

Study the effect of Dna2 helicase in the DM1 disease models

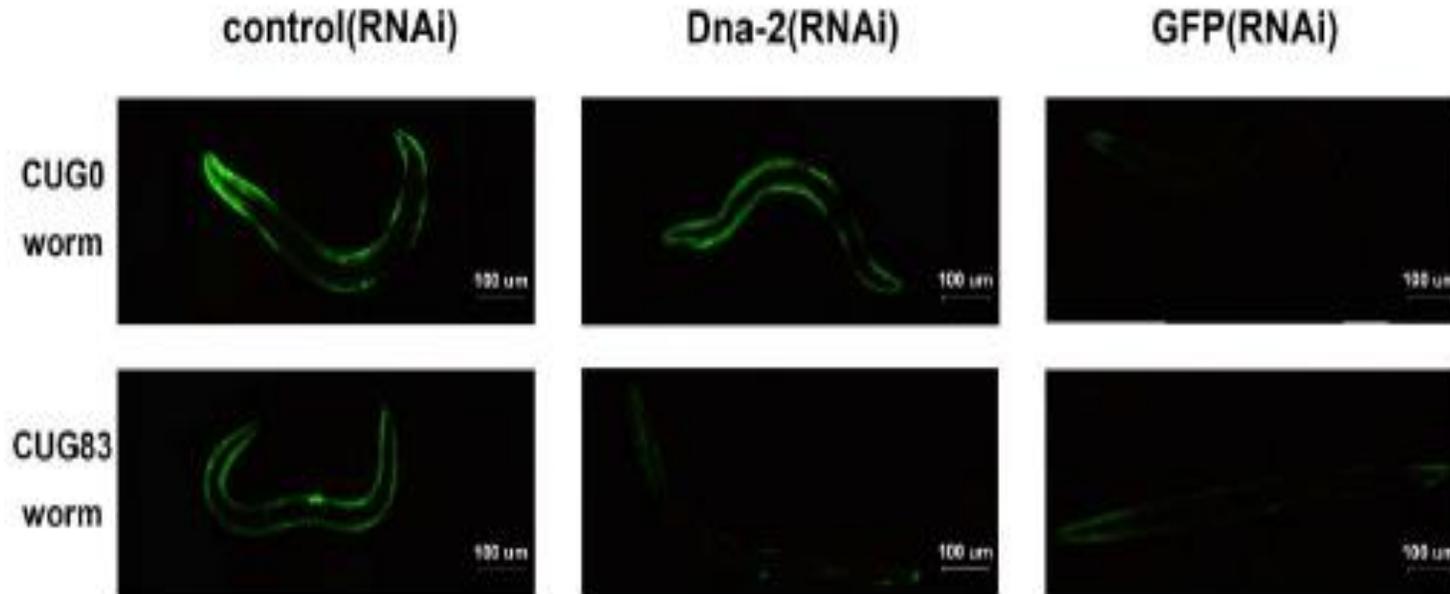
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DM1 disease

- ▶ DM1 is inherited in an autosomal dominant pattern, caused by an increase in the length of the number of **CTG repeats in the *DMPK* gene**. The expansion of the unstable region causes the features of the disorder to become more severe with each successive generation.
- ▶ The secondary structure of CUG repeat RNA recruits MNBL proteins in the nucleus to form **RNA foci** that compromises its **alternative splicing function** and leads to disease phenotypes.

Using RNAi library screen revealed several genetic modifier genes that could mitigate the disease phenotype in a DM1 model of transgenic *C. elegans* by expressing reporter-tagged CUG repeats.

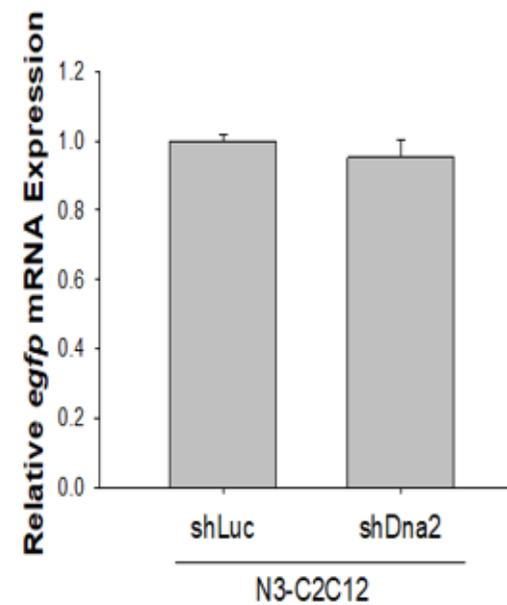
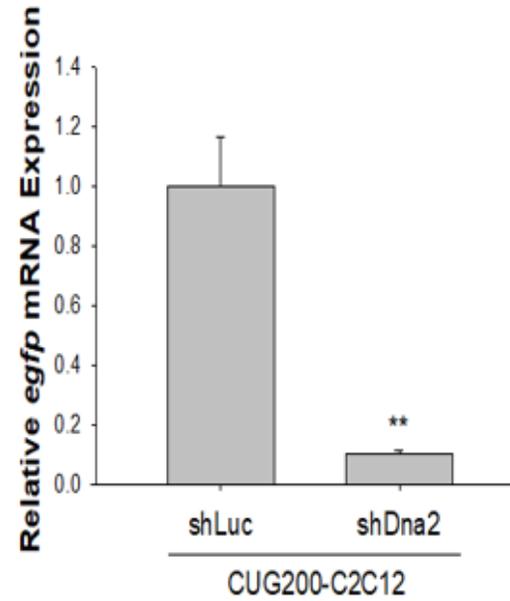
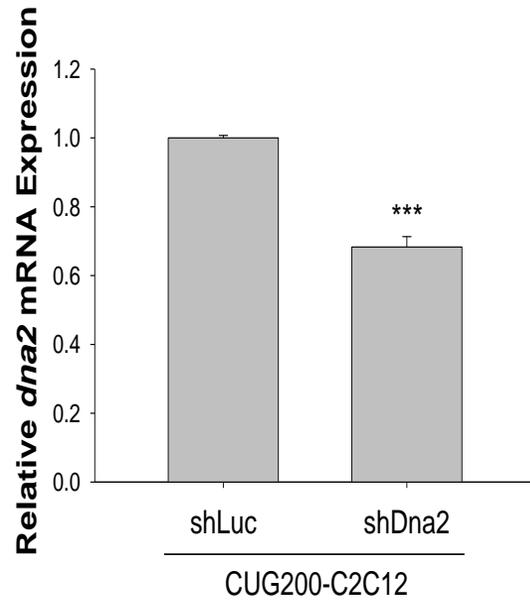


Dna2

(DNA replication ATP-dependent helicase/nuclease DNA2)

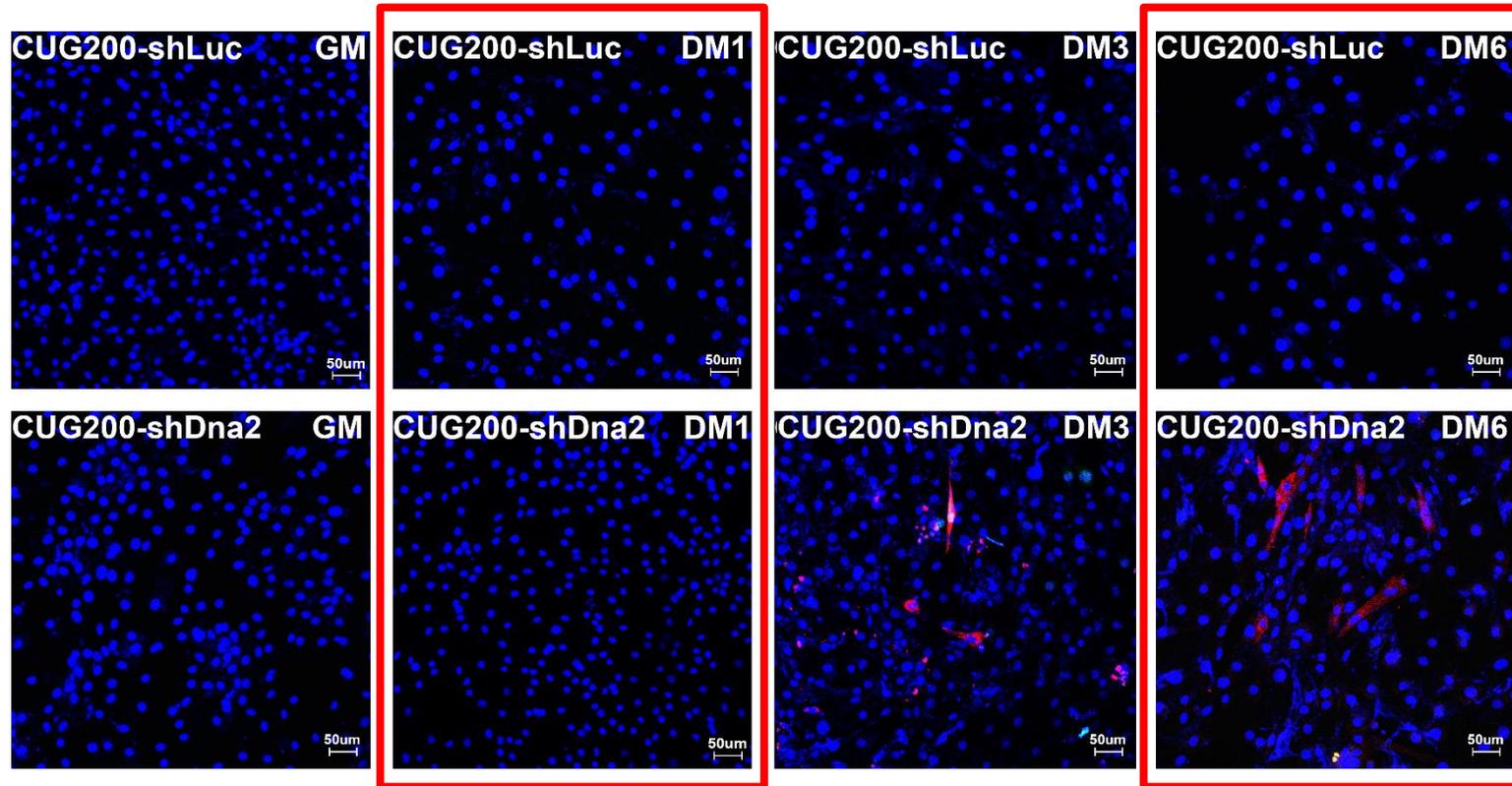
- ▶ Key enzyme involved in DNA replication and DNA repair in nucleus and mitochondrion. Involved in Okazaki fragments processing by cleaving long flaps that escape FEN1.
- ▶ Also involved in 5'-end resection of DNA during double-strand break (DSB) repair: recruited by BLM and mediates the cleavage of 5'-ssDNA, while the 3'-ssDNA cleavage is prevented by the presence of RPA. Also involved in DNA replication checkpoint independently of Okazaki fragments processing.

- Knockdown of Dna2 reduces the gfp expression in CUG200 C2C12 cells



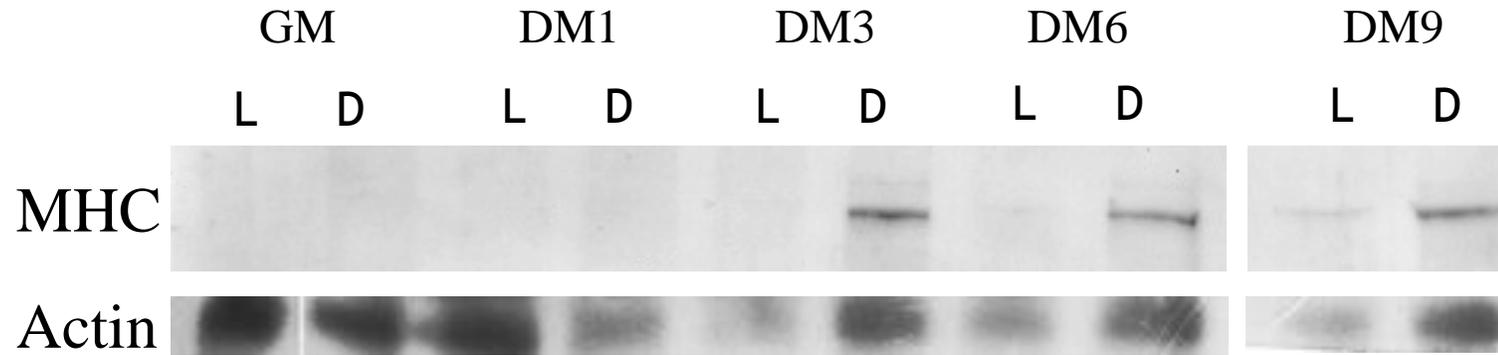
Knockdown efficiency

► Knockdown of Dna2 restored CUG200 C2C12 cell differentiation



Blue:DAPI Red:MHC(myosin heavy chain)

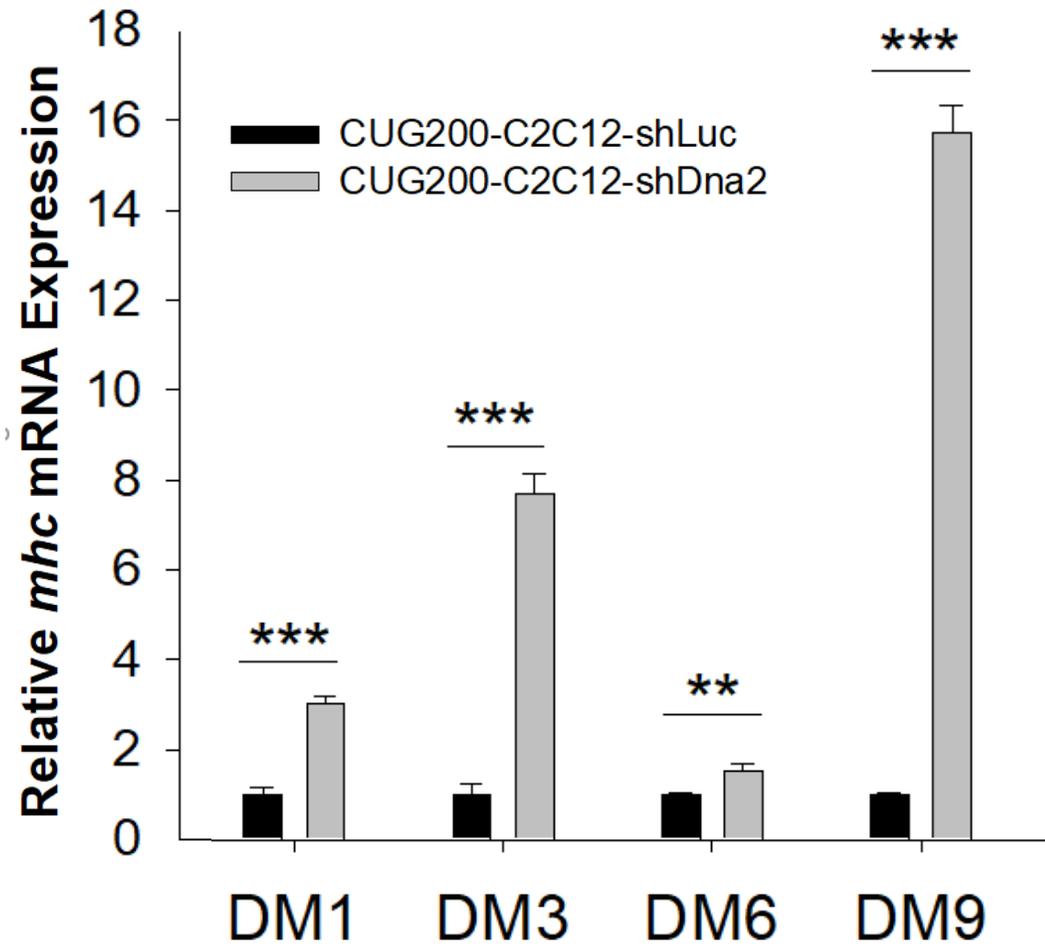
► Knockdown of Dna2 restored CUG200 C2C12 cell differentiation



L : CUG200-C2C12-shLuc

D : CUG200-C2C12-shDna2

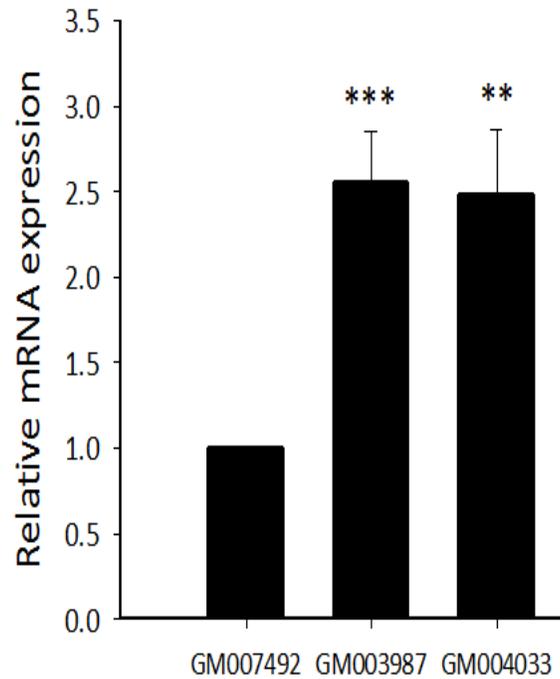
► Knockdown of Dna2 restored CUG200 C2C12 cell differentiation



Conclusion

- ▶ Knockdown of Dna2 in CUG200 C2C12 cells can restore cell differentiation.
- ▶ Dna-2 gene can function as a modifier of expanded CUG RNA toxicity by reducing its expression.

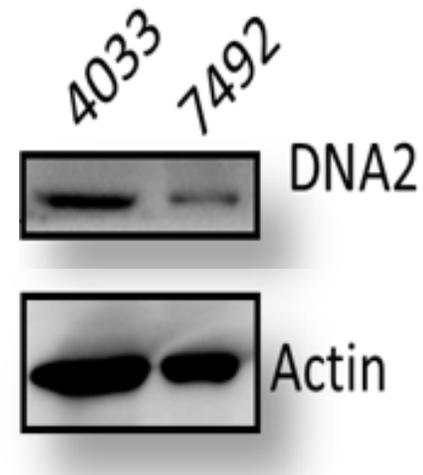
Human DM1 cell



GM7492: normal fibroblast

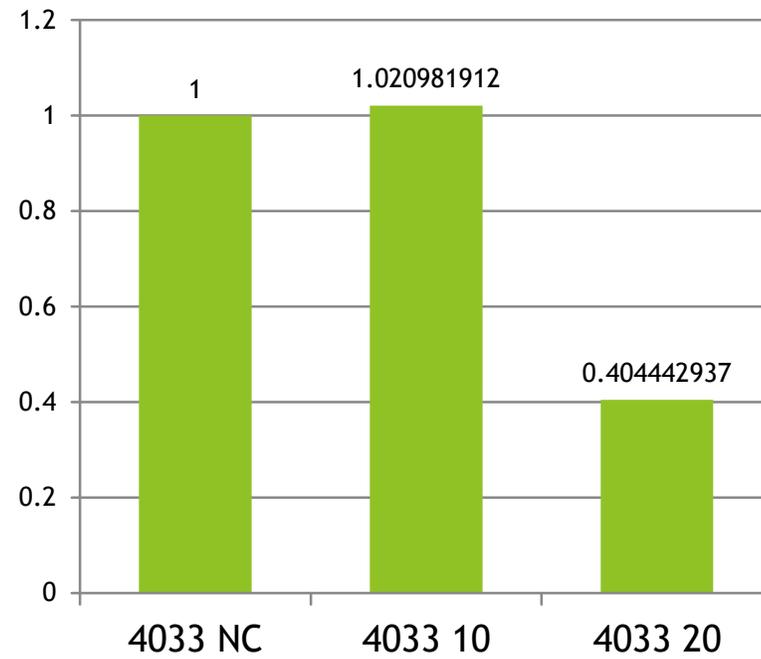
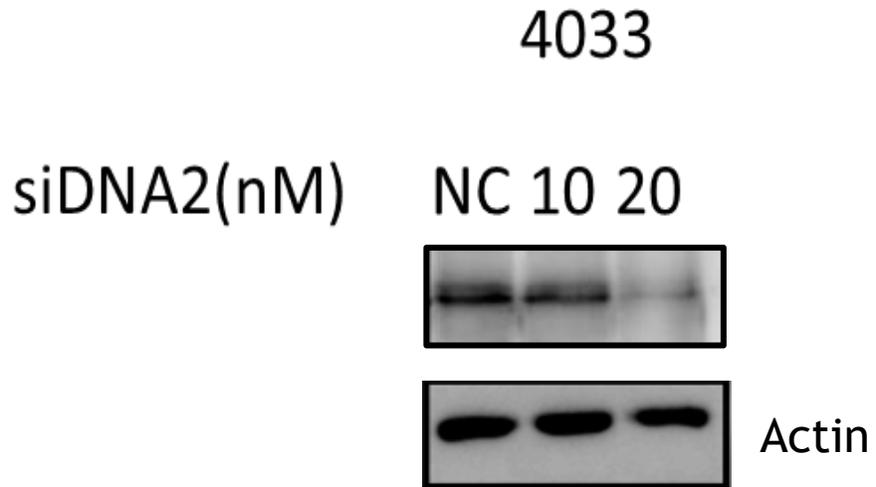
GM3987: DM1 patient's fibroblast(CUG500)

GM4033: DM1 patient's fibroblast(CUG1000)

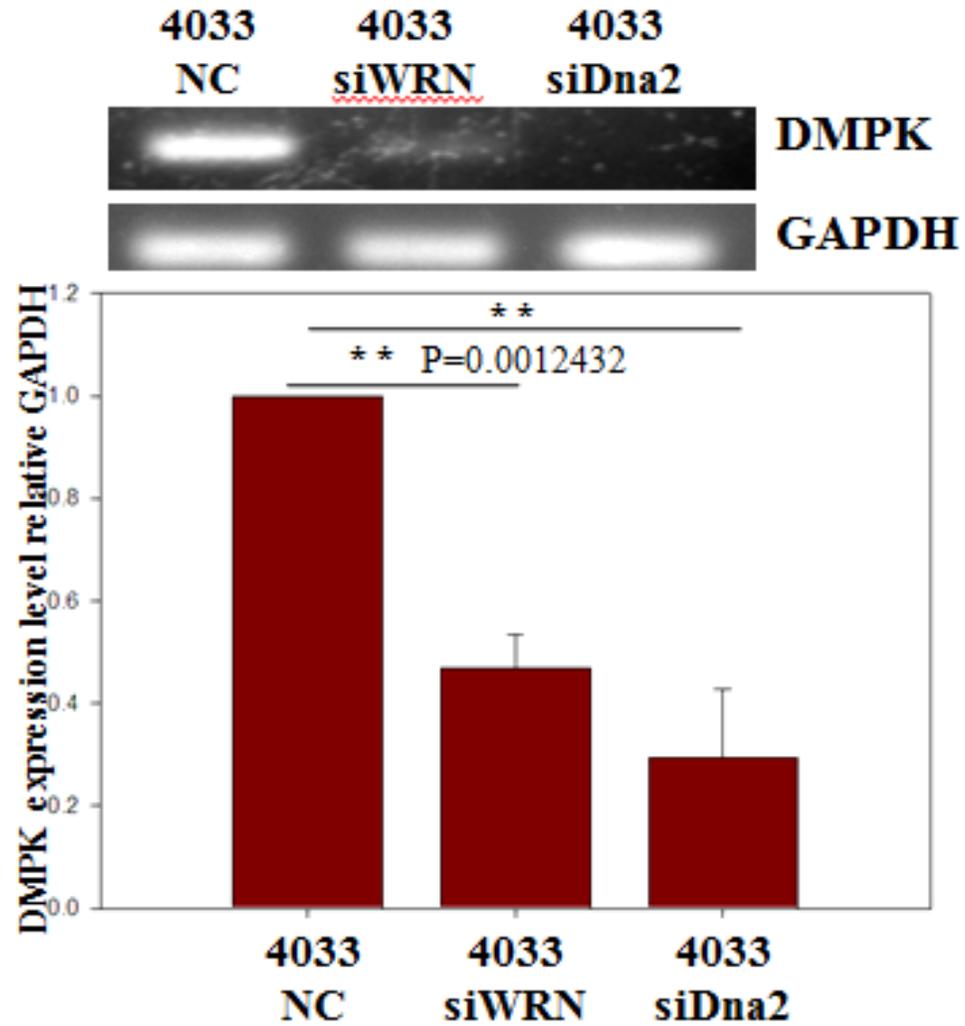


Expression of endogenous DNA2 in DM1 cell

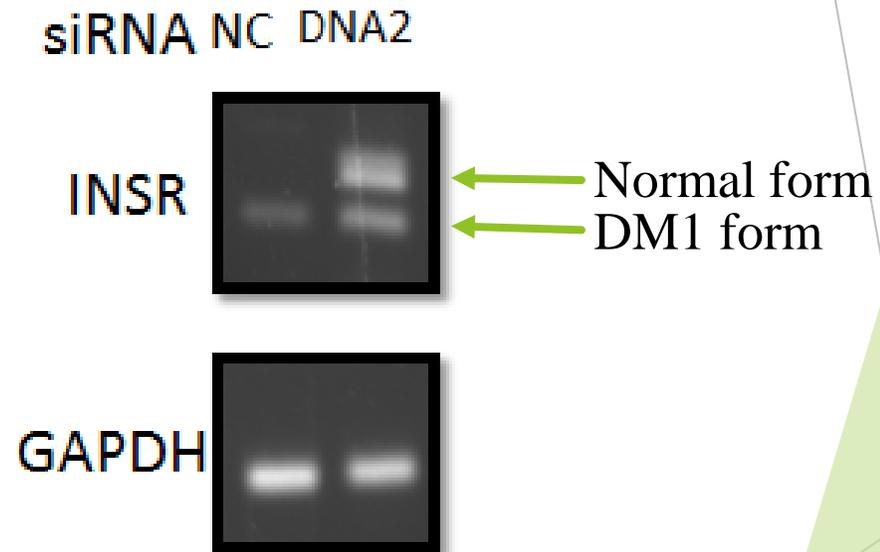
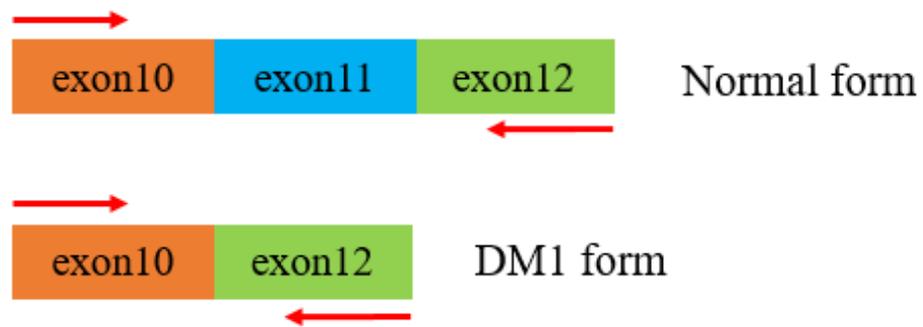
► Knockdown efficiency of Dna2 in DM1 cells.



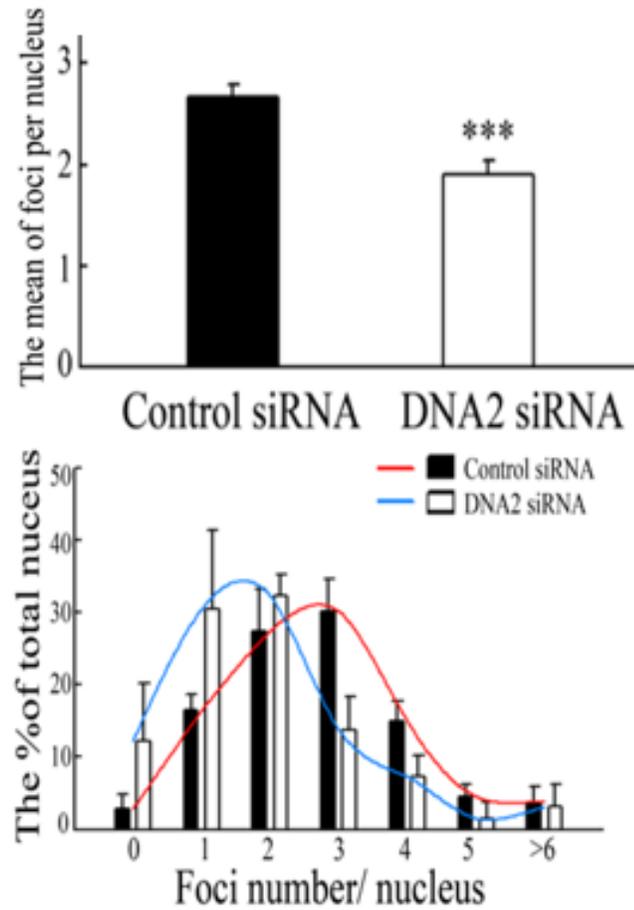
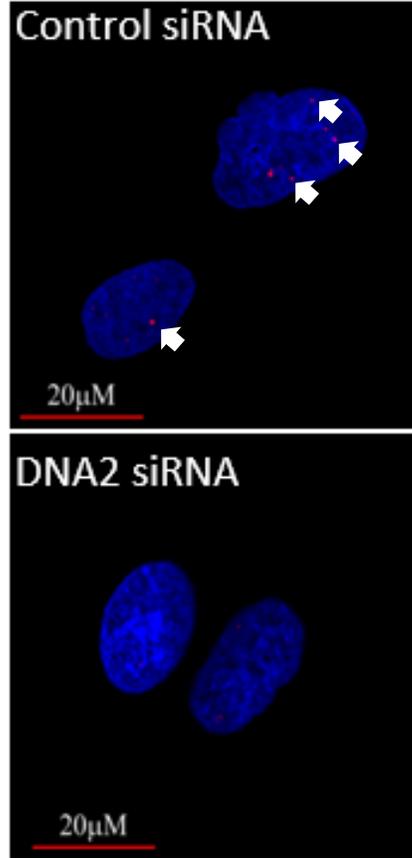
- ▶ Dna2 knockdown results in reduced DMPK expression



- ▶ Dna2 knockdown restores the normal splicing pattern in DM1 cell.



- Knockdown of Dna2 reduces RNA foci in DM1 cells.



Conclusion

- ▶ Knockdown of Dna2 in DM1 cells decrease *DMPK* expression.
- ▶ DNA2 knockdown restores the normal splicing pattern in DM1 cell.
- ▶ Knockdown of Dna2 reduces RNA foci in DM1 cells.

Zebrafish model

► Advantages:

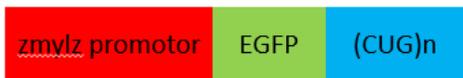
1. They are **cheaper** to maintain than mice.
2. Zebrafish produce **hundreds of offspring** at weekly intervals
3. They **grow at an extremely fast rate**, developing as much in a day as a human embryo develops in one month.
4. **As a vertebrate**, the zebrafish has the same major organs and tissues as humans.



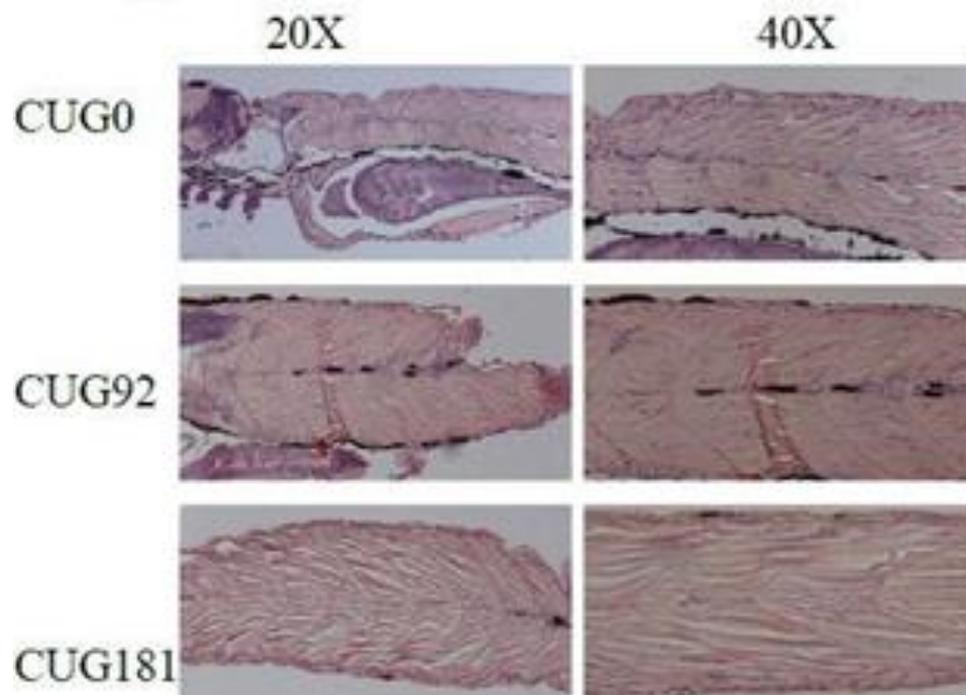
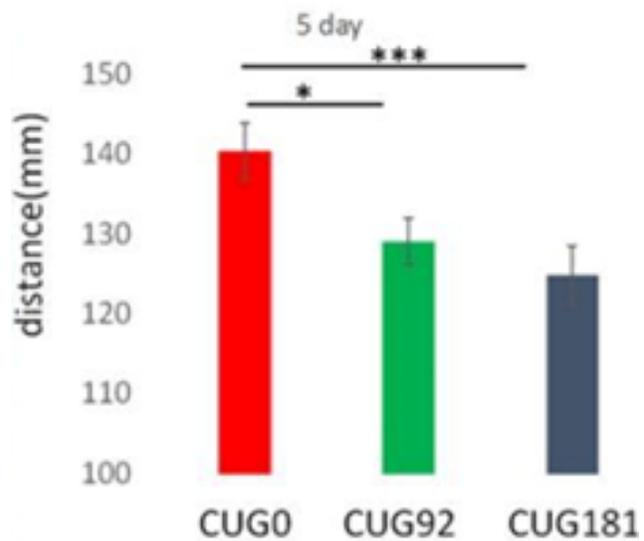
► Zebrafish model



Tg(myIz:EGFP-CTG0)

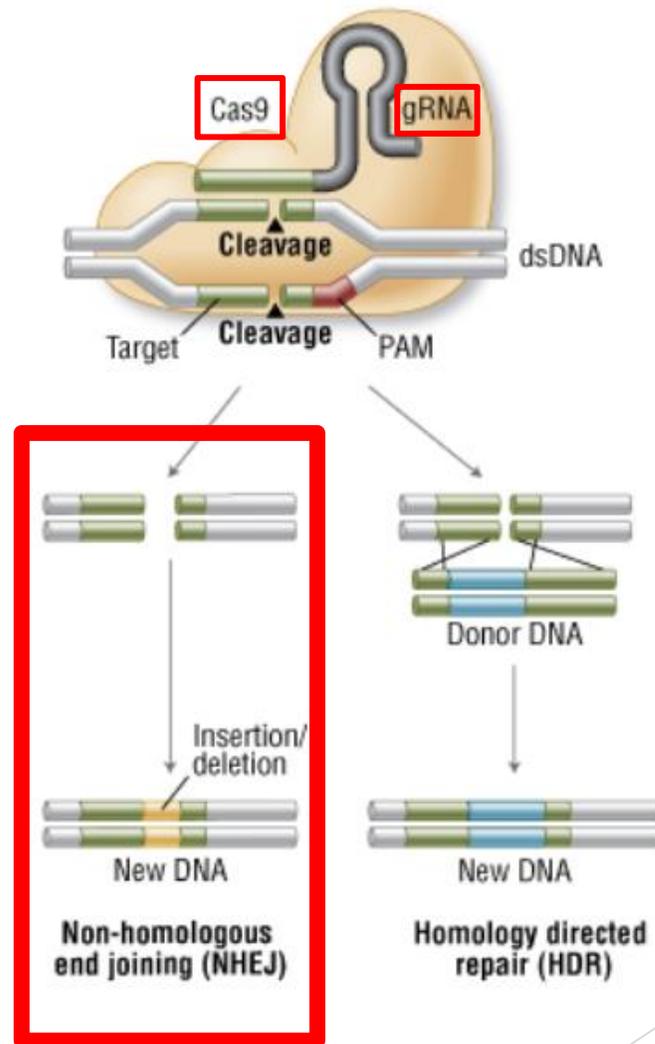


Tg(myIz:EGFP-CTG~)

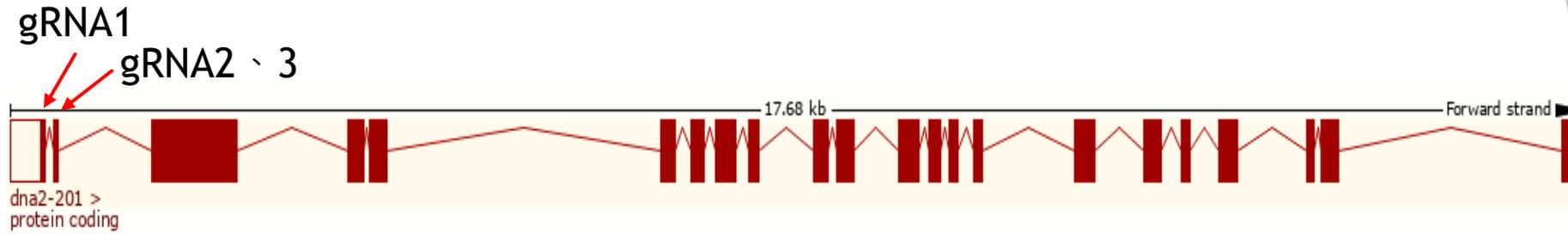


CRISPR/Cas9 knockout system

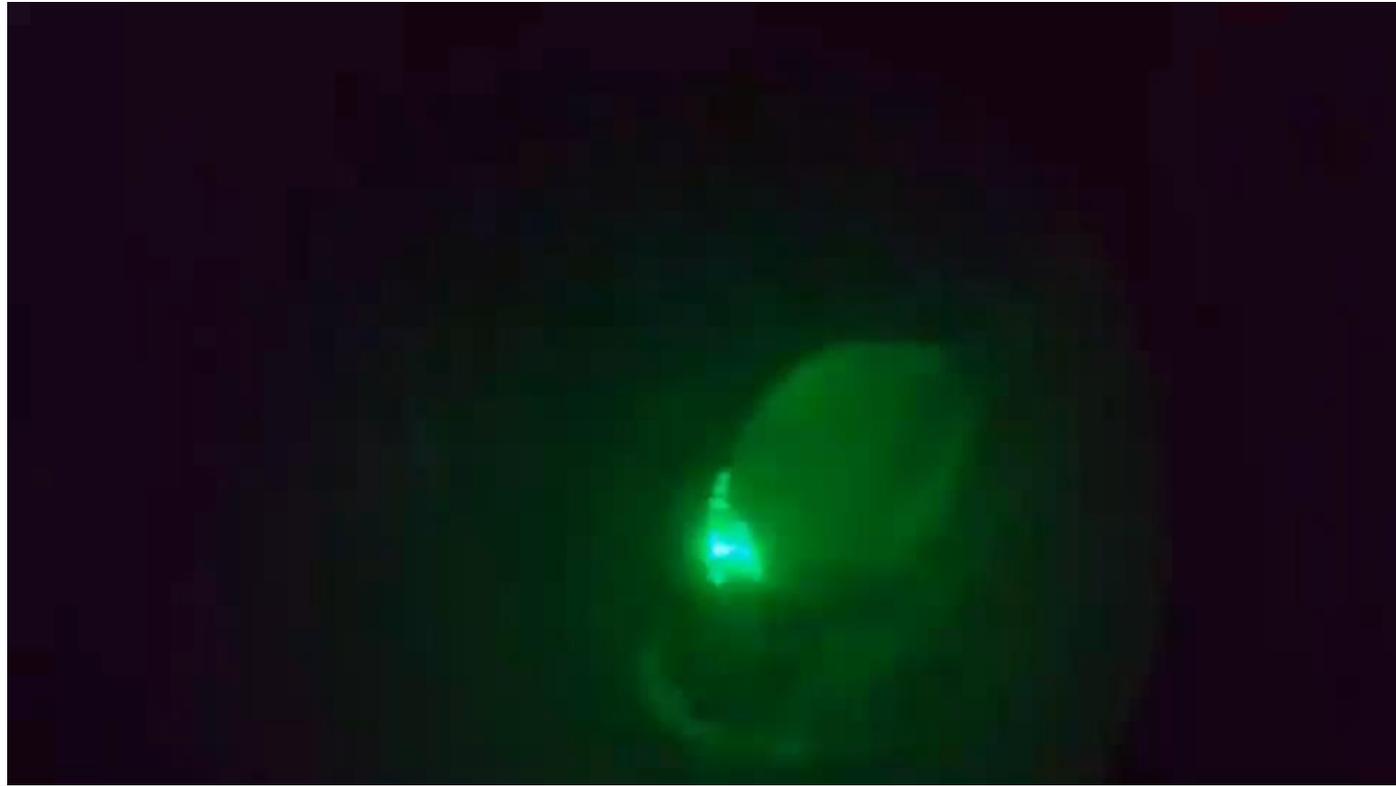
- ▶ The functions of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes are essential in **adaptive immunity** in select bacteria and archaea, enabling the organisms to **respond to and eliminate invading genetic material**.



► gRNA design on Dna2 helicase



Target gene	gRNA forward	gRNA reverse
Dna2	5'-GCCGATCATCTGTGACTATCGA-3'	5'- GATACTCACAGATGATCGGCGT-3'
	5'-GTTTAATCTTAGATGGTCAGGT-3'	5'-CTGACCATCTAAGATTAACGA-3'
	5'- CTCAAGGTAAGGACACGACGA-3'	5'- GTCGTGTCCTTACCTTTGAGGT-3'



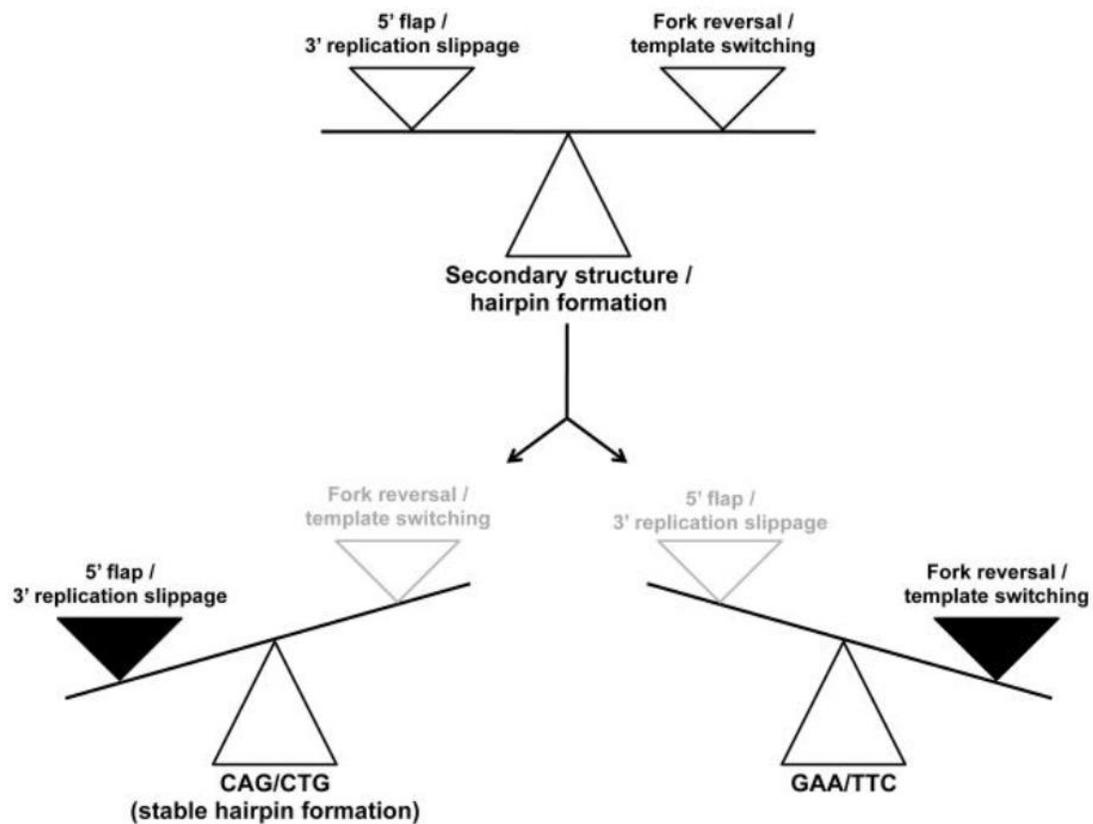
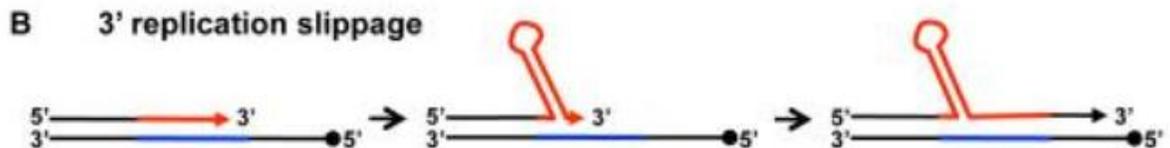
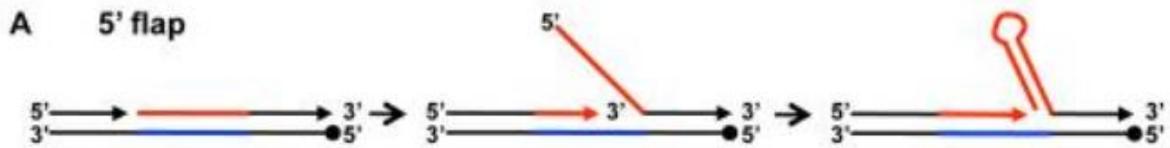
Future work

- ▶ Using T7E1 assay to check knockout efficiency
- ▶ Analysis after microinjection :
 - ▶ 24hr : Death rate 、 Spontaneous coiling
 - ▶ 48hr : Fluorescence rate 、 Escape response
 - ▶ 72hr~ : Western blot 、 Swimming pattern

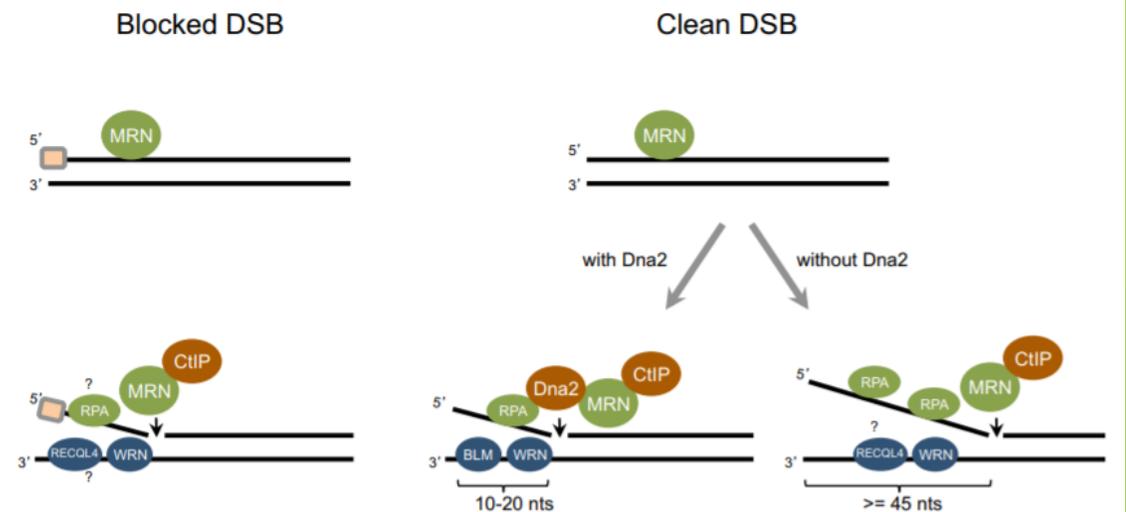


Restores swimming distance 、 muscle structure

Thanks for listening !

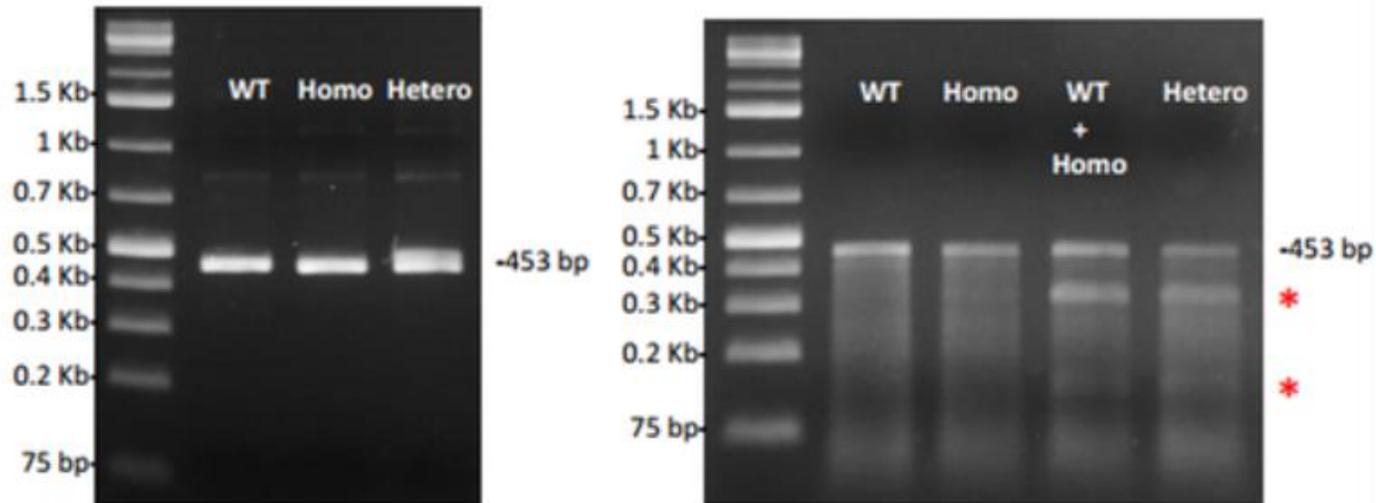
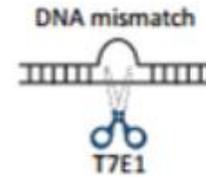


► Dna2 works with WRN and BLM in end section



► T7E1 Assay

T7 Endonuclease I digestion



T7 Endonuclease I recognizes and cleaves non-perfectly matched DNA, cruciform DNA structures, Holliday structures or junctions, heteroduplex DNA and more slowly, nicked double-stranded DNA.