

Last update: August, 2011.

What is this for?

I am in the process of preparing a new course on **biotechniques applied in plant pathology, biosecurity and microbial forensic for undergraduates. Teaching 'primer design workshops for PCR based diagnostics using Web-interface software' is an important component. Exercising primer design softwares allows me to select the appropriate educational materials, exercises, sequences, examples, and getting along with interesting questions. In general, facilitates knowing in advance about troubles to be circumvented by students. If you were a participant of a workshop or I had taught this to you in other way including by mouth to mouth basis, I am in tremendous debt with you. You made a contribution to my project. THANKS!!**

***Now... willing to organize a workshop?
Please contact me to explore ways to obtain the required funds.
It usually requires writing a proposal.***

Primer design workshops

by F. M. Ochoa Corona

Primer design for development of PCR based diagnostics is a Web-based learning workshop for graduate and undergraduate level, scientists or laboratory technicians requiring an introduction to PCR-based primer design for developing PCR based molecular diagnostics for agricultural, biosecurity and forensics applications. The workshop addresses the needs of students starting molecular biology studies and require to design oligonucleotides for PCR-based experimentation. This workshop is a new and practical Web-based learning experience that focuses on 'hands on' guided instruction that introduce students to self designing oligonucleotides primers. Students learn from accessing relevant and useful web-links and web-interface software based on accepted algorithms available free in the Web. This workshop also provides essential information, concepts and definitions related to important thermodynamic PCR parameters required for End Point (standard) and Real Time PCR primer design. Students are encouraged to follow exercises, respect and breaking golden rules, and working with own DNA sequences or projects of interest. This is a validated procedure that offers a Web-based strategy for primer design and addresses a basic procedure (the Primer3-mFOLD-BLASTn pathway) and tips for making more efficient the primer design. This primer design procedure is not universal, and students are encouraged to modify the strategy to their own working convenience by incorporating new molecular tools to the process. Students are encouraged to become open mind, this is a field that rapidly reflects the effects of technological innovation and the presented strategy may change in the near future. This workshop can be organized in English, Spanish or Italian.

Intended Learning Outcomes

- This workshop provides essential information, concepts and definitions related to nucleic acids and important thermodynamic parameters required for End Point

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(standard) and Real Time PCR primer design which is required for successful development of PCR based diagnostics.

- Learning primer design through web-interfaced software will improve student's proficiency in PCR applications, and will ultimately make them more efficient, productive and competitive.

The overall process at a glance

[1] The nucleotide sequences of interest are obtained from **Gene sequences repositories** or self-generated in the lab, and converted to 'Fasta' format using alternative Web-based software, and subsequently aligned using ClustalX or MAUVE also available through the Web at no cost.

[2] Alternatively, amino acid sequences of interest can be aligned by Block Maker to obtain conserved regions.

[3] The obtained sequence are blocked and copied into **PRIMER3** for primer design using optimal parameters. Alternative Web-software for primer design can also be used. Once the primers are generated, the generated primers sequences are copied into **mFOLD**. This Web-interface software allows the *in silico* analysis of the secondary structure and thermodynamic features.

[4] Finally, the primer sequences are copied into **BLASTn** (NCBI) and assessed for specificity.

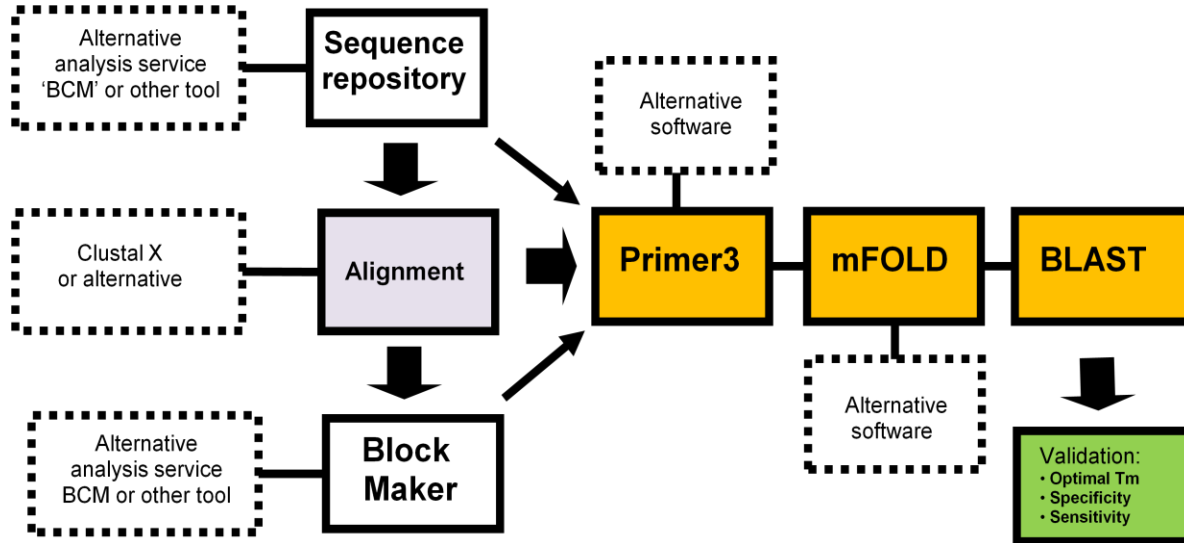


Fig. 1. Flow-chart showing the primer design process through Web interface software.

Citation:

F.M. Ochoa Corona, J. Tang, B.S.M. Lebas, B. Alexander. Validation of primer design for plant virus diagnostics using the Web-interface pathway Primer3-mFOLD-BLASTn. *Phytopathology* 97: (7) S86 (full article in preparation)

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- Next Workshops-

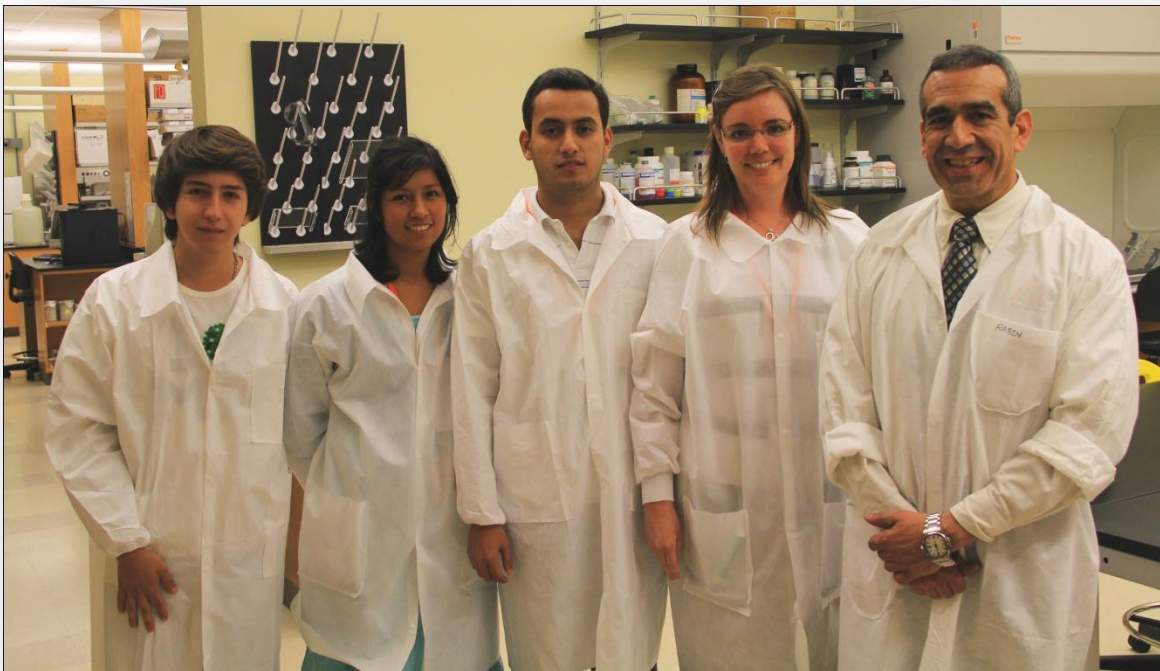
To be announced

Past Workshops

Oklahoma State University, NIMFFAB & Dpt. of Entomology and Plant Pathology. Stillwater, OK, USA.

June 7-9, 2011.

This is the second primer design workshop organized at OSU, Department of Entomology and Plant Pathology and the National Institute for Microbial Forensics and Food and Agricultural Biosecurity (NIMFFAB). The workshop took place from the 7th to the 9th of June, 2011. Participants included undergraduate students visiting from UPAEP, Puebla, Mexico.



Picture right to left, Raul Garcia, Areli Munive, Rodrigo Huevo, Prof. Maria Cristina, Miranda Vergara and myself. Not picture, a number of participants from NIMFFAB, the OSU department of Entomology & Plant Pathology and postdocs. From the top of my mind, Kerstien McMurl (undergraduate), Andrea Payne, Chris Timmons, Ahmed Abd-Elmagid, Hugo Castillo, Overall Lisa, Gabriela Orquera and others.

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**University of Food and Technologies, Plovdiv, Bulgaria
14-16 of September, 2010**

This primer design workshop was organized in Bulgaria at the University of Food and Technologies, Department of Organic Chemistry and Microbiology with the sponsorship of the 2009-2010 USDA's Foreign Agricultural Service (FAS) administered under the Borlaug International Agricultural Science and Technology Fellowship Program for Bulgaria. The title of the project is "Rapid detection of vegetable borne pathogens and spoilage microbes using real time PCR". This workshop was also possible thanks to the effort of Prof. Denica Blazheva and the IT skills of Prof. Aleksandar Slavchev, as well as the participation of my OSU colleague Prof. Carla Garzon. I was also glad and pleased visiting with Prof. Zapryana Denkova, director of Department of Organic Chemistry and Microbiology. I was impressed by the interest and receptivity shown by all participants and the potential to develop new diagnostics methods in Bulgaria for biosecurity applications and the solution of relevant food and technology problems. Not mentioning, I got also impressed by the beauty of 'Old Plovdiv's colorful houses and historic places. I'll be keeping in a special place of my heart the handful Bulgarian hospitality and sincere friendship, which will remain always fresh in my mind.



In the picture the participants of the workshop held at the University of Food and Technologies, Plovdiv, from left to right: Francisco Ochoa Corona, Denica Blazheva, Aleksandar Slavchev, Plamena Nedelcheva, Velichka Yanakieva and Rositsa Deukova.

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**Università degli study di Catania, Catania, Sicily, Italy
7-9 of September, 2010**

This is a primer design workshop organized in Italy with the support of the 'Dipartimento di Science e Tecnologie Fitosanitarie' (DISTEF), Facoltà di Agraria, and their International doctoral program (Dottorato Internazionale) which is coordinated by Prof. Gabriella Cirvilieri. I was impressed by the interest and receptivity shown by all participants as well as the research topics being addressed. I was also glad and pleased of visiting with Prof. Antonino Catara (director) and Prof. Rosa La Rosa who also contributed to make this workshop possible. Thanks to all for the genuine Italian hospitality, sincere friendship and unforgettable time.



In the picture the participants of the workshop held at the Università degli Study di Catania, from left to right: Yosra Ahmed, Sarra Ben Attia, Giuseppe Scuderi, Serena Rizza, Rosa Elena Spallino, Claudia Platania, Domenico Raspagliesi, Prof. Rosa La Rosa and Prof. Gabriella Cirvilieri. Not pictured, Giuliana Maria Ialacci.

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**Oklahoma State University, Stillwater, USA
22 of January, 2010**

This is first primer design workshop organized at OSU, Department of Entomology and Plant Pathology and the National Institute for Microbial Forensics and Food and Agricultural Biosecurity (NIMFFAB). The workshop took place the 22 of January, 2010, and I was glad of having graduate & undergraduate students, postdocs, and faculty members among the audience. It was indeed an enjoyable occasion. We shared experiences about primer design, projects, goals and how to address particular situations regarding primer design.



In the picture some of the participants. On the first row and from left to right, Mohammad Arif, and Dhiraj Gautam. Sitting on the second row: Francisco Flores, Donna Caasi, Patricia Garrido, Kazi Kader and Cesar Solorzano Torres. Standing on the back: Francisco Ochoa Corona, Rebecca Pace, Kaushal Maskey, Kelsy Thompson, Shefali Dobhal, Andrea Payne, Ahmed Abd-Elmagid, Ian Moncrief, Ioannis Oikonomakos, Sophia Kamenidou and Lakmini Wasala. Not pictured Professors Astri Wayadande and Li Ma.

Testimonials:

**Jason Shiller, from La Trobe University, Melbourne, Australia.
22 of September, 2010.**

“I remember you did some work on standardizing primer design methods using primer 3 mfold etc and you taught a lot of people how to do it (including myself). I have used your system many times with great success and passed on the knowledge when I have been able to.”