# Generation and characterization of *mecp2* mutant

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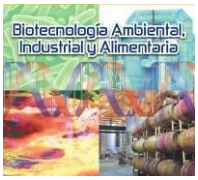


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## ABSTRACT

**Motivation:** *Mecp2* gene encodes the Methyl CpG binding protein (MeCP2). This protein is known for being an important regulator of gene expression that interacts with methylated and unmethylated genomic DNA regions, and enhances or silences transcriptional processes. Different mutations of the MeCP2 protein in humans can lead to a variety of symptoms in Rett syndrome (RTT), a rare disease which causes abnormalities during female brain development as well as acute mental and physical disability. These misfunctions are caused by mutations in one or two of the domains that can be found in the MeCP2 protein: the methyl binding domain (MBD) and the transcriptional repression domain (TRD). The aim of this study is the generation of a knock-out (KO) mutant of the *mecp2* gene in zebrafish by employing the CRISPR/Cas9 technique. Once we obtain the homozygous mutant, we will elucidate the significance of this mutation by deep RNA sequencing (RNA-Seq), focusing on the differences between transcripts present in wild type (WT) and homozygous mutant fish. These results will give us an indication of which genes are potentially regulated by the MeCP2 protein, which could render zebrafish as a useful model system aimed at understanding RTT etiology *in vivo.*

**Methods:** To create the *mecp2* KO, we designed sgRNA guides targeting the *mecp2* exon 2, using the *CRISPRscan* software. We then tested the sgRNAs in F0 by microinjection of single-cell stage zebra-fish embryos. The embryos were allowed to develop to 24-48 hours post fertilization (hpf), after which they were genotyped. Amplification of regions of interest by PCR followed by electrophoresis in agarose gel, showed us that the sgRNAs worked, as we could see a band different to that of the WT (471bp). After the raising of the F0, we genotyped the mutant individually to identify founders. The founders of interest were named as follows: (i) *mecp2 26♂*, with a deletion of 181bp, (ii) *mecp2 27♂*, with deletion of 15bp and (iii) *mecp2 13♂*, with a deletion of 8bp. These founders were out-crossed with WT zebra-fish to stabilize the mutation (F1), and the offspring was genotyped and raised. We then genotyped the F1 generation by extracting the DNA from fin tissue (fin-clip); it is expected that 25% of this generation should be heterozygous. Once we have sequenced the heterozygous candidates for the *mecp2* gene, we will in-cross a male and a female with the same mutation, in order to have a homozygous F2. Finally, we will genotype the F2 individually at 72h, extracting the DNA and RNA at the same time, using the *qiagen DNAeasy blood and tissue* kit for DNA and *Trizol* for RNA. The RNA of the confirmed homozygous mutants will be sent for RNA-Seq analysis.

**Results and conclusions:** We have generated heterozygous *mecp2* mutants, that we have to genotype, from the founders mentioned above (F1). The deletions sequenced cause the generation of a stop codon, so the MeCP2 protein should not be expressed in homozygous fish. The next step will be generating the homozygous generation in order to study the phenotype and perform the RNA-Seq at 72h of embryo development. Once we obtain the results of the RNA-seq analysis, we will be able to explain which are the genes whose expression is modulated by MeCP2 and that are potentially implicated in RTT.

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