

# Introduction to molecular identification

## 1. DNA extraction

Genomic DNA template is extracted and purified from cells

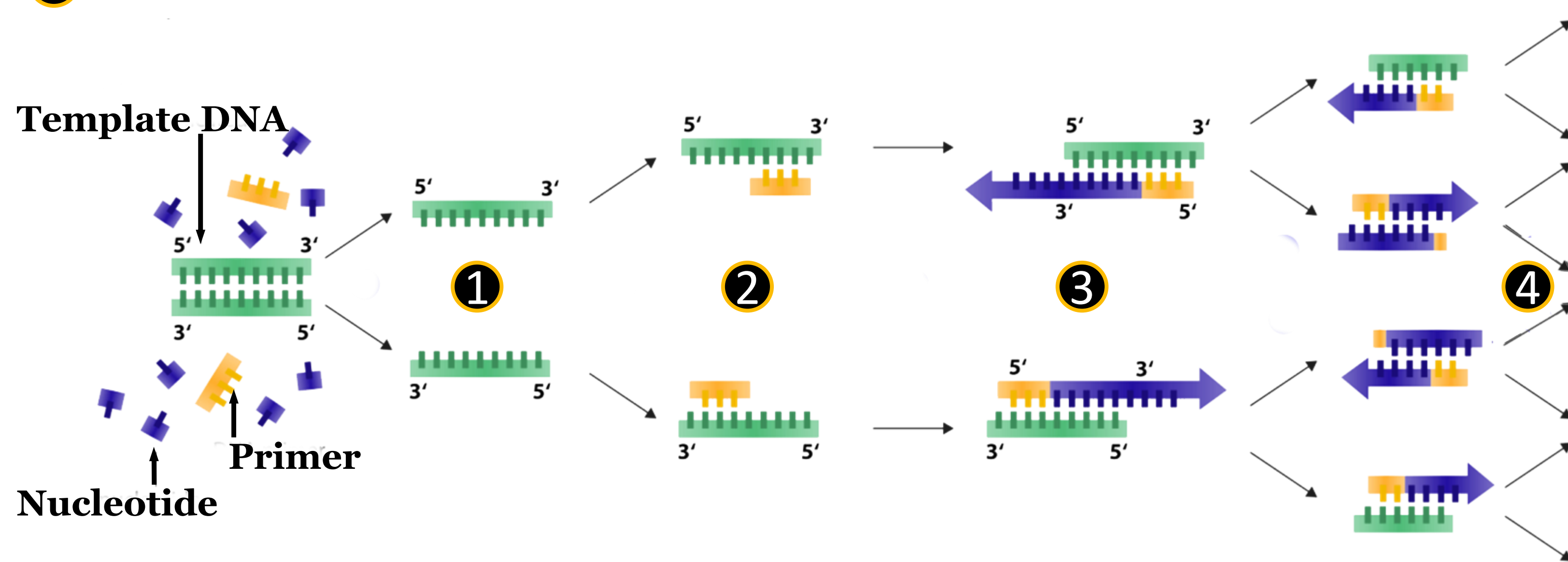
The Extract-N-Amplify extraction method:



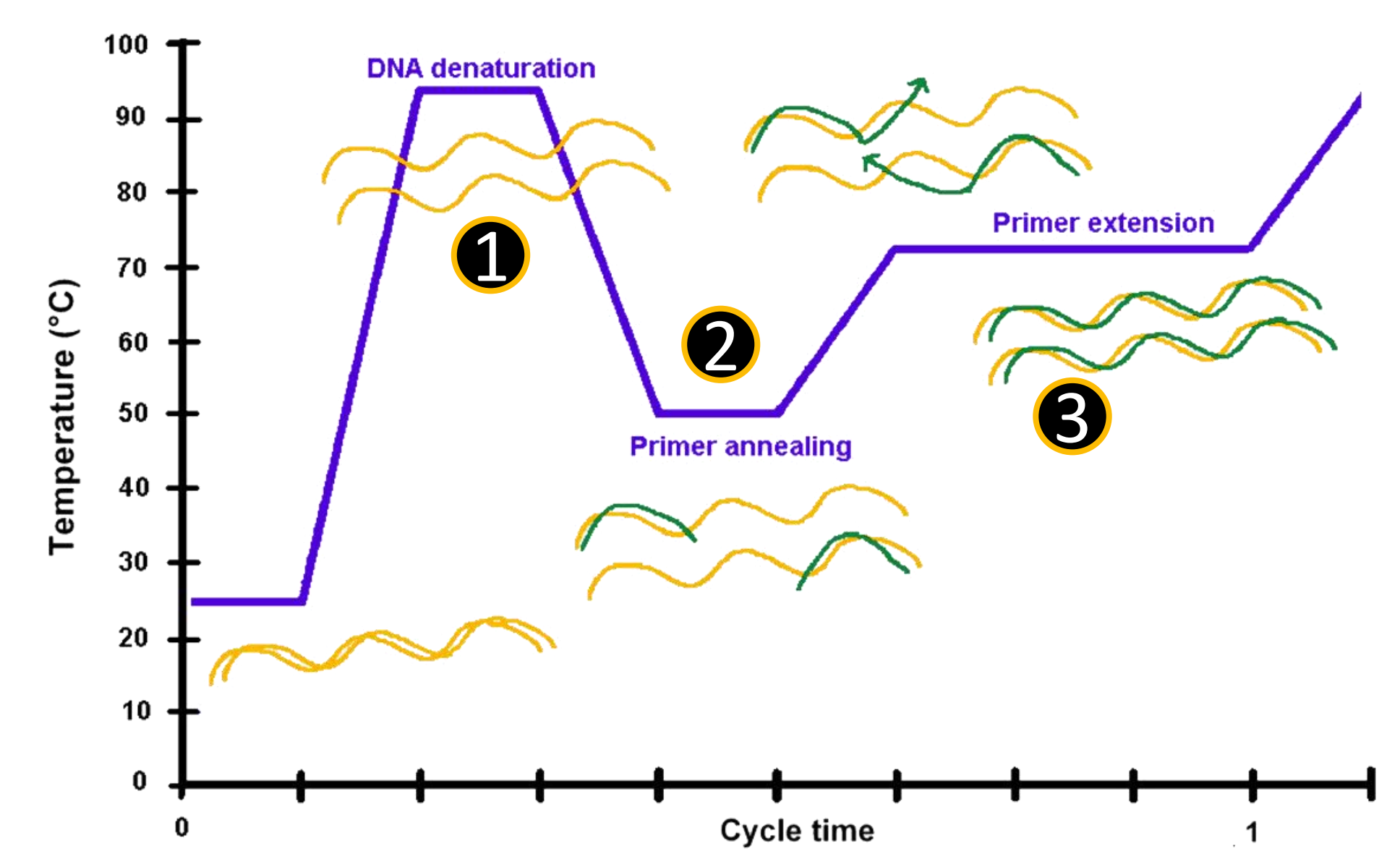
## 2. Polymerase Chain Reaction (PCR)

PCR is used to copy a single section, or multiple sections of DNA, millions of times.

- 1 DNA is split apart
- 2 Primers find a site to copy
- 3 Taq enzyme copies the DNA at the primer site
- 4 The cycle repeats to create millions of copies of a specific DNA sequence

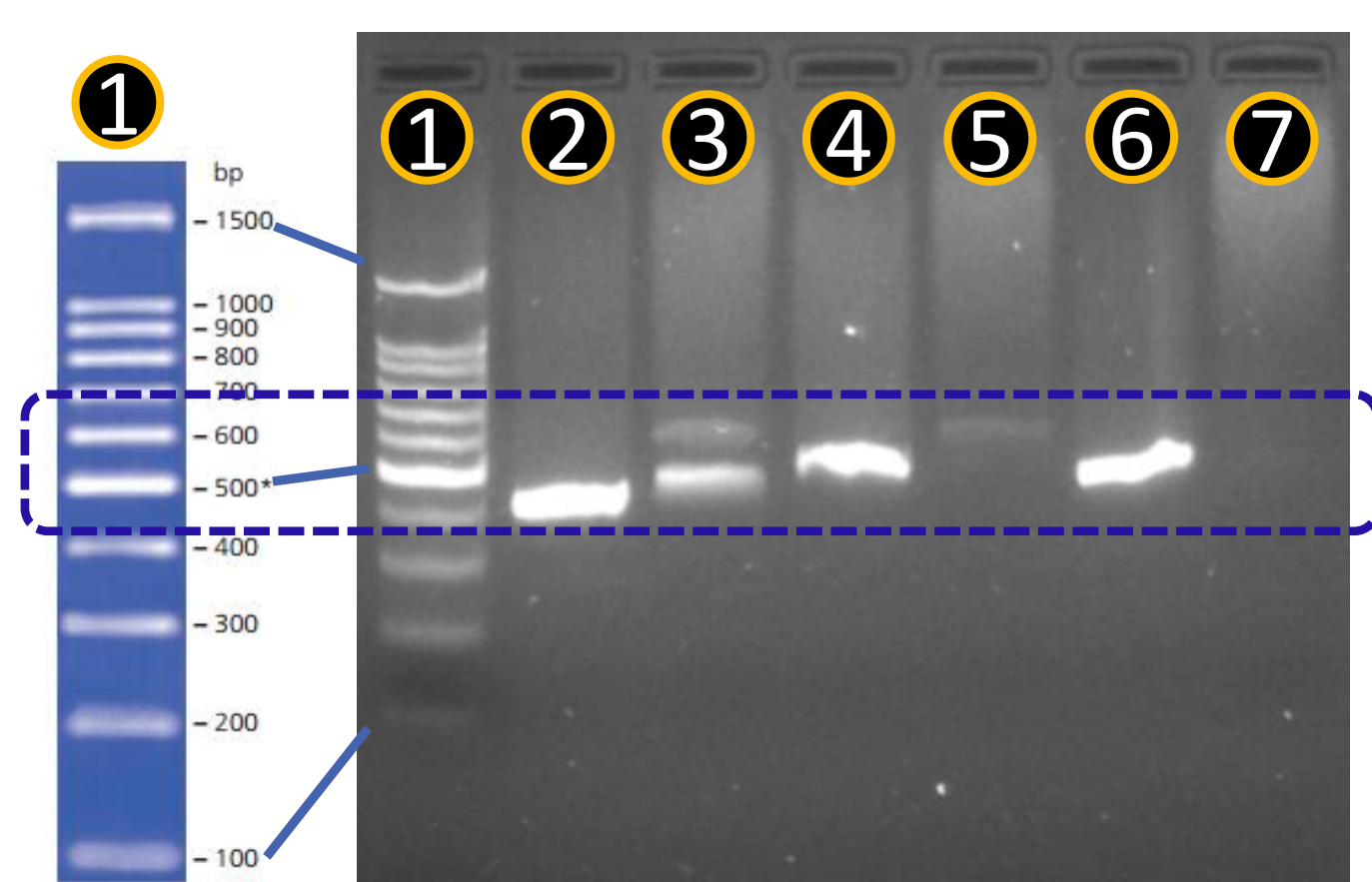


The thermocycler regulates the temperature:



## 3. Gel electrophoresis

Electrophoresis confirms if PCR worked by showing the length of a DNA sequence fragment



- 1 DNA ladder
- 2 ~450 base pair PCR amplicon (DNA fragment)
- 3 Double bands indicate contamination
- 4 ~500 base pair PCR amplicon
- 5 Less concentrated PCR amplicon
- 6 Positive control
- 7 Negative control

## 4. Sequencing

1. Purified PCR amplicons



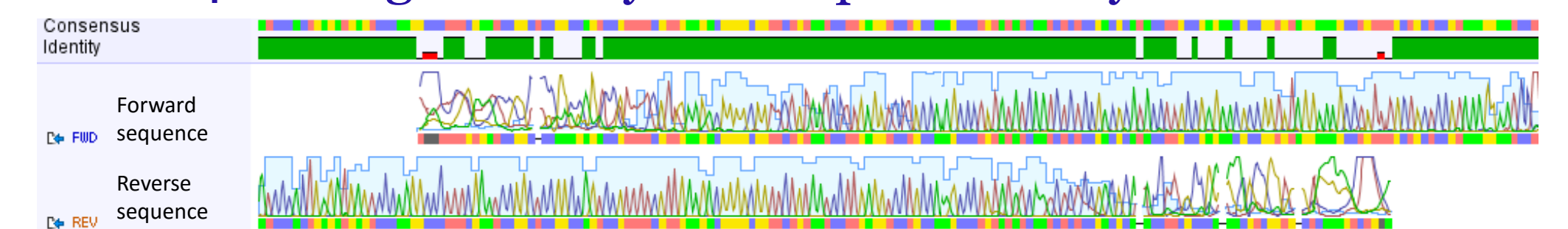
2. Shipping to sequencing facility



3. Performs Sanger sequencing



4. Contig assembly and sequence analysis



## 5. Phylogenetic analysis

Using BLAST will give an approximate identity of your organism, but creating a phylogeny is the only reliable way to identify it

BLAST results:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Hypothenemus hampei haplotyp SM1 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	739	739	100%	0.0	100%	GU133383.1
Hypothenemus hampei haplotyp AM-10N cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	734	734	100%	0.0	99%	GU133381.1
Hypothenemus hampei haplotyp CR1-CO1 cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	728	728	100%	0.0	99%	GU133382.1
Hypothenemus hampei haplotyp GUA-3N cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	728	728	100%	0.0	99%	GU133380.1
Hypothenemus hampei haplotyp NGO-2N cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	728	728	100%	0.0	99%	GU133343.1
Hypothenemus hampei haplotyp Bra-2N cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	723	723	100%	0.0	99%	GU133358.1
Hypothenemus hampei haplotyp Bra-3N cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	717	717	100%	0.0	99%	GU133359.1
Hypothenemus hampei haplotyp KAWB cytochrome oxidase subunit I (COI) gene, partial cds, mitochondrial	717	717	100%	0.0	99%	GU133344.1

Phylogenetic tree:

