

Scientific doctoral publications related to the thesis

Kasera, H., Sanghi, S., Singh, P*. PLK4 Homodimerization is Required for CEP152 Centrosome Localization and Spindle Organization, *Journal of Molecular Biology*, 2025 Apr 11; 437 (13): 169152. doi: 10.1016/j.jmb.2025.169152, PMID: 40222413

Kasera, H and Singh, P*. Harnessing Structure Prediction of Polo-Like Kinase 4 for Drug Repurposing. *Cytoskeleton (Hoboken)*. 2025 Mar 20. doi: 10.1002/cm.22020, PMID: 40110897

Kasera, H., Shekhawat, R.S., Yadav, P., Singh P*. Gene expression profiling and protein-protein network analysis revealed prognostic hub biomarkers linking cancer risk in type 2 diabetic patients. *Scientific Reports*, 2023, **13**, 22605. <https://doi.org/10.1038/s41598-023-49715-9>

Kasera, H., Kumar, S., & Singh, P. Yeast 2-hybrid assay for investigating the interaction between the centrosome proteins PLK4 and STIL. *Methods in Cell Biology*, 2022, 169, 97–114. <https://doi.org/10.1016/bs.mcb.2021.12.003>

Jaiswal, S*, **Kasera, H***, Jain, S., Khandelwal S., Singh P. Centrosome: A Microtubule Nucleating Cellular Machinery. *Journal of Indian Institute of Science*, 2021, 101, 5–18. <https://doi.org/10.1007/s41745-020-00213-1> (* Equal contribution)

Annexure A

Materials

A.1 Materials

Table A.1.1:- List of Primers

S. No.	Name of Primer	Sequence (5'-3')	Source
1	PLK4_BamH1_FP	ACCTGTACTTCCAGGGATCCATGGCGA CCTGCATCGGGG	Eurofins
2	PLK4_Xho1_RP	GTTTCTTTACCAGACTCGACCTCGAGTC AATGAAAATTAGGAGTCG	Eurofins
3	PLK4_571_BamH1 _FP	GGCCGGATCCATGAGCAAGACTAGGG GTATG	Eurofins
4	PLK4_570_Xho1_R P	GGCCCTCGAGTCACTGTTTCAGAAAGAG GATC	Eurofins
5	PLK4_271_Xho1_R P	GGCCCTCGAGTCATGTTGAAGAATTTC G	Eurofins
6	PLK4_265_BamH1 _FP	GGCCGGATCCATGTCCCGAAATTCTTC AAC	Eurofins
7	PLK4_820_Xho1_R P	GGCCCTCGAGTCAAGGAGGAGGTGAT AAGG	Eurofins
8	PLK4_880_BamH1 _FP	GGCCGGATCCATGTGTCTTCCTAAATC AGC	Eurofins
9	PLK4_814_BamH1 _FP	GGCCGGATCCATGAAGGCCTTATCACC TC	Eurofins
10	cMYC_oligo_HindI II_FP	AGCTTATGGAACAAAACTTATTAGCG AAGAAGATCTTG	Eurofins
11	cMyc_oligo_BamH 1_RP	GATCCAAGATCTTCTTCGCTAATAAGT TTTTGTTCCATA	Eurofins

12	PLK4_K_685_A_FP	CCAGAAAAATACTGGCGAGCATATCA ATATGCTTCCAGGTTT	Sigma
13	PLK4_K_685_A_R P	AAACCTGGAAGCATATTGATATGCTCG CCAGTATTTTTCTGG	Sigma
14	PLK4_E_774_K_FP	TATATGGACCATGCTAATAAGGGTCAT CGTATTTGTTTAGCA	Sigma
15	PLK4_E_774_K_RP	TGCTAAACAAATACGATGACCCTTATT AGCATGGTCCATATA	Sigma
16	PLK4_E_774_*_FP	TATATGGACCATGCTAATTAGGGTCAT CGTATTTGTTTAGCA	Sigma
17	PLK4_E_774_*_RP	TGCTAAACAAATACGATGACCCTAATT AGCATGGTCCATATA	Sigma
18	PLK4_774_Not1_R P	ATTGCGCGGCCGCATGGTCCATATACA TTTTTATC	BioReserve
19	PLK4_814_Not1_F P	ATTGCGGCCGCCTTATCACCTCCTCCTT CT	BioReserve
20	PLK4_K711A_FP	CTTATTTTACAAGATATGCTGCATGCAT TTTGATGG	BioReserve
21	PLK4_K711A_RP	CCATCAAAATGCATGCAGCATATCTTG TAAAATAAG	BioReserve
22	CEP152_BamH1_F P	GGCCGGATCCATGGGCTCATTAGACTT TGGCAG	Eurofins
23	CEP152_512_Xho1 _RP	GGCCTCGAGTTACAAATCCACATACG	BioReserve
24	EGFP_HindIII_FP	CGTTTAAACTTAAGCTTATGGTGAGCA AGGGC	Eurofins
25	EGFP_BamH1_RP	GGCATCAGGAACATGGATCCCTTGTAC AGCTCGTCC	Eurofins

Table A.1.2:- Source of plasmids and cDNAs

S. No.	Recombinant DNA	Source
1	pCDNA5/FRT/TO	Gift from Andrea Musacchio
2	pET Duet1	Gift from Andrea Musacchio
3	pGEX-6P1	Gift from Andrea Musacchio
4	siRNA-resistant PLK4 cDNA	Gift from Andrea Musacchio
5	siRNA-resistant CEP152 cDNA	Gift from Pierre Gözy
6.	pCDNA5 FRT/TO EGFP	Sonal Jaiswal
7.	pCDNA5 FRT/TO EGFP CEP152	Swati Jain
8.	pGEX GST CEP152 1-512	Karthik Srinivasan
9.	pGEX-6P1 GST CEP192 144-337	BioMatik, Canada

Table A.1.3:- List of plasmids generated for the thesis work

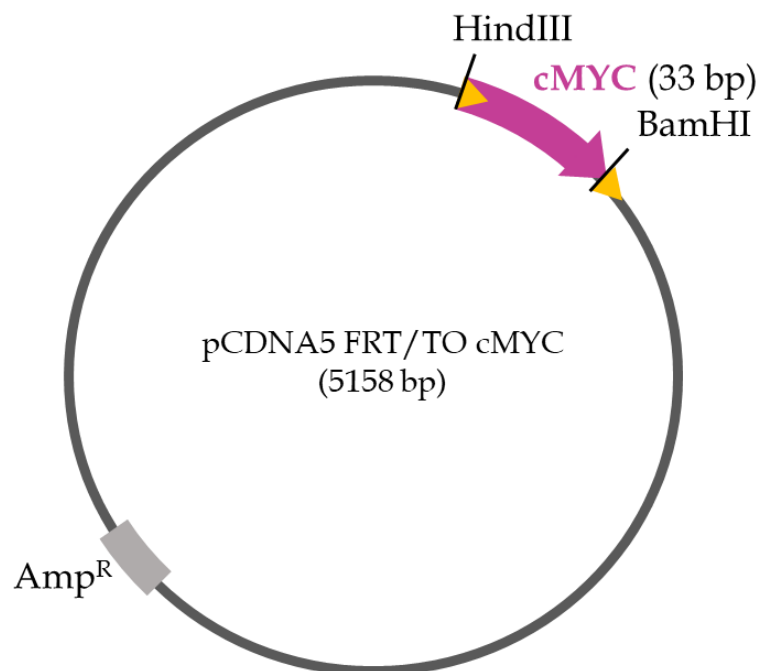
Entry No.	Name	Vector	Insert	Sites
1.	pET Duet1 His MBP PLK4 571-970	pET Duet1 His MBP	PLK4 571-970	BamHI and XhoI
2.	pET Duet1 His MBP PLK4 571-820	pET Duet1 His MBP	PLK4 571-820	BamHI and XhoI
3.	pET Duet1 His MBP PLK4 814-970	pET Duet1 His MBP	PLK4 814-970	BamHI and XhoI
4.	pET Duet 1 His MBP PLK4 571-970 E774K	pET Duet1 His MBP	PLK4 571-970 E774K	BamHI and XhoI
5.	pET Duet 1 His MBP PLK4 571-970 E774*	pET Duet1 His MBP	PLK4 571-970 E774*	BamHI and XhoI
6.	pET Duet 1 His MBP PLK4 571-970 K685A	pET Duet1 His MBP	PLK4 571-970 K685A	BamHI and XhoI
7.	pET Duet1 His MBP PLK4 571-970 K685/711A	pET Duet1 His MBP	PLK4 571-970 K685/711A	BamHI and XhoI
8.	pET Duet1 His MBP PLK4 571-774	pET Duet1 His MBP	PLK4 571-774	BamHI and NotI
9.	pET Duet1 His MBP PLK4 571-970 Δ 774-813	pET Duet1 His MBP	PLK4 814-970	NotI and XhoI
10.	pGEX GST PLK4	pGEX 6P1 GST	PLK4	BamHI and XhoI
11.	pGEX GST PLK4 571-970	6P1 GST	PLK4 571-970	BamHI and XhoI
12.	pGEX GST PLK4 571-970 E774*	6P1 GST	PLK4 571-970 E774*	BamHI and XhoI
13.	pCDNA5 FRT/TO EGFP PLK4	pCDNA5 FRT/TO EGFP	PLK4	BamHI and XhoI

14.	pCDNA5 FRT/TO EGFP PLK4 571-970	pCDNA5 FRT/TO EGFP	PLK4 571-970	BamHI and XhoI
15.	pCDNA5 FRT/TO EGFP PLK4 571-970 E774*	pCDNA5 FRT/TO EGFP	PLK4 571-970 E774*	BamHI and XhoI
16.	pCDNA5 FRT/TO EGFP PLK4 E774*	pCDNA5 FRT/TO EGFP	PLK4 E774*	BamHI and XhoI
17.	pCDNA5 FRT/TO EGFP PLK4 571-970 K685A	pCDNA5 FRT/TO EGFP	PLK4 571-970 K685A	BamHI and XhoI
18.	pCDNA5 FRT/TO EGFP PLK4 571-970 K685/711A	pCDNA5 FRT/TO EGFP	PLK4 571-970 K685/711A	BamHI and XhoI
19.	pCDNA5 FRT/TO eGFP PLK4 1-774	pCDNA5 FRT/TO EGFP	PLK4 1-774	BamHI and NotI
20.	pCDNA5 FRT/TO eGFP PLK4 Δ 774-813	pCDNA5 FRT/TO EGFP	PLK4 814-970	NotI and XhoI
21.	pCDNA5 FRT/TO cMYC	pCDNA5 FRT/TO	cMYC	HindIII and BamHI
22.	pCDNA5 FRT/TO cMYC PLK4	pCDNA5 FRT/TO cMYC	cMYC PLK4	BamHI and XhoI
23.	pCDNA5 FRT/TO cMYC PLK4 E774*	pCDNA5 FRT/TO cMYC	cMYC PLK4 E774*	BamHI and XhoI

A.2 Clone Maps

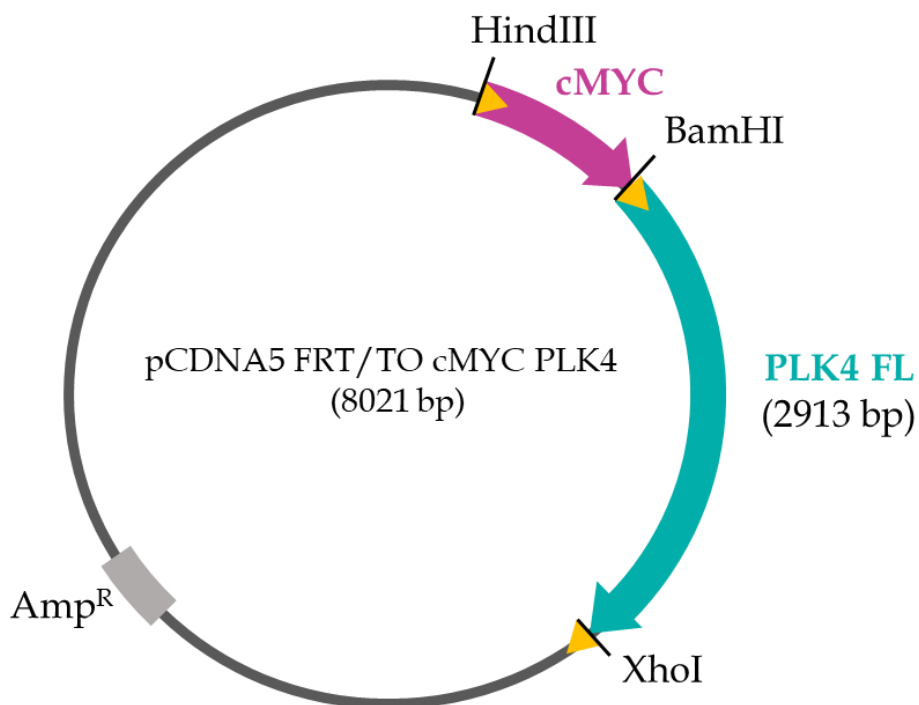
1. Clone map of pCDNA5 FRT/TO cMYC

Name of the Clone	pCDNA5_FRT/TO_cMYC
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	cMYC oligos were annealed and cloned in pCDNA5 FRT/TO plasmid between HindIII and BamHI restriction sites
Antibiotic resistance	Ampicillin
Vector	pCDNA5 FRT/TO
Insert	cMYC
Primers	FP- 5'- AGCTTATGGAACAAAACTTATTAGCGAAGAAGATCTTG -3' RP- 5'- GATCCAAGATCTTCTTCGCTAATAAGTTTTTGTTCATA -3'
Any other relevant Information	Construct codes for cMYC tag



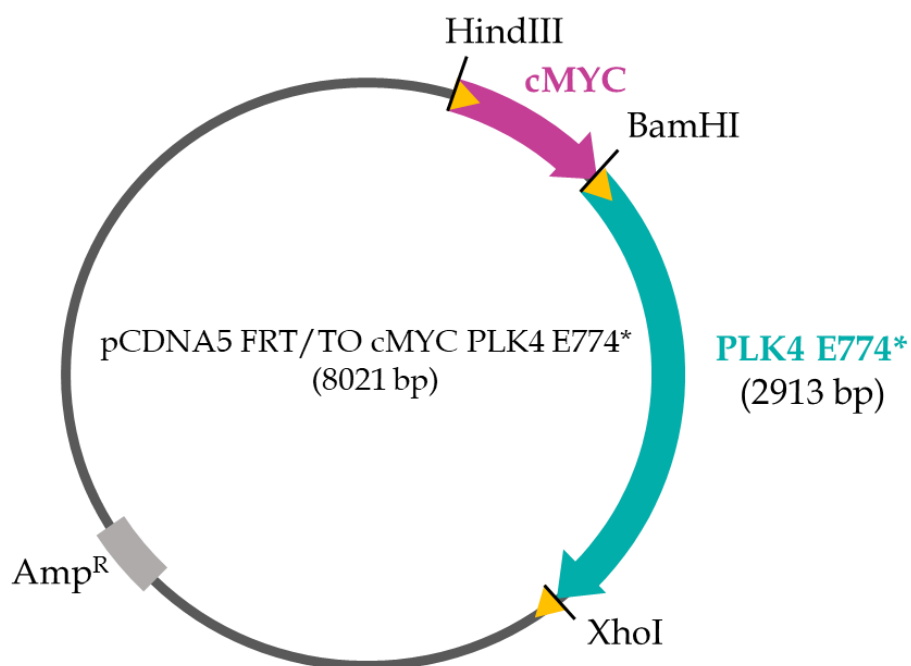
2. Clone map of pCDNA5 FRT/TO cMYC PLK4

Name of the Clone	pCDNA5 FRT/TO cMYC PLK4
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 full length cDNA was PCR amplified and cloned in pCDNA5 FRT/TO cMYC plasmid between BamHI and XhoI restriction sites
Antibiotic resistance	Ampicillin
Vector	pCDNA5 FRT/TO cMYC
Insert	PLK4
Primers	FP- 5'- ACCTGTACTTCCAGGGATCCATGGCGACCTGCATCGGGG -3' RP- 5'- GTTTCTTTACCAGACTCGACCTCGAGTCAATGAAAATTAGGAG TCG -3'
Any other relevant Information	Construct codes for PLK4 full length with N-terminal cMYC tag



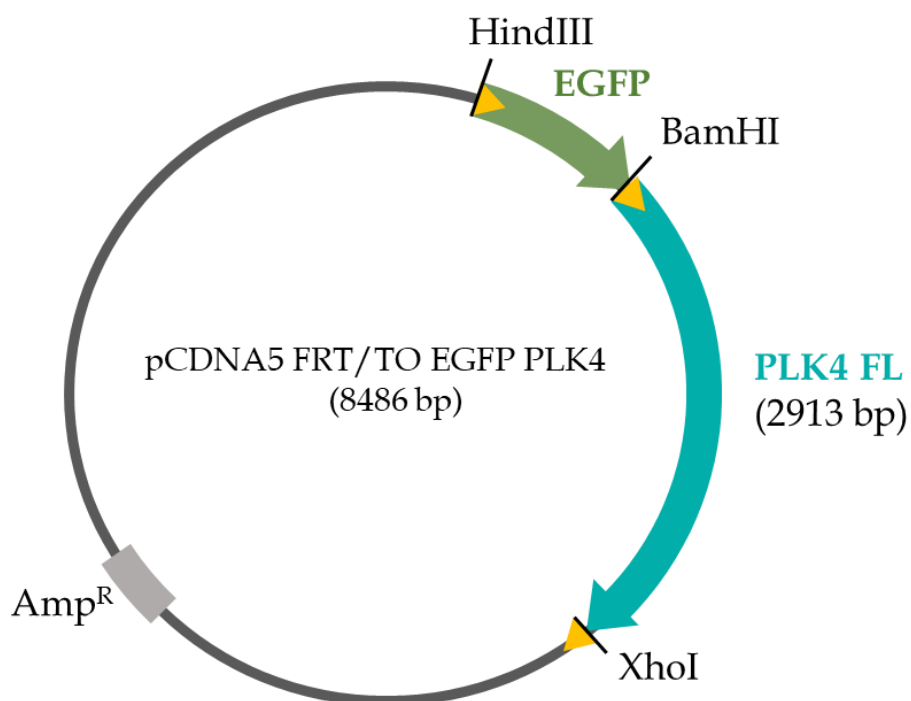
3. Clone map of pCDNA5 FRT/TO cMYC PLK4 E774*

Name of the Clone	pCDNA5 FRT/TO cMYC PLK4 E774*
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 E774* were generated by Site-directed mutagenesis. Plasmids were PCR amplified using mutagenesis primers, digested using Dpn1 and transformed in cells.
Antibiotic resistance	Ampicillin
Vector	pCDNA5 FRT/TO cMYC
Insert	PLK4 E774*
Primers	FP- 5'- TATATGGACCATGCTAATTAGGGTCATCG TATTGTTTAGCA -3' RP- 5'- TGCTAAACAAATACGATGACCCTAATTA GCATGGTCCATATA -3'
Any other relevant Information	Touch-down PCR was used to generate the mutations using iProof HF DNA polymerase (BioRad, #1725301)



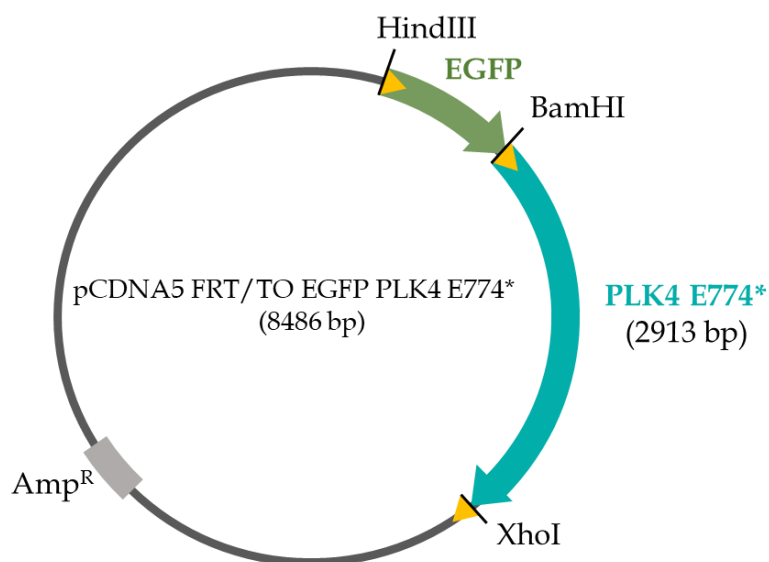
4. Clone map of pCDNA5 FRT/TO EGFP PLK4

Name of the Clone	pCDNA5 FRT/TO EGFP PLK4
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 full length cDNA was PCR amplified and cloned in pCDNA5 FRT/TO EGFP plasmids between BamHI and XhoI restriction sites
Antibiotic resistance	Ampicillin
Vector	pCDNA5 FRT/TO EGFP
Insert	PLK4
Primers	FP- 5'- ACCTGTACTTCCAGGGATCCATGGCGACCTGCATCGGGG -3' RP- 5'- GTTTCTTTACCAGACTCGACCTCGAGTCAATGAAAATTAGGAGT CG -3'
Any other relevant Information	Construct codes for PLK4 full length with N-terminal EGFP tag



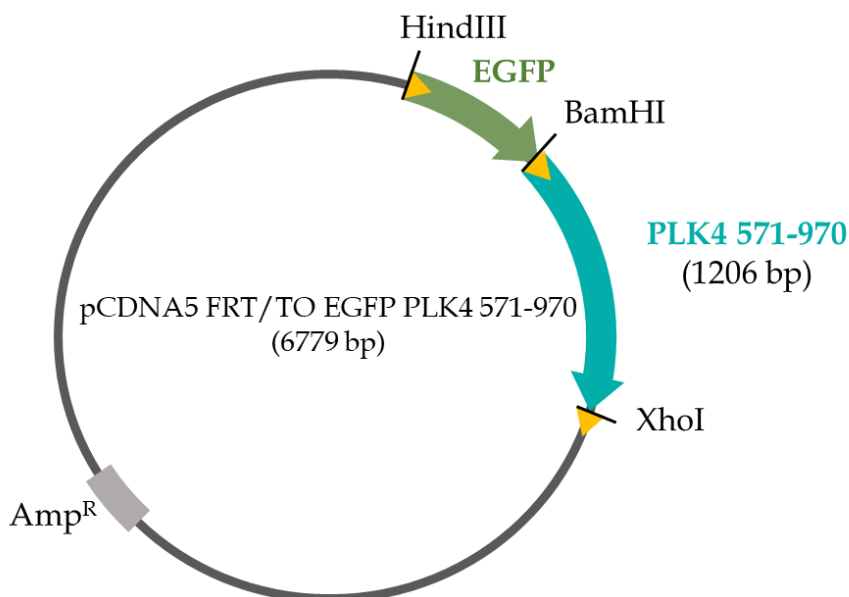
5. Clone map of pCDNA5 FRT/TO EGFP PLK4 E774*

Name of the Clone	pCDNA5 FRT/TO EGFP PLK4 E774*
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 E774* were generated by Site-directed mutagenesis. Plasmids were PCR amplified using mutagenesis primers, digested using Dpn1 and transformed in cells.
Antibiotic resistance	Ampicillin
Vector	pCDNA5 FRT/TO EGFP
Insert	PLK4 E774*
Primers	FP- 5'- TATATGGACCATGCTAATTAGGGTCATCGTATTTGTTTA GCA -3' RP- 5'- TGCTAAACAAATACGATGACCCTAATTAGCATGGTCCAT ATA -3'
Any other relevant Information	Touch-down PCR was used to generate the mutations using iProof HF DNA polymerase (BioRad, #1725301)



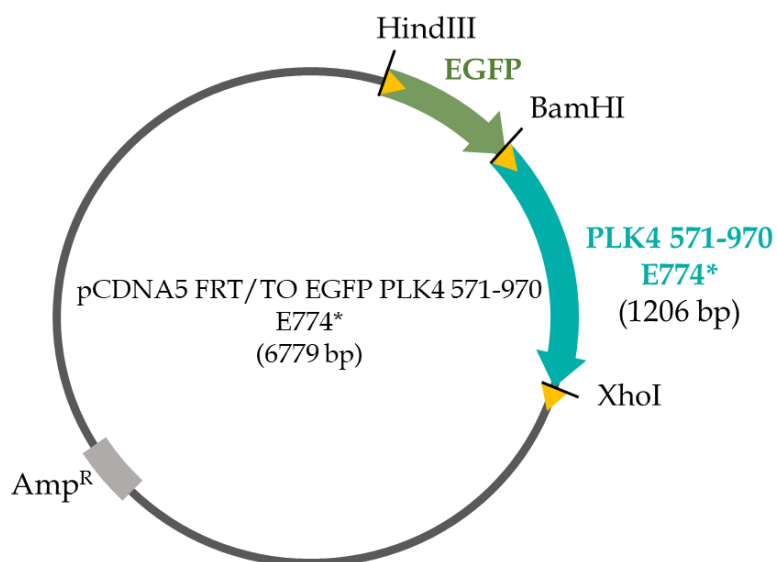
6. Clone map of pCDNA5 FRT/TO EGFP PLK4 571-970

Name of the Clone	pCDNA5 FRT/TO EGFP PLK4 571-970
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 571-970 fragment was PCR amplified and cloned in pCDNA5 FRT/TO EGFP plasmid between BamHI and XhoI restriction sites
Antibiotic resistance	Ampicillin
Vector	pCDNA5 FRT/TO EGFP
Insert	PLK4 571-970
Primers	FP- 5'-GGCCGGATCCATGAGCAAGACTAGGGGTATG-3' RP- 5'- GTTTCTTTACCAGACTCGACCTCGAGTCAATGAAAATTAGG AGTCG-3'
Any other relevant Information	Construct codes for PLK4 571-970 fragment with N-terminal EGFP tag



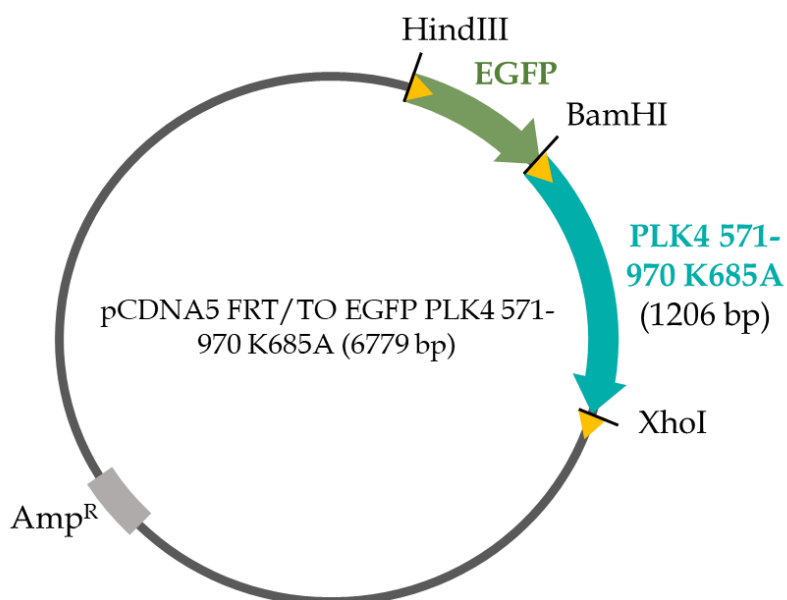
7. Clone map of pCDNA5 FRT/TO EGFP PLK4 571-970 E774*

Name of the Clone	pCDNA5 FRT/TO EGFP PLK4 571-970 E774*
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 571-970 E774* were generated by Site-directed mutagenesis. Plasmids were PCR amplified using mutagenesis primers, digested using Dpn1 and transformed in cells.
Antibiotic resistance	Ampicillin
Vector	pCDNA5 FRT/TO EGFP
Insert	PLK4 571-970 E774*
Primers	FP- 5'- TATATGGACCATGCTAATTAGGGTCATCGTATTTGTTTA GCA -3' RP- 5'- TGCTAAACAAATACGATGACCCTAATTAGCATGGTCCAT ATA -3'
Any other relevant Information	Touch-down PCR was used to generate the mutations using iProof HF DNA polymerase (BioRad, #1725301)



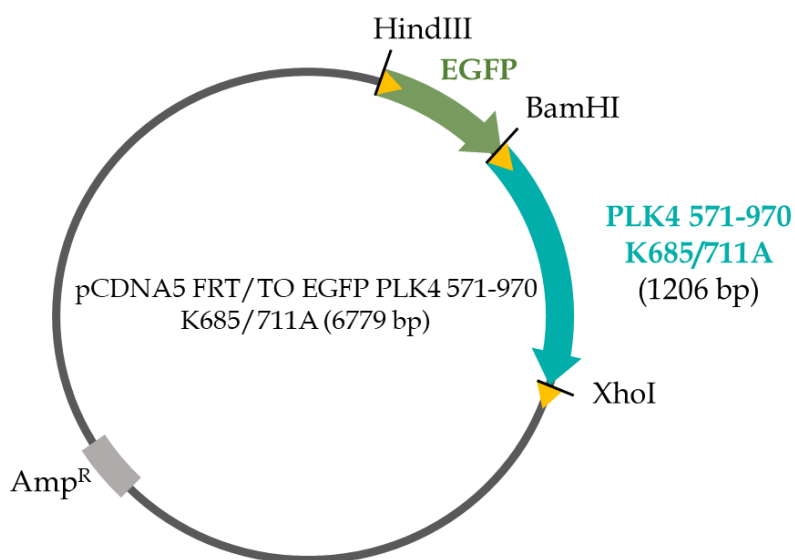
8. Clone map of pCDNA5 FRT/TO EGFP PLK4 571-970 K685A

Name of the Clone	pCDNA5 FRT/TO EGFP PLK4 571-970 K685A
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 571-970 K685A were generated by Site-directed mutagenesis. Plasmids were PCR amplified using mutagenesis primers, digested using Dpn1 and transformed in cells.
Antibiotic resistance	Ampicillin
Vector	pCDNA5 FRT/TO EGFP
Insert	PLK4 571-970 K685A
Primers	FP- 5'- CCAGAAAAATACTGGCGAGCATATCAATATGCT TCCAGGTTT -3' RP- 5'- AAACCTGGAAGCATATTGATATGCTCGCCAGTAT TTTCTGG -3'
Any other relevant Information	Touch-down PCR was used to generate the mutations using iProof HF DNA polymerase (BioRad, #1725301)



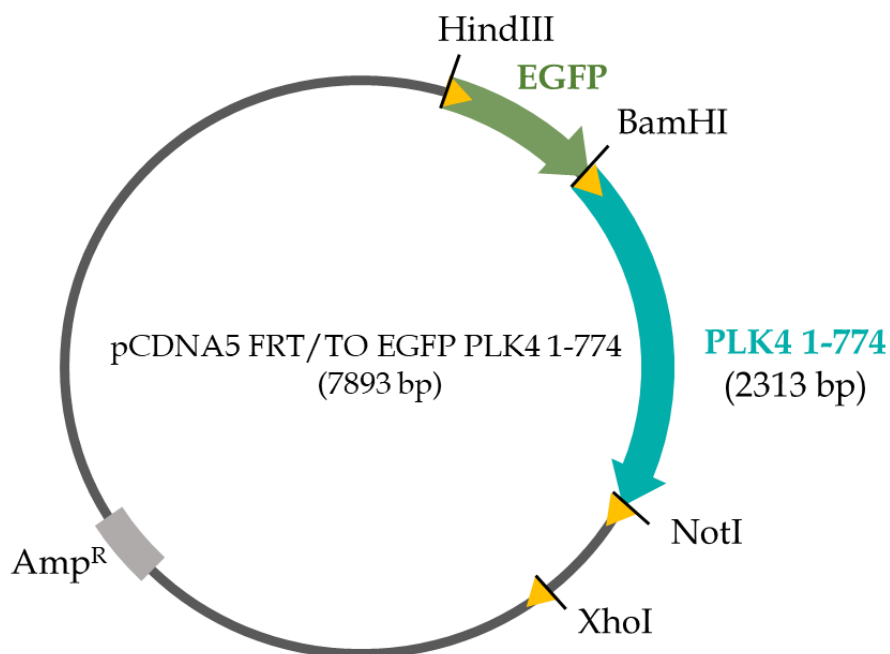
9. Clone map of pCDNA5 FRT/TO EGFP PLK4 571-970 K685/711A

Name of the Clone	pCDNA5 FRT/TO EGFP PLK4 571-970 K685/711A
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 571-970 K685/711A were generated by Site-directed mutagenesis. Plasmids were PCR amplified using mutagenesis primers, digested using Dpn1 and transformed in cells.
Antibiotic resistance	Ampicillin
Vector	pCDNA5 FRT/TO EGFP
Insert	PLK4 571-970 K685/711A
Primers	FP- 5'- CTTATTTTACAAGATATGCTGCATGCATTTTGATGG -3' RP- 5'- CCATCAAAATGCATGCAGCATATCTTGTAATAAG -3'
Any other relevant Information	Touch-down PCR was used to generate the mutations using iProof HF DNA polymerase (BioRad, #1725301)



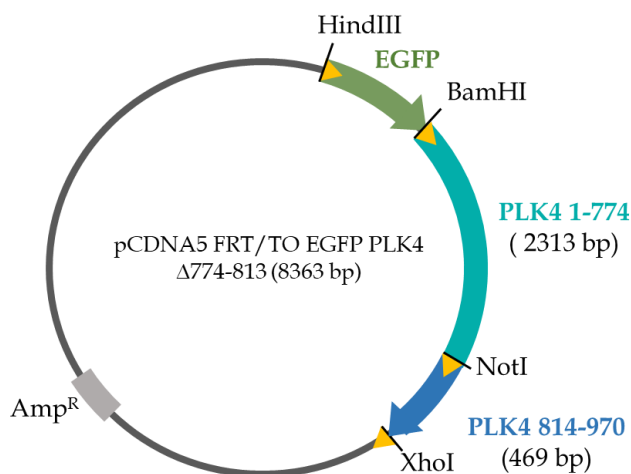
10. Clone map of pCDNA5 FRT/TO EGFP PLK4 1-774

Name of the Clone	pCNDNA5 FRT/TO EGFP PLK4 1-774
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 1-774 fragment was PCR amplified and cloned in pCDNA5 FRT/TO EGFP plasmid between BamHI and NotI restriction sites
Antibiotic resistance	Ampicillin
Vector	pCDNA5 FRT/TO EGFP
Insert	PLK4 1-774
Primers	FP- 5'- ACCTGTACTTCCAGGGATCCATGGCGACCTGCATCGGGG - 3' RP- 5'- ATTGCGCGGCCGCATGGTCCATATACATTTTATC -3'
Any other relevant Information	Construct codes for PLK4 1-774 fragment with N-terminal tag



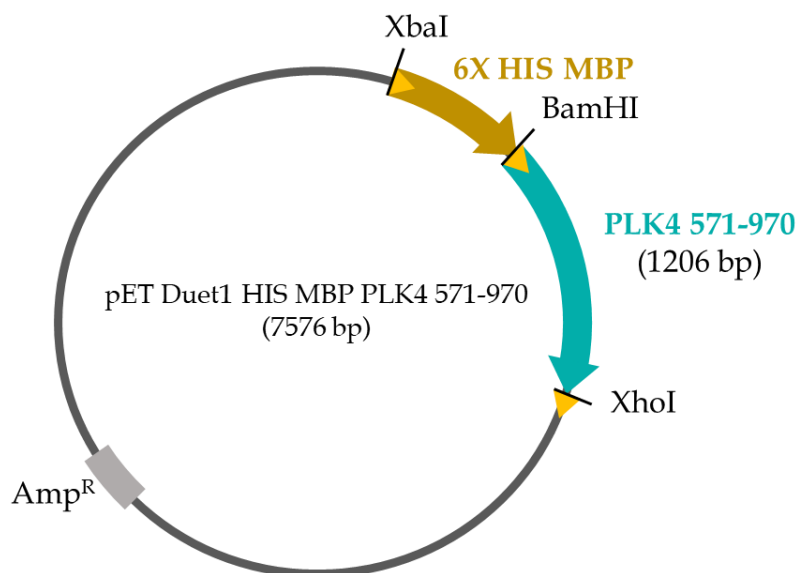
11. Clone map of pCDNA5 FRT/TO EGFP PLK4 Δ 774-813

Name of the Clone	pCDNA5 FRT/TO EGFP PLK4 Δ774-813
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 814-970 fragment was PCR amplified and cloned in pCDNA5 FRT/TO EGFP plasmid between BamHI and NotI restriction sites
Antibiotic resistance	Ampicillin
Vector	pCDNA5 FRT/TO EGFP
Insert	PLK4 814-970
Primers	FP- 5'- ATTGCGGCCGCGCTTATCACCTCCTCCTTCT -3' RP- 5'- GTTTCTTTACCAGACTCGACCTCGAGTCAATGAAAAT TAGGAGTCG -3'
Any other relevant Information	Construct codes for PLK4 Δ 774-813 fragment with N-terminal EGFP tag



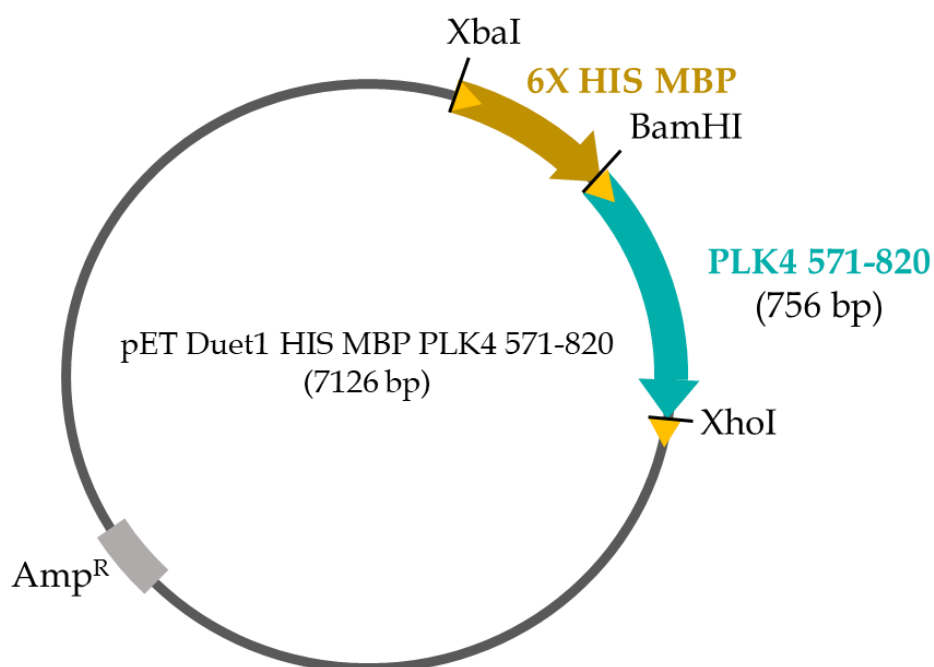
12. Clone map of pET Duet1 His MBP PLK4 571-970

Name of the Clone	pET Duet1 His MBP PLK4 571-970
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 571-970 fragment was PCR amplified and cloned in pET Duet1 HIS MBP plasmid between BamHI and XhoI restriction sites
Antibiotic resistance	Ampicillin
Vector	pET Duet1 His MBP
Insert	PLK4 571-970
Primers	FP- 5'-GGCCGGATCCATGAGCAAGACTAGGGGTATG-3' RP- 5'- GTTTCTTTACCAGACTCGACCTCGAGTCAATGAAAATT AGGAGTCG-3'
Any other relevant Information	Construct codes for PLK4 571-970 fragment with N-terminal HIS MBP tag



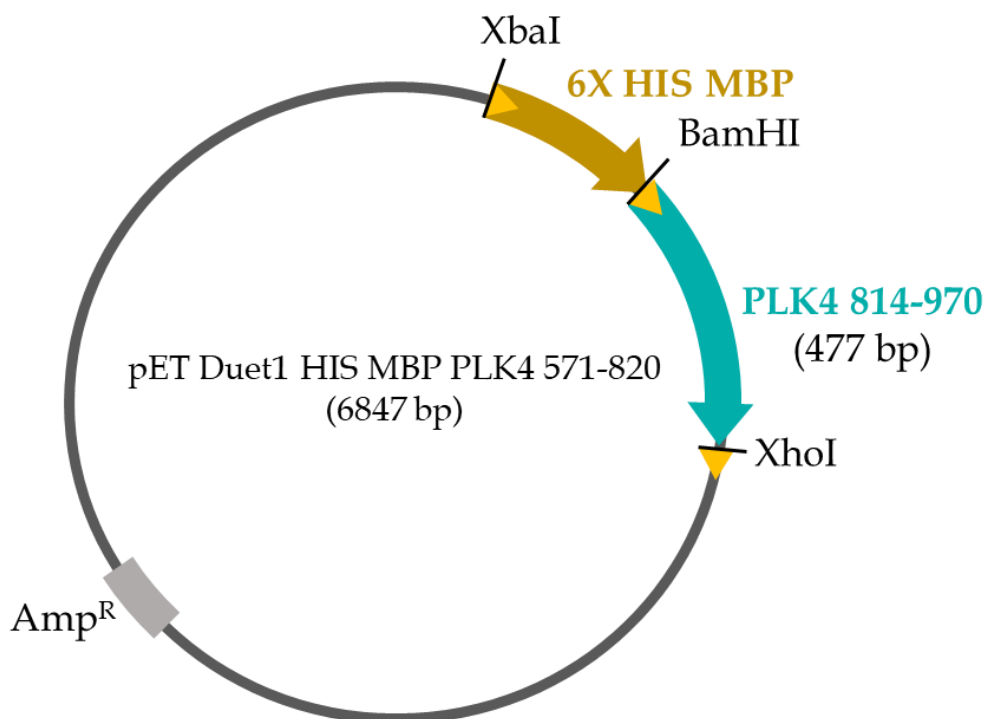
13. Clone map of pET Duet1 His MBP PLK4 571-820

Name of the Clone	pET Duet1 His MBP PLK4 571-820
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 571-820 fragment was PCR amplified and cloned in pET Duet1 HIS MBP plasmid between BamHI and XhoI restriction sites
Antibiotic resistance	Ampicillin
Vector	pET Duet1 His MBP
Insert	PLK4 571-820
Primers	FP- 5'-GGCCGGATCCATGAGCAAGACTAGGGGTATG-3' RP- 5'-GGCCCTCGAGTCAAGGAGGAGGTGATAAGG-3'
Any other relevant Information	Construct codes for PLK4 571-820 fragment with N-terminal HIS MBP tag



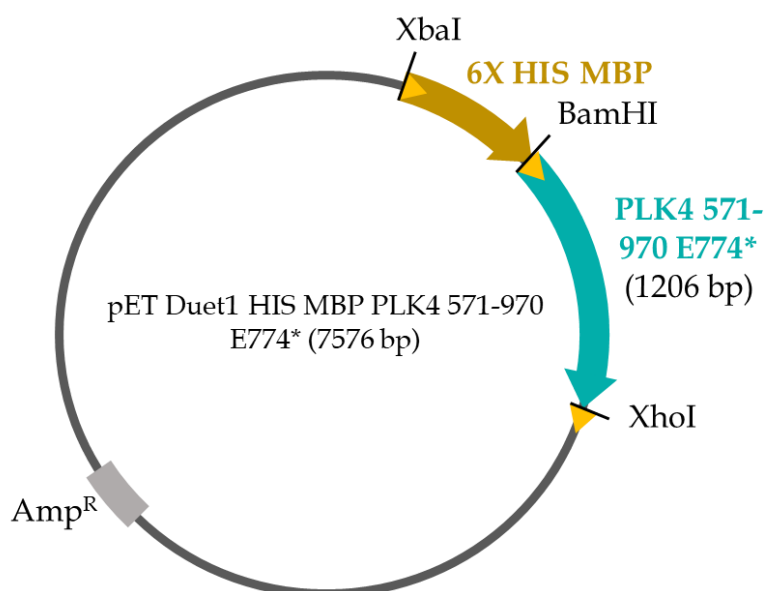
14. Clone map of pET Duet1 His MBP PLK4 814-970

Name of the Clone	pET Duet1 His MBP PLK4 814-970
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 814-970 fragment was PCR amplified and cloned in pET Duet1 HIS MBP plasmid between BamHI and XhoI restriction sites
Antibiotic resistance	Ampicillin
Vector	pET Duet1 His MBP
Insert	PLK4 814-970
Primers	FP- 5'-GGCCGGATCCATGAAGGCCTTATCACCTC-3' RP- 5'- GTTTCTTTACCAGACTCGACCTCGAGTCAATGAAAATTA GGAGTCG-3'
Any other relevant Information	Construct codes for PLK4 814-970 fragment with N-terminal HIS MBP tag



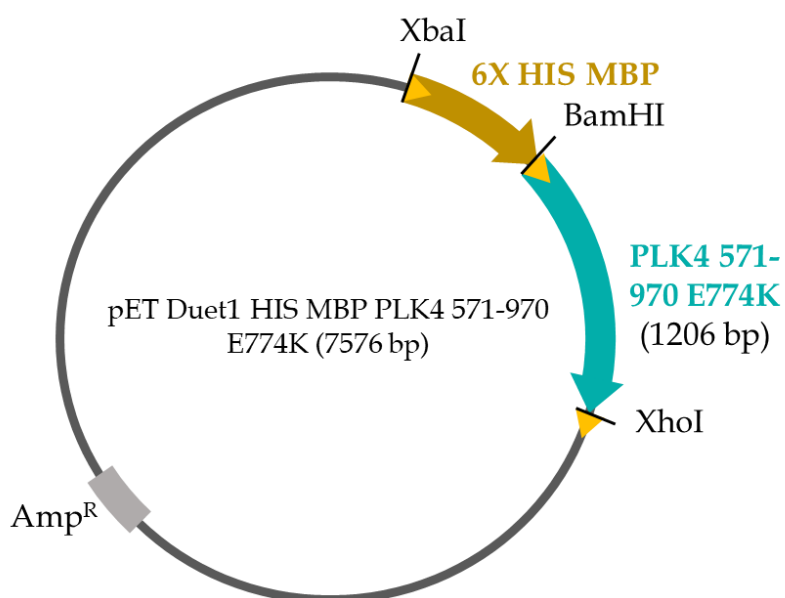
15. Clone map of pET Duet1 His MBP PLK4 571-970 E774*

Name of the Clone	pET Duet1 His MBP PLK4 571-970 E774*
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 571-970 E774* were generated by Site-directed mutagenesis. Plasmids were PCR amplified using mutagenesis primers, digested using Dpn1 and transformed in cells.
Antibiotic resistance	Ampicillin
Vector	pET Duet1 His MBP
Insert	PLK4 571-970 E774*
Primers	FP- 5'- TATATGGACCATGCTAATTAGGGTCATCGTATTGTGTTA GCA -3' RP- 5'- TGCTAAACAAATACGATGACCCTAATTAGCATGGTCC ATATA -3'
Any other relevant Information	Touch-down PCR was used to generate the mutations using iProof HF DNA polymerase (BioRad, #1725301)



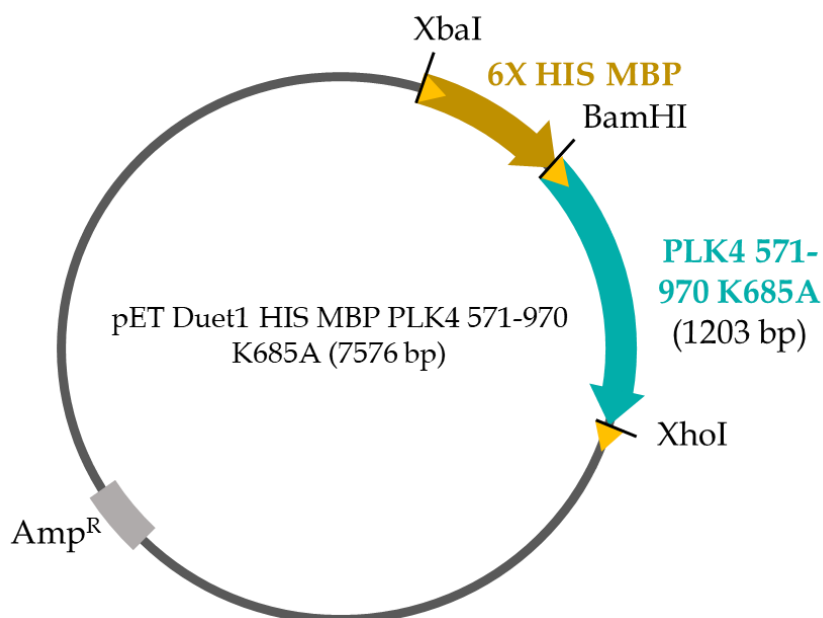
16. Clone map of pET Duet1 His MBP PLK4 571-970 E774K

Name of the Clone	pET Duet1 His MBP PLK4 571-970 E774K
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 571-970 E774K was generated by Site-directed mutagenesis. Plasmid was PCR amplified using mutagenesis primers, digested using Dpn1 and transformed in cells.
Antibiotic resistance	Ampicillin
Vector	pET Duet1 His MBP
Insert	PLK4 571-970 E774K
Primers	FP- 5'- TATATGGACCATGCTAATAAGGGTCATCGTATTG TTTAGCA -3' RP- 5'- TGCTAAACAAATACGATGACCCTTATTAGCATGGT CCATATA -3'
Any other relevant Information	Touch-down PCR was used to generate the mutations using iProof HF DNA polymerase (BioRad, #1725301)



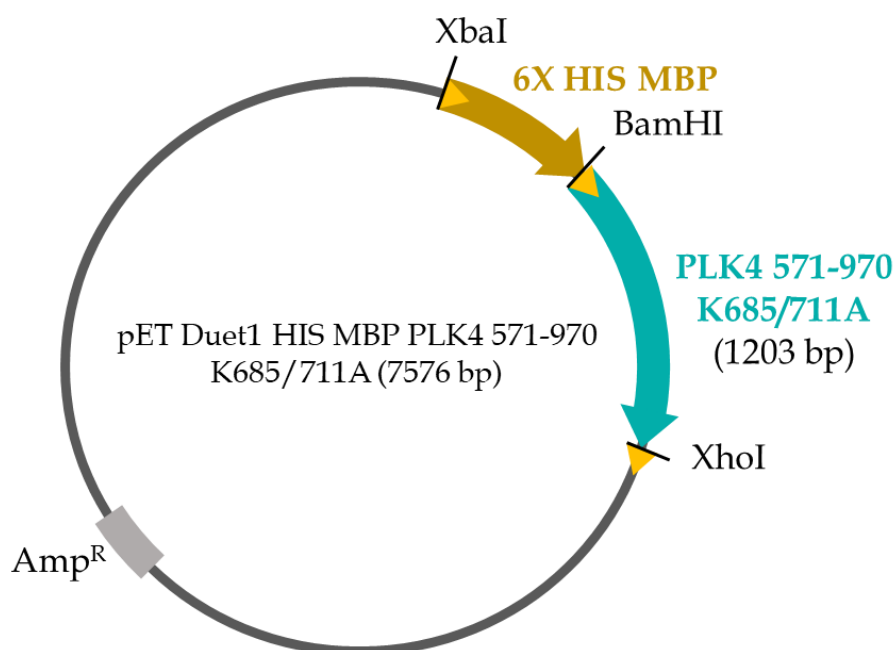
17. Clone map of pET Duet1 His MBP PLK4 571-970 K685A

Name of the Clone	pET Duet1 His MBP PLK4 571-970 K685A
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 571-970 K685A were generated by Site-directed mutagenesis. Plasmids were PCR amplified using mutagenesis primers, digested using Dpn1 and transformed in cells.
Antibiotic resistance	Ampicillin
Vector	pET Duet1 His MBP
Insert	PLK4 571-970 K685A
Primers	FP- 5'- CCAGAAAAATACTGGCGAGCATATCAATATGCTTCCAGG TTT -3' RP- 5'- AAACCTGGAAGCATATTGATATGCTCGCCAGTATTTTCT GG -3'
Any other relevant Information	Touch-down PCR was used to generate the mutations using iProof HF DNA polymerase (BioRad, #1725301)



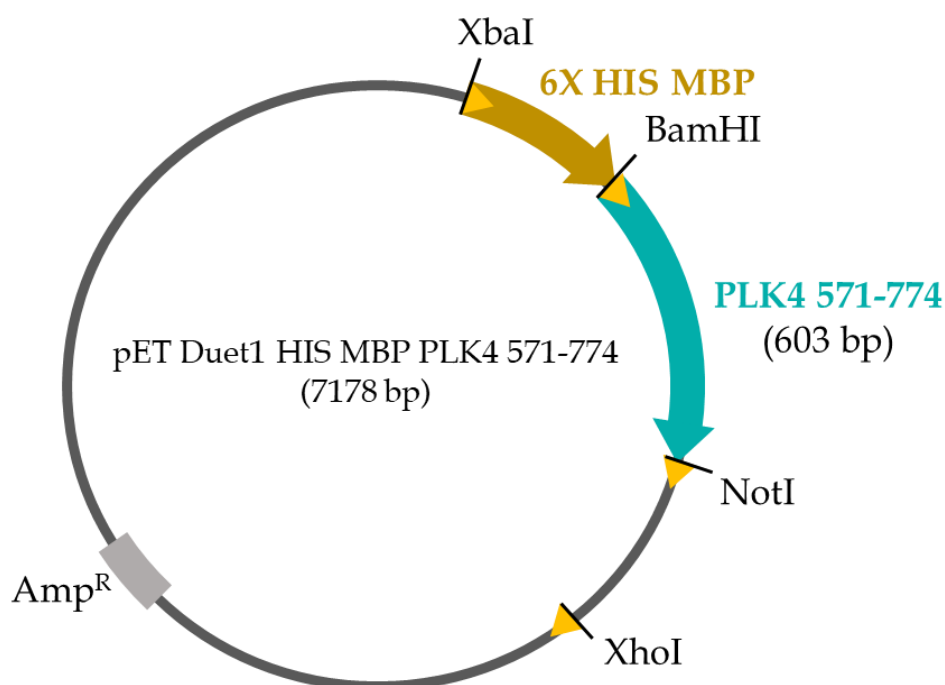
18. Clone map of pET Duet1 His MBP PLK4 571-970 K685/711A

Name of the Clone	pET Duet1 His MBP PLK4 571-970 K685/711A
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 571-970 K685/711A were generated by Site-directed mutagenesis. Plasmids were PCR amplified using mutagenesis primers, digested using Dpn1 and transformed in cells.
Antibiotic resistance	Ampicillin
Vector	pET Duet1 His MBP
Insert	PLK4 571-970 K685/711A
Primers	FP- 5'- CTTATTTTACAAGATATGCTGCATGCATTTTGATGG -3' RP- 5'- CCATCAAAATGCATGCAGCATATCTTGTAATAAAG -3'
Any other relevant Information	Touch-down PCR was used to generate the mutations using iProof HF DNA polymerase (BioRad, #1725301)



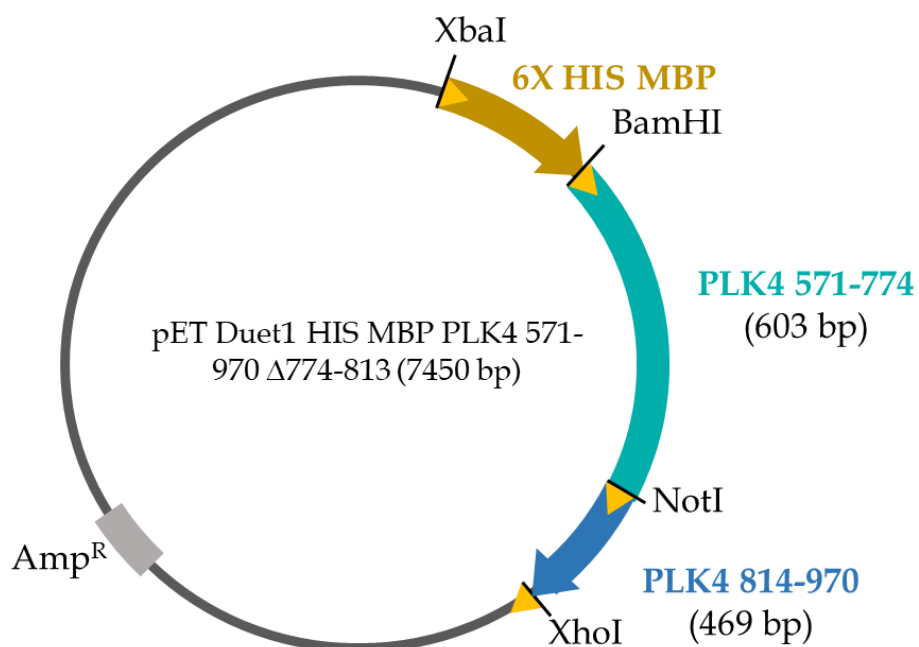
19. Clone map of pET Duet1 His MBP PLK4 571-774

Name of the Clone	pET Duet1 His MBP PLK4 571-774
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 571-774 fragment was PCR amplified and cloned in pET Duet1 HIS MBP plasmid between BamHI and NotI restriction sites
Antibiotic resistance	Ampicillin
Vector	pET Duet1 His MBP
Insert	PLK4 1-774
Primers	FP- 5'-GGCCGGATCCATGAGCAAGACTAGGGGTATG-3' RP- 5'-ATTGCGCGGCCGCATGGTCCATATACATTTTATC-3'
Any other relevant Information	Construct codes for PLK4 571-774 fragment with N-terminal tag



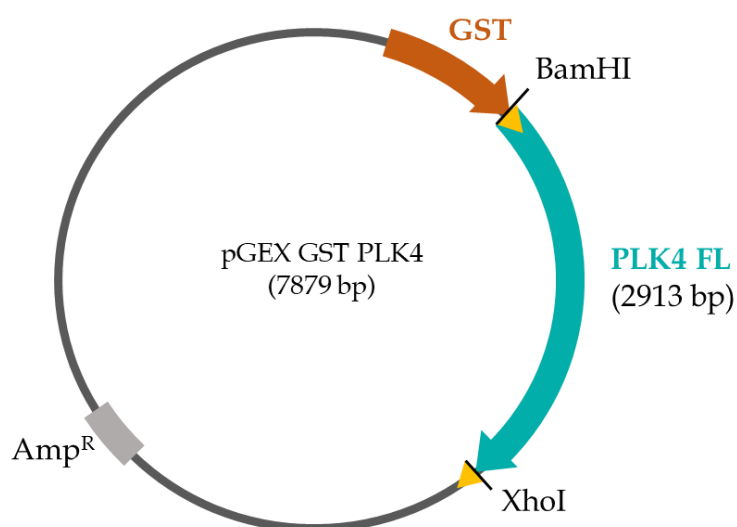
20. Clone map of pET Duet1 His MBP PLK4 571-970 Δ 774-813

Name of the Clone	pET Duet1 His MBP PLK4 571-970 Δ774-813
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 814-970 fragment was PCR amplified and cloned in pET Duet1 HIS MBP plasmid between BamHI and NotI restriction sites
Antibiotic resistance	Ampicillin
Vector	pET Duet1 His MBP
Insert	PLK4 814-970
Primers	FP- 5'- ATTGCGGCCGCCTTATCACCTCCTCCTTCT -3' RP- 5'- GTTTCTTTACCAGACTCGACCTCGAGTCAATGAAAATTA GGAGTCG -3'
Any other relevant Information	Construct codes for PLK4 571-970 Δ 774-813 fragment with N-terminal HIS MBP tag



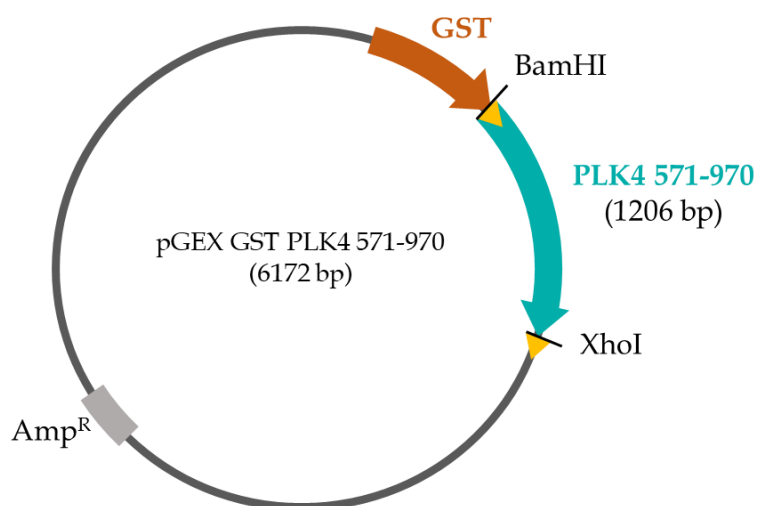
21. Clone map of pGEX GST PLK4

Name of the Clone	pGEX GST PLK4
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 full length cDNA was PCR amplified and cloned in pGEX GST plasmid between BamHI and XhoI restriction sites
Antibiotic resistance	Ampicillin
Vector	pGEX GST
Insert	PLK4
Primers	FP- 5'- ACCTGTACTTCCAGGGATCCATGGCGACCTGCATCGGG G -3' RP- 5'- GTTTCTTTACCAGACTCGACCTCGAGTCAATGAAAATT AGGAGTCG -3'
Any other relevant Information	Construct codes for PLK4 full length with N-terminal GST tag



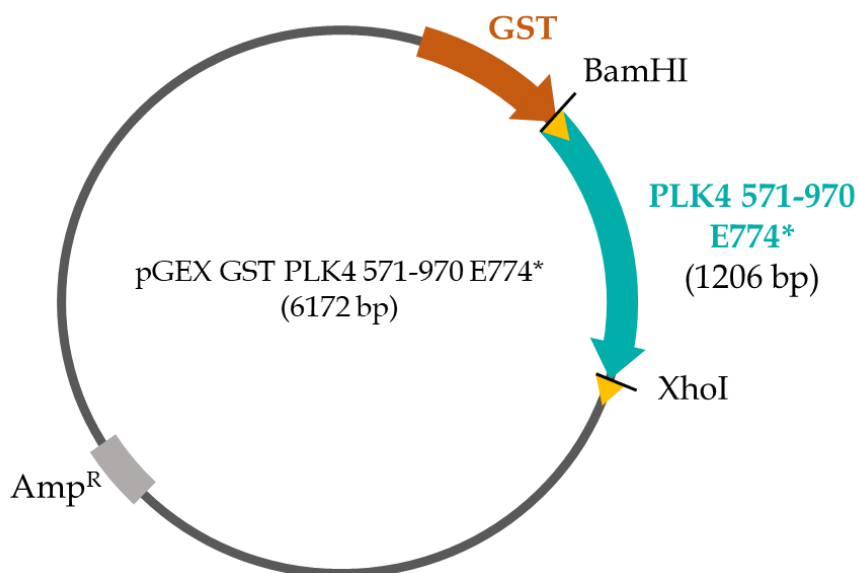
22. Clone map of pGEX GST PLK4 571-970

Name of the Clone	pGEX GST PLK4 571-970
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 571-970 fragment was PCR amplified and cloned in pGEX GST plasmid between BamHI and XhoI restriction sites
Antibiotic resistance	Ampicillin
Vector	pGEX GST
Insert	PLK4 571-970
Primers	FP- 5'-GGCCGGATCCATGAGCAAGACTAGGGGTATG-3' RP- 5'- GTTTCTTTACCAGACTCGACCTCGAGTCAATGAAAATT AGGAGTCG-3'
Any other relevant Information	Construct codes for PLK4 571-970 fragment with N-terminal GST tag

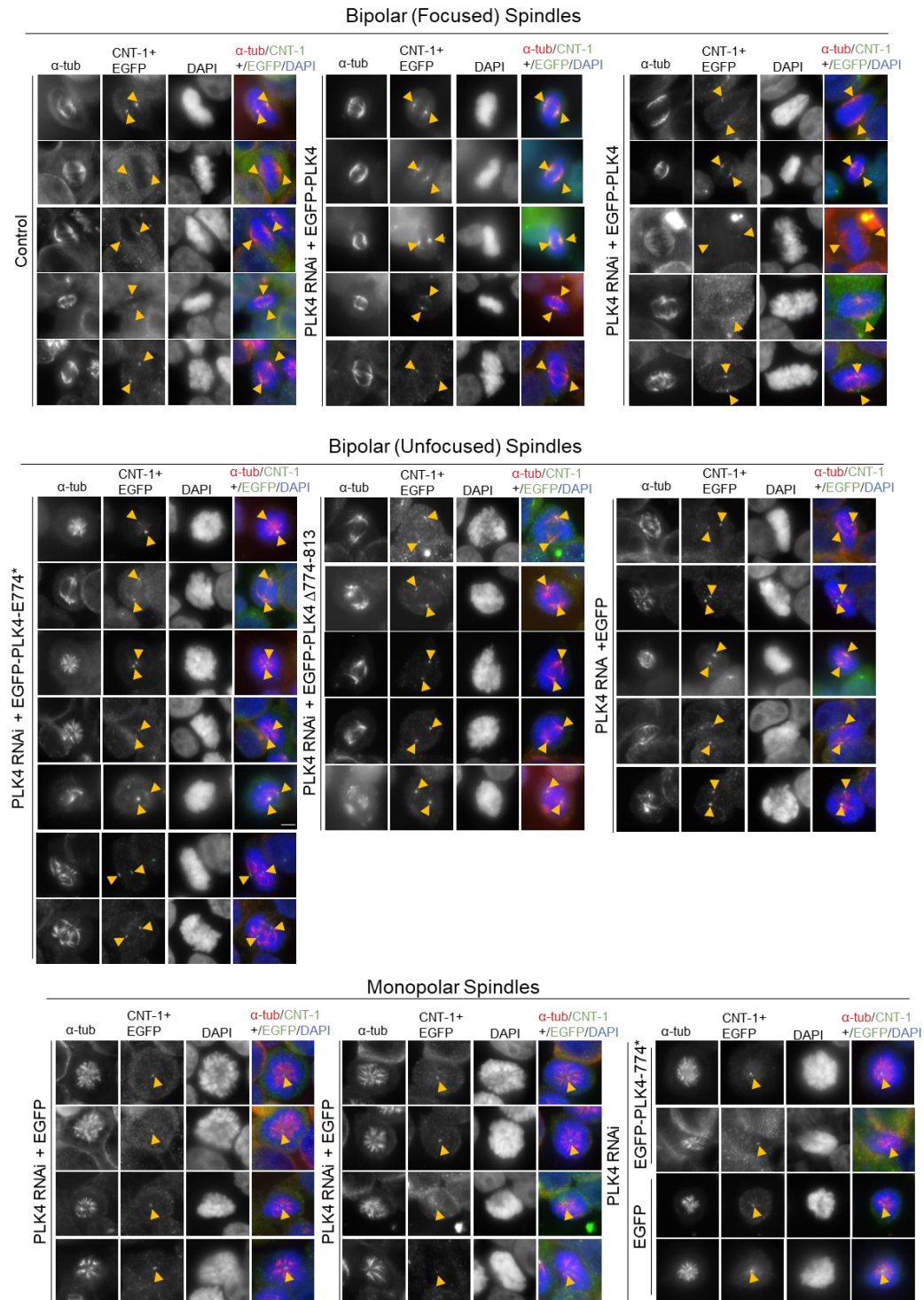


23. Clone map of pGEX GST PLK4 571-970 E774*

Name of the Clone	pGEX GST PLK4 571-970 E774*
Constructed by	Harshita Kasera
Place	IIT Jodhpur
Description	PLK4 571-970 E774* were generated by Site-directed mutagenesis. Plasmids were PCR amplified using mutagenesis primers, digested using Dpn1 and transformed in cells.
Antibiotic resistance	Ampicillin
Vector	pGEX GST
Insert	PLK4 571-970 E774*
Primers	FP- 5'- TATATGGACCATGCTAATTAGGGTCATCGTA TTTGTTTAGCA -3' RP- 5'- TGCTAAACAAATACGATGACCCTAATTAGC ATGGTCCATATA -3'
Any other relevant Information	Touch-down PCR was used to generate the mutations using iProof HF DNA polymerase (BioRad, #1725301)



A.3 Image gallery



A3. Image gallery to compare the bipolar (focused), bipolar (unfocused) and monopolar spindle phenotypes. The HeLa cells were stained for α -tubulin (red), centrin-1 (CNT-1, green) and DAPI (blue). The centrin-1 and EGFP signal for respective constructs were captured in the same channel.

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