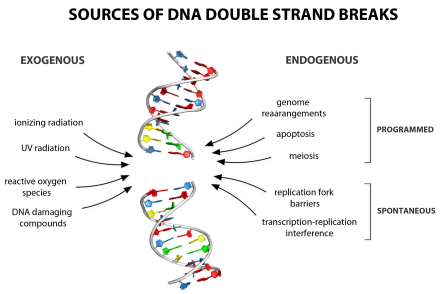
The main goal of this project is development of the new BLESS method for yeast cells and its application for genome-wide studying of double-strand DNA breaks in this eukaryotic model organism using combination of state of the art experimental and computational approaches, including molecular biology, genomics, next generation sequencing (NGS) and bioinformatics.

For an organism to function properly it is essential that its whole DNA (genome) stays integral. However, during the life span of a cell various factors can cause damage to its DNA. One of the most dangerous types of DNA damage are DNA double strand breaks (DSBs), that are characterized by simultaneous disruption of both strands of DNA double helix. Such breaks can cause chromosome loss or rearrangement, therefore even one DSB can result in cell death. DNA DSBs may lead to genomic instability, a hallmark of cancer cells, genetic disorders and cause of cancer predisposition. Thus it is crucial to determine detailed genome-wide map of DSBs and understand what is the mechanism of their emergence and how cells response to their formation. This questions remain still unresolved.



Recently, together with our collaborators, we have developed a new method for high resolution, genome-wide mapping of DSBs called BLESS. So far this method was optimized and validated only for mammalian cells. However, using yeast Saccharomyces cerevisiae, an eukaryotic model organism, for studying DSBs offers many advantages – ease genetic manipulations, availability of well known genome and vast comprehensive data from various studies. Therefore in this project we aim to 1) develop a new BLESS method designed for yeast cells, 2) validate the developed method to ensure its high specificity and sensitivity and 3) apply it to study genetic instability in yeast. In particular we want to take a deeper look at influence of natural pause sites on replication fork stalling and collapse, we will also examine the mechanism of replication checkpoint contribution to maintaining forks stability. In our research we will use combination of state of the art experimental and computational approaches, including molecular biology, genomics, next generation sequencing (NGS) and bioinformatics. We expect that this project will significantly contribute to moving the field from studying individual examples of gene instability to a systematic, genome-wide understanding of DSBs formation and quantification of their importance for RTCs and genome integrity and also will have an enormous impact on other related fields of scientific research.