Taq polymerase choice

High DNA barcoding production rates demand high success in amplification of the barcode region. One particularly critical element for PCR amplification is the polymerase enzyme. During the testing of DNA barcoding protocols across a broad range of taxonomic groups, from insects to mammals, it was clear that one higher-cost polymerase from Invitrogen™ (Platinum® *Taq* DNA Polymerase) delivered both greater intensity amplicons and amplification success in cases where standard *Taq* failed. Results indicated that Platinum® *Taq* offers the highest performance, and it is now the standard PCR enzyme used by the CCDB. Platinum® *Taq* also offers a number of benefits over standard *Taq* polymerases. It is a robust enzyme that needs less optimization compared to standard *Taq*. As Platinum® *Taq* requires a "hot start" for activation, there is less enzyme breakdown and fewer non-specific PCR amplicons. Platinum® *Taq* is also stable at room temperature, allowing for advanced preparation and storage of PCR plates for future use.

Addition of trehalose facilitates PCR and makes possible freezing of aliquoted master-mixes. Currently CCDB uses batch strategy for making PCR plates. Mixes are aliquoted directly into 96-well plates, using Biomek® FX robot, plates are covered with PCR film and stored at -20°C for up to 3 months. Each batch is labeled, recorded in the system and tested to assure performance. The combination of a thermostable Platinum® *Taq* with trehalose ensures high performance even after multiple freeze-thaws. Results with regular *Taq* polymerases may be less satisfactory.

Aliquots in tubes can be stored at -20°C for up to 3 months (1-3 freeze-thaw cycles don't affect performance). The content of a tube should be mixed by pipetting before use.

Consumables & Equipment for PCR amplification

- 10% trehalose: dissolve of 5 g D-(+)-trehalose dehydrate (Sigma, 90210), in 50 ml of total volume of molecular grade ddH_2O. Store at –20°C.
- 10X PCR Buffer for Platinum *Taq* (Invitrogen™). Store at –20°C.
- 50 mM $MgCl_2$ (Invitrogen™). Store at –20°C.
- 10 mM dNTP mix (New England Biolabs®). Store at –20°C in 100 µl aliquots.
- 100 µM primer stock: dissolve desiccated primer (Invitrogen™) in __ number of nmol x 10 µl ultrapure H_2O. Store at –20°C.
- 10 µM primer working solution: add 20 µl of 100 µM primer stock to 180 µl of molecular grade ddH_2O. Store at –20°C.
- Platinum *Taq* polymerase (Invitrogen™). Store at –20°C in 50 µl aliquots.
- Microplate (Eppendorf® plates).
- Cap strips (ABgene®) or Aluminum sealing film.
- Thermocycler (Mastercycler® ep gradient, Eppendorf®).

Basic recipe for Polymerase Chain Reaction (PCR)

PCR reagents per 12.5 µl reaction:

# of reactions	1	100
10% trehalose	6.25 µl	625 µl
ddH_2O	2 µl	200 µl
10X buffer	1.25 µl	125 µl
50 mM $MgCl_2$	0.625 µl	62.5 µl
10 µM primer A	0.125 µl	12.5 µl
10 µM primer B	0.125 µl	12.5 µl
10 mM dNTPs	0.0625 µl	6.25 µl
Polymerase (5 U/µl)	0.06 µl	6 µl
Total	**10.5 µl**	**1050 µl**
DNA template	2 µl per well	

Aliquot 1/8 of total mix volume in 8-tube PCR strip (if making more than one plate, pour mix into disposable container) and dispense desired volume (10.5 µl for 12.5 µl reactions) in 96-well plate and then add 1-2 µl of DNA extract. If you plan to fill several 96-well plates include extra volume to allow for pipetting mistakes and dead volume in the digital multichannel pipettor (e.g. for making 10 plates with 12.5 µl reactions each, include about 40 extra reactions).

General recommendations

- The use of filter tips is recommended for all PCR reagents to avoid contamination. Clean the bench top with alcohol before setting up reactions.
- Always use a sterile tip when removing *Taq* polymerase and the other reagents from their tubes.
- Keep DNA templates (i.e. other PCR products) away from the PCR reagents while you are setting up the reaction mixes. Add DNA after all of the reagents have been returned to the freezer.
- Always include a sample without template as a negative

By Natalia Ivanova and Chris Grainger
Canadian Centre for DNA Barcoding

control to check for contamination of the reagents. Include a positive control (a DNA sample that has amplified in the past) as well to test the effectiveness of the PCR reagents.

Tips for primer design

- Primers should be between 20-30 nt in length.
- Avoid complementarity within and between primers.
- The GC content should be approximately 50%.
- Avoid mono- or dinucleotide repetition within primers.
- The primer should end on a G or a C.
- Primers should end on the second (or first if necessary) position of a codon.
- The melting temperatures of primer pairs should be within 5°C of one another
- To design COI primers for a particular taxonomic group, try aligning as many COI genes from closely related taxa as possible (try surfing GenBank) for the desired species group. Design primers that are situated in regions that are conserved across all taxa.
- Primers could be tailed with M13 tails. However, some tailed versions can form strong primer dimers, reducing PCR efficiency (e.g. LepF1_t1 and LepR1_t1 used in a mammal cocktail, do not work well as stand alone primers).

PCR thermocycle program

Typical conditions for COI amplification include the initial denaturation at 94°C for 1 min, five cycles of 94°C for 30 sec, annealing at 45-50°C for 40 sec, and extension at 72°C for 1 min, followed by 30-35 cycles of 94°C for 30 sec, 51-54°C for 40 sec, and 72°C for 1 min, with a final extension at 72°C for 10 min, followed by indefinite hold at 4°C. Please, refer to original publications listed under primer references for more details on PCR conditions.

PCR product check

Invitrogen E-gel® 96 system and software

We now employ pre-cast agarose gels from Invitrogen™. This system is bufferless, so exposure to Ethidium Bromide is minimized. However, gloves should be worn when handling and loading the gel.

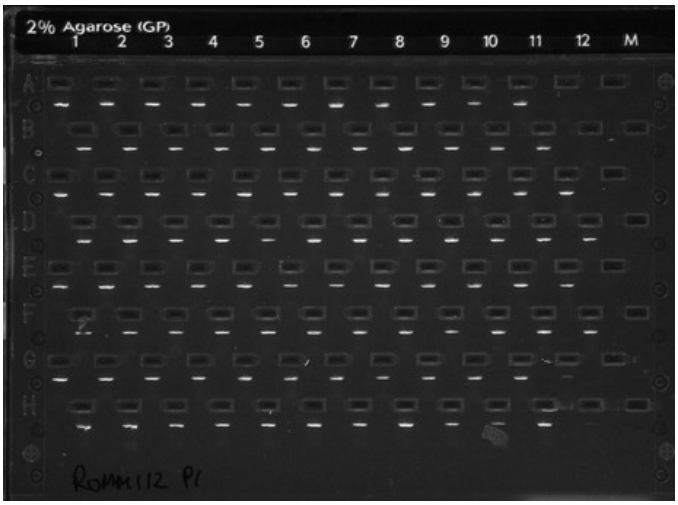
Loading and running E-gel® 96 gels

The recommended program for 2% Agarose E-gel® 96 gel is EG and the run time is 6-12 min. Plug the Mother E-Base™ into an electrical outlet. Press and release the pwr/prg (power/program) button on the base to select program EG.

- Remove gel from the package and remove plastic comb from the gel.
- Slide gel into the two electrode connections on the Mother or Daughter E-Base™.
- Load 16 µl of ddH_2O into wells with 8- or 12-multichannel pipettor.
- Load appropriate DNA markers in the marker wells.
- Load 4 µl of sample.
- To begin electrophoresis, press and release the pwr/prg button on the E-Base™. The red light changes to green.
- At the end of run (signaled with a flashing red light and rapid beeping), press and release the pwr/prg button to stop the beeping.
- Remove gel cassette from the base and capture a digital image of a gel on UV transilluminator equipped with digital camera.
- Analyze the image and align or arrange lanes in the image using the E-editor™ 2.0 software available at: http://www.invitrogen.com/egels/
- Incorporate E-gel image into lab spreadsheet for estimation of concentration and hit picking.

Typical E-gel image of COI amplification

Mammalian DNA was extracted with Glass Fiber method (refer to DNA extraction section) and amplified with Platinum *Taq* polymerase using M13-tailed Mammal cocktail (master mix contained 5% trehalose)



White bands indicate product; Square slots are the loading wells; A12, B12 – negative controls

PCR product clean-up

Currently we don't clean-up PCR products and proceed directly to sequencing.

References

Franks F (1990) Freeze drying: from empiricism to predictability. *Cryoletters,* **11,** 93-110.

Hajibabaei M, deWaard JR, Ivanova NV et al. (2005) Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society: Biological Sciences,* **360,** 1959-1967.

Ivanova N, Grainger C (2006) Pre-made frozen PCR and sequencing plates. *CCDB Advances, Methods Release No. 4*, December 1st, 2006.

Ivanova N, Grainger C, Hajibabaei M (2006) Increased DNA barcode recovery using Platinum® *Taq*. *CCDB Advances, Methods Release No. 2*, November 3, 2006.

Ivanova NV, deWaard JR, Hajibabaei M, Hebert PDN (2005) Protocols for high volume DNA barcoding. Draft submission to: DNA working group Consortium for the Barcode of Life. Published online at http://www.dnabarcoding.ca/

Spiess AN, Mueller N, Ivell R (2004) Trehalose is a potent PCR enhancer: Lowering of DNA melting temperature and thermal stabilization of *Taq* polymerase by the disaccharide trehalose. *Clinical Chemistry,* **50,** 1256-1259.