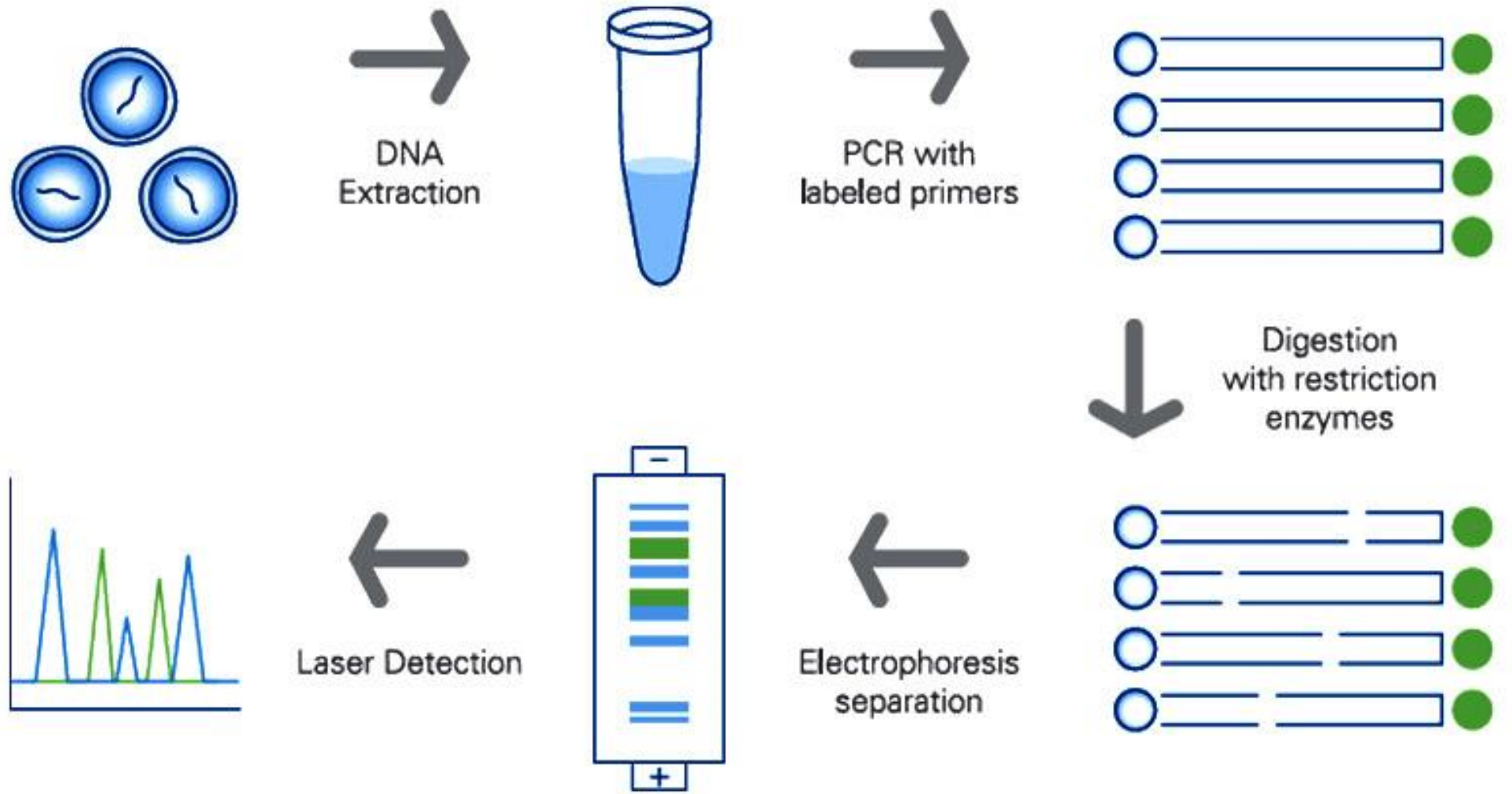
Aquaculture Biotechnology

Genetic Disease Mapping

Restriction Fragment Length Polymorphism (RFLP)

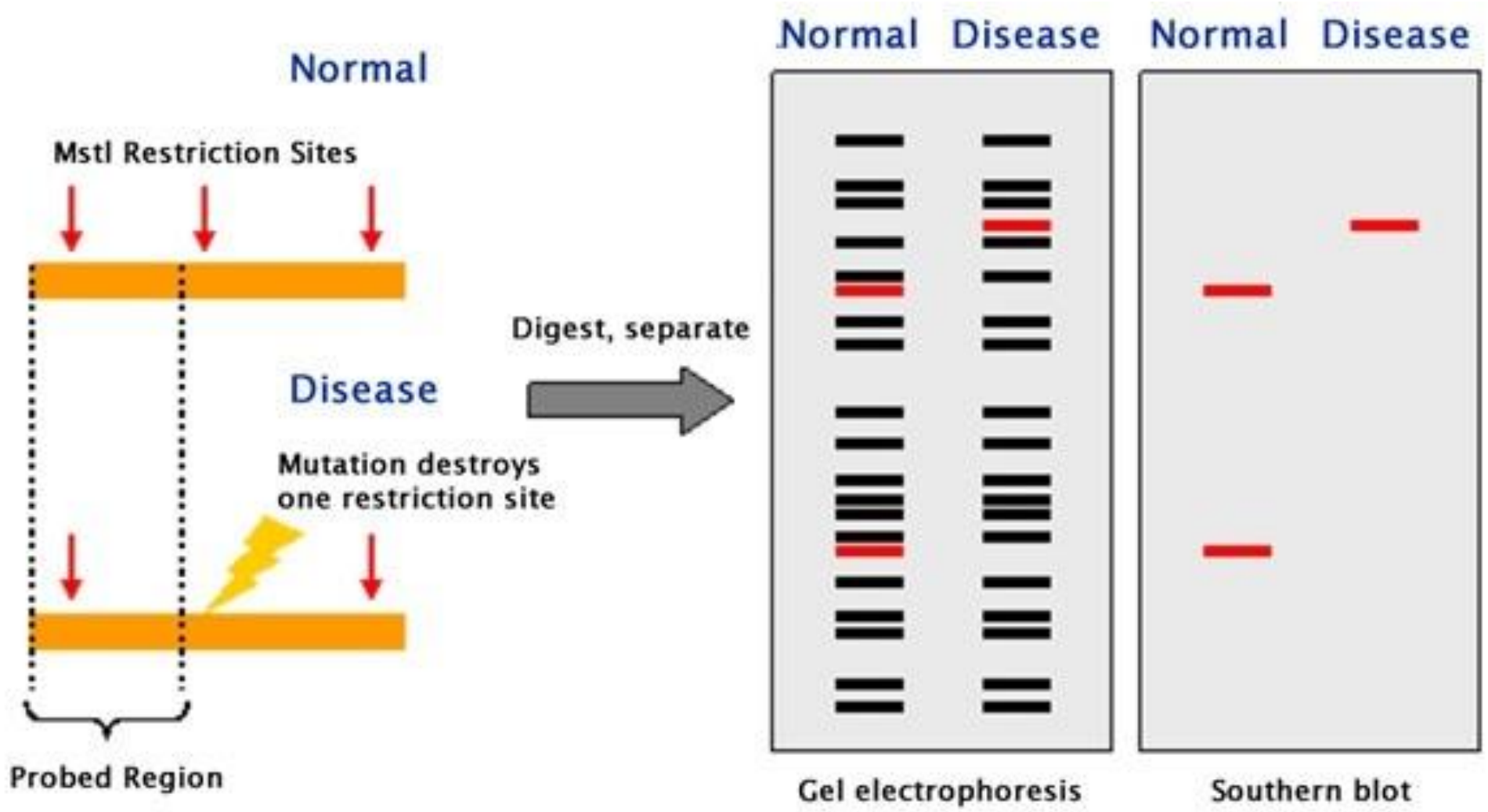
- is an enzymatic procedure for separation and identification of desired fragments of DNA.
- Using restriction endonuclease enzymes fragments of DNA is obtained and the desired fragment is detected by using restriction probes.
- May be used to differentiated two organism by analysis of patterns derived from cleavage of their DNA.
- RFLP analysis was formerly an important tool in genome mapping, localization of genes for genetic disorders, determination of risk for disease, and paternity testing.

RFLP technique



https://microbenotes.com/restriction-fragment-length-polymorphism-rflp/

RFLP technique



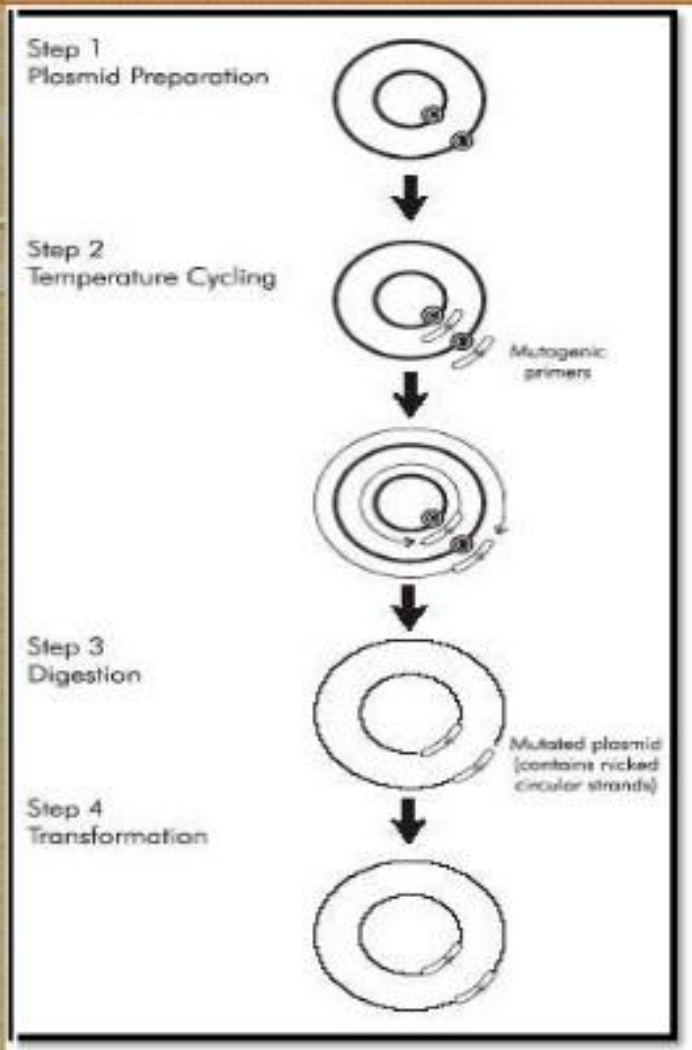
https://www.news-medical.net/image.axd?picture=2016%2F6%2FUntitled-25.jpg

Site-directed mutagenesis

- Site-directed mutagenesis is an invaluable tool to modify genes
- To study the structural and functional properties of a protein, based on the structure, function, catalytic mechanisms and catalytic residues of enzymes.
- Site-directed mutagenesis includes single and combinational mutations.

SITE DIRECTED MUTAGENESIS

BASIC MECHANISM OF SITE DIRECTED MUTAGENASIS



Gene in plasmid with target site mutation

Denature the plasmid and anneal the oligonucleotide.

primers containing the desired mutation
Using the non-strand-displacing action of *PfuTurbo* polymerase, extend and incorporate the mutagenic primers resulting in nicked circular strands

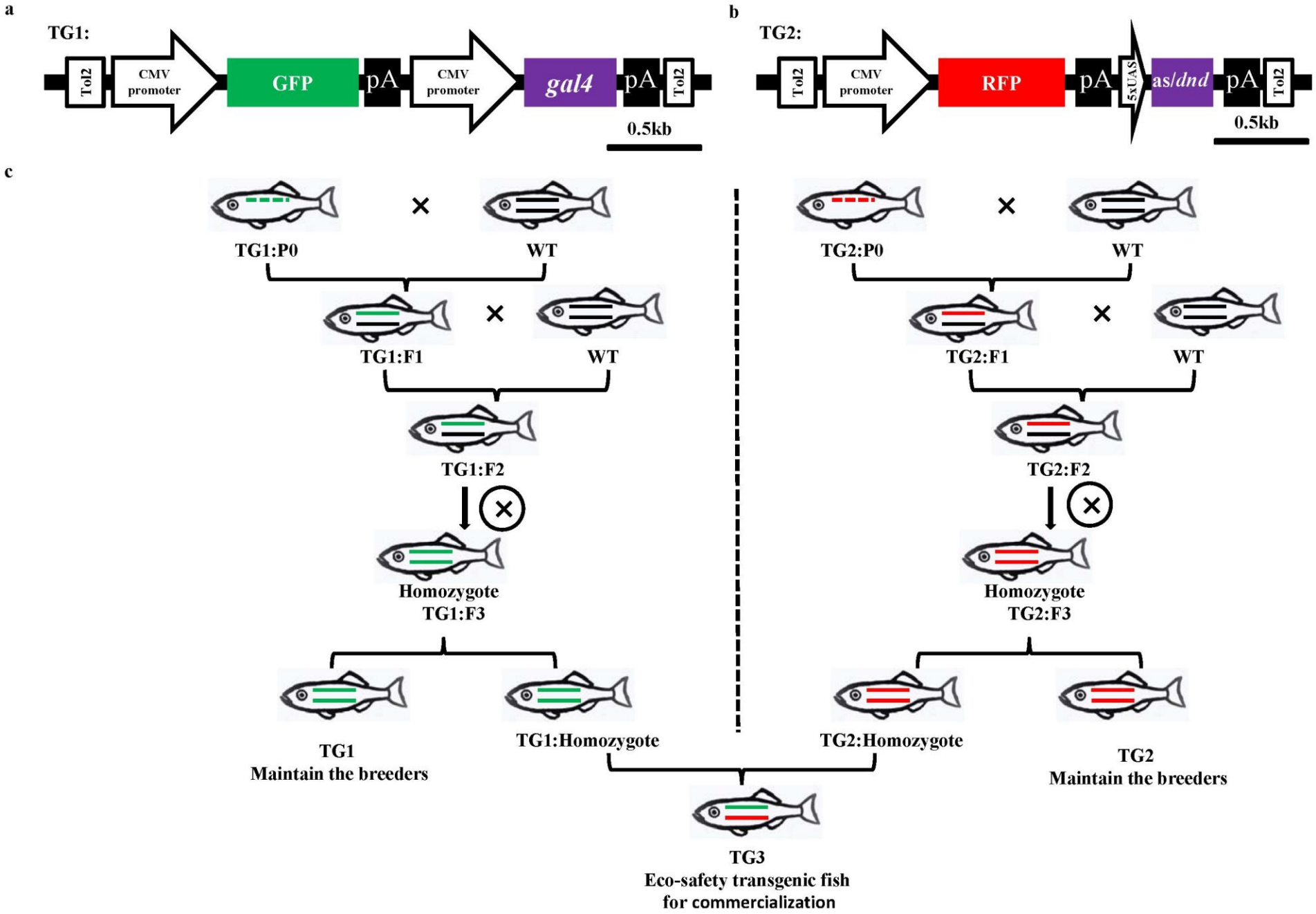
Digest the methylated, nonmutated parental DNA template with Dpn I

Transform the circular, nicked dsDNA into super-competent cells

After transformation the supercompetent cells repair the nicks in the mutated plasmid

Fig 1. Basic mechanism of site directed mutagenesis

https://image.slidesharecdn.com/2seminar-151207051846-lva1-app6891/95/site-directed-mutagenesis-7-638.jpg?cb=1449465565

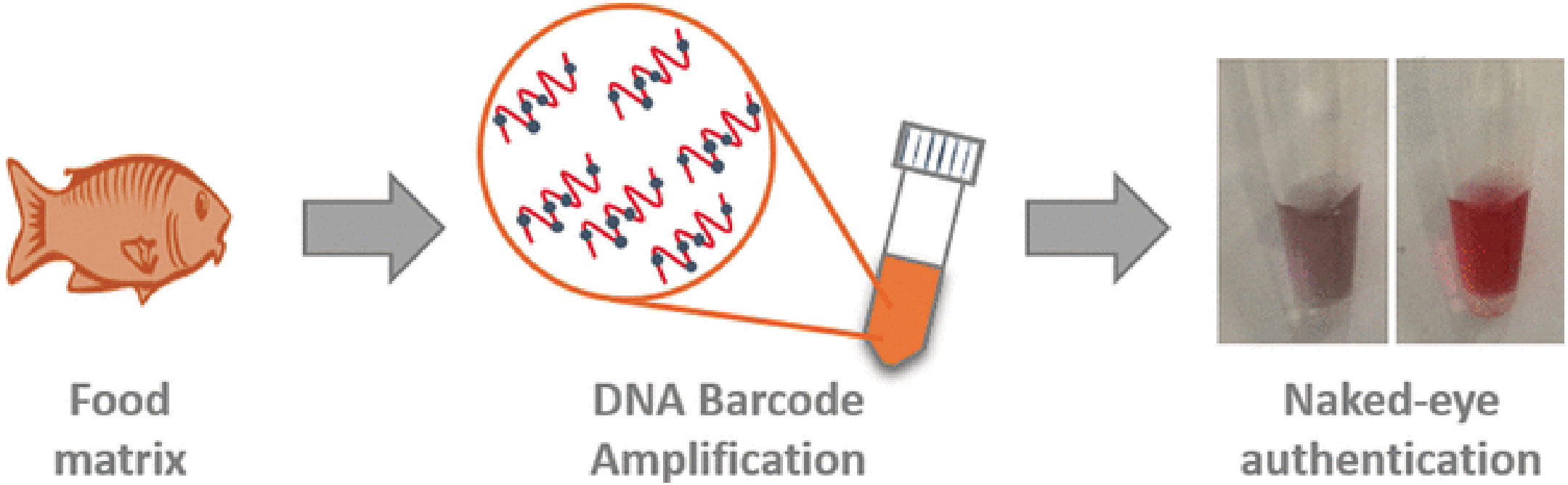


https://media.springernature.com/full/springer-static/image/art%3A10.1038%2Fsrep07614/MediaObjects/41598_2015_Article_BFsrep07614_Fig1_HTML.jpg

DNA fingerprinting

- is a method used to identify an organism from a sample of DNA by looking at unique patterns in their DNA.
- DNA fingerprinting is a technique that simultaneously detects lots of minisatellites in the genome to produce a unique pattern
- The probability of having organisms with the same DNA fingerprint that are not identical twins is very small.
- **DNA fingerprint methods can be applied in fish genetics for identification and conservation purpose**

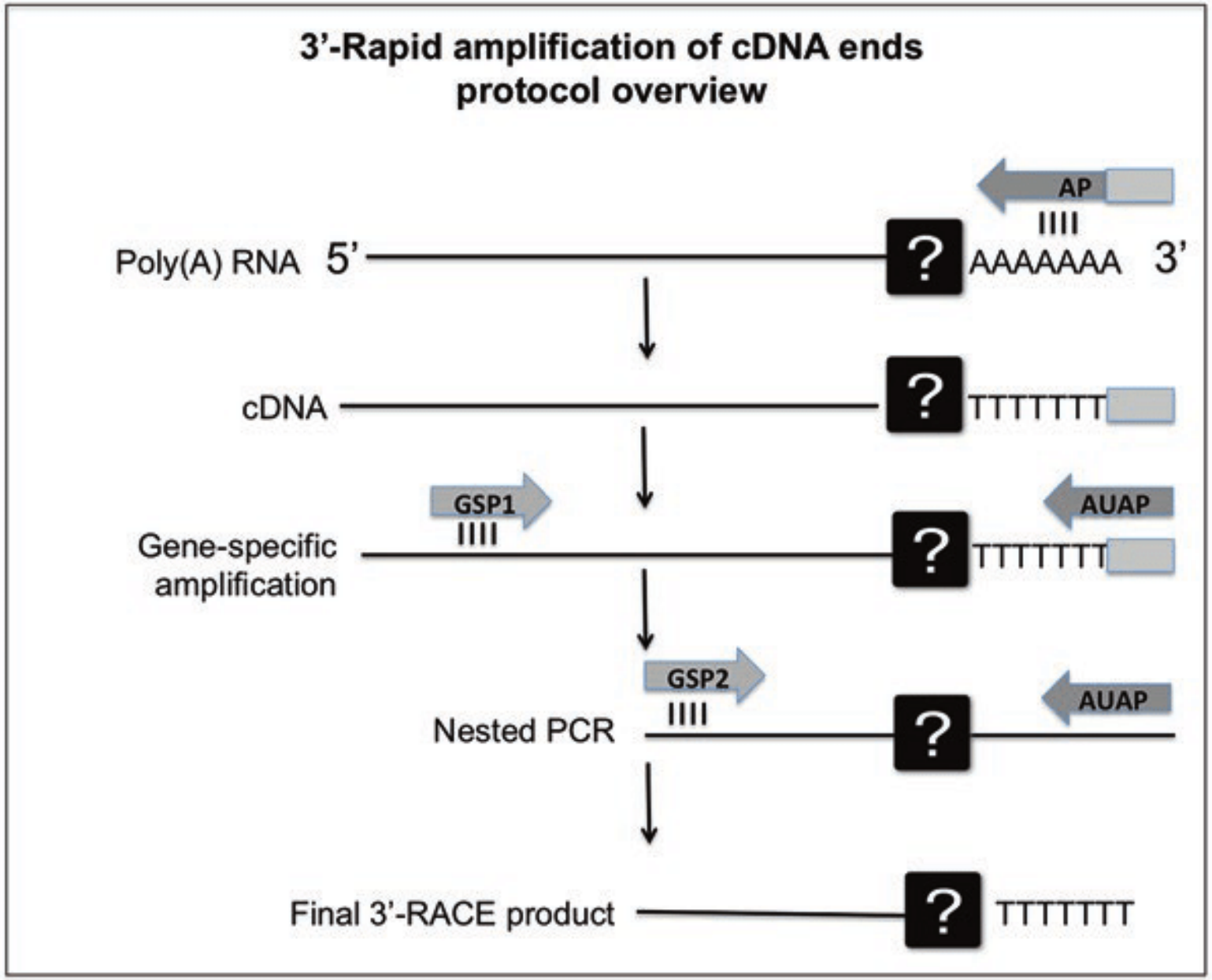
DNA fingerprinting for aquaculture



https://scx1.b-cdn.net/csz/news/800/2017/594236e3b001c.gif

Rapid Amplification of cDNA ends (RACE)

- Rapid Amplification of cDNA Ends (RACE) is a method for amplification of nucleic acid sequences from a messenger RNA template between a defined internal site and unknown sequences at either the 3' or the 5' -end of the mRNA.
- This is also known as “one-sided” PCR or “anchored” PCR .
- In this procedure, mRNAs are converted into cDNA using reverse transcriptase (RT) and an oligo-dT adapter primer.



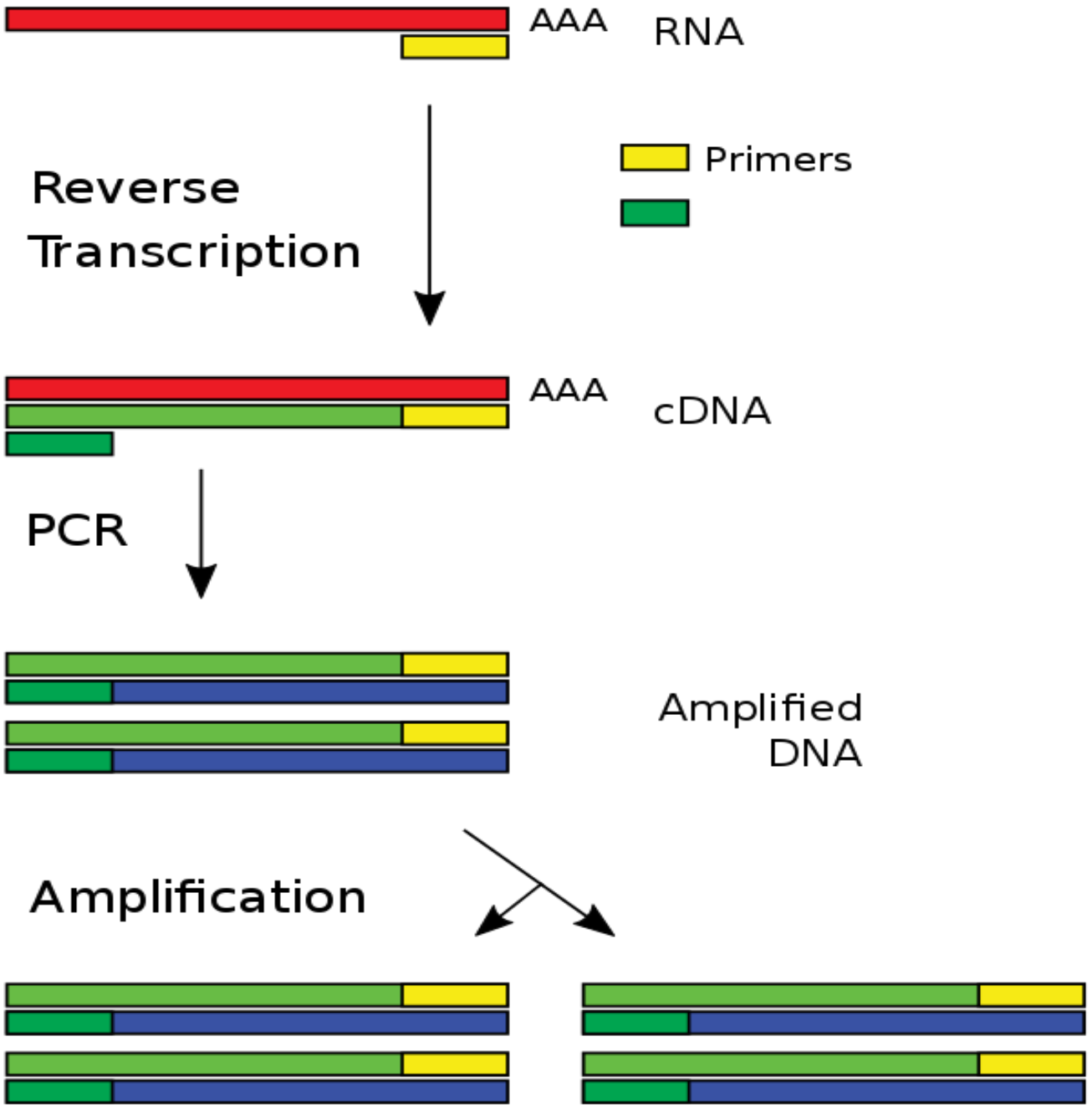
https://www.researchgate.net/profile/Chiara_Pastori/publication/315848247/figure/fig2/AS:611692518055937@1522850269086/Schematic-of-3-end-RACE-First-strand-cDNA-synthesis-is-initiated-at-the-polyA-tail-of.png

Reverse Transcription/Reverse Transcriptase

- Reverse transcription is a technique used to generate a complementary strand of DNA (cDNA) from RNA.
- The technology is based on a retroviral mechanism whereby enzyme reverse transcriptase can reverse transcribe RNA into DNA.
- In this procedure mRNA is isolated from the tissue and then by performing reverse transcription cDNA is produced.

Reverse Transcription/Reverse Transcriptase

- Reverse transcriptases (RTs) uses RNA template and a short primer complementary to the 3' end of the RNA to direct the synthesis of the first strand cDNA
- the first-strand cDNA can be made double-stranded using DNA Polymerase I and DNA Ligase.
- These reaction products can be used for direct cloning without amplification
- This is especially helpful when there is only small amount of sample present and cloning of a low copy gene is required.



Reverse Transcription

https://upload.wikimedia.org/wikipedia/commons/thumb/1/18/Reverse_transcription_polymerase_chain_reaction.svg/607px-Reverse_transcription_polymerase_chain_reaction.svg.png