

# QBOL: a new EU project focusing on DNA barcoding of Quarantine organisms\*

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In 2009 a new three year EU funded project (QBOL) started on DNA barcoding of important plant pests. An international consortium of 20 partners (universities, research institutes, and phytosanitary organizations) from around the world, coordinated by Plant Research International (Wageningen, the Netherlands), will collect DNA barcodes from many plant pathogenic quarantine organisms, store these sequences in a database accessible over the internet, develop a DNA bank and train end-users. All these activities should help National Plant Protection Services in the correct identification and detection of plant pathogenic quarantine organisms.

## Introduction

Development of accurate identification tools for plant pests is vital to support European Plant Health Policies. For this project Council Directive 2000/29/EC is important, listing some 275 organisms for which protective measures against their introduction into and their spread within the Community needs to be

taken. Those threats are now greater than ever because of the increase in the volumes, commodity types and origins of trade, the introduction of new crops, the continued expansion of the EU and the impact of climate change. Currently identifying pathogens (in particular new emerging diseases) requires a staff with specialised skills in all disciplines (mycology, bacteriology, etc.); which is only possible within large centralised laboratory facilities. Taxonomy, phytopathology and other fields which are vital for sustaining sound public policy on phytosanitary issues are threatened with extinction.

Modern molecular identification/detection techniques may tackle the decline in specialist skills since they are often more amenable for routine purposes and can be used for a whole range of different target organisms. Recently DNA barcoding has arisen as a robust and standardised approach to species identification. The aim of QBOL now is to make DNA barcoding available for plant health diagnostics and to focus on strengthening the link

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Project acronym: QBOL.

Project website: <http://www.QBOL.org>

Project full title: Development of a new diagnostic tool using DNA barcoding to identify quarantine organisms in support of plant health.

Those interested in collaborating with the consortium are invited to contact the coordinator.

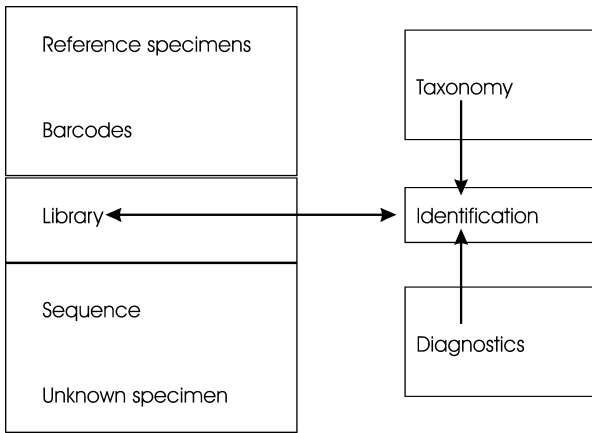


Fig. 1 Species identification using DNA barcode sequences.

between traditional and molecular taxonomy as a sustainable diagnostic resource (see Fig. 1).

Within QBOL collections harbouring plant pathogenic quarantine pests will be made available. Informative genes from selected species on the EU Directive and EPPO lists will be DNA barcoded from vouchered specimens. The sequences (Fig. 2), together with taxonomic features, will be included in a new internet-based database system. A validation procedure on developed protocols within the project and the database will be undertaken across worldwide partners to ensure robustness of procedures for use in a distributed network of laboratories across Europe.

### Objectives

The multinational QBOL consortium has a long-term vision of developing a sustainable diagnostic resource to enable ‘DNA-barcode identification’ ultimately for all quarantine plant pests or pathogens of statutory importance. The consortium aims to develop a DNA barcode database containing relevant reference DNA/RNA sequences of quarantine and regulated plant pathogens linked to appropriate taxonomic metadata. In addition to develop the tools and methods to enable the identification of all

quarantine and regulated plant pests and pathogens world wide by any plant diagnostic centre, national reference organisation and authority freely using the internet, to enable the implementation of Council Directive 2000/29/EC. To address these major challenges, QBOL has identified the major challenges in the chain for the *Development of new diagnostics methods in support of Plant Health Policy* and formulated four principal project objectives:

1. To obtain or produce relevant vouchered sequence data for individual pests or pest groups and position them in a correct taxonomic context. QBOL will determine which and how many genes (barcodes) are informative for correct species identification and what are the species limits for relevant quarantine pests and morphologically and/or taxonomically related organisms, to enable the accurate identification/diagnosis of all taxa on the EU Directive and EPPO A1 and A2 lists of pest recommended for regulation
2. To developing generic diagnostic tools based on these barcode sequences. The consortium will investigate bioinformatic tools to enable the correct identification of quarantine pests based on DNA barcode sequences, and develop a database that will enable the storage and searching of related diagnostic metadata, *to link vouchered sequence information to published biological information*
3. To develop strategic approaches and methodologies to enable the establishment of DNA banks and access to digital voucher specimens. The consortium will develop methods that enable the storage of DNA/RNA samples (a DNA bank) for the selected set of Q-organisms and their relatives to enable access of material to all national plant protection services for positive and negative controls
4. QBOL will support better collaboration between EU and non-EU countries diagnostic laboratories and also the international ‘DNA barcoding’ community.

### Work Packages

The four principal QBOL project objectives will be achieved through a range of measurable objectives, each one related to a

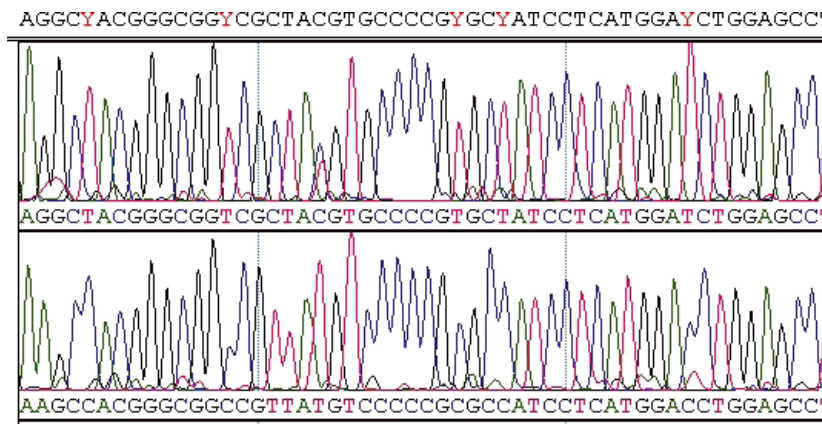


Fig. 2 A DNA sequence to be used as a DNA Barcode.

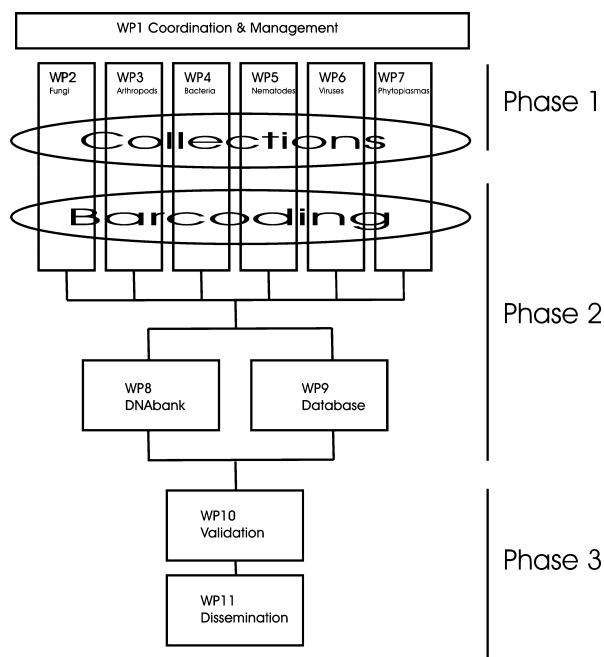


Fig. 3 All the WPs and their relationship.

specific Work Package (see Fig. 3) and will encompass all quarantine-group taxa within the fungi, nematodes, viruses, bacteria, arthropods and phytoplasmata.

The objectives of the eleven Work Packages are:

Work Package 1. To coordinate and manage the project.

Work Packages 2–7. To assemble all relevant collections of quarantine pests available in EU and third countries and make them available for the QBOL project. To investigate which and how many genes are required to identify quarantine pests of each type (fungi, arthropods, bacteria, nematodes, viruses and phytoplasmata) of species using barcode sequences and to generate these barcodes for the relevant taxa.

Work Package 8. To develop methods for effective amplification and storage of rare DNA samples.

Work Package 9. To develop bioinformatic tools and a database system to enable the storage of identification relevant metadata for relevant quarantine plant pathogens plus their relatives and allow links to the EMBL (European Molecular Biology Laboratory) and sister databases for storage of barcode sequences.

Work Package 10. To validate the protocols developed and the diagnostic use of DNA barcoding.

Work Package 11. To disseminate results obtained within the project and train mandated labs in EU and third countries in using protocols and database developed.

## The QBOL consortium will deliver:

### Culture collections and taxonomic experience

The consortium will bring together a worldwide network of relevant culture collections containing selected quarantine

pests and the taxonomic expertise related to these collections.

### Quarantine organisms EU Directive, EPPO list (the EPPO pest lists include 302 pests)

Many of the regulated organisms of the EU Directive/or pests recommended for regulation by EPPO list will be addressed. Priorities will be made based upon gaps in existing knowledge and availability of target specimens and closest relatives in different regions in the world. Besides providing barcodes, this work will also support legislation on quarantine pests by generating information on the correctness of the identity as named in the actual list of quarantine pests.

### Sequencing of Q-organisms

Multi gene analysis will be executed on selected quarantine pests. Sequencing will not be performed at a single central sequencing facility but will be centralized in each participating country, because organisms addressed are very diverse with many different genes, primers etc. to be used.

### Barcoding projects

Close contact with The Barcode of Life Data Systems (BOLD) is guaranteed and data will be shared with BOLD database. Links with other barcoding initiatives will be made, e.g. International Barcode of Life project (iBOL), European Consortium for the Barcode of Life (ECBOL), Netherlands Barcode of Life (NBOL). Paul Hebert (Canada), initiator of many barcoding projects, is participating in the advisory board.

### DNABank

Methods will be developed to enable the effective storage of DNA samples of barcoded quarantine pests and their taxonomically related species collected in the project, both for archive purposes and also for distribution to end users as control material. A DNA bank will be developed.

### Database

Databases created within the framework of the QBOL project and managed by the BioMICS software (BioAware, Hannut, Belgium) will have exportation and importation functionalities to and from CBOL (Consortium for the Barcode of Life), NCBI (The National Center for Biotechnology Information), EMBL (European Molecular Biology Laboratory) and GBIF (Global Biodiversity Information Facility). Data entry of new sequences of quarantine pests can be done by the users of the system using the free web interface of the software. Free MS-Windows based software will also allow remote addition of data and advanced curation of the databases by selected curators<sup>1</sup>

<sup>1</sup>Person in charge of validating the data entered in the database.

### Validation

The developed protocols and the system for identification to species using DNA barcodes will be validated by end-users. Protocols can be used as EPPO Diagnostic protocols for identification.

### Implementation-dissemination

A website will be built and a flyer will be distributed to stakeholders and end users. End-users will be trained, developed protocols and the database will be distributed and a workshop/symposium will be organized. Implementation of Database and DNAbank into an (inter)national platform within and outside EU laboratories will be performed.

### QBOL: un nouveau projet européen sur les codes-barres ADN des organismes de quarantaine

Récemment un nouveau projet financé par l'EU (QBOL) a débuté; il concerne les codes-barres ADN des organismes nuisibles importants pour les plantes. Un consortium international de 20 partenaires (universités, instituts de recherches et organisations phytosanitaires) de différentes parties du monde, coordonné par Plant Research International (Wageningen, Pays-Bas), collectera les codes-barres ADN de nombreux organismes de quarantaine pour les végétaux, conservera ces séquences dans une base de données accessible par Internet, développera une banque d'ADN et formera les utilisateurs finaux. Toutes

ces activités devraient aider les Services nationaux de Protection de végétaux pour l'identification et la détection correctes des organismes de quarantaine. Les personnes intéressées pour collaborer à ce consortium sont invitées à contact le coordinateur.

### QBOL: новый проект ЕС, нацеленный на штриховое кодирование ДНК карантинных организмов

Недавно был запущен новый, финансируемый Европейским Союзом, проект QBOL, ставящий своей целью штриховое кодирование ДНК важных патогенов растений. Международный консорциум, включающий 20 партнеров (университеты, научно-исследовательские институты и фитосанитарные учреждения) со всего мира при координации организацией Plant Research International (Вагенинген, Нидерланды), будет собирать штриховые коды ДНК для многих патогенных карантинных организмов, хранить эти последовательности в базе данных, доступной через Интернет, поддерживать банк ДНК и обучать конечных пользователей. Все эти меры должны помочь национальным службам карантина и защиты растений правильно определять и выявлять патогенные для растений карантинные организмы. Тем, кто проявляет интерес к сотрудничеству с этим консорциумом, предлагается связаться с координатором этого проекта.