

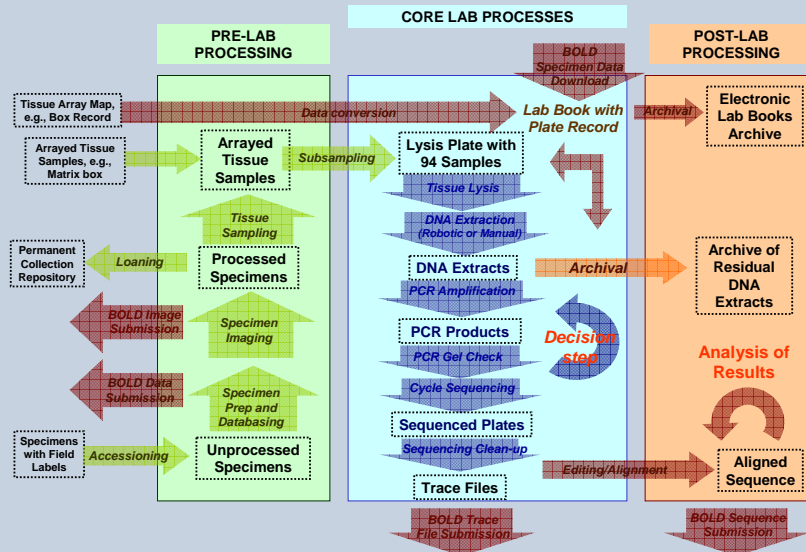
Methods for Streamlining High Throughput DNA Barcoding

Natalia V. Ivanova¹, Chris M. Grainger¹, Alex V. Borisenko¹

1- Canadian Centre for DNA Barcoding, Biodiversity Institute of Ontario, University of Guelph
579 Gordon Street, Guelph, ON, Canada, N1G 2W1



OUTLINE OF THE ANALYTICAL CHAIN



INTRODUCTION

The ability to generate DNA barcodes for high volumes of samples in an efficient and cost effective manner requires rigorous optimization of data flow and analytical protocols

Key events at CCDB in 2006-2007:

- Relocation to BIO
- Integration of automation
- Batch oriented processing (pre-made PCR and cycle sequencing plates)
- Conversion to more expensive, but reliable reagents at different analytical stages (e.g., Platinum® Taq for PCR, E-gel® for PCR check)
- Employing various home-made approaches (e.g. Glass Fiber DNA extraction protocol) to reduce cost of consumables
- More efficient data management with electronic lab book
- Optimization of workflow
- Defining analytical 'pipelines'

Outcome – increased production, enhanced QA/QC measures

ELECTRONIC LAB BOOK



Canadian Centre for DNA Barcoding
Electronic Lab Book Version 4.5.4 beta

Electronic Lab Book Features⁵

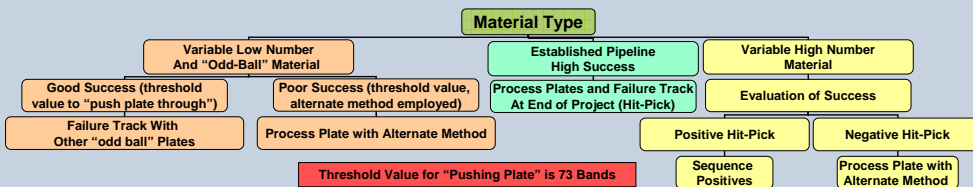
- Microsoft Excel based, user oriented, easy as 'click & copy'
- Tracking of analytical history of one 96-well plate
- Built-in data conversion tools, graphical interface
- Retrieval of BOLD Process ID
- Multiple PCR and multigene support
- Evaluation and scoring of E-gel® PCR results
- Output for DNA sequencer and robotic hit-picking
- Plate assembly from different sources
- Trace submission module
- FASTA-MEGA conversion module

CYCLE SEQUENCING

Sequencing Setup Milestones

- No PCR clean-up
- Pushing limits of BigDye dilution from 1/16 to 1/24
- Pre-made frozen cycle-sequencing plates and mixes with trehalose⁴
- Automated cycle-sequencing setup

DECISION MAKING – ANALYTICAL 'PIPELINES'



Sequencing Clean-up Milestones

- From manual to semi-automated Sephadex
- From Sephadex to automated magnetic bead cleanup (CleanSEQ®, Agencourt)

INTEGRATION OF AUTOMATION



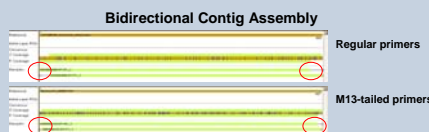
New Equipment and Upgrades:

- 2 Biomek FXP robots with dual-bridge systems (96 head with integrated gripper, Span-8), vacuum manifold, orbital shaker, tip wash station, refillable reservoirs, Cytomat hotel, barcode reader
- Software upgrade for Biomek NX
- New 3730xl, upgrade of existing 3730 to 3730xl

PCR PRIMER DESIGN

M13-tailed Primers and Primer Cocktails²

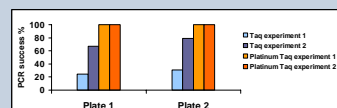
- Universal or taxon-specific
- Incorporation of inosine & degeneracy into primers
- M13-tails for easy sequencing
- Increased Contiguous Read Length (CLR) for better overlap of bidirectional reads



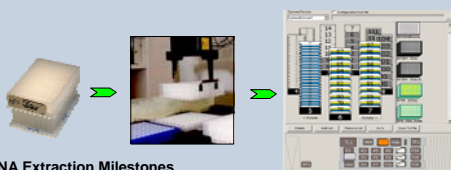
PCR

PCR Milestones

- Increased barcode recovery using Platinum® Taq³
- Pre-made frozen PCR plates with trehalose⁴
- Automated PCR setup
- From 30 min/person/plate to 5 min/person/plate
- M13-tailed primer cocktails²
- PCR check – E-gel® (Invitrogen)



DNA EXTRACTION



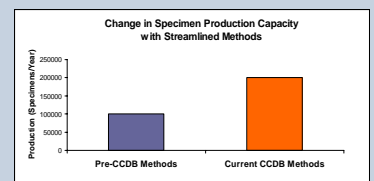
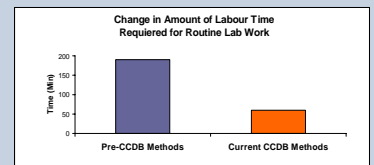
DNA Extraction Milestones

- From commercial kits to 'house-made' reagents – Glass Fiber DNA extraction protocol (\$0.50/sample)
- From centrifuge processing to robotic extraction
- From Biomek NX to Biomek FXP
- From 4 plates/person/day to 10 plates/person/day



Pre-cast, bufferless
Reuse twice
6 min per run

CONCLUSIONS



REFERENCES

- 1 Ivanova NV, deWaard JR & Hebert PDN (2006) *Mol. Ecol. Notes* 6, 998-1002
- 2 Ivanova NV, Zemlak TS, Hanner RH & Hebert PDN (2007) *Mol. Ecol. Notes*, doi: 10.1111/j.1471-8286.2007.01748.x
- 3 Ivanova N, Grainger C, Hajibabaei M (2006) *CCDB Advances, Methods Release No 2*, November 3, 2006.
- 4 Ivanova N, Grainger C (2006) *CCDB Advances, Methods Release No. 4*, December 1, 2006.
- 5 Borisenko A, Dooh R (2007) *CCDB Advances, Methods Release No. 8*, April 13, 2007.

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