

Accessing the Uncultivable: Unraveling Activities from Metagenomic Libraries Generated from Dry and Rain Forest Soils in Puerto Rico Using Functional and Sequence-based Approaches

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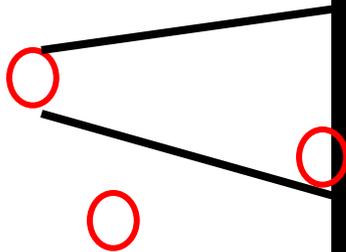
University of Puerto Rico at Mayagüez, ¹Industrial Biotechnology Program and ³Biology Department,
²University of Puerto Rico at Humacao, Biology Department

May 11, 2009

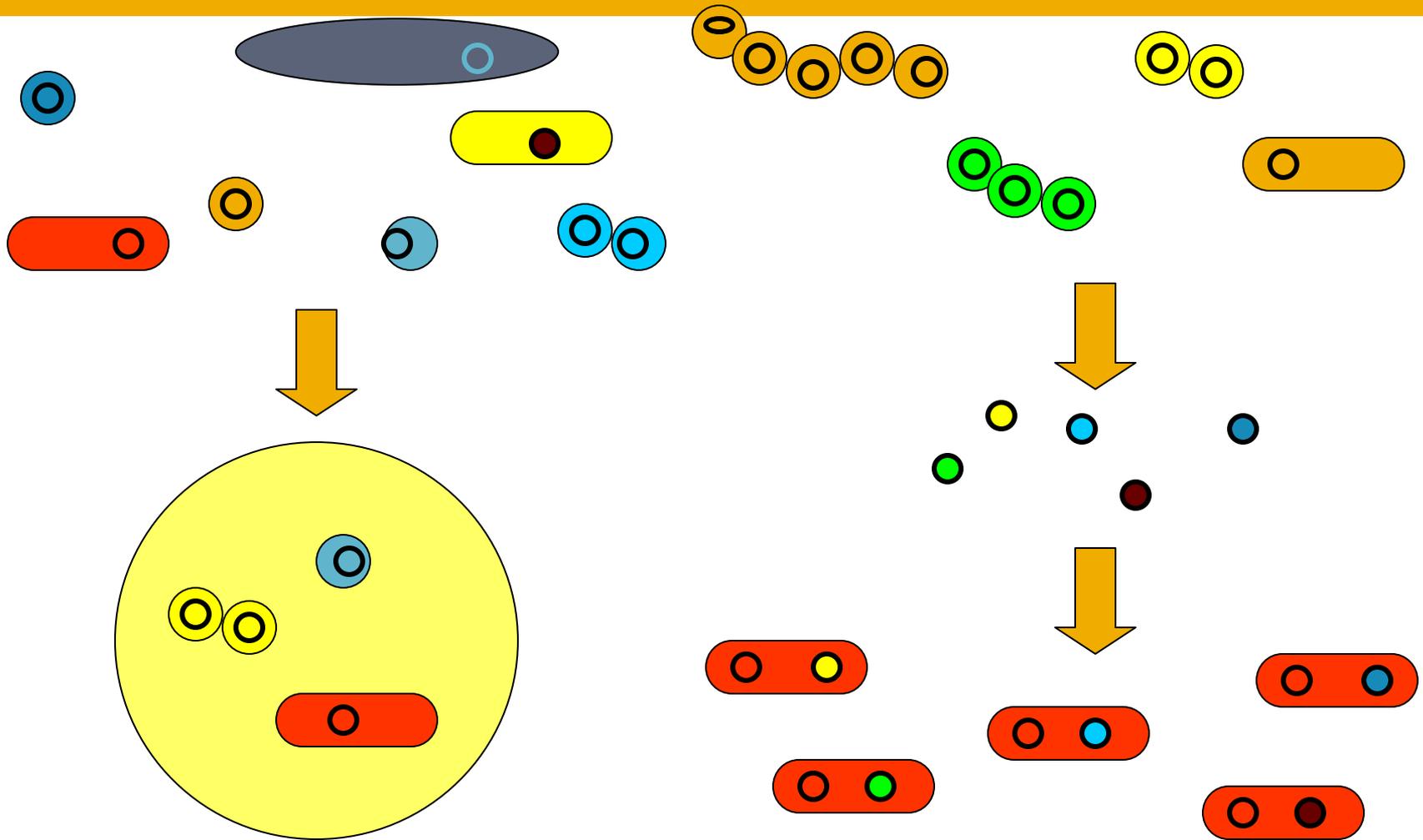
NCSS Conference at Las Cruces, NM



Soil: A valuable natural resource



Why metagenomics?



Access to uncultivable microorganisms

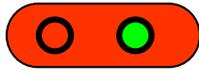
Why metagenomics?

Bacterial Clone

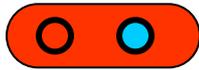
Novel Activity



None



Antimicrobial agents



Biodegradation enzyme(s)

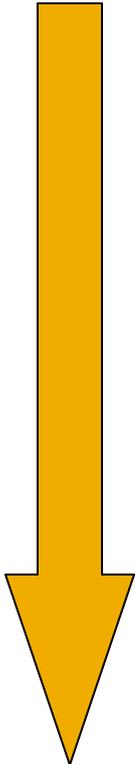


High temperature tolerance

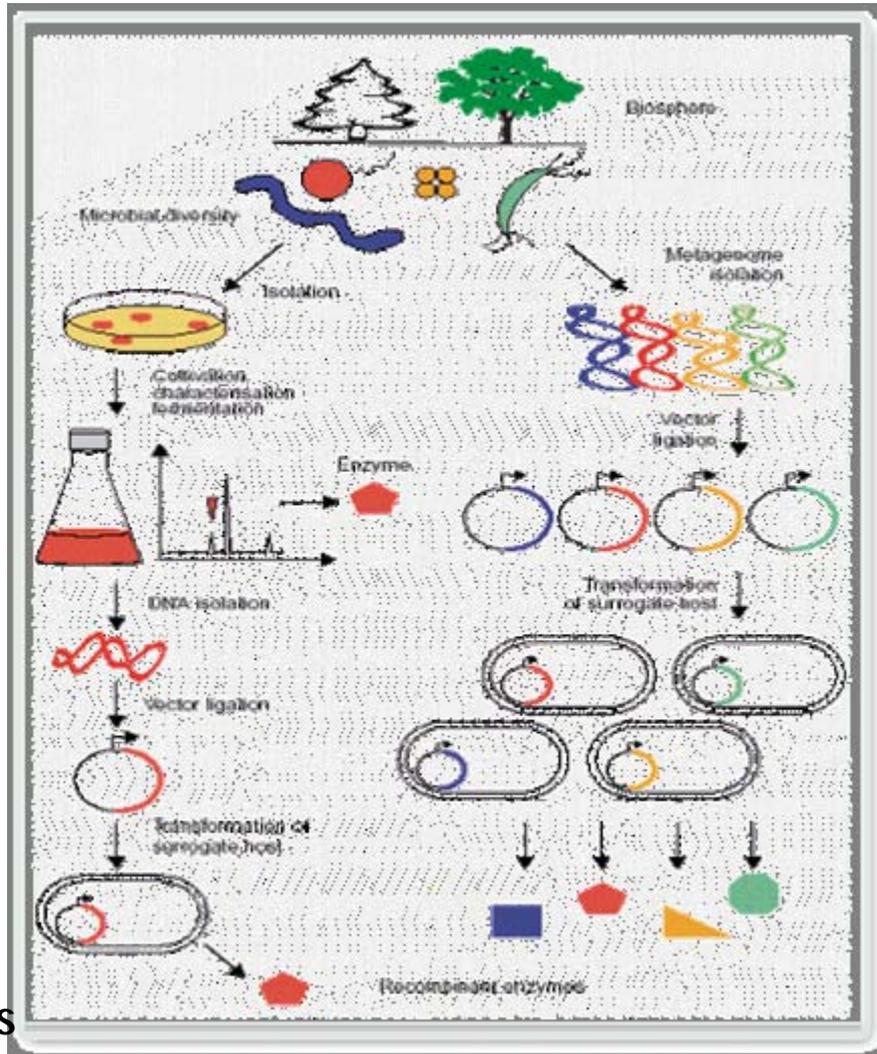
Unraveling new activities with medical and/or biotechnological applications

Why metagenomics?

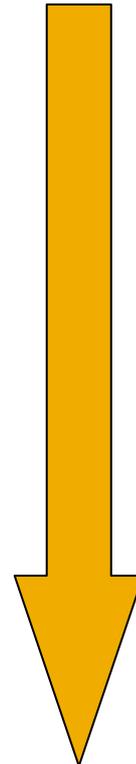
1%



Cultivating techniques



1% + 99%



Metagenomics

Monitoring: two approaches

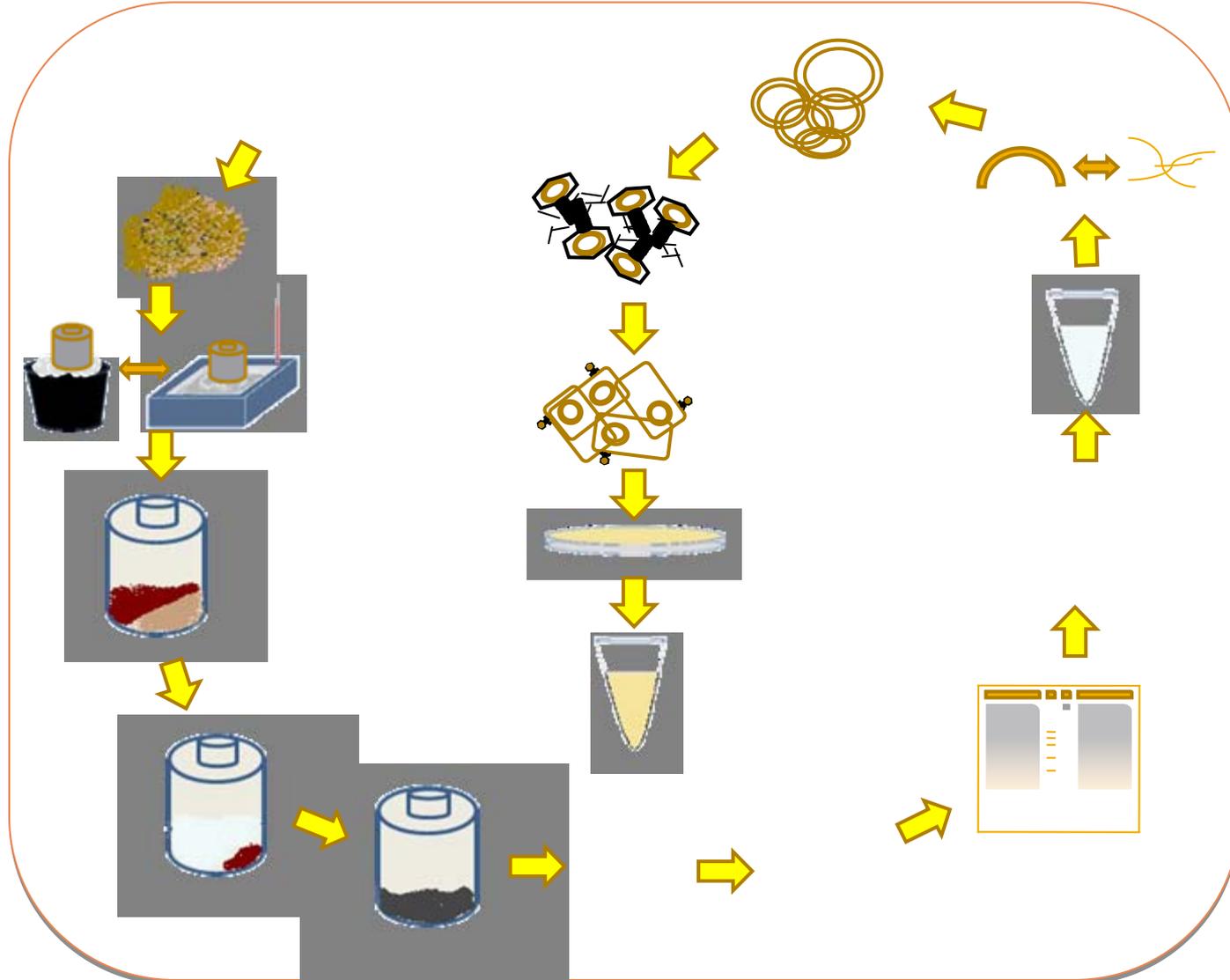
Functional metagenomics

- Antibiotic resistance activity
- Production of antimicrobial agents
- Detection of enzymatic activity

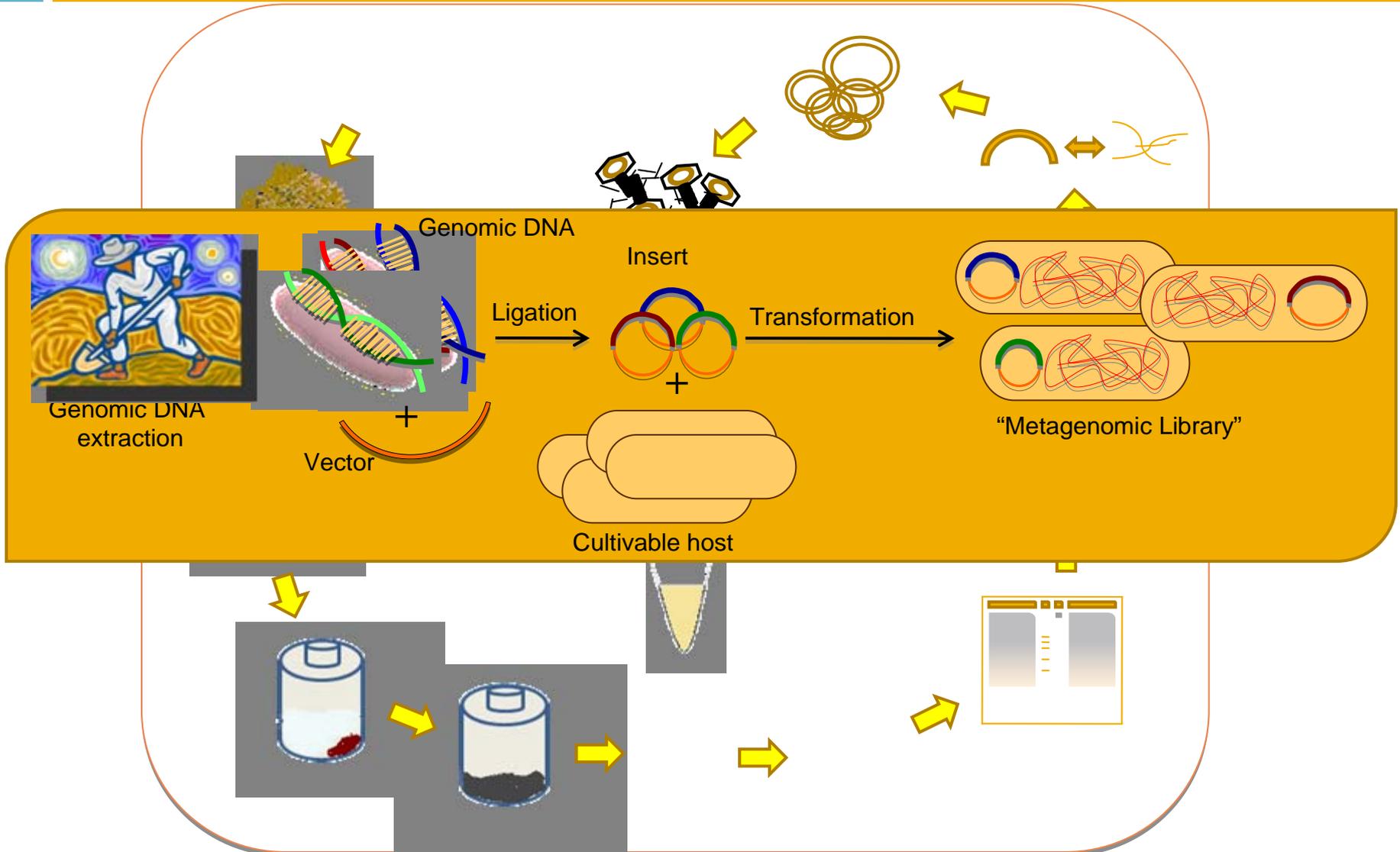
Sequence based metagenomics

- Microbial diversity
- Production of antimicrobial agents

Metagenomic library generation



Metagenomic library generation



Sampling Sites



<http://www.definitivecaribbean.com/images/TemplateImages/caribbeanmap.jpg>

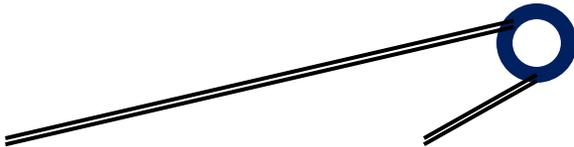
<http://www.aaa-calif.com/apps/travel/destinations/images/lonelyplanet/maps/wg-puerto-rico-2446-400x300.gif>

http://farm3.static.flickr.com/2038/1496561042_2b3b2eb84d.jpg

http://ammedia.tv/images/rain_forest3.jpg

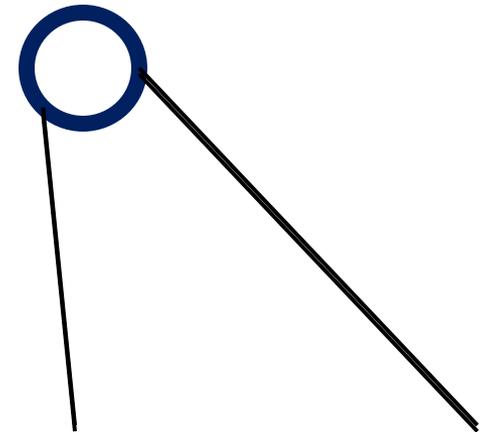
<http://www.sphaydenphotography.com/images/prrainmap.gif>

Sampling Sites



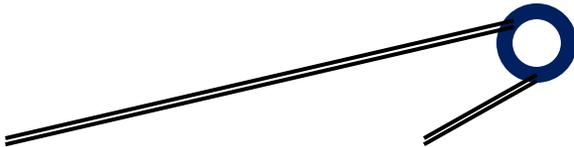
Guánica Dry Forest

<http://www.definitivecaribbean.com/images/TemplateImages/caribbeanmap.jpg>
<http://www.aaa-calif.com/apps/travel/destinations/images/lonelyplanet/maps/wg-puerto-rico-2446-400x300.gif>
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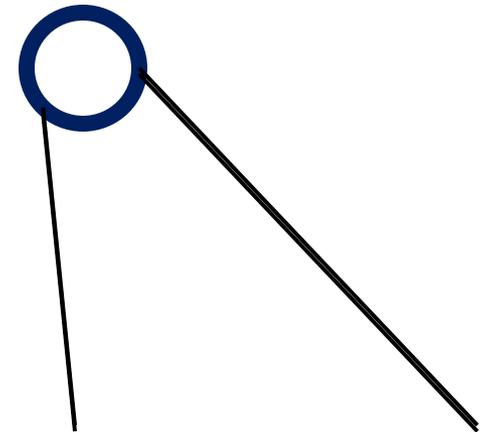
Yunque Rainforest

Sampling Sites



Guánica Dry Forest

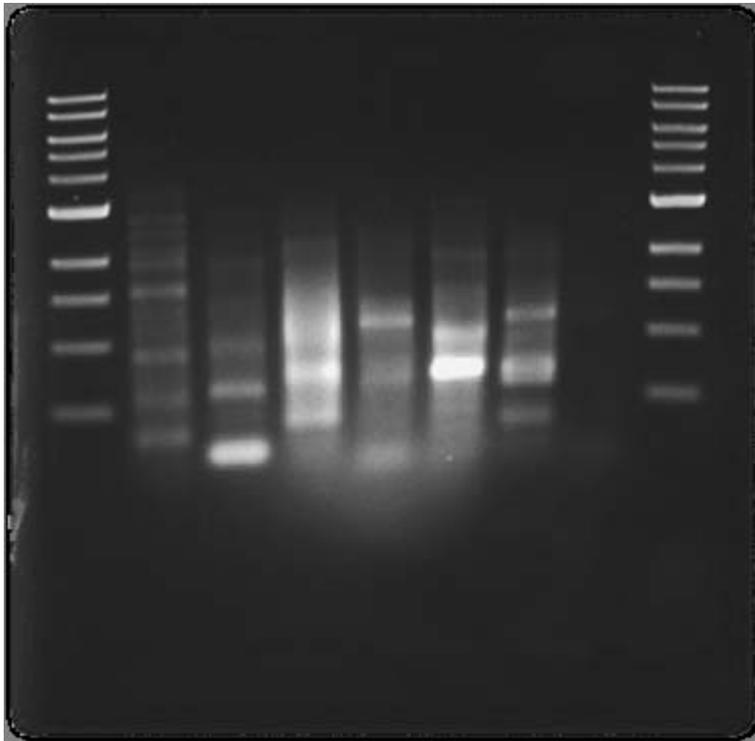
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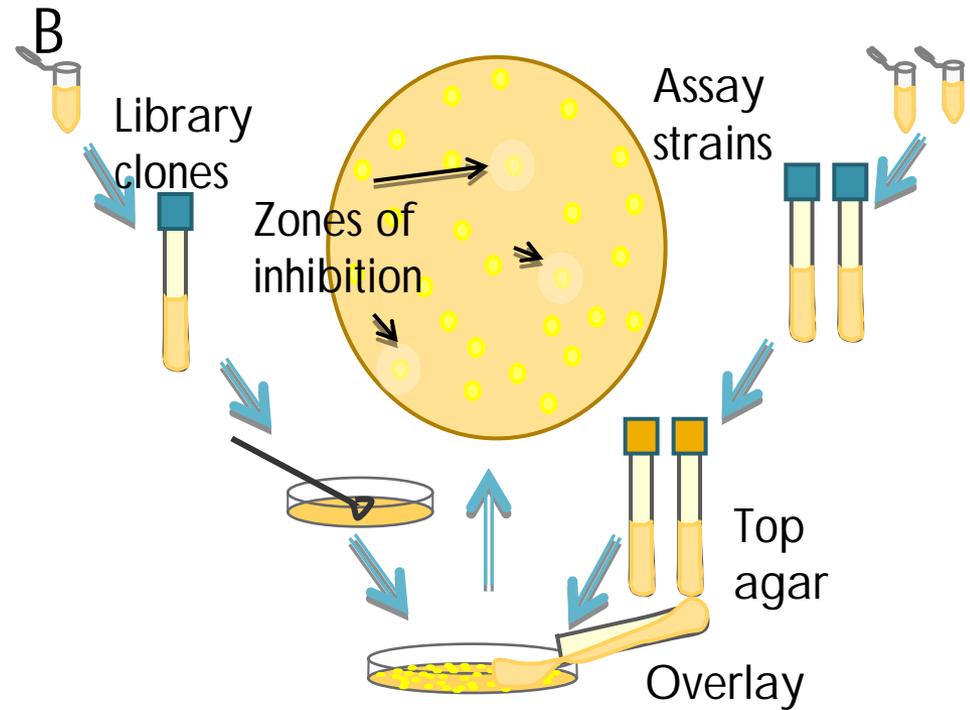
Yunque Rainforest

Antimicrobial activity

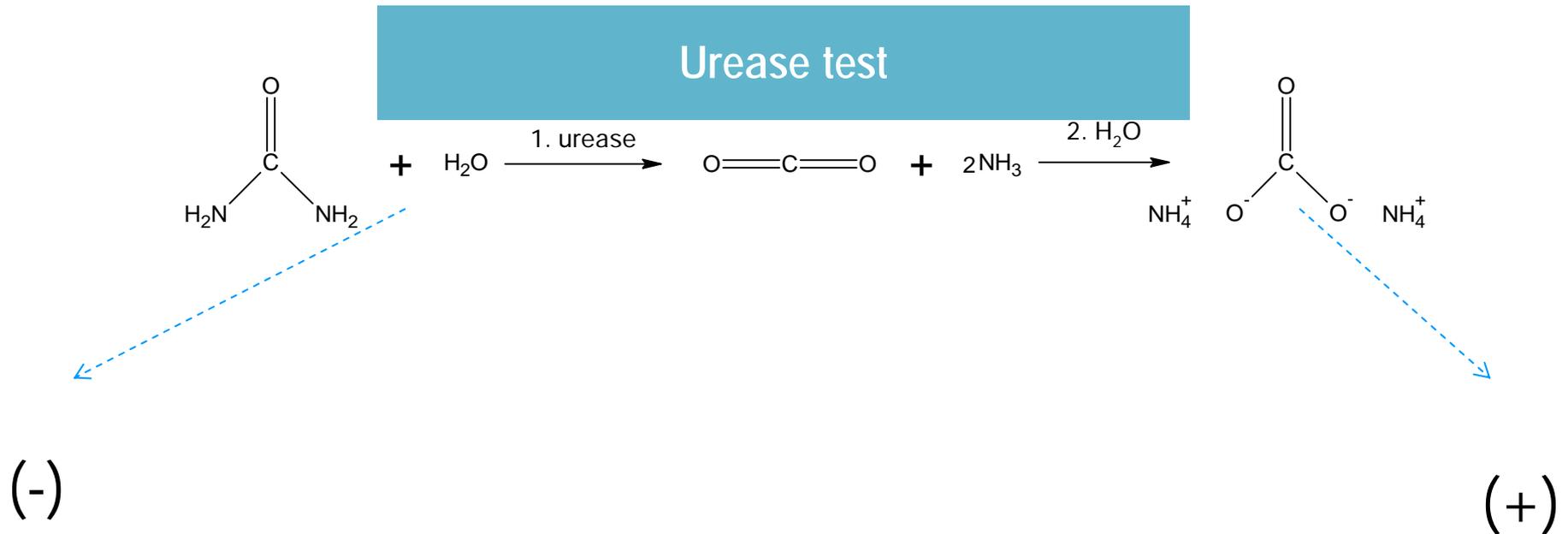
Polyketide Synthases amplifications



Overlay assays



Detection of urease using the urease test



Conclusions

- A total of 8 metagenomic libraries from two forests have been generated (~850,000 clones).
- There are differences in microbial diversity between the two forests.
- Through sequence-based analysis the presence of amplicons associated with production of antimicrobial agents has been detected.
- 4 examples of functional analyses have been used: production of antimicrobial agents, and epoxide hydrolase, urease, and antibiotic resistance activity.
- Ongoing experiments involve lipase detection, transposon mutagenesis to identify genes associated with activity, and further sequencing and screening efforts.

Acknowledgements

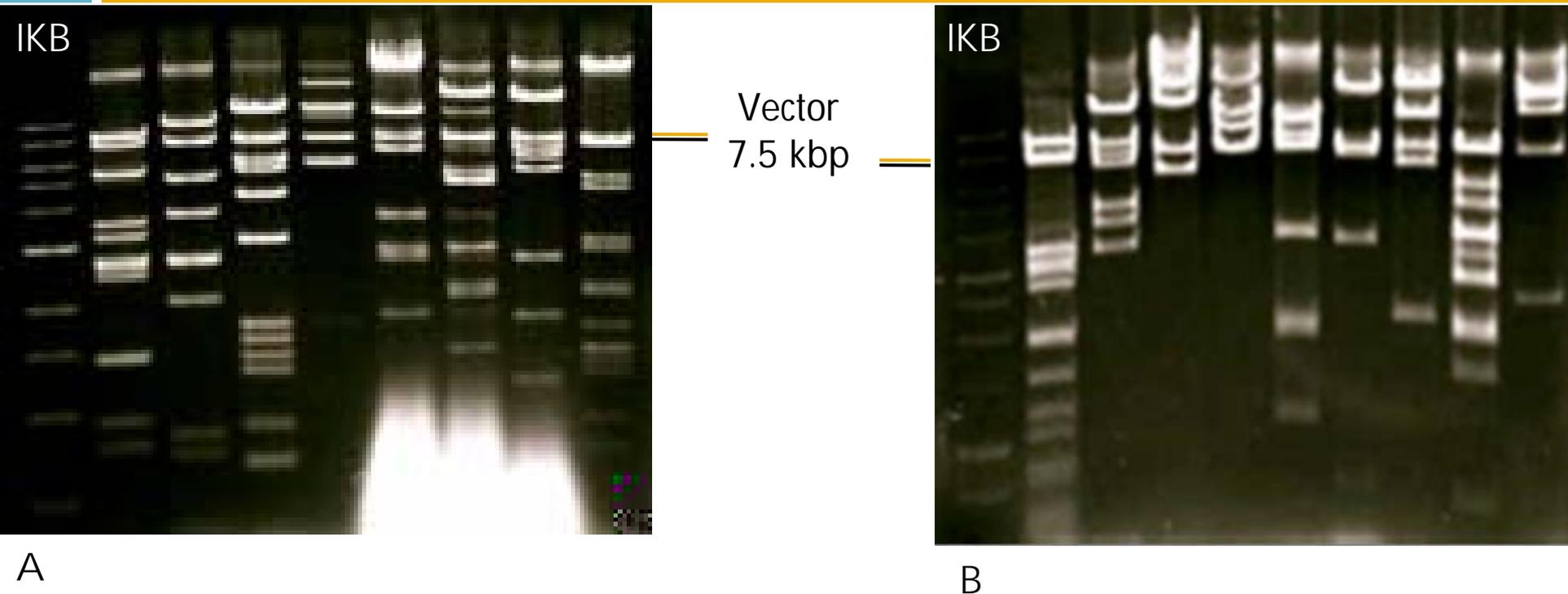
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- Mr. Dubiezel Medina
- USDA-CSREES 2007-02386
- PR-LSAMP UPRM



The TEAM

<http://www.cohemis.uprm.edu/gemspr/>

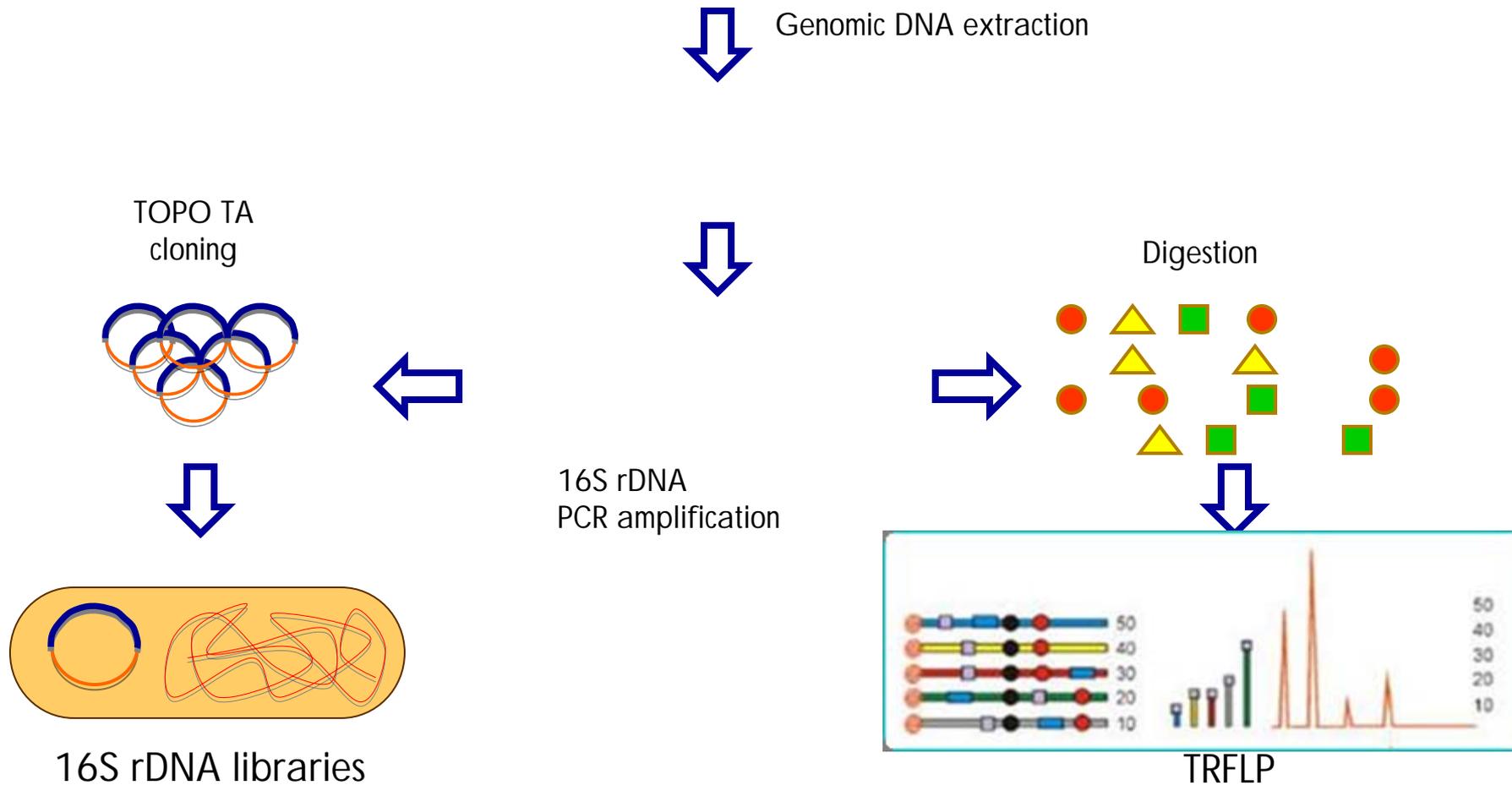
Results – Restriction digest analysis



Restriction digest analysis of (A) Guánica dry forest library clones and (B) Yunque rainforest library clones digested with I . The line indicates the fosmid vector. Comparing the amount of internal I recognition sites between both samples, sample(A) contained DNA inserts higher in GC content than sample (B).

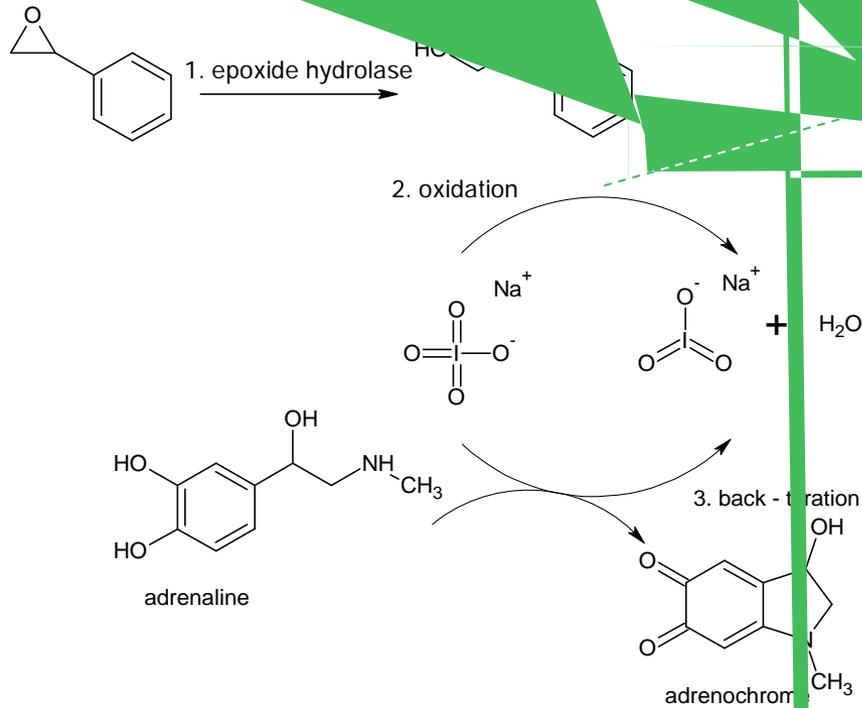
I recognition site 5'-GCGGCCGC-3'

Diversity analyses



Epoxide hydrolase detection adrenaline test

Epoxide hydrolase activity: adrenaline



Results - Epoxide hydrolase detection

Example of adrenaline test assay for Guánica dry forest library subpool 6. Odd rows indicate actual assay, even rows represent unassayed duplicates. Sample (A) above was induced, (B) was not. The well for the last column in row 8 for sample (B) is

Under these experimental conditions, it appears induction does not modify results significantly.

1
2
3
4
5
6
7
8

1
2
3
4
5
6
7
8

A

B

Results - Antimicrobial activity detection



(A) Example of 20 screened plates, for

(B) Example of 40 screened plates, 20 for
and 20 for

TRFLP

