BASICS ASPECTS OF MOLECULAR BIOLOGY AND DNA EXTRACTION.

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Regional Workshop on the prevention and diagnostic of Fusarium Wilt (Panama disease) of bananas and plantains caused by Fusarium oxysporum cubensis – Tropical Race 4 (TR4)
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Molecular Biology

“Study of the structure, function and composition of biologically important molecules”

Mainly concerns itself with understanding the interactions between the various systems of a cell, including the interactions between the different types of DNA, RNA and protein biosynthesis as well as learning how these interactions are regulated to get a refined cell functionability.
DNA Molecule
(Desoxirribonucleic acid)

Is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms and many viruses, being responsible of its hereditary transmission.
DNA Molecule

- Molecule of two strand strands coiled around each other to form a double helix
- Each strand is composed by:
  - Sugar
  - Phosphate
  - Base (adenine, thymine, cytosine, guanine)
1. Lysis or cells disruption:

Extraction buffer and lysis buffer and incubation at 65°C:

**NaCl (sodium chloride):** phosphate of DNA molecule repel one molecule from others. Na+ ions form an ionic bond with phosphates and neutralized the negative charge allowing DNA molecules grouping.

**EDTA (Ethylenediamine tetracetic acid):** chelating agent with high affinity to metallic ions of Mg, DNase cofactors (enzymes that degrade the DNA). EDTA bind to ions and overturn its effects.

**CTAB (Hexadecyl trimethyl-ammonium bromide):** detergent used to brake cellular membranes and remove lipids

Other stabilizer agents: Tris HCl, sorbitol, sodium bisulphite, DTT, detergents: SDS (remove lipids), sarkosyl, triton, PVP (bind to polyphenols- components of vegetable cellular wall-removing), 2-mercaptoethanol (denature proteins).
DNA EXTRACTION PROTOCOL

   Organic solvents, hydrophobics lysates keep trapped, eg. membrane lipids, proteins or polysacharids. Besides denature proteins.

3. Proteins are eliminated adding a protease and increasing the osmolarity (sodium acetate or ammonium acetate)
4. DNA precipitates with alcohol – usually pure and could ethanol or isopropanol (2-propanol). Because DNA is non-soluble in alcohol, precipitate and form a pellet in the bottom of the tube after centrifugation. This step also remove alcohol soluble salts.
5. DNA cleans with 70% ethanol, dry and dilute in TE buffer (protect DNA from degradation) or sterile distilled water.

DNA Extraction kits
DNA Quantification

a. Espectrophotometer/ nanometer

DNA/RNA absorb ultraviolet light with an absorption peak of 260 nm.
Detector register the light that pass trough the sample (↑ absorption of light - ↑ concentration of nucleic acids)

DNA/RNA is present
Results can be altered by contaminants (phenol, proteins)
260/280 = 1.8 stable
260/280 = 2.0 o > (contamination with proteins)
260/280 = 1.6 o < (contamination with con ARN)
b. Electrophoresis in agarose gels
Electrophoresis in agarose gels

Positive result: presence of the fungi in the sample

Negative result: NO presence of the fungi in the sample

M: molecular weight ladder (indicate the base pares number of the band)
Polymerase Chain Reaction (PCR)


Since then it’s included in more that 250000 scientific publications
It’s a machine that heat and cool down the reaction in short periods of time.
Reactive needed for a PCR reaction:

- **DNA template**: contains the DNA region (target) to be amplified. Can be used at different concentrations, but frequently at 10 ng.

- **Buffer solution (Buffer 10x)**: provide a suitable chemical environment for optimum activity and stability of the DNA polymerase.

- **MgCl₂**: It’s used at 25mM -s a required cofactor for thermo-stable DNA polymerases, and magnesium concentration is a crucial factor that can affect the success of the amplification.

- **Deoxynucleotide triphosphates** (dNTP’s: dATP, dGTP, dCTP, dTTP). Nucleotides containing triphosphate groups, the building-blocks from which the DNA polymerase synthesizes a new DNA strand

- **Primers**: Short sequences of 20-24 nucleotides in length, that are complementary to the 3´ (three prime) ends of each of the sense and anti-sense strand of the DNA target.

- **Taq Polimerasa**: DNA polimerasa de *Thermus aquaticus*
STEPS OF PCR REACTION:

1. **dATP**
2. **DNA**
3. **Primers**
4. **dCTP**
5. **dGTP**
6. **dTTP**

Process:
- DNA and Primers are mixed with dNTPs and Taq polymerase.
- The mixture goes through cycles of
  - 94°C for 30 seconds (denaturation)
  - 50-65°C for 30 seconds (annealing)
  - 72°C for 1 minute (extension)
- The final step is 4°C.

Example diagram:
- A test tube with DNA, Primers, dNTPs, and Taq polymerase.
- A thermocycler showing the cycle temperatures.
- A graph illustrating the heating and cooling phases.
Other analysis: Real-Time PCR or quantitative PCR

The amplified DNA is detected as the reaction progresses in "real time"

http://www.youtube.com/watch?v=QVeVIM1yRMU
Other analysis:

Sequencing:

- Amplicon generated in PCR is used

- Sample are cleaned or purified directly from PCR or band is extracted from the gel with special kits

- Websites like Gen Bank (www.ncbi.com) and Fusarium I-D (http://isolate.fusariumdb.org/index.php) allow to compare of sequence with other published in those sites (libraries)
Sequencing

unknown:  CGGTCATGGCCTAATTATGGCCCAAAAT

Pseudomonas sp.:  CGGTCATGGCCTAATTATGGCCCAAAAT

Agrobacterium sp.:  CGGTGTTGGCCTAACAAATGGCCCTCAAT

Burkholderia sp.:  AGGTCATCGCCTATGTTATGGGGAAAAT

Result: unknown DNA has 100% of similarity with Pseudomonas syringae sequences
THANK YOU

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