Electronic Supplementary Material (ESI) for Molecular BioSystems

Experimental Methods for:

Optimization of a Genetically Encoded Biosensor for Cyclin B1-Cyclin Dependent Kinase 1

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Experimental Methods

General Materials and Methods. Unless otherwise indicated all chemicals and reagents were purchased from Fisher Scientific (Ottawa, ON, Canada) or Sigma-Aldrich Canada (Oakville, ON, Canada). All primers (Supplementary Table 1) were purchased from Integrated DNA Technologies (Coralville, IA, USA). Polymerase chain reactions (PCRs) were performed using either Taq DNA polymerase (Life Technologies, Burlington, ON, Canada) or Pfu DNA polymerase (Fermentas, Burlington, ON, Canada) according to the provided manufacturer's protocols. Deoxyribonucleotide triphosphate (dNTP) solutions used in PCRs were purchased from Life Technologies. Fast Digest Endonucleases (Fermentas, Burlington, ON, Canada) were used for restriction digests of all PCR products and plasmids. PCR products and products of restriction digestion were purified by gel electrophoresis and extracted using GeneJet gel extraction kit (Fermentas, Burlington, ON, Canada). DNA ligation was performed using T4 DNA ligase (Life Technologies, Burlington, ON, Canada). *E. coli* ElectroMax DH10B (Life Technologies, Burlington, ON, Canada) was used for cloning, plasmid propagation, and protein expression. Dye terminator cycle sequencing using BigDye (Applied Biosystems, Burlington, ON, Canada) was used to confirm the complete DNA sequences of all fusion constructs. Sequencing reactions were analyzed at University of Alberta Molecular Service Unit (MBSU).

Construction of the PKB biosensor library. The pUADE (University of Alberta Dual Expression plasmid) dual expression plasmid[1] was modified to include restriction sites compatible with insertion of the genes for constitutively active PKB and its reporter BKAR. Consequently, a DNA fragment encoding *AvrII*-linker-*SalI*-linker-*SbfI*-Linker-*EagI* was PCR amplified, digested with *AvrII* and *EagI* restriction enzymes, and ligated into similarly digested pUADE. An *Xho1* site and a *HindIII* site were already present in the plasmid at the 5' and 3' ends of the insertions site, respectively. Successful ligation was confirmed by analytical double digestion. The gene encoding mTFP1 was PCR amplified, double

digested with *XhoI* and *AvrII* and ligated into this modified pUADE after it had been similarly digested. Site-directed mutagenesis by overlap-extension PCR was used to remove an undesirable *XhoI* restriction site in the phosphoserine/threonine binding domain FHA2. Two PCR reactions were carried out using the pRSET B-BKAR as a template (Addgene plasmid 14877). The first reaction used a forward primer carrying *AvrII* and a reverse primer that mismatch annealed to the *Xho1* site and carried a silent mutation to destroy the restriction site. The second reaction used a reverse primer carrying a *SalI* restriction site and a forward primer that was the reverse complement of the reverse primer in the first reaction. The two resulting PCR products were gel purified, mixed in equal proportions, and used as the template for a third PCR reaction with the *AvrII* and *SalI* containing primers. The resulting PCR product was double digested by *AvrII* and *SalI* and ligated into similarly digested modified pUADE containing mTFP1 as described above. Similar plasmid digestion and ligation steps with appropriately digested PCR product were used to insert the PKB substrate sequence and YFP between *SalI*/*SbfI* and *SbfI*/*HindIII* sites respectively. Finally the gene encoding constitutively active PKB (PKB-T308D, S473D) was PCR amplified from a template of Addgene plasmid 14751 (pcDNA3 backbone). The PCR product was doubly digested with *EcoRI* and *BglII* and ligated downstream of the P_{BAD} promoter in the modified pUADE from the last step.

To insert a variety of cyan FPs into the appropriate site of the biosensor scaffold library, we designed primers to introduce *XhoI* and *AvrII* sites at the 5' and 3' ends, respectively, of a selection of cyan FPs (CFP, TFP, CyPet, cpTFP193, and cpTFP207). Similarly, primers for yellow FPs (YFP, Ypet, mCitrine, cpVenus173) were designed to introduce an *SbfI* at the 5' end and a stop codon followed by *HindIII* at the 3' end. PCR amplifications were performed individually, and resulting PCR products for members of each group were mixed in equimolar amounts and subjected to double digestion with appropriate enzymes. Following ligation of the cyan FP group into similarly digested modified pUADE, 2 µl were used to transform 50 µl of electrocompetent *E. coli* and the whole reaction mixture was added to 4 ml LB medium containing 0.04% ampicillin and incubated at 37 °C with shaking at 220 rpm for 16 hours followed by plasmid DNA extraction. The extracted plasmid was then appropriately digested and ligated with the similarly digested pool of yellow FPs. The resulting plasmid library, with various combinations of CFPs and YFPs, was used to transform competent *E. coli*.

The cDNA encoding the substrate portion of BKAR was prepared by overlap PCR amplification using a forward primer appending *SalI* followed by the '218' linker and a reverse primer appending an *SbfI* restriction site. This cDNA was used as the PCR amplification using the same 3' primer and a variety of 5' primers. These different 5' primers coded for different lengths of linker at the 5' end. The PCR products were mixed in equimolar amounts, double digested, and ligated into the above mentioned biosensor library containing both the cyan pool and yellow pool. The resulting plasmid-based library was designated pUADE-PKB (Supplementary Fig. 1A).

Construction of CDK1 biosensor library. Further modification of pUADE was performed to ensure compatibility of the restriction sites with the constitutively active enzyme gene and its corresponding biosensor. An *XmaI* site was inserted between the *EcoRI* and *BglII* sites of the P_{tac} multiple cloning site. Polycistronic CDC28-CAK1-CKS1-CLB5 was PCR amplified from GEX6P-1/CDC28-CAK1-CKS1-CLB5 using primers that appended *XmaI* and *BglII* sites at 5' and 3' ends, respectively. The resulting PCR product was double digested and ligated into similarly digested pUADE (previously modified to contain the *XmaI* site). Introduction of the cyan FP gene pool was performed as described above. The cDNA for the CDK1 substrate region was constructed by an overlap PCR that left a *KpnI* restriction site at the 3' end. The yellow FP pool was PCR amplified with appropriate primers with *KpnI* and *SbfI* sites at 5' and 3' respectively. Single digestion of both the CDK1 substrate cDNA and the yellow FPs gene pool with *KpnI* was performed. These were then mixed in equimolar proportion and ligated for 1 hour. The ligated product was then used as a template for further PCR amplifications. Specifically, the 3' primer was the *SbfI*-containing primer mentioned above, and the 5' primer was a mixture of primers encoding various lengths of linker and ending in a *SalI* restriction at the 5' end. The resulting PCR products were purified, double digested with *SalI* and *SbfI*, and ligated with similarly digested modified pUADE that already carried the cyan FP library pool. The resulting plasmid-based library was designated pUADE-CDK1 (Supplementary Fig. 1B).

Primary library screen in colonies. As described above, we had prepared two distinct plasmid-based biosensor libraries, pUADE-PKB and pUADE-CDK1, in dual expression plasmids together with the corresponding enzymes that would catalyze their post-translational modification. *E. coli* was transformed with either pUADE-PKB or pUADE-CDK1, and transformants were plated on LB-agar with 0.04% ampicillin, 1 mM IPTG and 20 mM D-glucose in polystyrene Petri dishes and incubated at 37 °C overnight. The

imaging system used for colony screening in our lab has been previously described.[2] Briefly, it is composed of a 175 W xenon-arc lamp (Sutter instrument company, Novato, CA) as a source of excitation light; band-pass filters housed in a filter wheel for the wavelength selection of the excitation light; a bifurcated fibre optic bundle (Newport corporation, Stratford, CT) to guide the filtered excitation light to a compartment with a circular recession to fit a 10 cm diameter Petri dish; a second set of band-pass filters housed in a filter wheel for selection of emission wavelength positioned vertically above the illuminated Petri dish; and a Retiga 1300i 12-bit CCD camera (QImaging, Burnaby,BC) just behind the emission filter wheel to snap pictures of the fluorescent colonies grown in the Petri dish. For FRET imaging using this system, fluorescence images of the untreated plates were acquired in both donor (excitation 420-440 nm; emission 460-500 nm) and acceptor (excitation 420-440 nm; emission 520-550 nm) fluorescence channels. Plates were then sprayed with 1 M L-arabinose solution, left at room temperature for 2 hours and images were acquired again for both the donor and acceptor fluorescence channels. Custom macros running in Image Pro Plus (Media Cybernetics Inc., Silver Spring, MD) were used to process the acquired images and create a spreadsheet showing donor and acceptor intensities for each colony both pre- and post-spray. The spreadsheets were exported to Microsoft Excel where the ratio of emission intensity of acceptor to emission intensity of donor ($I_{Acceptor}/I_{Donor}$) was calculated for each colony pre- and post-spray. The emission ratio change was calculated as $\Delta R/R_{min}$ % and colonies showing the highest ratio changes (typically $\Delta R/R_{min} > 15\%$) were isolated, propagated, and their plasmid DNA extracted and sequenced.

Secondary library screen with purified protein. As a secondary screen of clones identified during the primary colony based screen, each clone was subjected to 2 separate protein expression conditions. In the first condition, the biosensor was co-expressed with constitutively active enzyme and in the second condition the biosensor was expressed alone. The first condition was obtained by inoculating 500 ml Lysogeny Broth (LB) medium supplemented with 0.04% ampicillin, 1 mM IPTG and 10 mM L-arabinose with a single colony of *E. coli* that had been transformed with pUADE carrying the gene for a specific biosensor variant. The second condition was obtained by inoculating 500 ml LB medium supplemented with 0.04% ampicillin, 1 mM IPTG and 30 mM D-glucose with an identical colony. All cultures were incubated at 37 °C for 24 hours with shaking at 250 rpm (Innova 4330 shaker, New Brunswick Scientific). Cells were harvested by centrifugation at 8000

rpm (Beckmann Rotor centrifuge) and re-suspended in lysis buffer (50 mM sodium phosphate, 300 mM sodium chloride, 10 mM imidazole, pH 7.5) and lysed by a cell disruptor (Constant Systems, Daventry, Northants, UK). Insoluble cell debris was separated by centrifugation at 14000 rpm, and biosensor proteins (carrying an N-terminal His_6 tag) were then purified by Ni-NTA affinity chromatography (Amersham, Amersham, Buckinghamshire, UK) and exchanged into PBS buffer (37 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH of 7.4) using Amicon Ultra-4 Centrifugal filter units (Millipore) with a molecular weight cut-off of 50 kDa. All protein expressions are done in at least 4 replicates to confirm reproducibility and statistical significance.

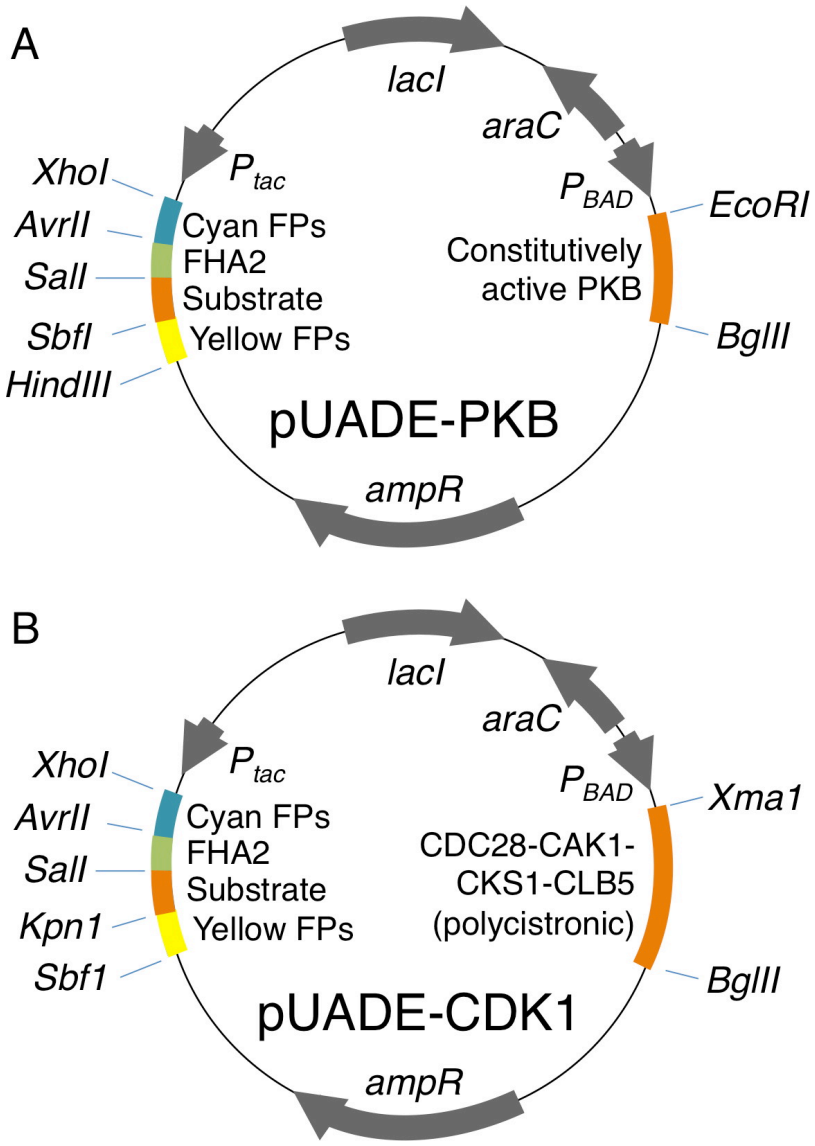
Gel electrophoresis and Western blotting. Gel electrophoresis and Western blotting was used to assess protein expression and degree of biosensor phosphorylation of expressed proteins. The concentration of proteins expressed as described above was determined using bicinchoninic acid (BCA) protein assay following the manufacturer's protocol (Pierce). Each of the expressed proteins (1 µg) was subjected to tricine-SDS-polyacrylamide gel electrophoresis as described earlier in two parallel experiments. The first gel was stained with Coomassie Brilliant Blue R-250 and the second gel was subjected to Western blotting. Western Blotting was perfomed via electroblotting the bands on the SDS-polyacrylamide gel into PVDF membrane (Millipore) followed by blocking the membrane by immersion in 5% bovine serum albumin (BSA) in TTBS Buffer (Tris-buffered saline pH 7.4 containing 0.1% tween-20) overnight at 4 °C. The membrane was then washed and incubated with rabbit anti-phosphoserine (Life Technologies catalog number 61-800) or rabbit anti-phosphothreonine (Life Technologies catalog number 71-8200) polyclonal primary antibody overnight at 4 °C. The membrane was then washed again and incubated with a horseradish peroxidase (HRP) conjugate of goat anti-rabbit IgG secondary antibody (Life technologies) for 2 hours at 4 °C. Bands were visualized using ECL-chemiluminescence substrate (Pierce) and ImageQuant RT ECL imager (GE Healthcare life sciences). In the lane for the protein expressed in the presence of L-arabinose we observed an extra band for constitutively active PKB enzyme (expected MW 54 kDa). The PKB enzyme contains an N-terminal His_6 tag so co-purifies with the biosensor during Ni-NTA affinity purification.

Live Cell Imaging: To construct a mammalian expression encoding the cyclin B1-CDK1 biosensor, the dual expression vector pUADE carrying the best variant was doubly digested with XhoI and HindIII. The appropriate restriction product was purified by gel

extraction and then ligated with similarly digested modified pcDNA3.1(-) vector (Life Technologies). Successful cloning was confirmed by DNA sequencing. HeLa S3 cells (ATCC; Manassas, VA) were cultured in a 1:1 mixture of Dulbecco's Modified Eagle's Medium (DMEM) and Ham's F12 supplemented with 12.5% Cosmic calf serum (Thermo Scientific; Waltham, MA) at 37 °C in a 5% CO_2 incubator. Cells were seeded directly onto 35 mm Delta-T culture chambers (Bioptechs; Butler, PA) at ~60-70% confluence and were transfected with 1 µg of plasmid DNA using Effectene (QIAGEN; Valencia, CA). Approximately 18 hours after transfection, cell division was synchronized with 2 mM thymidine for 24 hours. Cells were then washed with PBS and supplemented with fresh medium for 3 hours after which, 100 ng/mL nocodazole was added to the media for 12 hours. The cells were then washed with PBS, supplemented with fresh medium, and imaged immediately.

Live cell imaging was performed on an Olympus FV1000 confocal microscope (Olympus; Center Valley, PA) with an Olympus PLAPO 40× oil immersion objective (NA=1.0). A 458 nm argon-ion laser line and a 515 nm argon-ion laser line with a 458/515 nm dichroic mirror were used to excite the cyan and yellow FPs, respectively. The CFP emission channel spanned 475-500 nm and the YFP emission channel spanned 430-630 nm. Each imaging experiment was set to a scan speed of 8.0 µs/pixel and a pinhole size of 500 µm. Raw data was collected and analyzed with the Fluoview confocal microscope software (Olympus).

Supplementary Figure



Supplementary Fig. 1 Schematic representations of dual expression plasmids used for library screening. A) pUADE-PKB. B) pUADE-CDK1.

Supplementary Table

Supplementary Table 1 Sequences for all oligonucleotides used in this work.

Name	Sequence (5′ to 3′)
PKB-EcoRI-FD	CGCCTAGAATTCATGGACGACGTGGCCATCGT
PKB-BglII-BK	TAGGCGAGATCTTCAGGCCGTCGCGCTAG
YLS-FRET-FDprimer	CGCCTAGGTACCTCCGGACCTAGGTCCGGAGGGTCGACATCCGGA
YLS-FRET-BKprimer	TAGGCGGCCGGCCGTTCCGGAACCTGCAGGTCCGGATGTCGACCC
TFP-XhoI-FD	CGCCTACTCGAGCATGGTGAGCAAGGGCGAGGA
TFP-AvrII-BK	TAGGCGCCTAGGCTTGTACAGCTCGTCCATGCCGTC
TFP-BglII-BK	TAGGCGAGATCTCTTGTACAGCTCGTCCATGCCGTCGGT
XbaI-LK-TFP-FD	CGCCTATCTAGAGGAGGATCCGGAGGTATGGTGAGCAAGGGCGAGGAGACC
TFP194-FD-XhoI	TATTCTCGAGaATGCTGCCCGACTATCACTTTGTGGACCACCG
TFP193-BK-AvrII	AATACCTAGGCTTCACCGCCTTCTTGGCCCTGTAGAT
TFP-208-FD-XhoI	TATTCTCGAGaAACCACGACAAGGACTACAACAAGGTG
TFP-207-BK-AvrII	AATACCTAGGCAGGATCTCGATGCGGTGGTC
TFP-146-FD-XhoI	TATTCTCGAGaACCGGCTGGGACGCCTCC
TFP-145-BK-AvrII	AATACCTAGGGGTCTTCTTCTGCATCACGGGGCC
XhoI-CyPet-FD	CGCCTACTCGAGGATGTCTAAAGGTGAAGAATTATTCGGCGG
CyPet-AvrII-BK	GTTAACCTAGGTTTGTACAATTCATCCATACCATGGGTAATACC
mut-FHA2-sens	GAAGACAATAGGTTGTCACGAGTTCATTGCTTC
mut-FHA2-antisens	GAAGCAATGAACTCGTGACAACCTATTGTCTTC
FHA2-AvrII-FD	CGCCTACCTAGGAAGAAAGTTTGGACATTTGGTAGAAACCC
FHA2-SalI-BK	TAGGCGGTCGACTACACCAACGGTTATTTCATCACCTTG
YFP-SbfI-FD	CGCCTACCTGCAGGATGGTGAGCAAGGGCGAGGAG
YFP-stp-HindIII-BK	TAGGCGAAGCTTTTACTTGTACAGCTCGTCCATGCCGAG
SbfI-cp-Venus-FD	TATTCCTGCAGGAATGGACGGCGGCGTGCAGC
Cp-Venus-HindIII-BK	AATAAAGCTTTTACTCGATGTTGTGGCGGATCTTGAAGTTG
PKB-Sub-218link-SalI-FD	CGCCTAGTCGACGGTTCGACTAGCGGCAGTGGAAAGCCAGGATCTGGGGAA GGGTCAACAAAAGGTAGGAA
PKB-Sub-218link-SbfI-BD	TAGGCGCCTGCAGGGATTCCTAGAGTTCCAAGTCTGTCACGCTTCCTACCT TTTGTTGACCCTTCCCCAGA
SalI-SGLK1-PKB-Sub-FD	CGCCTAGTCGACGGCGGCAGCGGCGGCAGGAAGCGTGACAGACTTGGAACT CTAGGAATC
SalI-SGLK2-PKB-Sub-FD	CGCCTAGTCGACGGCGGCAGCGGCGGCGGCGGCAGCGGCGGCAGGAAGCGT GACAGACTTGGAACTCTAGGAATC
SalI-SGLK3-PKB-Sub-FD	CGCCTAGTCGACGGCGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGC GGCGGCAGGAAGCGTGACAGACTTGGAACTCTAGGAATC
SalI-SGLK4-PKB-Sub-FD	CGCCTAGTCGACGGCGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGC GGCGGCGGCGGCAGCGGCGGCAGGAAGCGTGACAGACTTGGAACTCTAG
SalI-LK0-PKB-Sub-FD	CGCCTAGTCGACAGGAAGCGTGACAGACTTGGAACTCTAGGAATC
SalI-LK2-PKB-Sub-FD	CGCCTAGTCGACATCAGGAGGAAGCGTGACAGACTTGGAACTCTAGGAATC
SalI-LK4-PKB-Sub-FD	CGCCTAGTCGACATCAACAGCAGGAGGAAGCGTGACAGACTTGGAACTCTA GGAATC
SalI-LK6-PKB-Sub-FD	CGCCTAGTCGACATCAACAGCAGGATCAGGAGGAAGCGTGACAGACTTGGA ACTCTAGGAATC
SalI-LK8-PKB-Sub-FD	CGCCTAGTCGACATCAACAGCAGGATCAGGAACAGGAGGAAGCGTGACAGA CTTGGAACTCTAGGAATC
SalI-LK10-PKB-Sub-FD	CGCCTAGTCGACATCAACAGCAGGATCAGGAACAGGAGCAGGAGGAAGCGT GACAGACTTGGAACTCTAGGAATC
SalI-LK14-PKB-Sub-FD	CGCCTAGTCGACATCAACAGCAGGATCAGGAACAGGAGCATCAGGTACAGC AGGAGGAAGCGTGACAGACTTGGAACTCTAGGAATC
SalI-LK20-PKB-Sub-FD	CGCCTAGTCGACATCAACAGCAGGATCAGGAACAGGAGCATCAGGTACAGC AGCAGGAAGAGGAACAGGAGGAGGAAGCGTGACAGACTTGGAACTCTAG
SalI-PKB-Sub-EA1-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAAGGAAGCGTGACAGACTTGGAACT CTAGGAATCCCT
SalI-PKB-Sub-EA2-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAAGGAAGCGT GACAGACTTGGAACTCTAGGAATCCCT
SalI-PKB-Sub-EA3-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAAGGAAGCGTGACAGACTTGGAACTCTAGGAATCCCT

SalI-PKB-Sub-EA4-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAAGGAAGCGTGACAGACTTGGAACTCTAGGA ATCCCT
SalI-PKB-Sub-EA5-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAAGGAAGCGTGACAGA CTTGGAACTCTAGGAATCCCT
SalI-PKB-Sub-EA6-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAA AGGAAGCGTGACAGACTTGGAACTCTAGGAATCCCT
SalI-PKB-Sub-EA7-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAA GAAGCGGCGGCGAAAAGGAAGCGTGACAGACTTGGAACTCTAGGAATCCCT
SalI-PKB-Sub-EA8-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAA GAAGCGGCGGCGAAAAGGAAGCGTGACAGACTTGGAACTCTAGGAATCCCT
SalI-PKB-Sub-EA9-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAA GAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAAGGAAG CGTGACAGACTTGGAACTCTAGGAATCCCT
SalI-PKB-Sub-EA10-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAA GAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCG GCGGCGAAAAGGAAGCGTGACAGACTTGGAACTCTAGGAATCCCT
EcoRI-XmaI-YPET-FD	CGCCTAGAATTCCCCGGGATGTCTAAAGGTGAAGAATTATTCACTGGTGTT GTCCCAATTTTG
Ypet-BglII-BK	TAGGCGAGATCTTTATTATTTGTACAATTCATTCATACCCTCGGTAATACC AGCAGCA
XmaI-polycis-FD	CGCCTACCCGGGATGAGCGGTGAATTAGCAAATTACAAAAGACTTGAGAAA GTCGGTG
Polycis-BglII-BK	TAGGCGAGATCTCTACTTAAGATTAAATAGATTTTGAAAGTTGCTATGCAT TTCGGATGTACACCACTTGAAAG
SalI-218LK-CDK-sub-FD	CGCCTAGTCGACGGTTCGACTAGCGGCAGTGGAAAGCCAGGATCTGGGGAA GGGTCAACAAAAGGTACCCCTGAGCCTATTTTGGTTGATACT
SalI-SGLK1-CDK-sub-FD	CGCCTAGTCGACGGCGGCAGCGGCGGCACCCCTGAGCCTATTTTGGTTGAT ACT
SalI-SGLK2-CDK-sub-FD	CGCCTAGTCGACGGCGGCAGCGGCGGCGGCGGCAGCGGCGGCACCCCTGAG CCTATTTTGGTTGATACT
SalI-SGLK3-CDK-sub-FD	CGCCTAGTCGACGGCGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGC GGCGGCACCCCTGAGCCTATTTTGGTTGATACT
SalI-SGLK4-CDK-sub-FD	CGCCTAGTCGACGGCGGCAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCAGC GGCGGCGGCGGCAGCGGCGGCACCCCTGAGCCTATTTTGGTTGATACT
SalI-LK0-CDK-sub-FD	CGCCTAGTCGACACCCCTGAGCCTATTTTGGTTGATACT
SalI-LK2-CDK-sub-FD	CGCCTAGTCGACATCAGGACCCCTGAGCCTATTTTGGTTGATACT
SalI-LK4-CDK-sub-FD	CGCCTAGTCGACATCAACAGCAGGACCCCTGAGCCTATTTTGGTTGATACT
SalI-LK6-CDK-sub-FD	CGCCTAGTCGACATCAACAGCAGGATCAGGACCCCTGAGCCTATTTTGGTT GATACT
SalI-LK8-CDK-sub-FD	CGCCTAGTCGACATCAACAGCAGGATCAGGAACAGGACCCCTGAGCCTATT TTGGTTGATACT
SalI-LK10-CDK-sub-FD	CGCCTAGTCGACATCAACAGCAGGATCAGGAACAGGAGCAGGACCCCTGAG CCTATTTTGGTTGATACT
SalI-LK14-CDK-sub-FD	CGCCTAGTCGACATCAACAGCAGGATCAGGAACAGGAGCATCAGGTACAGC AGGACCCCTGAGCCTATTTTGGTTGATACT
SalI-LK20-CDK-sub-FD	CGCCTAGTCGACATCAACAGCAGGATCAGGAACAGGAGCATCAGGTACAGC AGCAGGAAGAGGAACAGGAGGACCCCTGAGCCTATTTTGGTTGATACT
SalI-EA1-CDK-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAACCCCTGAGCCTATTTTGGTTGAT ACT
SalI-EA2-CDK-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAACCCCTGAG CCTATTTTGGTTGATACT
SalI-EA3-CDK-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAACCCCTGAGCCTATTTTGGTTGATACT

SalI-EA4-CDK-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAACCCCTGAGCCTATTTTGGTTGATACT
SalI-EA5-CDK-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAACCCCTGAGCCTATT TTGGTTGATACT
SalI-EA6-CDK-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAA ACCCCTGAGCCTATTTTGGTTGATACT
SalI-EA7-CDK-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAA GAAGCGGCGGCGAAAACCCCTGAGCCTATTTTGGTTGATACT
SalI-EA8-CDK-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAA GAAGCGGCGGCGAAAGAAGCGGCGGCGAAAACCCCTGAGCCTATTTTGGTT GATACT
SalI-EA8-CDK-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAA GAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAACCCCT GAGCCTATTTTGGTTGATACT
SALI-EA10-CDK-FD	CGCCTAGTCGACGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCG GCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAA GAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCGGCGGCGAAAGAAGCG GCGGCGAAAACCCCTGAGCCTATTTTGGTTGATACT
YFP-sTp-SbFI-BK	TAGGCGCCTGCAGGTTACTTGTACAGCTCGTCCATGCCGAG
Ypet-stp-sbfI-BK	TAGGCGCCTGCAGGTTATTTGTACAATTCATTCATACCCTC

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