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Characterization of different BRCA1 and BRCA2 variants and their interaction with the DNA damage tolerance pathway

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BRCA1 and BRCA2 proteins are involved in control of homologous recombination and double-strand break (DSB) repair in response to DNA damage. DSBs are generated naturally when replication forks encounter blocking lesions. Stalled replication forks can activate the DNA damage tolerance pathway that takes the replication machinery through the damaged site. In other case the replication fork can be rescued by recombination dependent mechanisms, which, however, have a potential for DNA rearrangements: nonhomologous end-joining and homologous recombination. In normal cells a delicate balance of damage bypass and homologous recombination can ensure cell survival and at the same time effectively prevent increased mutagenesis. However, mutation in genes affecting one of these pathways results in high degree of mutagenesis and frequent gross chromosomal rearrangements leading to cancer.

Mutations in *BRCA1* and *BRCA2* account for 20-40% of families with hereditary susceptibility to breast and ovarian cancer. Such mutations are located throughout the genes and typically result in premature translation termination. Structural and functional changes of mutated proteins caused by different *BRCA1* mutations are not identical and can lead to various phenotypes of cancers (genotype-phenotype correlations). For this reason, clinical presentations, outcome and response to treatment of tumours can differ significantly depending on the type of mutations. Therefore, there is currently a need to study the genotype–phenotype correlation among different mutations in *BRCA1* and *BRCA2* genes.

To face this challenge we developed a new method for next generation sequencing of *BRCA1* and *BRCA2* genes. The methodology relies on a multiplex PCR amplification of the two genes combined with enzymatic fragment library preparation. A training set of samples was used to optimize and to validate the performance of the workflow. The method was successfully validated being suitable for the detection of mutations, small insertions and deletions specific for the hungarian population. We also plan to characterize the unclassified and newly discovered mutations in sensitivity and mutagenesis assays. For this we use *BRCA1* and *BRCA2* mutant cell lines, in which we knock down the expression of DNA damage tolerance genes, and examine their sensitivity to various DNA damaging agents. Gaining more insight into the interaction of BRCA1 and BRCA2 with other players of DNA repair is important for understanding the molecular basis of genome stability and carcinogenesis.

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Regulation of gene expression by cis acting chromatin elements. Investigation of long-range promoter-enhancer interactions in the *Ubx* domain in *Drosophila*

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In the post-genomic era, one of the main challenges facing biology is answering the question of how different cell types and cell lineages, deriving from the zygote during development, utilize the very same genome differently. Part of the answer must be sought at the level of chromatin structure. The alteration of the chromatin structure is a substantial process of epigenetic regulation of gene expression. In *Drosophila* the *bithorax complex* (BX-C) is an exquisitely convenient model system to study epigenetic regulation.

In *Drosophila* the POLYCOMB group proteins are responsible for maintaining inactive chromatin conformation of numerous key regulator genes. The mechanism of repression and the *Drosophila* POLYCOMB group proteins are evolutionally conserved. The long-term silencing effect is amounted to the condensation of the target gene's chromatin structure. The *Polycomb* regulation depends on special DNA sequences called Polycomb Response Elements (PREs). The PREs are able to interact with each other over large distances; they were mostly studied in transgenic constructs. With a new gene conversion method developed in our laboratory, we are able to modify and study PREs *in situ*. We focus on the well-known *bxd PRE*.

Our aims: 1. to investigate the enhancer sets localized in the proximal and distal subdomain of the functionally divided *bxd cis*-regulatory region; 2. to study the effects of a built in boundary region and the separation of the PRE on the *Ubx* promoter and on the reporter gene; 3. to identify sequences on the homolog chromosome that affects the expression level of the integrated *Gal4-VP16* reporter gene and investigate the role of the *bxd* PRE in this *cis-trans* communication.

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