

BARCODES OF LIFE

DNA BARCODING ANIMAL LIFE USING COI

LABORATORY PROTOCOL:

COI AMPLIFICATION

GENERAL PRACTICES

- The basic recipe for a Polymerase Chain Reaction (PCR) is given in the table at the right. The amount of DNA used will depend on the concentration of the sample. It is best to keep the volume of DNA template as low as possible to avoid adding enzyme inhibitors that may be present, and to avoid illegitimate amplification of excess DNA.
- The use of plugged tips is recommended for all PCR reagents to avoid contamination. Clean the bench top with alcohol before setting up reactions.
- Always use a clean 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 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.
- The concentration of MgCl₂ will depend on the primers. Use the lowest concentration of MgCl₂ possible to give the cleanest product. The minimum concentration is 1.5 mM.
- Recipes for the individual reagents are given on the following page.

PCR REACTION MIX

Volumes for One Reaction (50 µl total)	
H ₂ O + DNA	41.5 µl
10X PCR Buffer	4.5 µl
50 mM MgCl ₂ (2.5 mM)	2.5 µl
10 mM dNTP	0.25 µl
10 µM Primer 1 (5 pmol)	0.5 µl
10 µM Primer 2 (5 pmol)	0.5 µl
Taq polymerase	0.2 µl

MULTIPLE SAMPLES

When doing multiple reactions with the same primer set, make a master mix that does not contain the DNA templates.

Example: you want to amplify 2 µl of 20 DNA samples in a 50 µl reaction. Remember to include one reaction for the negative control and "one extra for the pot". It is OK to round up volumes of dNTPs, primers and Taq to the nearest 0.5 µl.

Volumes for 22 Reactions		
	1 Tube	22 Tubes
DNA	2 µl	----
H ₂ O	39.5 µl	869 µl
10X PCR Buffer	4.5 µl	99 µl
50 mM MgCl ₂	2.5 µl	55 µl
10 mM dNTP	0.25 µl	5.5 µl
10 µM Primer 1	0.5 µl	11 µl
10 µM Primer 2	0.5 µl	11 µl
Taq polymerase	0.2 µl	4.5 µl

Aliquot 48 µl of the mix to each individual reaction tube and then add 2 µl of template.

PCR THERMOCYCLE PROGRAM

- A typical PCR program on the **MJ PTC100** thermocycler is shown at the right.
- Step 1 is an initial 94°C soak to completely denature the original template, particularly if it is genomic DNA.
- Step 2 is the denaturing step.
- Step 3 is the annealing step whose temperature will depend on the sequence of the primers. For COI, it is ideal to begin annealing at a low temperature (45°C) for a few initial cycles to allow the primers to bind to the template and then raise the temperature (51°C) to avoid excessive non-specific binding of primers.
- Step 4 is the extension step whose time depends on the length of the product. Generally, extension steps should be at least 1 min/1000 bp.
- Step 5 repeats steps 2, 3, and 4 five more times.
- Steps 6, 7, 8, and 9 denature, anneal at 51°C, and extend for 36 cycles.
- Step 10 is a soak at 72°C that will allow the Taq polymerase to complete any unfinished products.
- Step 11 is a 4°C soak that will last "forever" until someone cancels the program.

MJ PTC100 PCR Program for COI		
Step	Action	Time
1	94°C	1 min
2	94°C	30 sec
3	45°C	1 min, 30 sec
4	72°C	1 min
5	GOTO step 2	5 more times
6	94°C	30 sec
7	51°C	1 min, 30 sec
8	72°C	1 min
9	GO TO step 6	35 more times
10	72°C	5 min
11	4°C	00:00:00