**One shot method to create CDK1analogue-sensitive cell lines in human cell lines**

**Construction of plasmids**

**Vector for expression of CDK1as:** pT2/SVNeo, a gift from Perry Hackett (Addgene 26553 *– Cui et al., 2002*) containing the Sleeping Beauty transposon was modified as follows. pT2/SVNeo was digested with EcoRI and PfoI and then blunt ended. Analogue-sensitive mutant Xenopus laevis CDK1 cDNA (gift of Helfrid Hochegger, University of Sussex) linked to a Zeocin or puromycin resistance gene via a T2A peptide was digested with NruI and ApaI then blunt ended to create the insert. This Insert was ligated with the vector and correct orientation of the insert was confirmed by sequencing.

**Vector for expression of Sleeping Beauty transposase:** pCMV(CAT)T7-SB100, a gift from Zsuzsanna Izsvak (Addgene 34879 *– Mates et al., 2009*) encodes Sleeping Beauty transposase and was used without modification.

**Vector for inactivation of endogenous CDK1 genes:** pX330-U6\_Chimeric\_BB-cBh-hSpCas9, a gift from Feng Zhang (Addgene 42230 *– Cong et al, 2013*) encodes a backbone expressing a Guide RNA and Cas9. Double stranded Oligos (5’- CACCGATTTCCCGAATTGCAGTACT-3’ and 5’-AAACAGTACTGCAATTCGGGAAATC-3’) were inserted into the plasmid following the protocol provided at the Addgene site.

**Creation of a CDK1 analogue sensitive cell line**

1. Co transfection of three plasmids above (either choose puromycin or zeocin resistance plasmid according to the cell line of your choice, Equal amount of each plasmid were transfected but the ratio has not been optimised.)
2. Addition of antibiotics after 24-48 hours depending on the cell type.
3. Selection of colonies by the sensitivity to 1NMPP1 treatment (2-5 µM). 1NMPP1 would inhibit the entry into mitosis if the cells become CDK1as cells.
4. Protein samples can be obtained to evaluate the presence of the CDK1as mutant by Western blot using CDK1 antibody. Due to the presence of T2A and partial myc tag at the C-terminus of the protein, Xenopus laevis CDK1as protein migrates slower than the endogenous one.

**Note:** Cells can normally be treated with 1NMPP1 for a duration of one cell cycle without excessive cell death. However, longer G2 block would result in centrosome amplification. We did not see >2 centrosome up until 4h treatment. Contact Dr Kumiko Samejima ([Kumiko.samejima@ed.ac.uk](mailto:Kumiko.samejima@ed.ac.uk)) for further information.