



**CORSO TEORICO-PRATICO SIGA**  
**"Established and emerging gene editing techniques in Solanaceae and rice"**

UniNA – Dipartimento di Agraria  
Portici, 9-12 June 2025

**PROGRAMME**

**Monday, June 9<sup>th</sup> 2025**

**14:00 - 14:30** - Welcome addresses – *Prof. Silvio Salvi* (President, SIGA) – *Prof. D. Ercolini* (Director, DiA UniNA) – Welcome Coffee

**14:30 - 15:30** – Crop gene editing: present and future – *Prof. Vittoria Brambilla* (UniMi)

**15:30 - 16:30** – European and Italian legislative framework – *Dr. Teodoro Cardi* (CNR-IBBR)

**16:30 - 17:30** – CRISPR/CAS cloning strategies: The Golden Gate cloning system, from sgRNA guide design to construct assembly – *Dr. Fabio D’Orso* (CREA-GB)

**17:30 - 18:30** – *Dr. Fabio D’Orso* (CREA-GB) – *Dr. Alessandro Nicolìa* (CREA-OF, CNR-IBBR)

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

**Tuesday, June 10<sup>th</sup> 2025**

**09:00 - 10:30** – **Practice:** gRNA design and construct assembly *in silico*, bring your own gene! – *Dr. Fabio D’Orso* (CREA-GB)

**10:30 - 11:30** - Prime editing and base editing: concepts and elements for designing your guides – *Prof. Vittoria Brambilla* (UniMI)

**11:30 - 11:50** – Coffee Break

**11:50 - 12:40** - Tissue culture: Solanaceae (tomato and potato) – *Dr. Alessandro Nicolìa* (CREA-OF, CNR-IBBR), *Dr. Vincenzo D’Amelia* (UniNA)

**12:40 - 13:30** – Tissue culture: Rice – *Dr. Fabio Dobetti* (UniMI)

**13:30 - 14:30** - Lunch

**14:30 - 16:15** – **Practice 1:** Solanaceae tissue culture (tomato and potato) – *Dr. Marco Possenti* (CREA-GB), *Dr. Alessandro Nicolìa* (CREA-OF, CNR-IBBR), *Dr. Giorgia Batelli* (CNR-IBBR), *Lorenza Sannino* (CNR-IBBR)

- Preparation of explants 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:** Rice tissue culture - *Dr. Fabio Dobetti* (UniMI), *Prof. Vittoria Brambilla* (UniMI)

- calli removal from seeds
- transformation with Agrobacterium
- co-cultivation overnight

### **Wednesday, June 11<sup>th</sup> 2025**

**9:00 - 13:00** – **Practice 1:** Solanaceae tissue culture (tomato and potato) – *Dr. Marco Possenti* (CREA-GB), *Dr. Alessandro Nicolia* (CREA-OF, CNR-IBBR), *Dr. Vincenzo D’Amelia* (UNINA), *Antonella Vitiello* (CNR-IBBR)

- 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:** Rice tissue culture - *Dr. Fabio Dobetti* (UniMI), *Prof. Vittoria Brambilla* (UniMI)

- Agrobacterium removal, calli selection and plant regeneration

**16:00 - 16:30** – Coffee Break

**20:30** - Dinner

### **Thursday, June 12<sup>th</sup> 2025**

**9:00 – 10:00** – Methods of sequence analysis for editing/off-target evaluation– multiallelism, polyploidy *Dr. Fabio D’Orso* (CREA-GB)

**10:00 – 11:00** –Analysis of edited individuals through Sanger sequencing + ICE analysis

**11:00 – 11:30**– Coffee Break

**11:30 - 13:00** – 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)

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

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*La SIGA aderisce alla Federazione Italiana Scienze della Vita, alla Associazione Italiana Società Scientifiche Agrarie, alla European Association for Research on Plant Breeding e alla European Sustainable Agriculture Through Genome Editing*