

## MEICOM Marie Curie ITN 2018 ESR Progress Summary

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**Workpackage Title:** Developing and implementing methods that modify recombination patterning  
**RTP12:** Targeted meiotic DSB formation in Brassica crops

### Research aims and progress for the period:

- 1) Generation of constructs encoding fusion proteins between SPO11 or PRD2 from Brassica fused to ddCAS9
  - We selected the two Brassica genes (BnaC01g37870D and BnaC09g33470D) for cloning considering sequence analysis comparison to *Arabidopsis thaliana* genome and the application for both Brassica species: *Brassica napus* and *Brassica oleracea*.
  - We are generating the second mutation in the D10A dCas9 to obtain a ddCas9 in which both D10A and H840A sites are mutated to Ala.
- 2) Grow Brassica plants and prepare DAPI slides and immunostaining slides.
  - We are practicing cytogenetic skills in Brassica plants and testing antibodies from Arabidopsis.
- 3) Investigation of DNA double strand break (DSB) proteins interactions
  - Cloning of tagged DSB proteins to the pF3K WG vector for *in vitro* expression.
  - *In vitro* expression of DSB proteins.
  - Western blot to check protein expression.
  - Pull down assays to check protein interactions.
- 4) Generation of PRD2 CRISPR-CAS9 null mutant
  - Selection of 10 T1 plants with CAS9 activity
  - DNA extraction from rosette and cauline leaves of T2 plants.

### Skills Training received:

- Priming your PhD course (3 weeks full-time course)
- Introduction to microscopy
- TIRF microscopy and laser safety
- Mass Spec workshop
- Statistics with R course
- R as a language course
- Lab Journal Club (every week)
- Monday seminar at Vienna BioCenter (every week progress presentation of PhD students)

### Meetings attended:

- 9<sup>th</sup> Central European Genome Stability and Dynamics
- 16th VBC PhD Symposium: Metamorphosis

### Outreach activity: