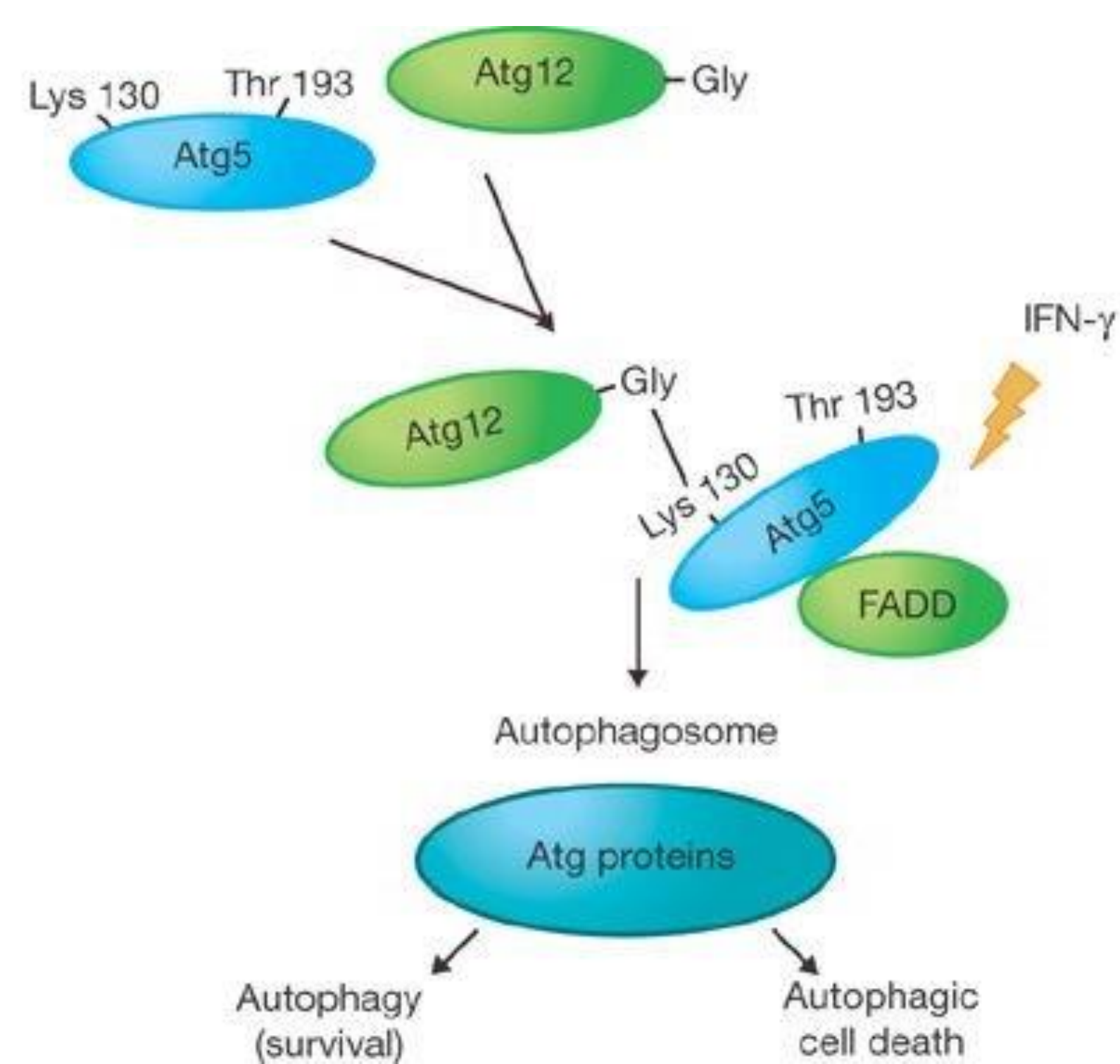


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## ABSTRACT



Autophagy is a self-degradation process that is important for balancing sources of energy in response to nutrient stress.

Autophagy can act as both tumor suppressor and tumor promotor, it is usually detrimental in early stages and beneficial in later stages for cancer cells.

Key players in autophagy are the AuTophagy-related (ATG) proteins.

ATG5 is one of those key players and it plays an important role in one of the essential steps to start autophagy which is the step of conjugation with ATG12. If ATG5 is unable to conjugate with ATG12, autophagy will be inhibited.

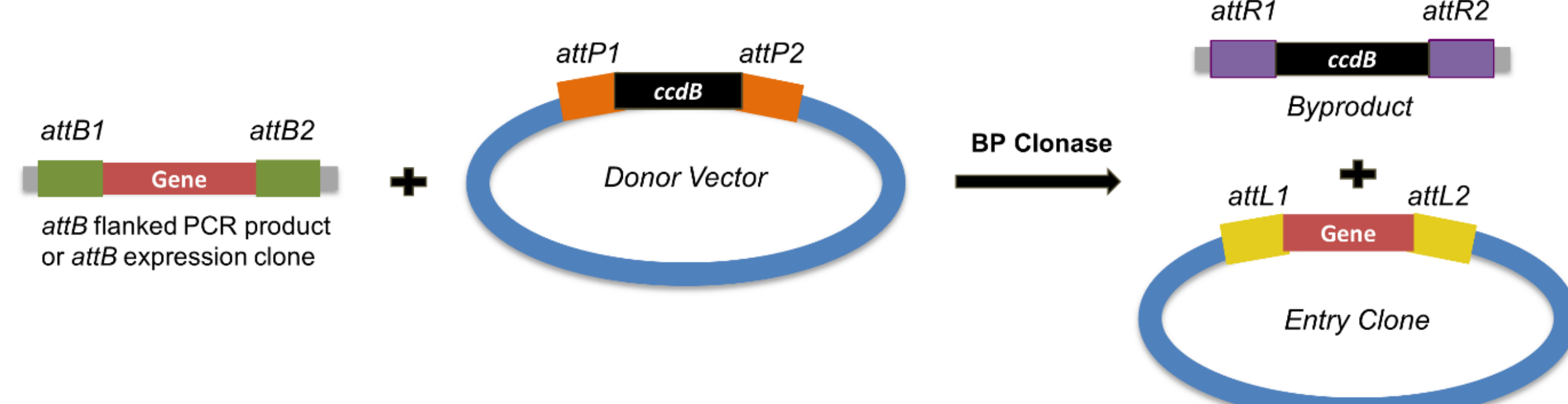
To understand the non-autophagic role of ATG5, ATG5 K130R mutant will be used since it is unable to conjugate with ATG12.

## OBJECTIVE

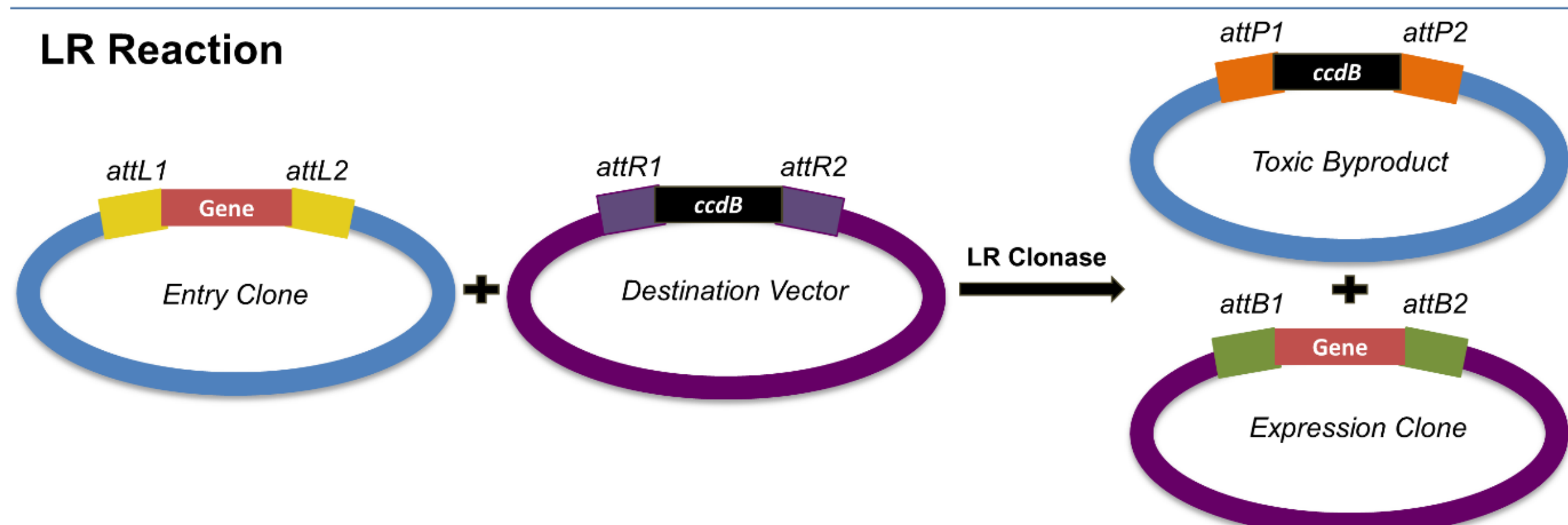
Understanding the non-autophagic role of ATG5 in HeLa cells.

## PROJECT DETAILS

### BP Reaction

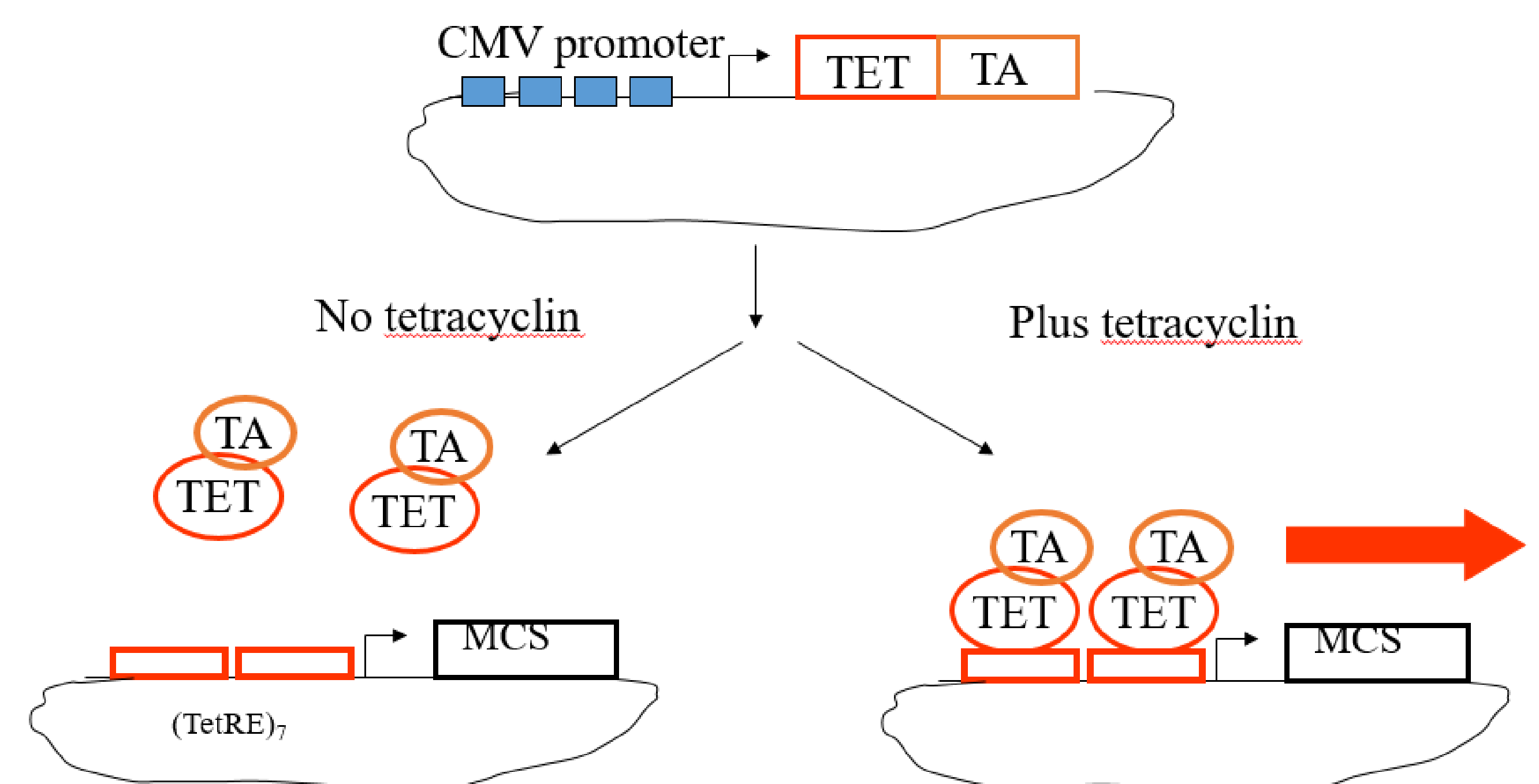


### LR Reaction



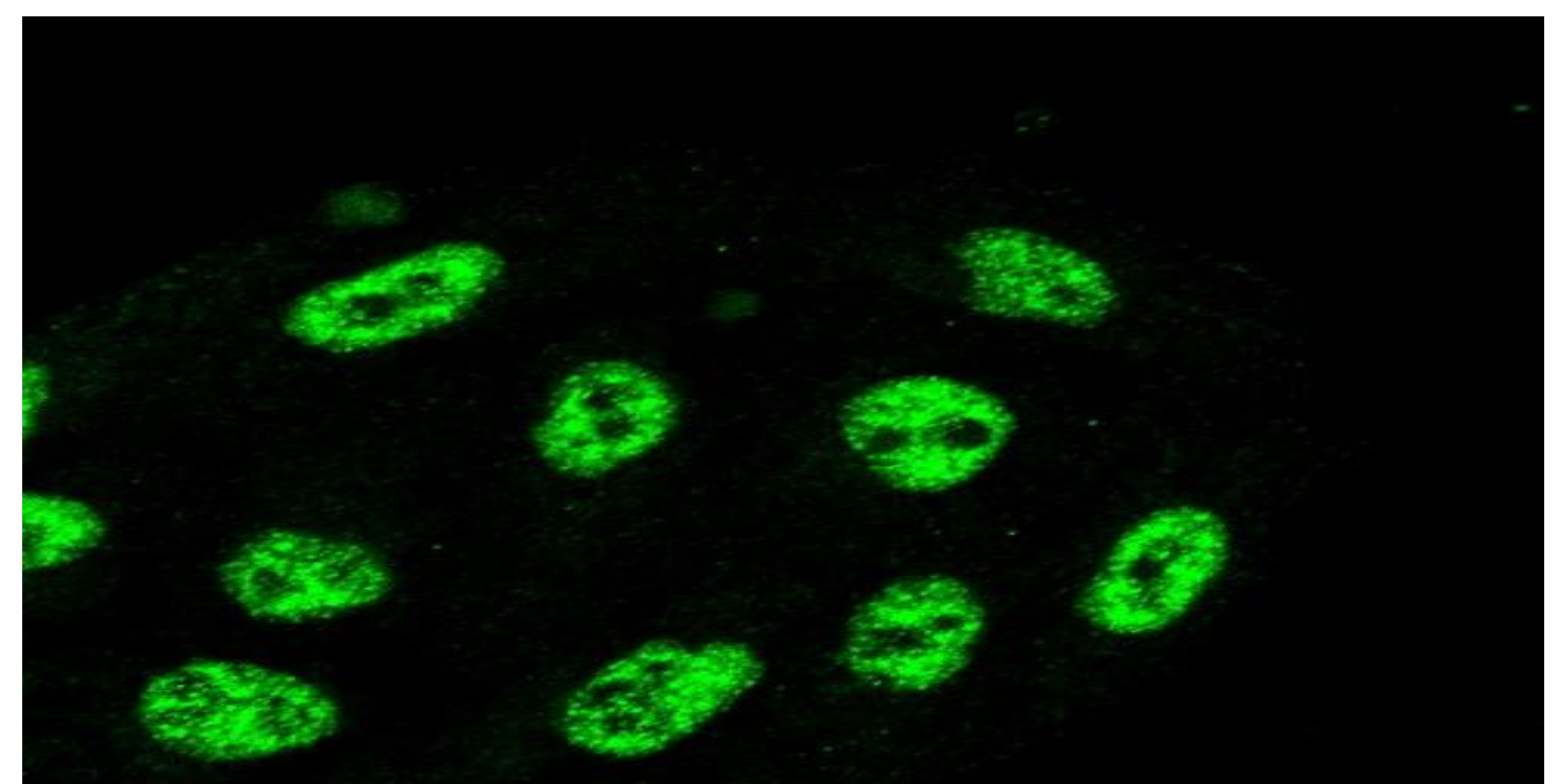
In the first part of the project mutant ATG5 K130R gene will be cloned with the gateway method. This method has two main reactions, BP and LR. Before going into BP reaction, PCR is performed to add attB1/2 flanking regions to our gene of interest. With the added attB1/2 regions, donor vector's attP1/2 regions can recombine and the gene of interest will get into the plasmid. Then this plasmid will be grown in bacteria and after the growth it will be isolated for LR reaction. In LR reaction, again specific regions will be recognized and recombined so that in the end the gene of interest is in the inducible expression vector which is the vector that will be given to the HeLa cells.

## PROJECT DETAILS 2



In the second part of the project, inducible expression vector will be given to HeLa cells which are derived from cervical cancer cells. After cells get the vector, cell activities will be observed to understand the effects of ATG5 K130R on cells. The reason to use inducible expression vector is to control the gene expression. In this project we will use TET-ON system and this system will enable us to control gene expression by changing the presence of tetracycline in the environment of HeLa cells. With the presence of tetracycline, gene will be expressed and if it is not present, gene will not be expressed. With this system we will compare the cell activities and try to understand the difference to find out ATG5's non-autophagic role.

## CONCLUSIONS



There are some findings about the non-autophagic role of ATG5 but not all of the roles are found and there are still much to find about. With this project role of ATG5 in a non-autophagic way will be understood to an extent on the human cancerous cells.

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