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IBCE&W 2018 WORKSHOP

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Outlines

1. Introduction/Purpose
2. Gel Electrophoresis
3. Utilization/usefulness
4. Types of Gel
5. SDS-PAGE
6. Recent work
7. Applications

Gel Electrophoresis

- Separates:

i. DNA molecules
ii. RNA molecules
iii. Proteins

according to their sizes, electric charges, or other properties through a gel in an electric field.

- ✓ It is a standard method of separation, identification and purification of the molecules mentioned above.
- ✓ Different gel types and conditions are used for different molecules and types of applications.

- Samples are placed in a block of gel and an electric current is applied which causes the samples to move through the gel
- Smaller samples are less slow down by the gel matrix and hence will move faster through the gel
- This causes samples of different sizes to separate as they travel at different speeds

Usefulness

- Molecular biology
- Forensics
- Biochemistry
- Microbiology
- Genetics
- Agriculture
- Medicine

Analysing Gel

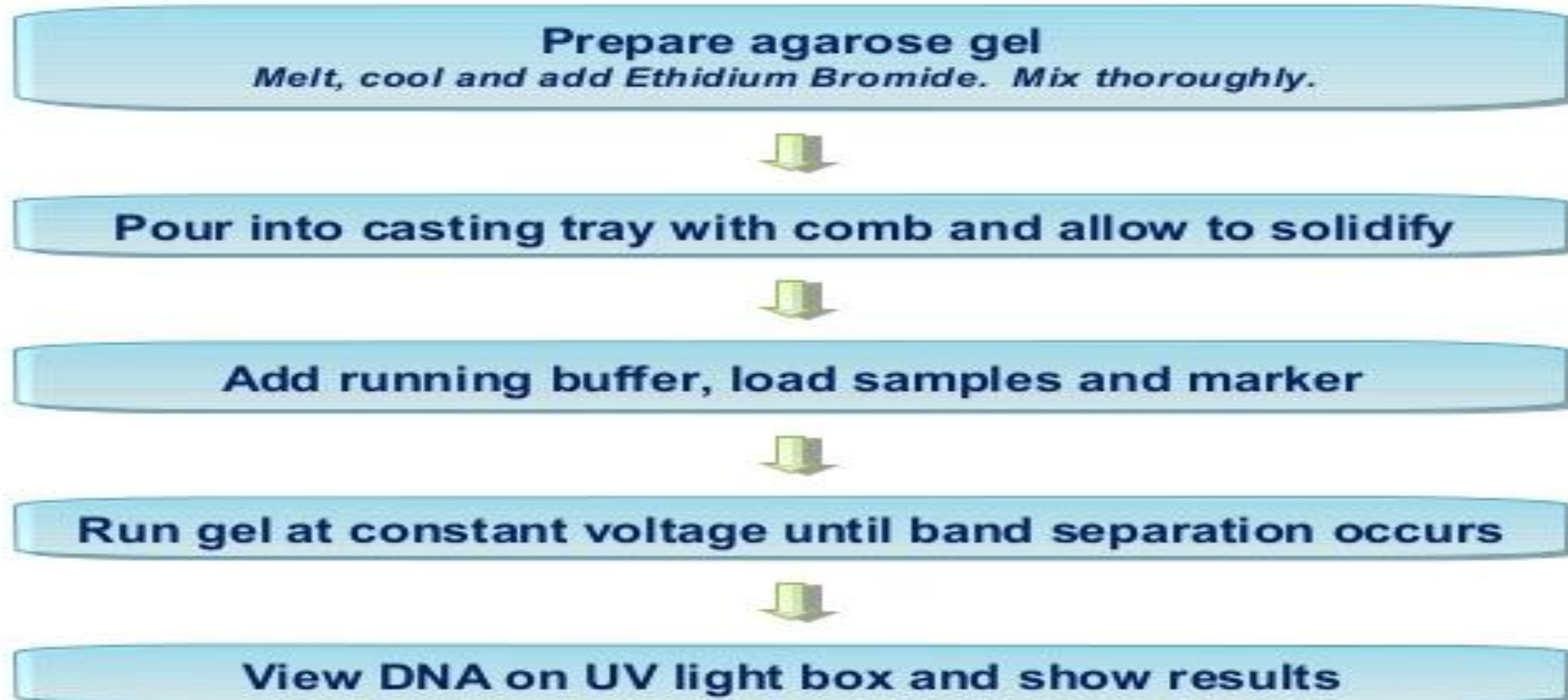
- Visualizing the gel with UV light and a gel imaging device;
- Analyse the intensity of the band or the measure of the spot of interest.

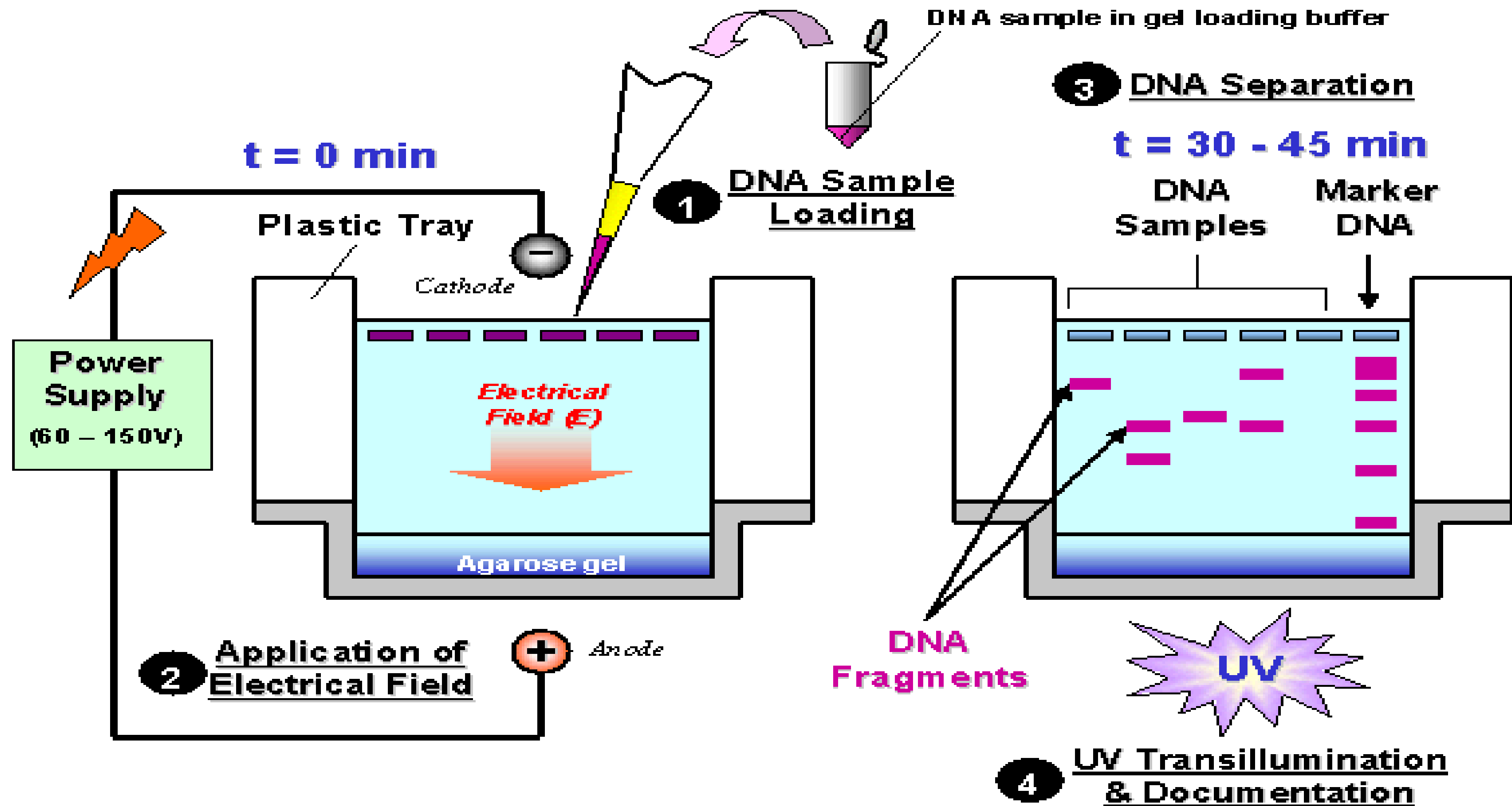
Types of Gels

1. Agarose gel: DNA separation

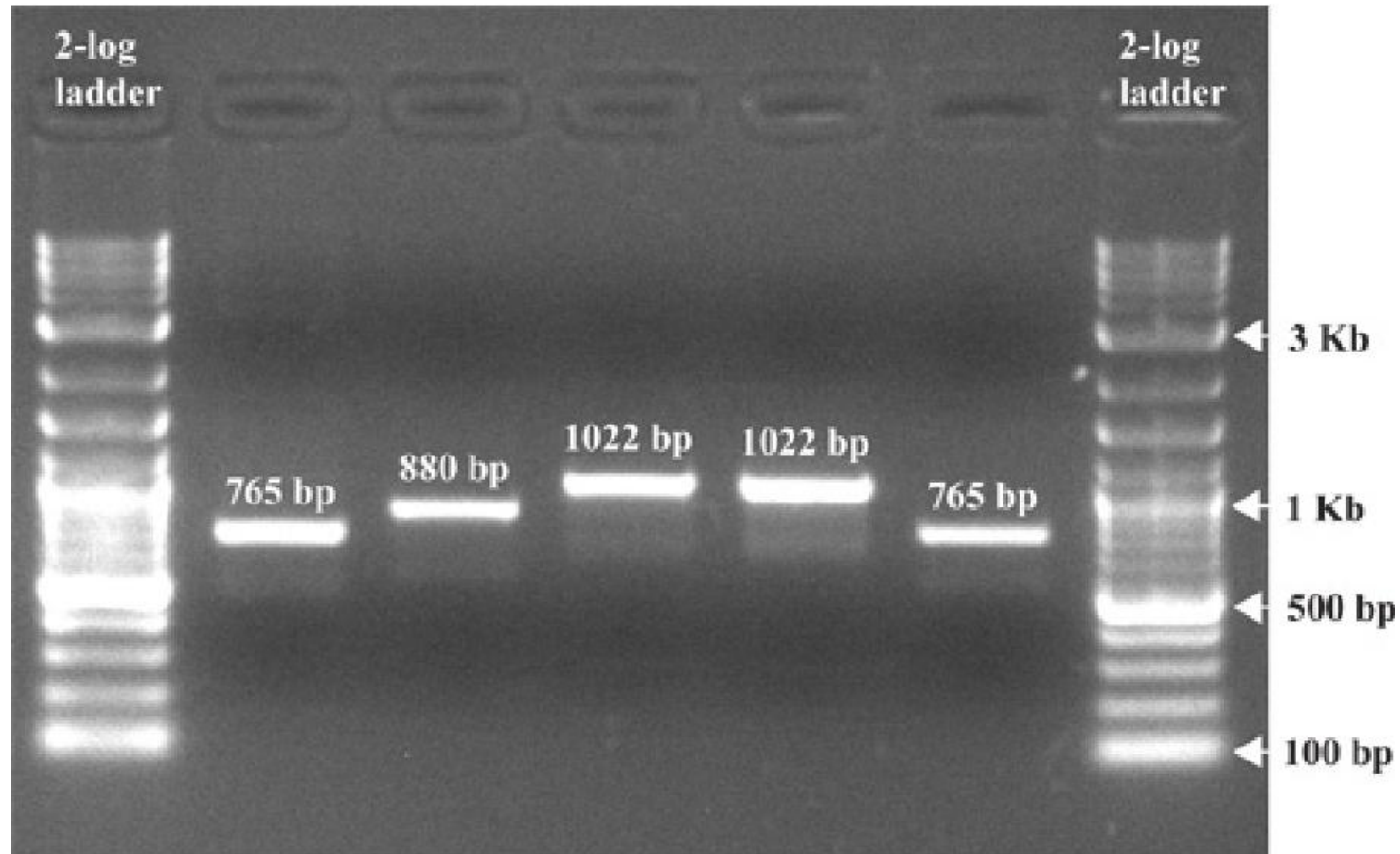
- DNA may be cut into fragments using restriction endonuclease – different DNA samples will generate different fragment lengths
- Fragments separate because DNA is negatively charged due to the presence of a phosphate group (PO_4^{3-}) on each nucleotide
- DNA samples are placed into an *agarose* gel and fragment size estimated by comparing against known industry standards
- Specific sequences can be identified by incorporating a complementary radiolabelled hybridisation probe, transferring the separated sequences to a membrane and then visualising via autoradiography (Southern blotting)

Separation of DNA Fragments by Agarose Gel Electrophoresis




DNA sample in gel loading buffer
3 DNA Separation
t = 30 - 45 min
DNA Samples
Marker DNA
t = 0 min
1 DNA Sample Loading
Plastic Tray
Cathode
Power Supply
(60 – 150V)
Electrical Field (E)
Agarose gel
2 Application of Electrical Field
Anode
DNA Fragments
UV
4 UV Transillumination & Documentation
Graphics©E Schmid/2001

A typical result



2. Protein Separation

- SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).
- Proteins may be folded into a variety of shapes (affecting size) and have positive and negative regions (no clear charge)
- Why do we have to treat proteins with an anionic detergent (SDS)?

- Protein samples are placed into a *polyacrylamide* gel and sizes compared against known industry standards
- Separated proteins are transferred to a membrane and then target proteins are identified by staining with specific monoclonal antibodies (Western blotting)

SDS-PAGE

- To estimate the molecular weights of proteins.
- Merit: speed to analyze a large number of samples, and
- simultaneously exploits differences in molecular size to resolve proteins differing by as little as 1 % in their electrophoretic mobility.

Recent Work:

- Assessment of genetic diversity through the seed storage proteins in Bambara groundnut
- Protein profiling and content of some accessions of cassava leaves as leafy vegetable in Nigeria.

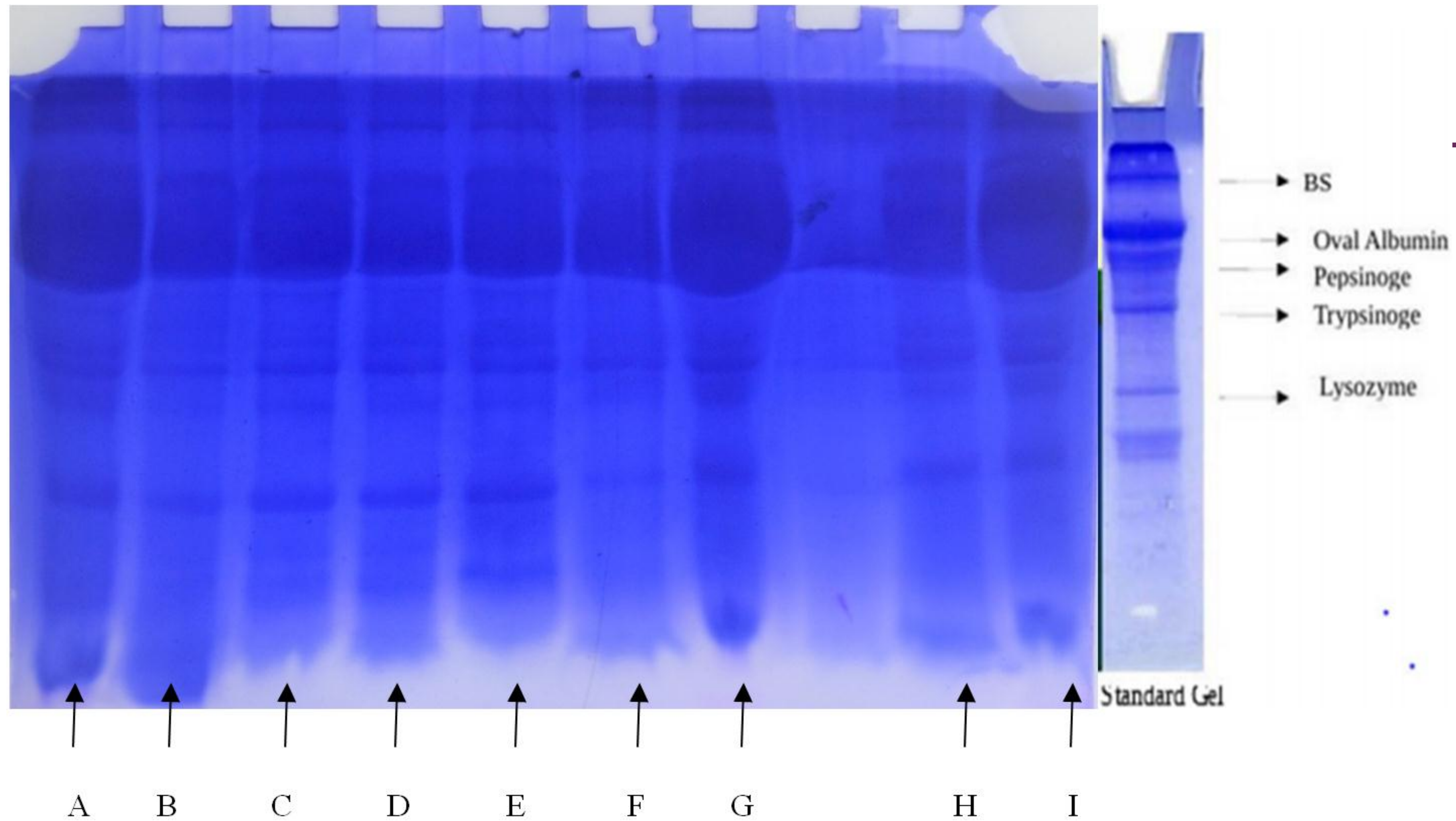


Figure 1: SDS-PAGE of the nine accessions in comparison with “standard gel”. A – I represents the sample number

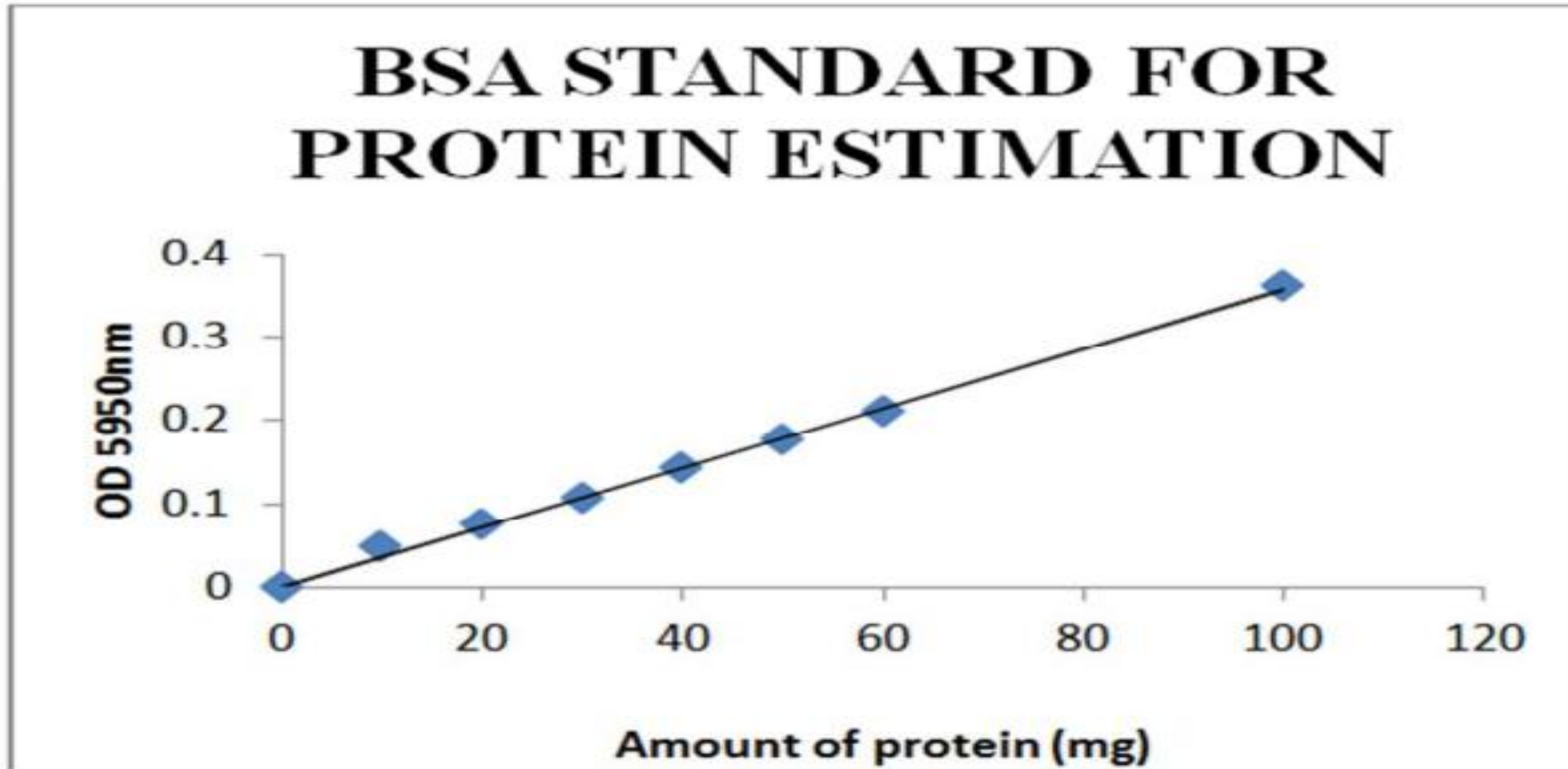


Chart 1: Bovine Serum Albumin (BSA) standard for estimation of proteins. The use of this standard is based on estimating the concentration of proteins in each sample by comparing an unknown protein concentration with a known concentration of BSA. Standard Protein Estimation Graph.

Table 1: Determination of Protein at Optical Density (O.D) 595Nm.

S/N	SAMPLE NUMBER	PROTEIN CONCENTATION (mg/ml)
A	NGB01494	0.066
B	NGB01497	0.053
C	NGB01499	0.065
D	NGB01501	0.060
E	NGB01245	0.062
F	NGB01489	0.062
G	NGB01311	0.063
H	NGB01496	0.061
I	NGB01495	0.061

Table 2: Characterization five protein bands generated from the nine accessions of Bambara groundnut studied.

Well lane	Protein group by molecular weight (kDa)						
	Samples	14-23	24-32	33-44	45-65	66-100	Number of bands
1	NGB01494	Abs	Low	Med	Low	Med	6
2	NGB01497	Abs	Low	Med	Low	Med	6
3	NGB01499	Med	Abs	Med	Low	Med	7
4	NGB01501	Med	Abs	Med	Low	Med	8
5	NGB01245	Med	Abs	Med	Low	Med	7
6	NGB01489	Abs	Med	Low	Low	Med	6
7	NGB01311	Abs	Low	Med	Low	Med	6
8	NGB01496	Abs	Low	Med	Low	Med	6
9	NGB01495	Abs	Low	Med	Low	Low	5
Total					Mono		57
%		24.56%	12.3 %	29.8 %	15.79%	29.8 %	

Interpretations

- Based on molecular weight of the proteins, five distinct groups were identified as follows:
- 14 to 23 corresponds to (lysozyme),
- 24-32 kDa to (Trypsinogen)
- 33 – 44 kDa (Pepsinogen),
- 45 – 65 kDa (Oval albumin) and
- > 66 kDa (BSA)

Applications

- Estimation of the size of DNA and protein molecules.
- Analysis of PCR products, i.e. in molecular genetic diagnosis or genetic fingerprinting,/genetic diversity studies
- Separation of restricted genomic DNA or of RNA.

Thank you.