Efficiency of Homologous Recombination at Heterochromatic Double Strand Breaks

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RESULTS

bility, potentially leading to more targeted treatment for genome-instability ultimately help identify the mechanisms of heterochromatin and genome stability. Using the innovative approaches described here, this study would ul-

Figure 1: Model of the DSB response in heterochromatin. (A) Heterochromatic repair sites (foci) relocalize to the euchromatic space before completing homologous recombination repair. (B) Direct ligation is used for repair in non-homologous end joining.

AsiSI targeting to the nucleus

γH2Av

FHA-Lig4

Figure 6: (Top) Immunofluorescence analysis of the HP1 domain with the heterochromatin enriched H3K9me3 marker. (Bottom) Immunofluorescence analysis of NHEJ repair component Ligase IV.

Figure 4: AsiSI expression induces DSB in Drosophila heterochromatin with high efficiency. (A) Quantification of γH2Av foci shows a sharp induction of DSBs with a plateau at 2h from 4-OHT addition. (B) ChIP-qPCR analysis of euchromatic and heterochromatic AsiSI sites selected with the ChIP-seq results shows significant enrichment in γH2Av signals at 2 or 5 hours after AsiSI induction (+ AsiSI 2hr, 5hr), compared with untreated conditions (- AsiSI).

CONCLUSION AND PERSPECTIVES

• AsiSI restriction enzyme efficiently induces DSBs in both euchromatin and heterochromatin.
• 3x Flag-HA tagged repair components for HR and NHEJ were cloned and transfected for analysis.
• ChIP-qPCR can quantify the efficiency of protein recruitment to determine the preferred pathways in both domains.
• The resection assay can be used to quantify the efficiency of HR in both DNA domains.

Figure 7: FHA-Lig4 enrichment in response to AsiSI-induced DSBs is detected.

Figure 8: Model of resection assay to quantify the efficiency of resection with qPCR.

Figure 3: AsiSI restriction site

Figure 5: Schematic of various cloned constructs of repair proteins for each respective pathway.