Introduction to Molecular Biology and Bioinformatics Workshop

Biosciences eastern and central Africa - International Livestock Research Institute Hub (BecA-ILRI Hub), Nairobi, Kenya
May 5-16, 2014

Rob Skilton
Team Leader-Capacity Building, BecA-ILRI Hub
Content

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• Lab groups
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• Lab manual
Introductions

• **Resource persons**
  – Val Aloo

• **Health and safety trainer**
  – Jeridah Sinange

• **Mol Biol Lab trainers**
  – Tina Kyalo
  – Moses Njahira
  – Wilson Kimani
  – Solomon Maina
  – Dr Roger Pelle
  – Dr Rob Skilton
  – Eunice Machuka

• **BFX trainers**
  – Joyce Njuguna
  – Dr Mark Wamalwa
  – Dr Anne Jores
  – Dedan Githae

• **SegoliP Unit**
  – Ben Kiawa

• **Lab Management**
  – Timothy Kingori

• **Participants:** name; position; institute; country; your expectations of the workshop; how you plan to use the acquired skills in your research
<table>
<thead>
<tr>
<th>Animal</th>
<th>Lab Groups</th>
<th>Plant</th>
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General Information

- **Please be punctual**
  - Bus leaves Pride Inn at 0745 each day
  - Strictly observe times for breaks and lunch

- Always wear your badge on campus
- Few lab access cards available

- Molecular biology: Lab 3 – Mon 5th to Sat 10th
- Bioinformatics: JVC – Mon 12th to Fri 16th

- Travel/accommodation/per diem/health: Val Aloo
- Course issues: Rob Skilton or your group tutor

- Wireless internet available throughout campus
General Information

- Do not leave laptops or personal effects unattended
- Phones on silent
- Avoid taking calls in the laboratory and lectures

- Tea/coffee in tent
- Lunch at poolside
- Breakfast and dinner at Pride Inn
- Bathrooms
- Bus leaves from ILRI reception

- Dinner on Saturday 11\textsuperscript{th}, 1600-1830
- Rest Day on Sunday 12\textsuperscript{th}
- BBQ Dinner at ILRI on Friday 17\textsuperscript{th}, 1800-2000

- Security
General Information

• Make the workshop interactive

• Don’t be afraid to ask questions. Asking questions is not a sign of stupidity! Those who ask lots of questions will gain more from the workshop.

• All participants will be expected to ‘present’ their group results and discuss them.

• We will have a QUIZ at end of each day: be prepared to answer questions and to ask questions.

• Keep a laboratory note book: A4 hardback notebook is provided. Keep it up to date. Record everything you do in the lab as you do it, methods, your thoughts and ideas, your results, and interpretation of results. We will display examples of participants’ lab books during the workshop.
No One Left Behind!

• Support each other in your group!
• Everyone on the bus!
All posters to be submitted to Val Aloo by end of today!
Workshop objectives

• Provide practical skills and concepts in basic molecular biology and bioinformatics
• Experience the discovery process as a team
• Provide skills to establish basic molecular biology and bioinformatics at your home institute
• Understand basic concepts of molecular biology and bioinformatics for understanding various contemporary areas of research and their applications and for communicating with other researchers in these fields
• Help establish links between researchers and with BecA
DNA barcoding: a new diagnostic tool for rapid species recognition, identification, and discovery.

DNA barcoding is based on a simple concept.

DNA barcoding is a taxonomic method that uses a specific short genetic marker in an organism's DNA to identify it as belonging to a particular species.

In 2003, Paul D.N. Hebert from the University of Guelph, Canada, proposed the compilation of a public library of DNA barcodes that would be linked to named specimens. This library would "provide a new master key for identifying species, one whose power will rise with increased taxon coverage and with faster, cheaper sequencing".
How Barcoding works

Organism is sampled

DNA is extracted

a small region of DNA (the Barcode DNA) is PCR amplified

ACGAGTCGGTAGCTGCCCTCTGACTGCATCGAA
TTGTCCCCCTACTACGTATATGCCTACCGTACG
TCGTACGAAGATTATATAGCTAGCTAGCTACTAG
CCCTATTACGATAACTAGCTACGATTATAGCTACA

The PCR product is sequenced

Sequenced DNA is compared with sequences in a barcode database to identify the organism
DNA barcoding

Barcode of Life Database:
Sequence statistics (as of April 28, 2014)

Barcode Sequences  3,008,123

Species coverage (formally described)
Animals            144,560
Plants             52,767
Fungi & Other Life 15,370

http://www.boldsystems.org/
Once a handheld barcode reader is available for examining a tissue sample and is connected to a database, scientists foresee many practical uses:

- Biologists could identify organisms in the field to quickly assess biodiversity.
- Public health authorities could identify mosquitoes carrying infectious agents, such as West Nile virus, and other disease vectors, enabling timely application of targeted control methods.
- Restaurant owners and consumers could check fish to be sure what they are buying is what is advertised.
- Taxonomists could spot genetically distinct specimens, speeding up cataloguing of new species before they become extinct.
- Farmers could identify pest species invading their fields, and port inspectors could intercept shipments harboring harmful species at borders.
- Doctors could rapidly diagnose fungal pathogens and parasites, such as the one that causes malaria.
- Museums could analyze the large backlogs of collected specimens, helping them find undescribed species lurking in museum drawers.
- Regulatory agencies could test animal feed for forbidden items likely to spread illnesses such as mad cow disease.
Caterpillars (*photographs, below left*) of the skipper butterfly (*Astraptes fulgerator*) in Costa Rica differ in appearance, habitat and favored foods, but the adults all look very similar (*below right*), and scientists had long thought they belonged to a single species. Barcoding tells a different story, however. Because variation in the CO1 gene correlates with appearance, lifestyle and chosen foods of the caterpillars, researchers determined that, despite the outward appearance of the adults, the butterflies actually divide into 10 separate species.
Applications of DNA barcoding

(1) Facilitating identification and recognition of named (described) species:
- Plant leaves (when flowers or fruit are not available)
- Linking life history stages, genders
- Differentiating cryptic species
- Identifying gut contents (e.g. ticks, mosquitoes, biting insects)
- Human and plant disease vectors
- Agricultural pests
- Biosecurity: detecting invasive insect pests at port of entry
- Inventory of species in genebanks
- Food, herbal medicines (market substitution)
- Bushmeat and illegally sold products of endangered species

(2) Surveying biodiversity; e.g., flagging potentially new (undescribed) species.
Learning molecular biology and bioinformatics through DNA barcoding

- Animal: Muscle (Unidentified species)
  - Mitochondrial cytochrome c oxidase subunit 1 (CO1) gene

- Plant: Leaf (Unidentified species)
  - Ribulose-1, 5-bisphosphate carboxylase oxygenase large subunit gene (rubisco; rbcL) from the plastid genome

Polymerase Chain Reaction (PCR)

DNA

Analyse DNA sequence to identify species
Workshop plan

Tissues

Purify DNA
- Analyse DNA (Nanodrop/Gel)

PCR
- Analyse PCR products (Gel)

PCR Optimisation

Purify PCR product
- Analyse purified PCR product (Nanodrop/Gel)

Restriction

Gel

DNA sequencing

Bioinformatics
Workshop plan

Day 1

Tissues

Purify DNA

Analyse DNA (Nanodrop/Gel)

PCR

PCR Optimisation

Analyse PCR products (Gel)

Purify PCR product

Analyse purified PCR product (Nanodrop/Gel)

Restriction

DNA sequencing

Gel

Bioinformatics
Workshop plan

Day 2

Tissues
- Purify DNA
  - Analyse DNA (Nanodrop/Gel)
- PCR
  - Analyse PCR products (Gel)
  - Purify PCR product
    - Analyse purified PCR product (Nanodrop/Gel)
  - PCR Optimisation
- Restriction
- Gel
- DNA sequencing
  - Bioinformatics
Workshop plan

Day 1
Day 2
Day 3
Day 4
Day 5
Day 6

WEEK 1

Molecular biology (Lab 3)

Day 7

Rest Day

WEEK 2

Day 8
Day 9
Day 10
Day 11
Day 12

Bioinformatics (JVC)
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<tr>
<th>Time</th>
<th>Activity</th>
<th>Location</th>
<th>Trainers/resource persons</th>
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<tr>
<td>0730</td>
<td>Bus leaves Pride Inn for ILRI</td>
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<tr>
<td>0800-0815</td>
<td>Registration</td>
<td>N’Dama Lounge</td>
<td>Val Alag, Marvin Wasonga</td>
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<td>0815-0830</td>
<td>Workshop Opening</td>
<td>N’Dama Lounge</td>
<td>Dr Appolinaire Dijkeng, Director, BecA-ILRI Hub</td>
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<td>0830-0930</td>
<td>Introduction to the course</td>
<td>N’Dama Lounge</td>
<td>Dr Rob Skilton</td>
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<td>• Introducing the participants and trainers</td>
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<td>• Housekeeping issues</td>
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<td>• Objectives and expectations</td>
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<td>• Workshop outline</td>
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<td>0930-1000</td>
<td>Pre-workshop test</td>
<td>N’Dama Lounge</td>
<td>Dr Rob Skilton</td>
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<td>1000-1015</td>
<td>Tea/coffee</td>
<td>N’Dama Lounge</td>
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<td>1015-1115</td>
<td>Seminar</td>
<td>N’Dama Lounge</td>
<td>Ephr Khaembma</td>
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<td></td>
<td>• Laboratory Health and Safety</td>
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<td>1115-1145</td>
<td>Lab</td>
<td>Lab 3</td>
<td>Ephr Khaembma</td>
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<td>• Laboratory Health and Safety</td>
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<td>1145-1245</td>
<td>Lab</td>
<td>Lab 3</td>
<td>Tina Kyalo, Moses Njahira, Wilson Kimani, Dr Roger Pelle, Dr Rob Skilton, Solomon Maina, Eunice Machuka</td>
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<tr>
<td></td>
<td>• Pipetting skills</td>
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<td>• Introduction to lab equipment</td>
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<td>1245-1330</td>
<td>Lunch</td>
<td>Poolside</td>
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<td>1330-1430</td>
<td>Seminar</td>
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<td>Dr Rob Skilton</td>
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<td></td>
<td>• Molecular biology and DNA structure</td>
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<td>• Introduction to PCR</td>
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<td>• Genomic DNA purification</td>
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<td>• Agarose gel electrophoresis</td>
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<td>1430-1530</td>
<td>Lab</td>
<td>Lab 3</td>
<td>Tina Kyalo, Moses Njahira, Wilson Kimani, Dr Roger Pelle, Dr Rob Skilton, Solomon Maina, Eunice Machuka</td>
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<td>• Genomic DNA (gDNA) purification (plant and animal tissue)</td>
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<td>1530-1545</td>
<td>Tea/coffee</td>
<td>N’Dama Lounge</td>
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<td>1545-1800</td>
<td>Lab</td>
<td>Lab 3</td>
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<td>• Continued: Genomic DNA (gDNA) purification</td>
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<td>• Prepare 1% agarose gels</td>
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<td>• NanoDrop spectrophotometry of DNA</td>
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<td>1800-1830</td>
<td>Review of Day 1 activities and Quiz</td>
<td>Lab 3</td>
<td>Moses Njahira</td>
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<td>1830</td>
<td>Bus leaves ILRI for Pride Inn</td>
<td>ILRI Reception</td>
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Lab Manual

• Using a Micropipette
• Lab Math
• Step by step guide to all methods
• Internet resources and further reading
Bioinformatics software download

• Download CLC Main Workbench
  Download > Get a Free Trial

• Download user manual
Acknowledgements
Thank you
Pre-workshop test

• Go to IMBB workshop website:

http://hpc.ilri.cgiar.org/beca/training/IMBB_2014/welcome.html

• Go to Programme > Workshop Test

• Add your name and date

• Complete all 22 multiple choice questions

• Submit