGT CTC CGC GAT ACT 3’
K 180 R Downstream primer	5’ AGT ATC GCG GAG ACC TGG TCT GAA ATC GTC ATC 3’
K 180 D Upstream primer	5’GAT GAC GAT TTC AGA CCA GGT CTC GAC GAT ACT 3’
K 180 D Downstream primer	5’ AGT ATC GTC GAG ACC TGG TCT GAA ATC GTC ATC 3’
K180 G Upstream primer	5’GAT GAC GAT TTC AGA CCA GGT CTC GGG GAT ACT 3’
K 180 G Downstream primer	5’ AGT ATC CCC GAG ACC TGG TCT GAA ATC GTC ATC 3’
K180A+R176A Upstream primer	5’ CCT GAT GAC GAT TTC GCC CCA GGT CTC GCC GAT ACT AAG 3’
K180A+R176A Downstream primer	5’ CTT AGT ATC GGC GAG ACC TGG GGC GAA ATC GTC ATC AGG 3’
K180A+D181A Upstream primer	5’GAT GAC GAT TTC AGA CCA GGT CTC GCC GCC ACT AAG CTA TTG 3’
K180A+D181A Downstream primer	5’CAA TAG CTT AGT GGC GGC GAG ACC TGG TCT GAA ATC GTC ATC 3’
D181A+ R176A Upstream primer	5’CCG GAT GAC GAT TTC GCC CCA GGT CTC AAA GCC ACT AAG CTA 3’
D181A+ R176A Downstream primer	5’TAG CTT AGT GGC TTT GAG ACC TGG GGC GAA ATC GTC ATC AGG 3’
P177A+K180A Upstream primer	5’ GAT GAC GAT TTC AGA GCG GGT CTC GCA GAT ACT AAG CTA TTG 3’
P 177A+K180A Downstream primer	5’ CAA TAG CTT AGT ATC TGC GAG ACC TGC TCT GAA ATC GTC ATC 3’
R176A+K180A+D181A Upstream primer	5’ CCT GAT GAC GAT TTC GCG CCA GGT CTC GCG GCG ACT AAG CTA 3’
R176A+K180A+D181A Downstream primer	5’ TAG CTT AGT CGC CGC GAG ACC TGG CGC GAA ATC GTC ATC AGG 3’