



CORSO TEORICO-PRATICO SIGA
"New Genomic Techniques: CRISPR/Cas9 in tomato and wheat"

CREA – Genomica e Bioinformatica
Roma, 2-5 July 2024

PROGRAMME

Tuesday, July 2nd 2024

14:00 - 14:30 - Introduction to the course and welcome addresses – *Prof. Silvio Salvi* (President, SIGA) – *Dr. Luigi Cattivelli* (Director, CREA-GB) – Welcome Coffee

14:30 - 15:30 - New Genomic Techniques – *Prof. Stefania Masci* (UniTUS)

15:30 - 16:30 – Cloning systems available for gene editing– *Dr. Fabio D’Orso* (CREA-GB)

16:30 - 17:30 – Tissue culture: Wheat – *Prof. Domenica Nigro* (UniBA), *Dr. Chiara D’Attilia* (UniTUS)

17:30 - 18:30 - Tissue culture: Tomato – *Dr. Alessandro Nicolìa* (CREA-OF)

Wednesday July 3rd 2024

09:00 - 10:00 – The Golden Gate cloning system, workflow to obtain your plasmids – *Dr. Fabio D’Orso* (CREA-GB)

10:00 - 11:30 - *Dr. Fabio D’Orso* (CREA-GB) – *Dr. Alessandro Nicolìa* (CREA-OF) – *Dr. Chiara D’Attilia* (UniTUS)

- Concepts to design specific and efficient RNA guides;
- RNA guide selection and off-target evaluation using *ad hoc* softwares (CRISPOR, CRISPR-P, CRISPR RGEN softwares)
- Virtual construct assembly through Golden Gate cloning tools (Geneious software)
- Systems for guide validation (*In vitro* validation, agroinfiltration, protoplasts, hairy roots)

11:30 - 12:00 – Coffee Break

12:00 - 13:30 - Practice: gRNA design and construct assembly *in silico*, bring your own gene!

13:30 - 14:30 - Lunch

14:30 - 16:15 – Practice 1: Tomato tissue culture – *Dr. Marco Possenti* (CREA-GB), *Dr. Alessandro Nicolìa* (CREA-OF), *Dr. Giorgia Batelli* (CNR-IBBR), *Dr. Valentina Forte* (CREA-GB)

- Preparation of explants from cotyledons for transformation with *Agrobacterium* and protoplast isolation
- Co-culture with *Agrobacterium*
- Protoplast isolation: digestion with cell wall-degrading enzymes

16:15 - 16:45 – Coffee Break

16:45 - 18:30 – Practice 2: Wheat tissue culture - Dr. Chiara D'Attilia (UniTUS), *Prof. Domenica Nigro* (UniBA)
- Spike threshing, seed sterilization

Thursday, July 4th 2024

9:00 - 13:00 – Practice 1: Tomato tissue culture – *Dr. Marco Possenti* (CREA-GB), *Dr. Alessandro Nicolìa* (CREA-OF), *Dr. Giorgia Batelli* (CNR-IBBR), *Dr. Valentina Forte* (CREA-GB)

- transfer of calli, shoot excision, transfer on rooting media
- protoplast isolation: filtration, washes, description of transfection steps
- visualization of protoplasts transformed with fluorescent markers

11:00 - 11:30 - Coffee Break

13:00 - 14:00 - Lunch

14:00 - 18:00 - Practice 2: Wheat tissue culture - *Dr. Chiara D'Attilia* (UniTUS), *Prof. Domenica Nigro* (UniBA)

- embryo isolation, axis removal, stereoscope visualization
- Co-culture with *Agrobacterium*
- biolistic transformation
- transfer of calli and putative transformants

16:00 - 16:30 – Coffee Break

20:30 - Dinner

Friday, July 5th 2024

09:00 - 10:30 – Whole genome sequencing vs. targeted (amplicon sequencing) approaches for analysis of edited individuals, off-target identification and T-DNA free plants *Dr. Riccardo Aiese Cigliano* (Sequentia Biotech)

10:30 – 11:00– Coffee Break

11:00 – 12:00 – Methods of sequence analysis for editing/off-target evaluation– multiallelism, polyploidy *Dr. Fabio D'Orso* (CREA-GB), *Dr. Chiara D'Attilia* (UniTUS)

12:00 – 13:00 –Analysis of edited individuals through Sanger sequencing + ICE analysis

13:00 - 13:30 - Questionnaire, Learning assessment (upon request), Closure of course