

'Just like human with their fingerprint, every species has its own DNA barcode.'

DNA barcoding is a method of **species identification** using the information of one or a few standardized genetic markers of DNA. Different of DNA regions are used to identify organisms:

- 1. COI or COX1 (Cytochrome C Oxidase I): The most used barcode region for animals and some protists. It can be found in mitochondrial DNA.
- 2. ITS rRNA (Internal Transcribed Spacer): It often used for fungi.
- 3. RuBisCO: It used for plants.

Workflow

Step 1

DNA Extraction

4. 16S rRNA: It is widely used in identification of prokaryotes.

Step 2

Amplification

DNA barcoding is used for more than just research, applications of DNA barcoding include identification of species, safety assessment of food, detection of alien species, identification of endangered and threatened species, securing intellectual property rights for bioresources, framing global management plans for conservation strategies. DNA barcode markers can be applied to address basic questions in systematics, ecology, evolutionary biology and conservation, including community assembly, species interaction networks, and taxonomic discovery. **Saraswanti Genomics Institute** (SGI) offers SGI – **DNA Barcoding Service** with broad-range standardized universal primer.

Step 3

Sequencing

Step 4

Comparing

Sample requirements

Sample Type			Concentration	Minimum Volume
	fresh bacterial liquid			1 ml
Bacterial Culture	overnight culture		if the culture only cultured for 4 hours, please put your culture into a 15 ml Tube and make a statement for them. We will continue culturing it.	2-5 ml
	cell sedimentation of bacterial liquid			1-2 ml
Plasmid			50 ng/µL	
Plasmid big clone (>30Kb)			200 ng/µL	
Purified PCR product		< 500 bp	5 ng/μL	20 μl 1st reaction $$ + 5 μl per add' 1 reaction
		500 - 1300 bp	15 ng/μL	
		> 1500 bp	30 ng/μL	
Unpurified PCR product			Same requirement as Purified PCR product (see above)	30 μ l 1st reaction + 5 μ l per add' 1 reaction
Primer			5 pmol/μl	10 μl 1st reaction $$ + 2 μl per add' 1 reaction
Premixed with primer			Template and primer should follow the concentrations listed in the above prior to mixing	6 μl Template + 6μ L Pimer

- All the DNA submitted should be in deionized water only.
- Quantitation of template by gel is highly recommended.
- For unpurified PCR products, run an agarose gel to make sure there is a single band.

Tips for designing primer

- A melting temperature (Tm) between 40-60°C.
- GC content is 40-60% .
- The length of the primer should be 18-25 bases.
- It is recommended that primer binding site should be 50-100 bases before the start of your sequence of interest. This is because the first 30-50 based of sequence are usually messy and unreliable.
- High Purity.

What will you get :

- Sequencing data.
- Two data files: " .ab1 " file and " .seq" file.

TAT : 1 – 2 weeks

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