Identification of meiotic recombination hotspots in Tomato

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The global population is estimated to reach 9 billion by 2050, emphasizing the need to increase food security. Delivering improvement and sustainability in crop production will largely remain based on classical breeding, using meiotic homologous recombination to introduce genetic variation through the formation of reciprocal genetic exchanges, called crossovers (COs). Enhancement of crops could be provided by introduction of genes from related wild species but due to sequence divergence, recombination between species (homeologous recombination) is limited as compared to homologous recombination. To improve the transfer of genes that convey e.g. tolerance to changing climate conditions or pathogen resistance from wild to cultivated species, a better understanding and control of meiotic recombination is crucial.

Meiotic recombination events are not randomly distributed along the genome, but tend to occur in hotspots: 1-2kb wide locations that show higher meiotic homologous recombination than surrounding regions. In mammals, specific consensus sequences associated with hotspots have been identified; in *A.thaliana* hotspots are mostly found in proximity of gene transcriptional start and open chromatin areas. What defines meiotic recombination hotspots in tomato (*S. lycopersicum*) is as of yet unknown. To allow a comprehensive analysis of hotspot associated DNA sequences, we aim to analyze the genome-wide distribution of hotspots in tomato, its wild relative *S.pimpinellifolium* and their F1 hybrid using Chromatin ImmunoPrecipitation (ChIP). The amount of meiocytes per anthers makes tomato an attractive model for ChIP analysis, however the existing ChIP protocols need to be adapted for tomato meiocytes. We are presenting here ChIP workflow and its preliminary results.

Chromatin immunoprecipitation targeting proteins involved in meiotic recombination

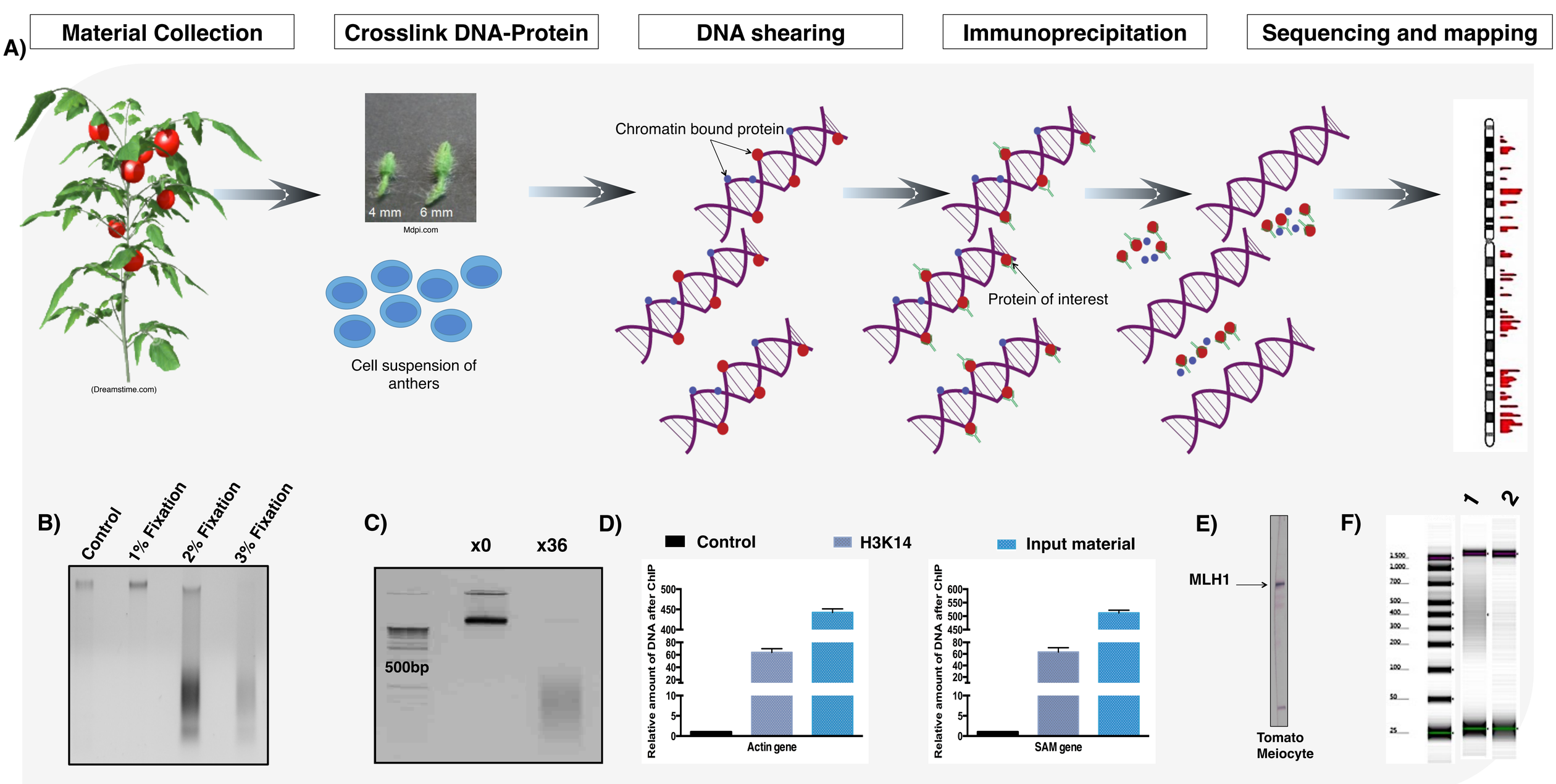

Figure: **Protocol for ChIP on tomato meiocytes and optimization**. **A) Workflow**. After purification of the DNA, the fragments will be sequenced and mapped to the tomato genome. **B) Fixation with 1% formaldehyde is the optimal condition**. Tomato anthers are fixed with different concentrations of formaldehyde and decrosslinked overnight. Control is non-crosslinked DNA. **C) 36 Cycles of sonication yields DNA fragments of 200-600bp.** Shearing of the DNA by sonication x0: no sonication, x36: 36 cycles. **D) Validation of the optimized ChIP protocol on tomato meiocytes**. qPCR on ChIP against H3K14 acetylation, control is ChIP without antibody. **E) Validation of the α-MLH1 antibody.** Western blot on tomato anthers, the MLH1 protein appears around 90kDa. **F) DNA fragments isolated from the ChIP using the antibody MLH1** visualized by TapeStation Agilent System. 1: ChIP performed on tomato anthers. 2: ChIP performed on tomato leaves.

Summary

- Anthers of the appropriate meiotic stage can be selected based on size
- Fixation conditions are: 1% formaldehyde
- Sonication conditions: 36 cycles
- Adapted protocol is suitable for ChIP

Future Plans

- Redo the ChIP targeting other meiotic proteins
- Antibody validation using immunocytology via collaboration
- Using the first MLH1-ChIP, we will create a library and develop a first map of recombination hotspot
- We aim to optimize our method of sample collection

Acknowledgement

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RIJK ZWAAN

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