

# EDUCATIONAL PRODUCTS GENOMIC

EDUCATIONAL

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Ouchterlony Double Diffusion (ODD) Teaching Kit  
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Ouchterlony Double Diffusion (ODD) Teaching Kit  
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**Immunoprob ing Techniques**

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**Labelling Techniques**

Antibody-HRP Conjugation Teaching Kit

**Protein Chromatography Techniques**

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Ion Exchange Chromatography Teaching Kit

Affinity Chromatography Teaching Kit

Immunoglobulin G. Isolation Teaching Kit

GeNei™ Thin Layer Chromatography Teaching Kit

Recombinant Protein Purification Kit

Hydrophobic Chromatography Teaching Kit

**Protein Electrophoresis Techniques**

SDS PAGE Teaching Kit

2-D PAGE Teaching Kit

**Protein Analysis Techniques**

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Elpho Kit for PAGE & Electro transfer (ETS3)

Elpho Kit for PAGE (ETS4)

Elpho Kit for Submarine Electrophoresis & Electrotransfer (ETS5)

Elpho Kit for 2-D PAGE (ETS6)

Elpho Kit for Submarine Electrophoresis & Capillary Transfer (ETS7)

## Educational Products

Genei ushers in a wide range of educational kits dealing in frontier areas of biology. Our association and experience with products for biological research is over a decade old. Therefore, we take pleasure in extending this service/experience to the teaching fraternity. Integrating Genei's education kits along with the curriculum at under and postgraduate level will certainly give synergistic effect to the quality of the course currently conducted.

### Microbiology



## Why Genei Teaching Kits.....???

- ◆ Genei kits have been developed with continuous interaction with faculty members in colleges/academic institutions
- ◆ Wide range of kits-from the very basic to the advanced biological techniques
- ◆ Easy to handle, complete with detailed instruction manual
- ◆ Can be performed within the allotted practical time & facility
- ◆ Basic storage facility is sufficient
- ◆ Kits are designed & developed by trained scientific staff
- ◆ Consistent & reproducible
- ◆ Undergoes elaborate quality checks & strict quality checks criteria
- ◆ Crystal clear results without any ambiguity
- ◆ All components manufactured in-house which ensures continuous supply
- ◆ Good technical support staff to guide the faculty & students
- ◆ Stable for a period of six months.

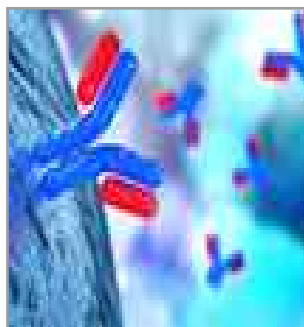
### Molecular



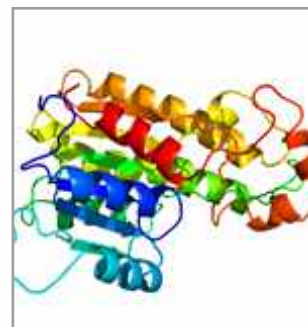
## On-site Training Program

- ◆ Exclusive demonstration and hands on training is extended for faculty & student levels
- ◆ Choice of comprehensive list of bio-techniques to enhance your hands on skills
- ◆ Techniques taught by team of experienced scientists
- ◆ Good platform for Academia-industry interaction
- ◆ Flexible and custom-made training slots available
- ◆ Techniques ranging from serial dilution to cutting edge techniques Affordable packages

### Immunology



### Proteomics



## Techware



- ◆ Good technical support staff to guide the faculty & students
- ◆ Stable for a period of six months.

Selection Guide: Molecular Biology Educational Products

## BASIC MOLECULAR BIOLOGY TECHNIQUES

Cat. No	PI No.	Product Description
<b>BASIC MOLECULAR BIOLOGY TECHNIQUES</b>		
6113000031730	KT130	GeNei™ Agarose Gel Electrophoresis Teaching Kit
6100100011730	KT01	GeNei™ Restriction Digestion Teaching Kit
6100800011730	KT08	GeNei™ Ligation Teaching Kit
6106600011730	KT66	GeNei™ DNA Molecular Size Determination Teaching Kit
6110300011730	KT103	GeNei™ Restriction Mapping Teaching Kit
<b>NUCLEIC ACID ISOLATION AND PURIFICATION</b>		
6100600011730	KT06	GeNei™ Plasmid Preparation Teaching Kit
6102800011730	KT28	GeNei™ Genomic DNA Extraction Teaching Kit (from Bacteria)
6106700011730	KT67	GeNei™ Genomic DNA Extraction Teaching Kit (from Leaves)
6112500011730	KT125A	GeNei™ Fungal Genomic DNA Extraction Teaching Kit
6112500011730	KT129B	GeNei™ Whole Blood DNA Extraction Teaching Kit
6113200011730	KT132	GeNei™ Total RNA Extraction Teaching Kit
6112600011730	KT126A	GeNei™ Plasmid DNA Purification Teaching Kit (using DNA Binding Membrane)
6112800011730	KT128A	GeNei™ Genomic DNA Purification Teaching Kit (using DNA Binding Membrane)
6104300011730	KT43B	GeNei™ Gel Extraction Teaching Kit (Solution Based)
6104300011730	KT134A	GeNei™ Gel Extraction Teaching Kit (membrane based)
6114800011730	KT148A	GeNei™ Total RNA Extraction Teaching Kit
<b>CLONING &amp; EXPRESSION TECHNIQUES</b>		
6106000011730	Kt60	GeNei™ GFP Cloning Teaching Kit
6106900011730	KT69	GeNei™ Bacterial Gene Expression Teaching Kit (using Lac Promoter System)
6113100011730	KT131A	GeNei™ In-vitro Transcription Teaching Kit
<b>PCR RELATED TECHNIQUES</b>		
6104400011730	Kt44	GeNei™ Student PCR Teaching Kit
6110000021730	KT100B	GeNei™ RAPD Application Teaching Kit (Rice Varieties Identification)
6110200011730	KT102A	GeNei™ Genotyping Analysis Teaching Kit
6113600011730	KT136	GeNei™ Student RT PCR Teaching Kit
6110700011730	KT107A	GeNei™ Multiplex PCR Teaching Kit
6110800011730	KT108A	GeNei™ GMO Detection Teaching Kit (Simulation Study)
<b>BLOTTING TECHNIQUES</b>		
6109600031730	KT96	GeNei™ Southern Hybridization Teaching Kit
6113800011730	KT138	GeNei™ Northern Blotting Teaching Kit

## BASIC MOLECULAR BIOLOGY TECHNIQUES

Cat. No	PI No.	Product Description
<b>DNA FINGERPRINTING TECHNIQUES</b>		
6109400011730	KT94	GeNei™ DNA Fingerprinting Teaching Kit (Using RFLP technique)
6109500011730	KT95B	GeNei™ DNA Fingerprinting Teaching Kit (Using RAPD technique)
6109900011730	KT99A	GeNei™ Single Nucleotide Polymorphism (SNP)
6114700011730	KT147A	GeNei™ AFLP Teaching Kit
<b>MICROBIAL GENETICS</b>		
6100500011730	KT05	GeNei™ Phage Titration Teaching Kit
6119000021730	KT90	GeNei™ Plasmid Curing Teaching Kit
6119000021730	KT45	GeNei™ Bacterial Conjugation Teaching Kit
6112300011730	KT123	GeNei™ Bacterial Transduction Teaching Kit
6112400011730	KT124B	GeNei™ Bacterial Transposons Teaching Kit
<b>BASIC MICROBIOLOGY TECHNIQUES</b>		
6112400011730	KT46	GeNei™ Bacterial Growth Curve Teaching Kit
6106800011730	KT68	GeNei™ Bacterial Antibiotic Sensitivity Teaching Kit
6109000011730	KT90	GeNei™ Isolation and Identification of Soil Bacteria Teaching Kit

### Selection Guide:

### Immunology Educational Products Teaching Kits

Cat. No	PI No.	Product Description
<b>IMMUNOPRECIPITATION TECHNIQUES</b>		
6101100011730	KT11	GeNei™ Quantitative Precipitin Assay (QPA) Teaching Kit
6112200011730	KT122	GeNei™ Immuno-precipitation Teaching Kit
<b>IMMUNODIFFUSION TECHNIQUES</b>		
6107000011730	KT70	GeNei™ ODD (For Antigen Antibody Patterns) Teaching Kit
6100900011730	KT09S	GeNei™ ODD (For Antibody Titration) Teaching Kit
6101000011730	KT10S	GeNei™ Radial Immuno Diffusion Teaching Kit
<b>IMMUNOELECTROPHORESIS TECHNIQUES</b>		
6102000031730	KT20	GeNei™ Immunoelectrophoresis Teaching Kit
6104700011730	KT47	GeNei™ Rocket Immunoelectrophoresis Teaching Kit
6102900011730	KT29	GeNei™ Counter Current Immunoelectrophoresis Teaching Kit
<b>AGGLUTINATION TECHNIQUES</b>		
6105300011730	KT53	GeNei™ Latex Agglutination Teaching Kit ELISA based Techniques
<b>ELISA TECHNIQUES</b>		
6101200011730	KT12S	GeNei™ Dot ELISA Teaching Kit
6105000011730	KT50	GeNei™ Antigen Capture ELISA Teaching Kit
6105100011730	KT51	GeNei™ Antibody Capture ELISA Teaching Kit
6105200011730	KT52	GeNei™ Sandwich ELISA Teaching Kit



Cat. No	PI No.	Product Description
<b>KITS FOR IMMUNOPROBING</b>		
6102100031730	KT21	GeNei <sup>TM</sup> Western Blotting Teaching Kit
<b>LABELLING TECHNIQUES</b>		
6104800011730	KT48	GeNei <sup>TM</sup> Antibody-HRP Conjugation Teaching Kit
<b>BASIC MICROBIOLOGY TECHNIQUES</b>		
6112400011730	Kt46	GeNei <sup>TM</sup> Bacterial Growth Curve Teaching Kit
6106800011730	KT68	GeNei <sup>TM</sup> Bacterial Antibiotic Sensitivity Teaching Kit
6109000011730	KT90	GeNei <sup>TM</sup> Isolation and Identification of Soil Bacteria Teaching Kit

### Selection Guide: Protein Purification and Analysis Techniques Teaching Kits

Cat. No	PI No.	Product Description
<b>PROTEIN CHROMATOGRAPHY TECHNIQUES</b>		
6103900011730	KT39	GeNei <sup>TM</sup> Gelfiltration Chromatography Teaching Kit
6104000011730	KT40	GeNei <sup>TM</sup> Ion Exchange Chromatography Teaching Kit
6104100011730	KT41	GeNei <sup>TM</sup> Affinity Chromatography Teaching Kit
6110600011730	KT106	GeNei <sup>TM</sup> Immunoglobulin G Isolation Teaching Kit
6120300011730	KT203	GeNei <sup>TM</sup> Recombinant Protein Purification Kit
6120200011730	KT202	GeNei <sup>TM</sup> Hydrophobic Chromatography Teaching Kit
<b>PROTEIN ANALYSIS TECHNIQUES</b>		
6119290021730	KT192	GeNei <sup>TM</sup> Thin Layer Chromatography Teaching Kit with TLC Chamber
6108900011730	KT89	GeNei <sup>TM</sup> Enzyme Kinetics Teaching Kit
6102100031730	KT21	GeNei <sup>TM</sup> Western Blotting Teaching Kit
6119600011730	KT196	GeNei <sup>TM</sup> Protein Fingerprinting Teaching Kit
<b>PROTEIN ELECTROPHORESIS TECHNIQUES</b>		
6103000011730	KT30	GeNei <sup>TM</sup> SDS-PAGE Teaching Kit
6114500011730	KT145	GeNei <sup>TM</sup> 2D - PAGE Teaching Kit

## EDUCATIONAL PRODUCTS GENOMICS

### Agarose Gel Electrophoresis Teaching Kit

#### Description : ▶

Electrophoresis is a technique used to separate charged molecules. DNA is negatively charged at neutral pH and when electric field is applied across the gel, the DNA migrates towards the anode. Migration of DNA through the gel is dependent upon:

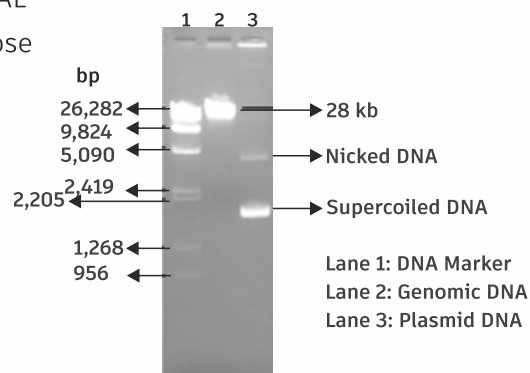
- ♦ Molecular size of DNA
- ♦ Agarose concentration
- ♦ Conformation of DNA
- ♦ Applied current

Matrix of agarose gel acts as a molecular sieve through which DNA fragments move on application of electric current. Higher concentration of agarose gives firmer gels, i.e., spaces between cross-linked molecules is less and hence smaller DNA fragments easily crawl through these spaces. As the length of the DNA increases, it becomes harder for the DNA to pass through the spaces. Lower concentration of agarose helps in the movement of larger DNA fragments as the spaces between the cross-linked molecules is more. The progress of gel electrophoresis is monitored by observing the migration of a visible dye (tracking dye) through the gel. Two commonly used dyes are Xylene cyanol and Bromophenol blue that migrate at the same speed as double stranded DNA of size 5000 bp and 300 bp respectively. These tracking dyes are negatively charged, low molecular weight compounds that are loaded along with each sample at the start of electrophoresis, when the tracking dye reaches towards the anode, electrophoresis is terminated. As the voltage applied to a gel is increased, larger fragments migrate proportionally faster than the smaller fragments. Best resolution of fragments is attained by applying no more than 5 volts per cm to the gel. Different buffers have been recommended

for electrophoresis of DNA. The most used are TAE (Tris-Acetate-EDTA) and TBE (Tris-Borate EDTA). DNA fragments migrate at somewhat different rates in these two buffers due to differences in their ionic strength. Buffers not only maintain the pH but also provide ions to support conductivity. Visualisation of DNA Fragments: Since DNA is not naturally coloured, it is not visible on the gel. Hence the gel, after electrophoresis, is stained with a dye specific to the DNA. Discrete bands are observed when there is enough DNA material present to bind the dye to make it visible, otherwise the band is not detected. The gel is observed against a light background wherein DNA appears as dark coloured bands. Alternatively, an intercalating dye like Ethidium Bromide (EtBr) is added to agarose gel and location of bands determined by examining the gel under UV light. The DNA bands appear fluorescent.

#### Kit Contents : ▶

- ♦ Genomic DNA
- ♦ Plasmid DNA
- ♦ DNA Marker
- ♦ 2.5X GLB
- ♦ 50X TAE
- ♦ Agarose



Mobility of two types of DNA on 1% Agarose Gel

#### Ordering Information:

Cat. No	PI No.	Product Description
6113000011730	KT130A	GeNei™ Agarose Gel Electrophoresis Teaching Kit, 10 expts.
6113000031730	KT130	GeNei™ Agarose Gel Electrophoresis Teaching Kit with ETS1,10 expts.

## Restriction Digestion Teaching Kit

### Description : ▶

Restriction digestion is a defense mechanism developed by bacteria against bacteriophages in the form of enzymes called endonucleases that cleave any foreign DNA. The restriction endonuclease cleavage is a precise DNA-excising process that occurs at specific sites on the DNA sequence.

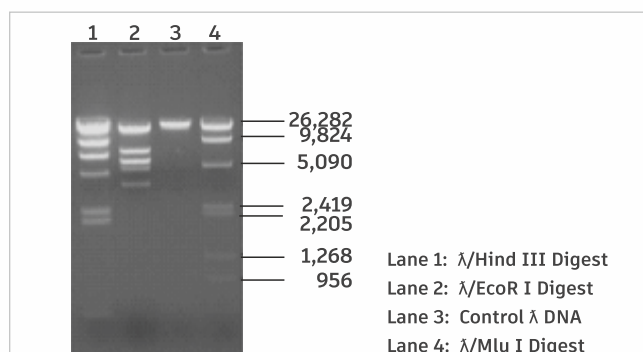
This mechanism has made way for the discovery of different types of restriction enzymes which help in cloning.

#### This kit demonstrates.

- ♦ The action of restriction enzymes-, *EcoR* I and *Hind* III, which selectively cleaves Lambda ( $\lambda$ ) DNA a linear double stranded DNA, 48502 base pairs at specific sites.
- ♦ The size of the fragments is determined when electrophoresed along with a standard DNA marker on an agarose gel.

### Kit Contents : ▶

- ♦ 2X Assay Buffer
- ♦  $\lambda$ /Mlu I Digest
- ♦ Gel Loading Buffer
- ♦ Lambda DNA (substrate)
- ♦ Restriction Enzymes: *EcoR* I, *Hind* III
- ♦ Control  $\lambda$  DNA
- ♦ Agarose
- ♦ 50X TAE
- ♦ 1.5 ml Vials



$\lambda$  DNA digested with *EcoR* I and *Hind* III, electrophoresed on 1% Agarose gel (stained with EtBr)

### Ordering Information:

Cat. No	PI No.	Product Description
6100100011730	KT01A	GeNei™ Restriction Digestion Teaching Kit, 5 expts.
6100100021730	KT01B	GeNei™ Restriction Digestion Teaching Kit, 20 expts.

## GeNei™ Restriction Mapping Teaching Kit

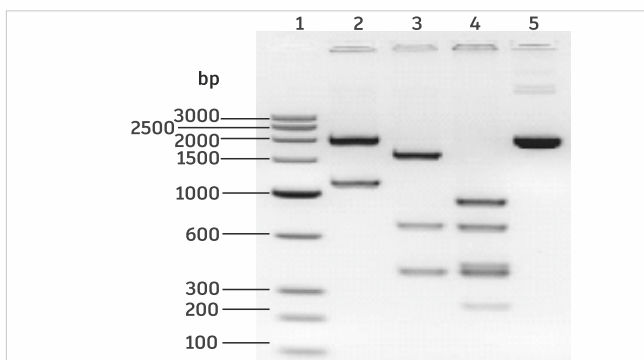
### Description : ▶

Restriction enzymes are endonucleases that cleave both strands of DNA at specific sequences of bases called recognition sites. The location of the cleavage site is important for mapping of DNA and molecular cloning experiments. DNA mapping involves the determination of the relative positions of the Restriction enzyme cleavage sites (RE sites) in a plasmid molecule. This is done by determining the size of the DNA fragments generated by single or combination of Restriction enzymes and subsequent construction of the DNA map. A Restriction map is a description of restriction endonucleases cleavage sites within a DNA sequence. Generating such a map is usually the first step in characterizing an unknown DNA and a prerequisite for manipulating it for other purposes.

Using this kit, students will perform the restriction digestion of plasmid DNA with two different enzymes i.e. *Ssp* I and *Bgl* I.

### Kit Contents : ▶

- ♦ Plasmid DNA
- ♦ Restriction Enzymes: *Bgl* I and *Ssp* I
- ♦ Assay Buffer
- ♦ Low Range DNA Ruler (Ready to use)
- ♦ 2.5X Gel Loading Buffer
- ♦ Agarose
- ♦ 50X TAE
- ♦ 1.5 ml vials
- ♦ Instruction Manual



Lane 1: Low Range DNA Ruler      Lane 4: Bgl I/Ssp I Digest  
Lane 2: Bgl I Digest              Lane 5: Control DNA  
Lane 3: Ssp I Digest

## Ordering Information:

Cat. No	PI No.	Product Description
6110300011730	KT103A	GeNei™ Restriction Mapping Teaching Kit, 5 expts.
6110300021730	KT103B	GeNei™ Restriction Mapping Teaching Kit, 20 expts.

## GeNei™ Ligation Teaching Kit

### Description : ▶

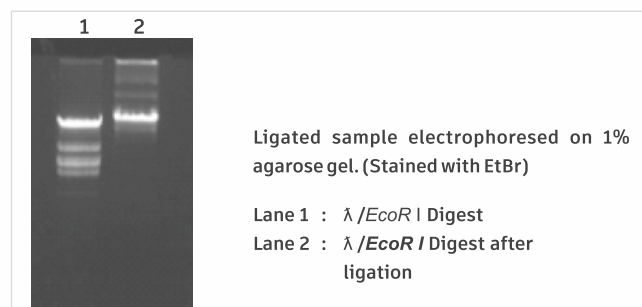
Construction of recombinant DNA molecule is dependent on the ability to covalently seal single stranded nicks in the DNA. This process is accomplished both in vivo and in vitro by the enzyme DNA ligase. This enzyme catalyzes the formation of phosphodiester bonds between juxtaposed 5' phosphate and a 3' hydroxyl terminus of double stranded DNA. It can repair single stranded nicks in double stranded DNA and join double stranded DNA restriction fragments having either blunt ends or homologous cohesive ends. (Fig. 1). E. coli ligase and T4 DNA ligase are the two DNA ligases used in nucleic acid research. They differ in their requirement of energy source and in their ability to ligate blunt ends. T4 DNA ligase is approximately 60000 dalton (60 kD) protein produced by Bacteriophage T4 requiring ATP as the energy source. T4 DNA ligase has the unique ability to join sticky and blunt ended fragments. Cohesive

end ligation is carried out at 12°C to 16°C to maintain a good balance between annealing of ends and activity of the enzyme. If reaction is set at higher temperatures annealing of the ends become difficult, while lower temperatures diminish the ligase activity.

In this kit, λ/EcoR I digest supplied, is obtained by digestion of λ DNA with EcoR I. EcoR I having 5 sites on λ DNA, produces 6 fragments of varied sizes with cohesive ends. By the action of ligase enzyme, the fragments will be joined end to end. Following ligation, the samples will be analyzed by agarose gel electrophoresis, wherein the ligated sample will appear as a single band as against 6 bands of λ/EcoR I digest.

### Kit Contents : ▶

- ♦ λ/ EcoR I Digest
- ♦ 2X Ligase Assay Buffer
- ♦ T4 DNA Ligase
- ♦ Gel Loading Buffer
- ♦ Agarose
- ♦ 50X TAE
- ♦ 1.5 ml vials



Ligated sample electrophoresed on 1% agarose gel. (Stained with EtBr)  
Lane 1 : λ/EcoR I Digest  
Lane 2 : λ/EcoR I Digest after ligation

From the gel, one can observe that the six double stranded fragments formed by digestion of λ DNA with EcoR I are ligated by T4 DNA Ligase to give a single band.

## Ordering Information:

Cat. No	PI No.	Product Description
6100800011730	KT08A	GeNei™ Ligation Teaching Kit, 5 expts.
6100800021730	KT08B	GeNei™ Ligation Teaching Kit, 20 expts.

# GeNei™ DNA Molecular Size Determination Teaching Kit

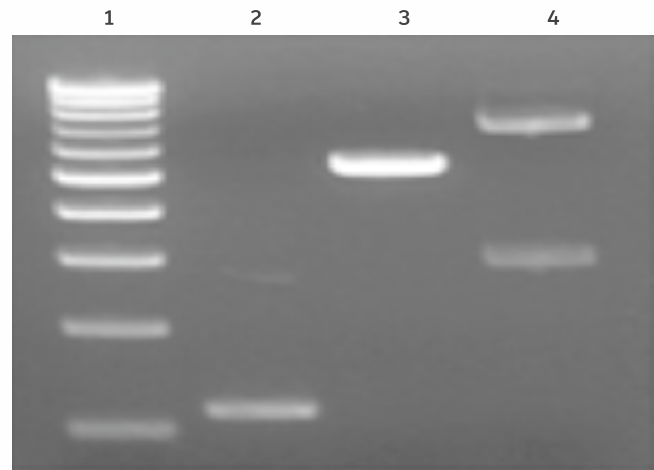
## Description : ▶

Agarose gel electrophoresis is a commonly used technique for resolving nucleic acids. It can also be used to determine the size of linear double stranded DNA molecule by comparing its electrophoretic mobility with that of Standard molecular weight DNA marker. The relative molecular size of a gel fractionated macromolecule (band) is determined from a standard curve that is based on a set of macromolecules of known relative molecular size (DNA marker) that covers the separation range of the gel system and is loaded on one or both of the outer lanes of the same gel as the samples. The log of the relative molecular size of a DNA marker is related to its relative mobility ( $R_f$ ) through a gel. The value  $R_f$  is defined as the distance traveled by a band divided by the distance traveled by the tracking dye. The relationship between the logarithm of the relative molecular size of each band of Standard DNA marker and its  $R_f$  value is plotted. Using this standard curve, molecular size of test samples can be determined. The standard DNA markers are included in the same gel as the test samples, as the extent of mobility of a macromolecule(s) varies from one electrophoretic run to the next.

Using this kit, students will determine the molecular size of four linear double stranded DNA fragments. These samples are electrophoresed along with a Standard molecular weight DNA marker. The marker is a 500 bp ladder wherein 10 bands are visualized ranging from 500 bp to 5000 bp. By measuring the distances migrated from the well by each of the test samples, marker and dye, the size of the test samples will be determined.

## Kit Contents : ▶

- ◆ StepUp™ 500 bp DNA Ladder (Ready to use)
- ◆ Test sample 1
- ◆ Test sample 2
- ◆ Test sample 3
- ◆ 6X Staining dye
- ◆ 50X TAE Agarose

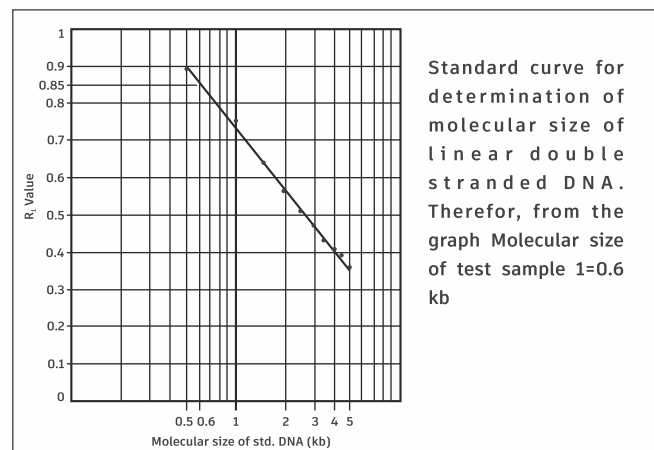


StepUp™ 500 bp DNA ladder & Test samples electrophoresed on a 1% agarose gel. (Stained with EtBr)

Lane 1 : StepUp™ 500 bp DNA Ladder      Lane 3 : Test Sample 2  
Lane 2 : Test Sample 1                      Lane 4 : Test Sample 3

SI No.	Molecular size of Standard (in kb)	Distance traveled by DNA (in cm)	$R_f$ Value
1	0.5	7.4	0.89
2	1.0	6.3	0.75
3	1.5	5.35	0.64
4	2.0	4.7	0.57
5	2.5	4.3	0.51
6	3.0	3.9	0.47
7	3.5	3.65	0.43
8	4.0	3.45	0.41
9	4.5	3.3	0.39
10	5.0	3.1	0.36
11	Test sample	7.15	0.85

$R_f$  values of Standard molecular weight DNA marker.



## Ordering Information:

Cat. No	PI No.	Product Description
6106600011730	KT66A	GeNei™ DNA Molecular Size Determination Teaching Kit, 5 expts.

## DNA Quantitation Teaching kit

### Description : ▶

After isolation of DNA, quantification and analysis of quality are necessary to ascertain the approximate quantity of DNA obtained and the suitability of DNA sample for further analysis. This is important for many applications including digestion of DNA by restriction enzymes or PCR amplification of target DNA. The most used methodologies for quantifying the amount of nucleic acid in a preparation are:

- ◆ Agarose Gel Electrophoresis Method DNA can be quantitated in an agarose gel by comparing the intensity of the fluorescence emitted by an ethidium bromide-stained DNA sample relative to a dilution series of a DNA standard of known concentration
- ◆ Determination of DNA Concentration Analysis of UV absorption by the nucleotides provides a simple and accurate estimation of the concentration of nucleic acid in a sample. Purines and pyrimidines in nucleic acid show absorption maxima around 260nm (eg., dATP: 259nm; dCTP: 272nm; dTTP: 247nm) if the DNA sample is pure without significant contamination from proteins or organic solvents (DNA concentration can be estimated by measuring the absorbance at 260nm (A<sub>260</sub>), adjusting the A<sub>260</sub> measurement for turbidity (measured by absorbance at 320nm), multiplying by the dilution factor, and using the relationship that an A<sub>260</sub> of 1.0 = 50µg/ml pure DNA.
- ◆ Colorimetric Determination of DNA Concentration: Diphenylamine Reaction DNA and RNA are nucleic acids made of nucleotide subunits. One major difference between DNA and RNA is their sugar: DNA contains deoxyribose, whereas RNA contains ribose. DNA can be identified chemically with the Dische diphenylamine test. The reaction between the Dische reagent and 2-deoxypentose results in the development of a blue color. The reaction depends on the conversion of the pentose to -

hydroxylaevalinic aldehyde which then reacts with diphenylamine to give a blue colored complex. The intensity of the blue color is proportional to the concentration of DNA. Dische reagent does not react with the ribose sugar in RNA and does not form a blue-colored complex. The concentration of colored product is proportional to the initial concentration of the DNA being assayed. Since optical density is proportional to the concentration of colored substances, the optical density will also be proportional to the concentration of the DNA being assayed.

In this kit students will determine the concentration of DNA by colorimetric method and spectrophotometric method. In colorimetric method the Standard DNA Solution and the Test DNA Solution will be made to react with Diphenylamine (DPA) which results in the hydrolysis of DNA to produce a blue colored compound. The intensity of this colored compound will be measured at 660nm in a spectrophotometer or colorimeter and a standard curve will be plotted using the absorbance values of the Standard DNA Solution. The concentration of the Test DNA Solution will be determined by comparing with a standard curve. In spectrophotometric method the Test DNA Solution will be diluted, and its concentration will be determined by measuring the intensity of absorbance of the Test DNA Solution at a wavelength of 260 nm in a spectrophotometer.

### Kit Contents : ▶

- ◆ Diphenylamine (DPA)
- ◆ Standard DNA Solution
- ◆ Test DNA solution

### Ordering Information:

Cat. No	PI No.	Product Description
6121900011730	KT219	DNA Quantitation Teaching kit, 5 expts.

## NUCLEIC ACID ISOLATION AND PURIFICATION:

### GeNei™ Plasmid Preparation Teaching Kit

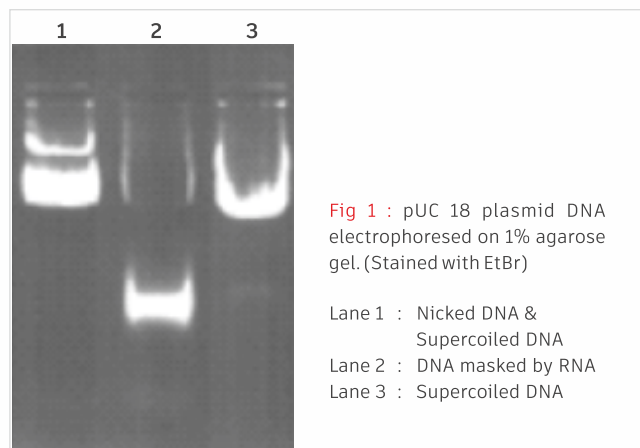
Plasmids carry genes that offer bacterial genetic advantages, such as resistance to antibiotics. During bacterial cell division, all the plasmids within the cell are duplicated, ensuring that each daughter cell inherits a copy of every plasmid. This mechanism has marked its significance in recombinant DNA technology.

#### This Kit demonstrates.

- ◆ The mini preparation of pUC<sub>18</sub> plasmid using the alkali lysis method from E. coli strain. It is a commonly used cloning vector of size 2686 bp with an Ampicillin resistance gene.
- ◆ Following isolation and RNase treatment, samples will be analyzed by agarose gel electrophoresis.
- ◆ Interpretation of the quality and quantity of the extracted plasmid DNA.

#### Kit Contents : ▶

- ◆ Ampicillin
- ◆ Control DNA (Ready to Use)
- ◆ Solution I, II and III
- ◆ 2.5X Gel Loading Buffer
- ◆ Host ( Lyophilised Vial)
- ◆ RNase A
- ◆ 1X TE
- ◆ Agarose
- ◆ Solution IV
- ◆ 50X TAE
- ◆ LB Broth
- ◆ Agar
- ◆ 1.5 ml Vials



#### Ordering Information:

Cat. No	PI No.	Product Description
6100600011730	KT06A	GeNei™ Plasmid Preparation Teaching Kit, 25 expts. (5 x 5 preps)
6100600021730	KT06B	GeNei™ Plasmid Preparation Teaching Kit, 100 expts. (20 x 5 preps)

### GeNei™ Plasmid DNA Purification Teaching Kit, 10 expts. (Binding Membrane)

#### Description : ▶

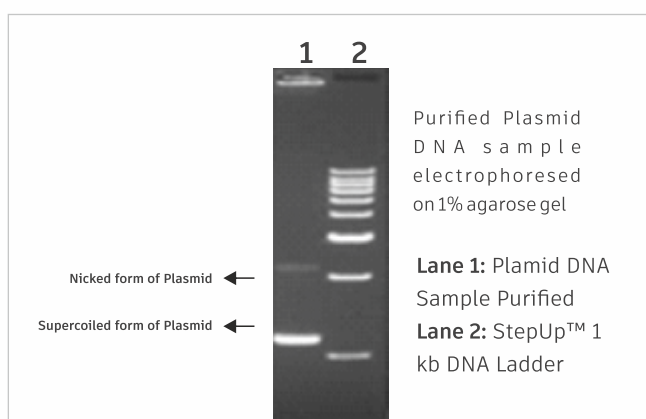
Silica gel membrane technology yields high purity nucleic acids suitable for most molecular biology applications such as restriction digestion, ligation, labeling, PCR, hybridization etc. Silica gel membrane selectively binds either RNA or DNA and separates nucleic acids. Optimized buffers (Binding Buffer and Wash Buffer) are used to obtain maximum separation between the nucleic acids during adsorption and washing steps. The purification steps involve a simple bind wash-elute procedure. Nucleic acids are adsorbed to the silica-gel membrane in the presence of high concentrations of chaotropic salts. Polysaccharides and proteins do not adsorb and are removed in the wash step of the

procedure. Nucleic acids are eluted under low salt conditions in small volumes, and these are ready to be used in any downstream applications without further concentration.

The kit contains reagents and silica membrane columns for purification of plasmid DNA from E.coli strain. Bacterial cells are harvested, lysed by alkaline lysis method using solution G1, G2, G3 buffers and the lysate is cleared by centrifugation. The lysate is then applied to the silica-gel membrane column where plasmid DNA adsorbs to the silica gel membrane. Impurities are washed away and pure DNA is eluted in a small volume of elution buffer.

### Kit Contents : ▶

- ◆ Solution G1,G2,G3
- ◆ Wash Buffer I
- ◆ Wash Buffer II
- ◆ Elution Buffer
- ◆ Spin Column
- ◆ RNase A
- ◆ 2 ml Collection Tubes
- ◆ Lyophilized Strain
- ◆ Ampicillin
- ◆ 1.5 ml Vials Agarose 50X TAE Control DNA
- ◆ Instruction Manual



### Ordering Information:

Cat. No	PI No.	Product Description
6112600011730	KT126A	GeNei™ Plasmid DNA Purification Teaching Kit, 10 expts. (Binding Membrane)

## GeNei™ Genomic DNA Extraction Teaching Kit, (from Bacteria)

The genetic material (genome) in bacteria is not very well organized as compared to the eukaryotic genome, which is highly condensed and is present as nucleosomes. Hence extraction of bacterial genomic DNA is simple. Three major types of techniques or a combination of them are employed in isolation of nucleic acids: differential solubility, adsorption methods or density gradient centrifugation. The choice of the method depends on the source of DNA being isolated and its application. A major goal of nucleic acid isolation is removal of proteins. This is accomplished due to differences in their chemical properties.

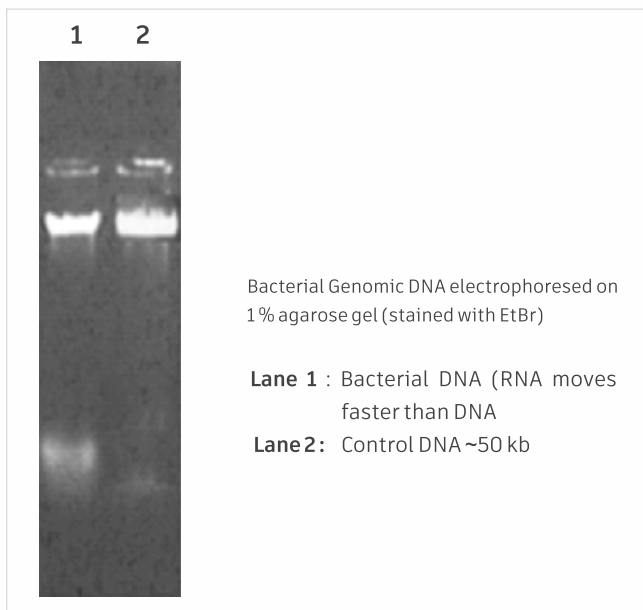
Most nucleic acid isolation protocols involve the following steps:

- ◆ Cell lysis step
- ◆ Enzymatic treatment
- ◆ Differential solubility (phenol extraction or adsorption to solid support) and
- ◆ Precipitation

### Kit Contents : ▶

- ◆ Bacterial Lyophilized vial
- ◆ Control DNA (Ready to use)
- ◆ LB Broth
- ◆ Agar
- ◆ Solution A
- ◆ Solution B
- ◆ Alcohol
- ◆ Agarose
- ◆ Gel Loading Buffer
- ◆ 50X TAE
- ◆ 1.5 ml Vials





centrifugation. The choice of the method depends on the type of the DNA being isolated and the application. The Major goal of nucleic acid isolation is removal of proteins, which is accomplished due to their different chemical properties. Most nucleic acid isolation protocols involve:

- ◆ Cell lysis step
- ◆ Enzymatic treatments
- ◆ Differential solubility (phenol extraction or adsorption to solid support)
- ◆ Precipitation.

### Kit Contents : ▶

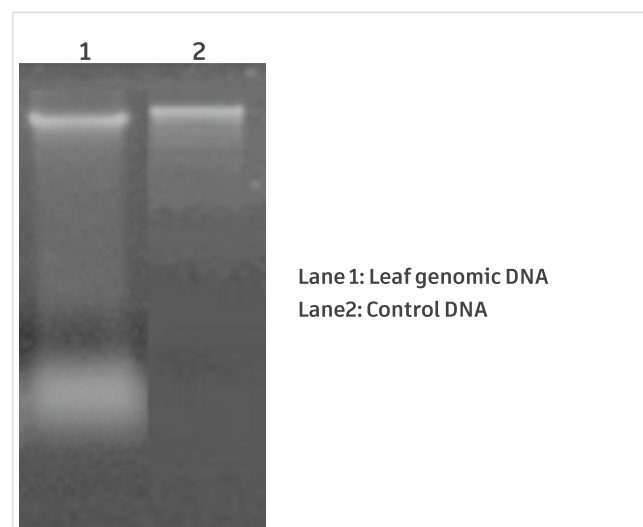
- ◆ Solution A
- ◆ Solution B
- ◆ Sodium acetate
- ◆ Alcohol
- ◆ Control DNA (ready to use)
- ◆ Gel Loading Buffer
- ◆ Agarose
- ◆ 50X TAE

Cat. No	PI No.	Product Description
6102800011730	KT28A	GeNei™ Genomic DNA Extraction Teaching Kit, (from Bacteria) 10 expts
6102800021730	KT28B	GeNei™ Genomic DNA Extraction Teaching Kit, (from Bacteria) 20 expts

## GeNei™ Genomic DNA Extraction Teaching Kit (from leaves)

### Description : ▶

Plants contain three types of DNA: nuclear, mitochondrial and chloroplast. Although quite elaborate methods exist for the isolation of each type of DNA, most experiments require only a simple preparation of total DNA. The two main problems in isolating DNA from plants are the presence of DNases that degrade the DNA and the presence of other macromolecules (polysaccharides, polyphenols) that co-purify with or polymerize to the DNA during isolation procedure. Three major techniques or a combination of them are employed in isolation of nucleic acids: differential solubility, adsorption methods or density gradient



### Ordering Information:

Cat. No	PI No.	Product Description
6106700011730	KT67A	GeNei™ Genomic DNA Extraction Teaching Kit (from leaves) 5 expts
6112500011730	KT125A	GeNei™ Fungal Genomic DNA Extraction Teaching Kit, 10 expts.

## GeNei™ Fungal Genomic DNA Extraction Teaching Kit

### Description :▶

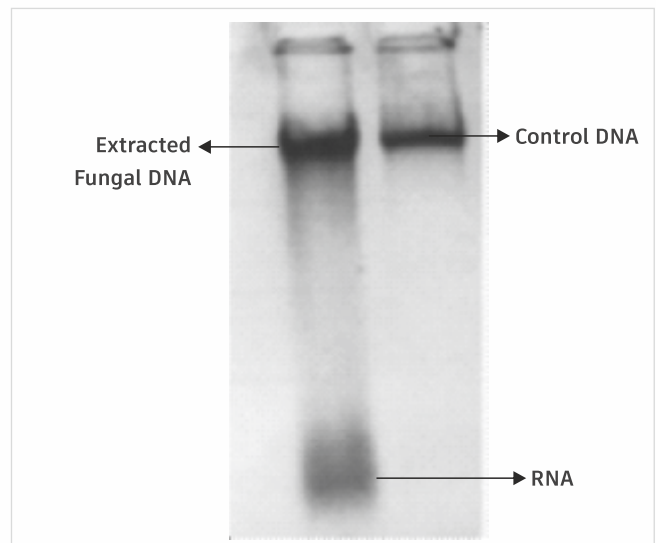
To analyze the complex genome of eukaryotes, it is necessary to prepare pure and high molecular weight genomic DNA. Varied protocols are available for the purification of genomic DNA from fungal mycelium. The principle involves breakage of cells to release nuclei and subsequent treatment with detergents and salt to degrade most of the contaminating proteins. Fungal species are known to produce a lot of proteins and exo-polysaccharides which interfere with the extraction of DNA. The contaminating molecules are effectively removed by Cetyl trimethylammonium bromide (CTAB). CTAB complexes with both polysaccharides and residual proteins and these are effectively removed by

chloroform:isoamyl alcohol extraction. The DNA is further recovered by alcohol precipitation. This procedure is effective in producing a good yield of genomic DNA with minimal shearing.

In this kit, Fungal Cell Pellets are provided which will be lysed using the Lysis Buffer containing CTAB and detergent (SDS), using a tissue grinder. The Lysis buffer apart from cell lysis, effectively removes proteins and polysaccharides by forming a complex. This complex is removed by a mixture of Chloroform: Isoamylalcohol. The DNA is then precipitated with alcohol. The DNA will then be solubilized in solution D and the extracted DNA will be analysed by agarose gel electrophoresis along with Control Genomic DNA provided.

### Kit Contents :▶

- ◆ Fungal Cell Pellet
- ◆ Control DNA
- ◆ Lysis Buffer A
- ◆ Solution B
- ◆ Solution D
- ◆ 2.5X Gel Loading Buffer
- ◆ Alcohol
- ◆ Agarose
- ◆ 50X TAE
- ◆ Tissue Grinder



### Ordering Information:

Cat. No	PI No.	Product Description
6112500011730	KT125A	GeNei™ Fungal Genomic DNA Extraction Teaching Kit, 10 expts.

## GeNei™ Whole Blood DNA Extraction Teaching Kit

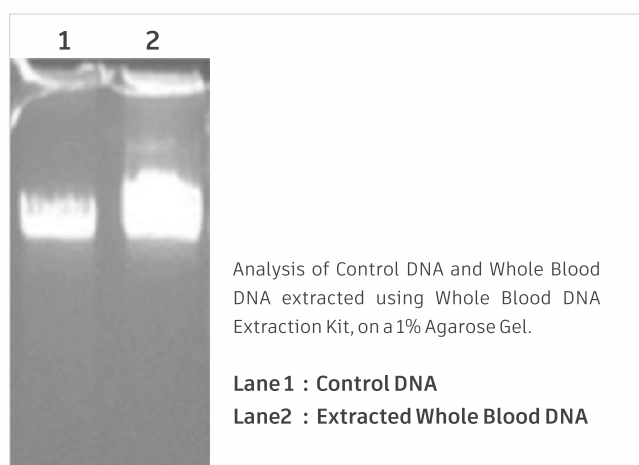
### Description : ▶

Blood is a complex mixture of cells, proteins, metabolites, and many other substances. About 56% of human blood volume is comprised of cells, more than 99% of which are erythrocytes. Human erythrocytes and thrombocytes platelets (which constitutes 0.5% of blood components) do not contain nuclei and are unsuitable for preparation of genomic DNA, the only blood cells that contain nuclei are leukocytes (0.3% of cellular blood components). The number of leukocytes / WBC vary in different blood samples depending upon the health of the donor (human/animal). Healthy blood samples contain fewer leukocytes compared to infected blood samples. Different protocols are available for the extraction of genomic DNA from blood for various downstream applications. This kit provides an easy, safe, reproducible method of purification. Blood sample is used for extraction from which the serum is separated by centrifugation. RBC lysis buffer supplied contains detergents and salts which creates a hypertonic condition resulting in lysis of RBC. The white pellet (ie WBC) then obtained is lysed with a buffer containing Guanidium thiocyanate salt and detergents which effectively remove the contaminating proteins releasing the DNA. The DNA is precipitated by alcohol and rehydrated in Tris buffer.

In this kit all the reagents required to extract Genomic DNA from fresh blood are provided. Use EDTA coated tubes to collect blood sample. Blood is centrifuged to remove the serum followed by lysis of RBCs by Solution A. Whitish pellet of WBC obtained is lysed by Solution B which is a Guanidium thiocyanate-based buffer. DNA is then precipitated by alcohol and the DNA pellet rehydrated using Solution C (Tris buffer). Extracted DNA is then analysed by agarose gel electrophoresis and compared along with control DNA provided.

### Kit Contents : ▶

- ◆ Solution A (3X)
- ◆ Solution B
- ◆ Solution C
- ◆ Control DNA (Ready to use)
- ◆ Alcohol
- ◆ 2.5X Gel Loading
- ◆ Buffer EDTA
- ◆ Agarose
- ◆ 50X TAE
- ◆ 1.5 ml vials
- ◆ Instruction Manual



### Ordering Information:

Cat. No	PI No.	Product Description
6112900011730	KT129A	GeNei™ Whole Blood DNA Extraction Teaching Kit, 10 expts.

## GeNei™ Plant Mitochondrial DNA Isolation Teaching Kit

### Description : ▶

Mitochondrial DNA (mtDNA) is a small, maternally inherited molecule which has been used to study genetic variation, particularly the relationships between different taxa. In order to screen large sample sizes a single and inexpensive method of mitochondrial DNA isolation is essential. Mitochondrial DNA isolation is done by

conventional Cesium chloride gradient centrifugation. In the basic method mitochondrial DNA is contaminated by the nuclear DNA and DNA from other organelles. Nuclear DNA can be removed by DNase treatment, Cesium chloride gradient separation or physical separation e.g. by using a column.

Using this kit the students will isolate mitochondrial DNA (mtDNA) from plant samples. Reagents are provided to perform DNA isolation from plant mitochondria, starting from leaf or cotyledons. The mtDNA isolated would be further amplified using mitochondrial and/or nuclear specific primers for confirmation of the mtDNA along with plant nuclear DNA provided, as control. The amplified products are then analyzed on 1.5% agarose gel along with a marker. The students will observe a single band with mitochondrial primers for mitochondrial DNA but not with nuclear DNA supplied as control. StepUp™ DNA marker is a 100bp ladder wherein, 10 bands are seen ranging from 100bp to 1Kb.

### Kit Contents : ▶

- ▶ Dnase I
- ▶ Control DNA
- ▶ Taq DNA polymerase
- ▶ 10X Taq DNA Buffer
- ▶ 10mM dNTP mix
- ▶ Primer Set I - FP
- ▶ Primer Set I - RP
- ▶ Primer Set II - FP
- ▶ Primer Set II - RP
- ▶ Nuclease Free Water
- ▶ StepUp™ 100 bp DNA Ladder
- ▶ 2.5X Gel Loading Buffer
- ▶ Buffer Mft - 1
- ▶ Buffer Mft - 2
- ▶ Buffer Mft - 3
- ▶ Buffer Mft - 4
- ▶ Buffer Mft - 5
- ▶ Buffer Mft - 6
- ▶ Buffer Mft - 7
- ▶ Agarose
- ▶ Mineral Oil
- ▶ 50X TAE
- ▶ PCR Tubes



**Lane 1 :** Test DNA with Primer Set I  
**Lane 2 :** Control DNA with Primer Set II  
**Lane 3 :** Control DNA with Primer Set I  
**Lane 4 :** StepUp™ 100 bp DNA ladder

Analysis of PCR products of plant mitochondrial DNA on a 1.5% agarose gel.

Lane # 1 shows a band (350 bp) specific for a house keeping gene of Mitochondrial DNA. The amplified product in lane # 2 shows amplification of a nuclear housekeeping gene (700 bp). The condition has been optimized to give highly specific products of 350 bp (for mitochondrial specific locus) and 700 bp (for nuclear specific locus). This shows that mitochondrial DNA was successfully isolated from plant tissue.

### Ordering Information:

Cat. No	PI No.	Product Description
6114800011730	KT148A	GeNei™ Plant Mitochondrial DNA Isolation Teaching Kit, 5 expts.

## GeNei™ Total RNA Extraction Teaching Kit

### Description : ▶

Ribonucleic acid (RNA) is a nucleic acid polymer consisting of covalently bound ribonucleotides which contain ribose sugar, a phosphate and a base (uracil or adenine and guanine or cytosine) RNA is transcribed from DNA, and it serves as the template (mRNA) for translation of genes into proteins, transferring amino acids to the ribosome to form proteins (tRNA) and also translating the transcript into proteins (rRNA). Living cell whether prokaryotic/eukaryotic contain three major types of RNA: ribosomal RNA (rRNA), transfer RNA (tRNA) & messenger RNA (mRNA). For eg: A single mammalian cell contains a total of approximately  $1 \times 10^{-5}$   $\mu$ g of RNA of which 80 - 85% is ribosomal RNA (rRNA) with three subunits of 28s, 18s and 5s. 15-20% is composed of a variety of low molecular weight species transfer RNA (tRNA), small nuclear RNAs etc and 1-5% is messenger RNA (mRNA).

The basic steps involved in the isolation of RNA are:

- ◆ Disruption of cells or tissue
- ◆ Denaturation of nucleic acid: protein complexes.
- ◆ Inactivation of endogenous ribonucleases.

- ◆ Purification of the RNA from contaminating DNA and protein.

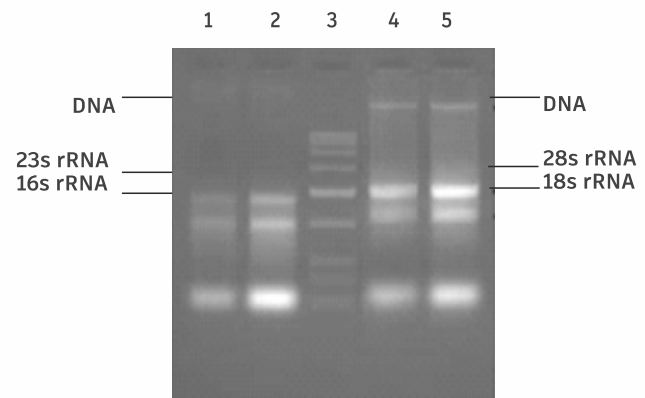
RNA is generally isolated by using strong denaturant like guanidine thiocyanate and phenol-chloroform with a reducing agent, β-mercaptoethanol, to inhibit the RNase activity. These methods work well but require the use of toxic compounds and are cumbersome. Most methods do not yield DNA free RNA and would further require the samples to be treated with DNase I. Other methods include the use of Lithium chloride to selectively precipitate RNA

Using this kit, students will isolate total RNA from a prokaryote (*E. coli* strain) and a eukaryote (green gram sprout). Protocol used in this kit does not involve use of toxic chemicals and can be completed within a short time. However, the method employed does not remove DNA completely and hence it is seen as a contaminating band (as indicated in figure 1). The purpose of the kit is only to show the different steps involved in extracting total RNA using this kit include:

- ◆ Homogenization
- ◆ Phase Separation
- ◆ RNA Precipitation
- ◆ RNA Wash
- ◆ RNA Solubilization

### Kit Contents : ▶

- ◆ Proteinase K
- ◆ DTT
- ◆ Low Range DNA Ruler (Ready to Use)
- ◆ Nuclease Free Water
- ◆ 2.5X Gel Loading Buffer
- ◆ Extraction Buffer
- ◆ 3M Sodium acetate
- ◆ E.coli (Lyophilized vial)
- ◆ Alcohol
- ◆ Tissue Grinder
- ◆ Agarose
- ◆ 50X TAE 40
- ◆ LB Broth
- ◆ Agar



Lane 1 & 2 : Total RNA from *E. coli* (4 µl and 8 µl)  
 Lane 3 : Low Range DNA Ruler  
 Lane 4 & 5 : Total RNA from green gram sprout (2 µl and 4 µl)

Total RNA extracted from *E. coli* and green gram sprout, electrophoresed on 1.5% agarose gel stained with EtBr.

**Note:** Low Range DNA Ruler supplied is not for sizing the rRNA bands

### Ordering Information:

Cat. No	PI No.	Product Description
6113200011730	KT132A	GeNei™ Total RNA Extraction Teaching Kit, 10 expts.

## GeNei™ Gel Extraction Teaching Kit (Solution Based)

### Description : ▶

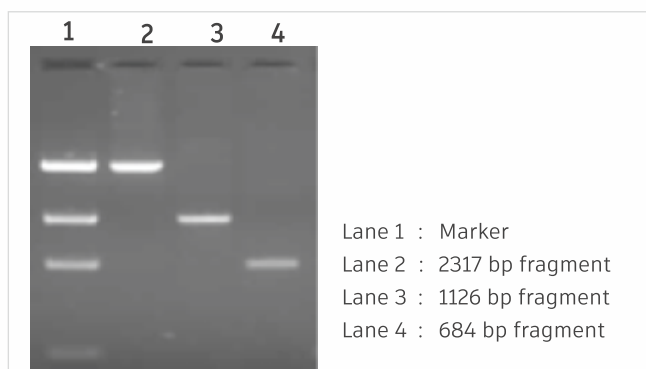
binds Electrophoresis of DNA using polyacrylamide or agarose gels is one of the core techniques used in molecular biology. This method is used to separate, identify and purify DNA fragments. Agarose gels can be used to effectively separate fragments from 50 bp to several thousand bases in length by varying the porosity of the gel and application of current. Migration of DNA through the pores of gel depends upon the size and conformation of DNA. Electrophoresed DNA can be purified from gels by a number of methods such as: Electroelution, electrophoresis onto DEAE Cellulose / Nitrocellulose (NA45) paper, using β-Agarase (from Low Melting Agarose) or using glass beads / silica etc. In this kit, DNA is purified from agarose gel using silica / glass powder of a specified size. It is based on the fact that DNA to silica under specific conditions of salt and pH. This method works best for purification of fragments between approximately 500 bp to 5000

bp as DNA of smaller sizes (< 500 bp) bind strongly to silica thereby resulting in lower yields, while larger DNA fragments (> 5 kb) tend to get sheared by glass beads. DNA binds to silica in presence of a chaotropic salt, Sodium iodide (NaI) independent of its base composition and topology. Chaotropic salts are known to disrupt the hydrogen bonds of water, thus increasing the solubility of non-polar substances in water. Hence, due to dehydration of phosphodiester backbone by chaotropic salts, the exposed phosphate residues of DNA adsorb to silica. Once adsorbed, double stranded DNA remains in either native / partially denatured state and cannot be eluted from the matrix by solvents that displace other biopolymers such as RNA / carbohydrates / proteins. When rehydrated with aqueous buffer, DNA is eluted.

In this kit, students are provided with a DNA Marker, having 4 fragments of sizes 2317 bp, 1126 bp, 684 bp and 234 bp. These fragments will be separated by electrophoresis, excised out of the gel and only first 3 fragments will be purified individually using silica. Each purified fragment will then be analyzed on agarose gel, along with Marker

### Kit Contents : ▶

- ◆ DNA Marker
- ◆ Silica
- ◆ Sodium iodide Solution
- ◆ 1X TE
- ◆ 2.5X Gel Loading Buffer
- ◆ Wash Buffer
- ◆ Agarose
- ◆ 50X TAE
- ◆ 6X Staining Dye
- ◆ 1.5 ml Vials



Purified Marker fragments electrophoresed on 1% agarose gel (Stained with EtBr)

### Ordering Information:

Cat. No	PI No.	Product Description
6104300011730	KT43A	GeNei™ Gel Extraction Teaching Kit (Solution Based), 5 expts.

## GeNei™ Gel Extraction Teaching kit (membrane based)

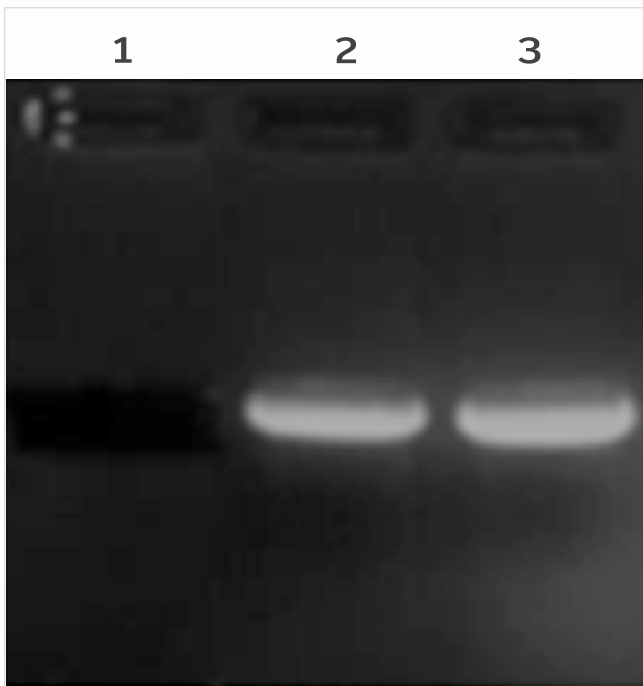
### Description : ▶

Electrophoresed DNA can be purified from the gel by a number of methods such as electroelution, electrophoresis onto DEAE Cellulose / Nitrocellulose (NA45) paper, using β-Agarase (from low melting agarose) or using glass beads / silica etc. DNA binds to silica in presence of a chaotropic salt, independent of its base composition and topology. Chaotropic salts are known to disrupt the hydrogen bonds of water, thus increasing the solubility of non-polar substances in water. Hence, due to dehydration of phosphodiester backbone by chaotropic salts, the exposed phosphate residues of DNA adsorb to silica. Once adsorbed, double stranded DNA remains in either native / partially denatured state and cannot be eluted from the matrix by solvents that displace other biopolymers such as RNA/carbohydrates/proteins. When rehydrated with aqueous buffer, DNA is eluted out. There is no shearing of DNA. Purification of DNA fragment from agarose gel using silica membrane in spin column format is an improvisation over silica solution. This technique is useful in rapid purification of DNA fragments from agarose gel.

In this kit, students are provided with DNA digest (Ready to use), which has 1 fragment of size 2686 bp. This fragment will be separated by electrophoresis on an agarose gel. The band of interest will be purified using spin columns. The purified fragment will then be analyzed on agarose gel, along with DNA digest.

**Kit Contents : ▶**

- ◆ DNA Digest (ready to use)
- ◆ 2.5X Gel Loading Buffer
- ◆ Gel Solubilizer
- ◆ Wash Buffer (conc. 4X)
- ◆ Diluent for Wash Buffer
- ◆ Binding Buffer
- ◆ Elution Buffer
- ◆ Spin Columns
- ◆ Collection Tubes
- ◆ Agarose
- ◆ 50X TAE
- ◆ 1.5 ml Vials



Analysis of the Purified / Eluted DNA fragment with the control.

Lane 1 : DNA Digest  
 Lane 2 : Eluted DNA  
 Lane 3 : Control DNA Digest

**CLONING & EXPRESSION  
 TECHNIQUES**

**GeNei™ GFP Cloning  
 Teaching Kit**

**Description : ▶**

Molecular cloning, or gene cloning, is a process that encompasses the insertion of a DNA fragment, often a gene of interest, into a cloning vector. The resulting recombinant is then introduced into a compatible host strain, thereby generating the desired clones based on screening.

The GFP Cloning Teaching Kit empowers students with the cloning GFP gene in the following steps.

- ◆ Preparation of competent cells
- ◆ Ligation of the Green Fluorescent Protein (GFP) gene into the linearized pUC vector
- ◆ Transformation of the above ligation mixture.
- ◆ Screening of recombinants observed as luminescent green colonies when exposed to UV light.
- ◆ Calculation of the transformation efficiency

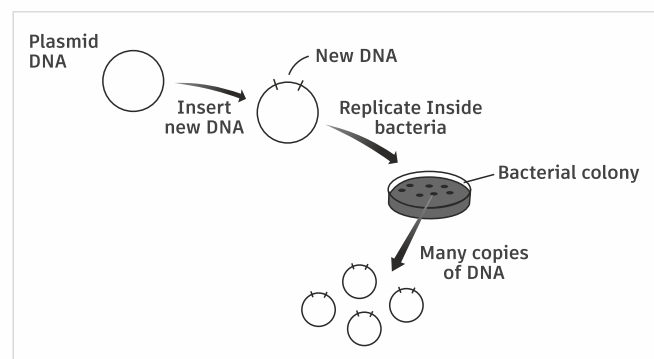


Figure: Illustrating DNA cloning -Ligation and Transformation

**Ordering Information:**

Cat. No	PI No.	Product Description
6113400011730	KT134A	GeNei™ Gel Extraction Teaching kit (membrane based), 5 expts

**Ordering Information:**

Cat. No	PI No.	Product Description
6106000011730	KT60	GeNei™ GFP Cloning Teaching Kit, 5 expts.

## GeNei™ Bacterial Gene Expression Teaching Kit

### Description ▶

Molecular cloning or gene cloning involves insertion of a DNA segment of interest into an autonomously replicating DNA molecule, i.e., a cloning vector. Transforming the vector into a suitable host organism results in the production of large amounts of the inserted DNA fragment. For expression of genes, the insert DNA should be flanked by correctly oriented control sequences for RNA and protein synthesis. Hence, one uses an expression vector, such that the host produces large quantities of RNA and subsequently the protein which can be isolated and purified. Five major expression systems have been developed:

- ◆ Bacterial expression system
- ◆ Yeast expression system
- ◆ Bacillus expression system
- ◆ Baculovirus expression systems
- ◆ Mammalian expression system

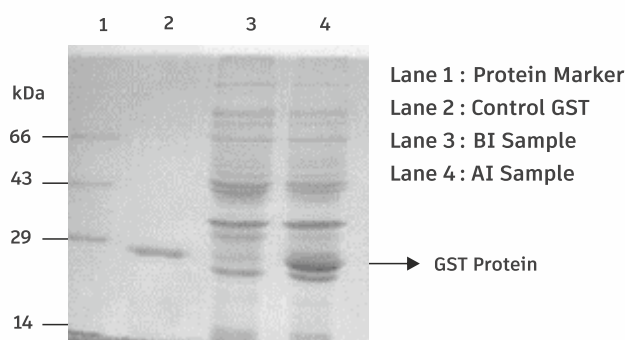
Bacterial expression vectors contain necessary elements for bacterial transcription and translation, including a strong bacterial promoter with appropriate recognition sequences for RNA polymerase. A suitable ribosome binding site (Shine Dalgarno sequence) is required for efficient translation initiation. These sequences are placed in the plasmid at an appropriate distance upstream from the inserted DNA fragment. It is important that the gene is inserted into the plasmid in a proper reading frame to ensure expression of the right protein.

The kit supplies an *E.coli* strain having a recombinant expression vector. Glutathione-S-transferase (GST) gene is cloned into the expression vector having *tac* (*lac* + *trp*) promoter and *lac* I operator elements. Students will grow recombinant cells to mid-log phase and induce it with IPTG to

allow expression of GST protein. Cells will then be lysed and the GST protein (26 kDa) analysed by SDS-PAGE. The expression will be verified by comparing samples before and after induction for the amount of GST protein produced.

### Kit Contents : ▶

- ◆ *E.coli* strain (Lyophilized)
- ◆ Ampicillin
- ◆ 0.1M IPTG
- ◆ Control GST Protein
- ◆ Cell Lysis Buffer
- ◆ Tris-SDS pH 8.8
- ◆ Sample Loading Buffer
- ◆ Protein Marker
- ◆ 30% Acrylamide mix
- ◆ Ammonium persulphate
- ◆ TEMED
- ◆ 10X Reservoir Buffer (Tris Glycine SDS Buffer)
- ◆ Stainer (Ezee blue)
- ◆ LB Broth
- ◆ 1.5 ml vials



GST Protein analyzed on SDS-PAGE before and after IPTG induction.

### Ordering Information:

Cat. No	PI No.	Product Description
6106900011730	KT69A	GeNei™ Bacterial Gene Expression Teaching Kit, 5 expts.



## GeNei™ In-Vitro Transcription Teaching Kit

### Description : ▶

Transcription is the process of RNA synthesis from a DNA template. It is an important step as the genetic information encoded in the DNA is passed onto RNA for synthesis of proteins in vivo. The ability to synthesize RNA in the laboratory is critical to many techniques and is referred to as In-vitro transcription. In-vitro transcription requires a pure linear DNA template containing a promoter, ribonucleotide triphosphates (rNTP), a buffer that includes Dithiothrietol (DTT) and magnesium ions, and an appropriate phage RNA Polymerase. RNA Polymerases are DNA template-dependent with distinct and very specific promoter sequence requirements. The common RNA Polymerases used in in-vitro transcription reactions are SP6, T7 and T3 Polymerases, representing the bacteriophages from which they were cloned. The bacteriophage promoters, T7, T3 and SP6 consist of 23 base pairs numbered -17 to +6, where +1 indicates the first base of the coded transcript. An important observation is that, of the +1 through +6 bases, only the base composition of +1 and +2 are critical and must be a Guanine (G) and purine, respectively, to yield an efficient transcription template.

T7 RNA Polymerase binds to its double stranded DNA promoter and Polymerase separates the two DNA strands to initiate transcription at a specific base in the template. It uses the 3'-5' strand as template for the synthesis of a complementary 5'-3' RNA strand with good fidelity until the end of the lower DNA strand is reached.

Using this kit, students will carry out In-vitro-transcription with two different DNA templates. One of these templates has the T7 promoter region which

will give an RNA transcript while the other template lacking the T7 promoter region will not yield RNA transcript. Following transcription with T7 RNA Polymerase students will analyze the samples on agarose gel, stain the gel then destain with water. On visualizing the gel under UV transilluminator, DNA will be seen as a sharp green fluorescent band & RNA transcript as a diffused orange band.

### Kit Contents : ▶

- ◆ Template 1
- ◆ Template 2
- ◆ Rnase Inhibitor
- ◆ RNase Inhibitor
- ◆ T7 RNA Polymerase
- ◆ 2.5X Gel Loading Buffer
- ◆ 5X T7RNA Polymerase Buffer
- ◆ rNTP Mix
- ◆ Nuclease Free Water
- ◆ Rinse Buffer
- ◆ Staining Solution
- ◆ 50X TAE
- ◆ Agarose

### Ordering Information:

Cat. No	PI No.	Product Description
6113100011730	KT131A	GeNei™ In-Vitro Transcription Teaching Kit, 10 expts.

## PCR RELATED TECHNIQUES

### GeNei™ Student PCR Teaching Kit

PCR-Polymerase Chain Reaction is an in vitro method of enzymatic synthesis of specific DNA sequences. This technique was developed by Kary Mullis in 1983. PCR encompasses the utilization of brief synthetic DNA fragments, known as primers, to target and select the specific genomic segment for amplification. Multiple rounds of DNA synthesis are then executed to magnify the chosen segment. PCR consists of three basic steps:

- ◆ Denaturation: During this step, the two strands of DNA melt open to form single stranded DNA and all enzymatic reactions stop. This is generally carried out at 92°C – 96°C.
- ◆ Annealing: Annealing of primers to each original strand for new strand synthesis is carried out between 45°C – 55°C.
- ◆ Extension: The polymerase adds 2'-deoxynucleoside-5'-triphosphates (dNTPs) complementary to the template at the 3' end of the primers. Since both strands are copied during PCR, there is an exponential increase in the number of copies of the gene.

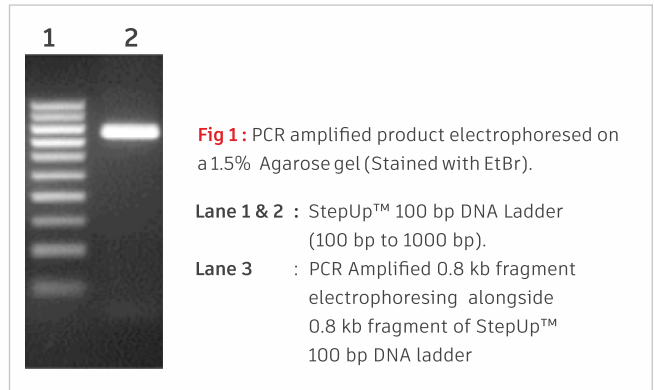
This Kit demonstrates the:

- ◆ PCR amplification of specific target sequence from genomic DNA.
- ◆ Analysis of the amplified product by agarose gel electrophoresis.

#### Kit Contents :▶

- ◆ Taq DNA Polymerase
- ◆ 10X Assay Buffer
- ◆ dNTP Mix
- ◆ StepUp™ 100 bp DNA Ladder (Ready to Use)
- ◆ Template DNA
- ◆ Forward Primer
- ◆ Reverse Primer

- ◆ Nuclease Free Water
- ◆ Mineral Oil
- ◆ Agarose
- ◆ Gel Loading Buffer
- ◆ 50X TAE
- ◆ PCR Tubes
- ◆ dInstruction Manual



#### Ordering Information:

Cat. No	PI No.	Product Description
6104400011730	KT44A	GeNei™ Student PCR Teaching Kit, 10 expts.
6104400021730	KT44B	GeNei™ Student PCR Teaching Kit, 20 expts.

### GeNei™ Multiplex PCR Teaching Kit

#### Description :▶

PCR – Polymerase Chain Reaction is an in vitro method of enzymatic synthesis of specific DNA sequences. This technique was developed by Kary Mullis in 1983. It is a very simple and inexpensive technique for characterizing, analyzing and synthesizing any specific piece of DNA or RNA from virtually any living organism (plant / animal / virus / bacteria). It exploits the natural function of the polymerases, present in all living things, to copy genetic material or perform “Molecular Photocopying.”

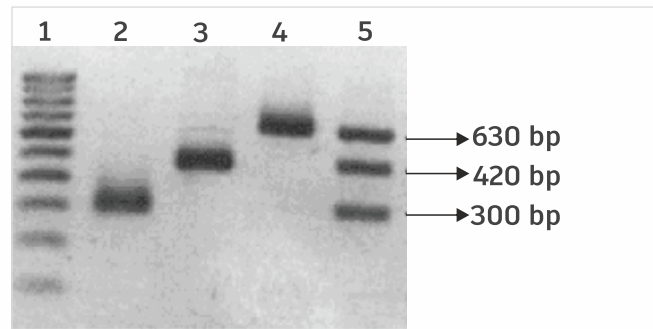
The basic principle of multiplex PCR is the same as PCR, with the variation that two or more loci i.e. 4 or more primers are used to amplify multiple regions at a time. Especially important for the successful multiplex PCR assays are the

- ◆ Relative concentration of primers at the various loci
- ◆ Concentration of PCR buffer
- ◆ Cycling temperature Balance between the MgCl<sub>2</sub> and deoxynucleotide triphosphate concentration.

This kit provides reagents to perform multiplex PCR using DNA template containing the three loci considered for amplification. Using this kit, students will carry out 4 PCR reactions of which 3 primers will give a single product and the fourth will give all the 3 product in multiplex. Six primers (3 forward and 3 reverse) are used to amplify 3 regions of the template that result in three fragments of 300bp, 420bp and 630bp, respectively. The amplified products are then analyzed on a 1.5% agarose gel along with a marker. The DNA marker is a StepUp™ 100bp DNA Ladder wherein, 10 bands are seen ranging from 100bp to 1Kb.

**Kit Contents : ▶**

- ◆ Taq DNA Polymerase
- ◆ 10X Assay Buffer
- ◆ dNTP Mix
- ◆ Template DNA
- ◆ Primer Set #1
- ◆ Primer Set #2
- ◆ Primer Set #3
- ◆ Nuclease Free Water
- ◆ StepUp™ 100 bp DNA Ladder (Ready to Use)
- ◆ 2.5X Gel Loading Buffer
- ◆ Agarose 5g
- ◆ RT Mineral Oil
- ◆ 50X TAE
- ◆ PCR Tubes



PCR amplified product analyzed on 1.5% Agarose gel (Stained with EtBr)

Lane # 1 : StepUp™ 100 bp DNA Ladder  
 Lane # 2 : Primer set # 1  
 Lane # 3 : Primer set # 2  
 Lane # 4 : Primer set # 3  
 Lane # 5 : All the 3 primers

As observed on agarose gel, PCR amplification of the template using specific primers results in a product of a particular size. The PCR conditions have been optimized to give highly specific product of 300, 420, 630 bp as is observed by the absence of any non-specific product. In lane 5 all the primers when used in single reaction gives all three products size.

**Ordering Information:**

Cat. No	PI No.	Product Description
6110700011730	KT107A	GeNei™ Multiplex PCR Teaching Kit, 5 expts.

**GeNei™ GMO Detection Teaching Kit (Simulation Study)**

**Description : ▶**

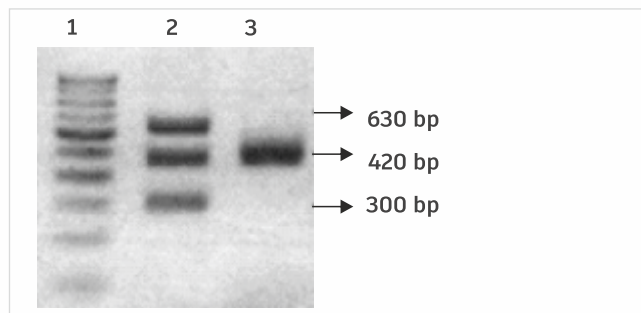
Genetically modified organisms (GMOs) have recently attracted the attention of agricultural, medical, food scientists and governments of many countries in the world due to an increasing concern that the recombinant gene (s) inserted into an organism may result in unforeseen effects. Therefore, there is a need to regulate each transgenic event so that the officially approved events will be the only products for commercial use. However, for controlling the unauthorized use of the unregulated transgenic events, their early detection is necessary. These detection methods are primarily based on identifying the inserted DNA sequence (DNA-based techniques) or the specific proteins

resulted from the inserted gene (protein-based techniques). The DNA based techniques are currently the major detection methods that are widely used due to their ease and accuracy. However, detection for the presence or absence alone is not sufficient for regulation of GMOs. Rather, identification of the transgenic event (authorized or not) and their amount in a given lot should also be quantified to determine the threshold level. Hitherto, PCR (polymerase chain reaction)-based approaches are the most reliable methods for the quantification of genetic modification both in raw as well as processed products. For every country expected to use genetically engineered crops or food products resulted from them, detection and quantification capacity should be readily available.

In this kit reagents are provided to perform multiplex PCR using DNA containing the three loci considered to be amplified as a template. Six primers (3 forward and 3 reverse) are used to amplify 3 regions of the template that result in three bands, 300 bp 420 bp and 630 bp respectively. The amplified products are then analyzed by electrophoresis on 1.5% agarose gel along with a marker. The DNA marker is a StepUp™ 100bp DNA ladder wherein, 10 bands are seen ranging from 100bp to 1Kb.

### Kit Contents : ▶

- ◆ Taq DNA Polymerase
- ◆ 10X Assay Buffer
- ◆ 10 mM dNTP Mix
- ◆ Template DNA
- ◆ Control DNA
- ◆ Primer Set #1
- ◆ Primer Set #2
- ◆ Primer Set #3
- ◆ Nuclease Free Water
- ◆ StepUp™ 100bp DNA Ladder (Ready to Use)
- ◆ Gel Loading Buffer (2.5X)
- ◆ Agarose
- ◆ Mineral Oil
- ◆ 50X TAE
- ◆ PCR Tubes



PCR amplified product electrophoresed on 1.5 % agarose gel (stained with EtBr)

Lane 1 : StepUp™ 100 bp DNA Ladder  
 Lane 2 : Template DNA  
 Lane 3 : Control DNA

Lane 3 shows a single band of the control while PCR product in lane 2 shows amplification of all the 3 bands in a single tube, thus explaining the concept of multiplexing to detect the GMO sample. The condition has been optimized to give highly specific products of 300 bp, 420 bp and 630 bp.

### Ordering Information:

Cat. No	PI No.	Product Description
6110800011730	KT108A	GeNei™ GMO Detection Teaching Kit (Simulation Study), 5 expts.

## GeNei™ RAPD Application Teaching Kit

### Description : ▶

A DNA fingerprint can be called a genetic photograph of an individual. RAPD is a very general method for obtaining a molecular fingerprint of a species or strain. Low stringency PCR amplification of genomic DNA using a single short primer (10-22 mer) of arbitrary sequence is used to generate a set of fragments that is characteristic of the variety from which the DNA was isolated. Each fragment in the profile is the result of hybridization and extension of a primer on opposite DNA strands in the appropriate orientation and separated by such a distance allowing efficient PCR. This random primer is able to bind under appropriate conditions to a number of partially or perfectly complementary sequences at unknown locations in the genome of an organism. If the binding sites occur in a spacing

and orientation that allows amplification of DNA fragments, fingerprinting patterns are produced. By performing similar experiments with different primers and many crop varieties, quantitative data can be derived which can be then used to prepare dendrograms for taxonomic studies. A particular band can also be considered to be a "Mendelian" trait of an organism and can be used as a molecular marker to study the segregation of other traits of economic significance.

This kit demonstrates the sensitivity of the RAPD technique, which differentiates one rice variety from the other. The kit provides 4 samples of rice genomic DNA (4 different varieties), random primer and components for setting up a PCR reaction. The PCR can be performed and then analyzed by electrophoresis of the product on an agarose gel and visualizing the RAPD pattern

All the 4 varieties of rice DNA have generated a signature RAPD profile with minor variations within themselves. The study establishes the robustness of RAPD technique that can distinguish genomes even with minor variations. This variation is seen as a difference in either intensities or number (presence or absence) of DNA bands.

## Ordering Information:

Cat. No	PI No.	Product Description
6110000011730	KT100A	GeNei™ RAPD Application Teaching Kit, 5 expts.
6110000021730	KT100B	GeNei™ RAPD Application Teaching Kit, 20 expts.

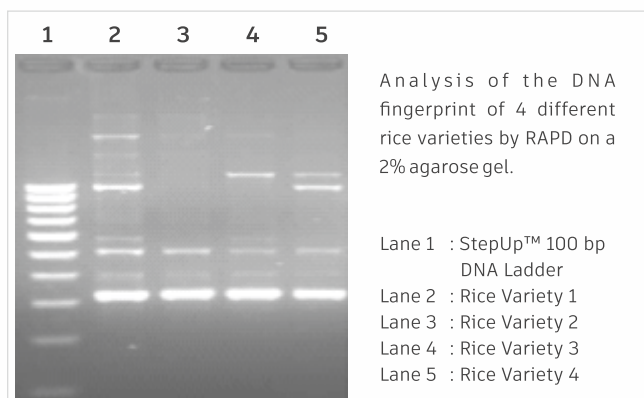
## Kit Contents : ▶

- ◆ Genomic DNA (4 Varieties of Rice DNA)
- ◆ Random Primer
- ◆ Taq DNA Polymerase
- ◆ 10X Assay buffer
- ◆ 10mM dNTP Mix
- ◆ 2.5X Gel Loading Buffer
- ◆ Nuclease Free Water
- ◆ StepUp™ 100bp DNA Ladder (Ready to Use)
- ◆ Mineral Oil
- ◆ 50X TAE
- ◆ Agarose
- ◆ PCR Tubes

## GeNei™ PCR Application Teaching Kit

## Description : ▶

PCR – Polymerase Chain Reaction is an in vitro method of enzymatic synthesis of specific DNA sequences. This technique was developed by Kary Mullis in 1983. It is a very simple and inexpensive technique for characterizing, analyzing and synthesizing any specific piece of DNA or RNA from virtually any living organism (plant / animal / virus / bacteria). It exploits the natural function of the polymerases, present in all living things, to copy genetic material or perform “Molecular Photocopying.” PCR consists of three basic steps: Denaturation: During this step, the two strands melt open to form single stranded DNA and all enzymatic reactions stop. This is generally carried out at 92°C – 96°C. Annealing: Annealing of primers to each original strand for new strand synthesis is carried out between 45°C – 55°C. Extension at 72°C: The polymerase adds 2'-deoxy ribonucleoside-5' triphosphate (dNTPs) complementary to the template at the 3' end of the primers. Since both strands are copied during PCR, there is an exponential increase in the number of copies of the

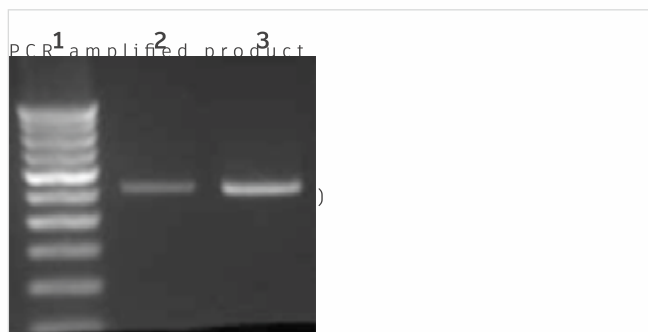


gene. These 3 steps are repeated 20-30 times in an automated thermocycler that can heat and cool the reaction mixture in tube within a very short time. This results in exponential accumulation of specific DNA fragments, ends of which are defined by 5' ends of the primers.

This kit demonstrates the use of a simple and non-invasive protocol for isolation and purification of Human genomic DNA from cells of the buccal mucosa. The Human genomic DNA thus isolated from cells of buccal mucosa is used as a template for the amplification of the 500 bp Interferon gene by PCR using gene specific primers 1 and 2. The amplified product is then analyzed by electrophoresis on a 1.5% agarose gel along with a marker. The DNA marker provided is StepUp™ 100 bp DNA Ladder, with 10 bands ranging from 100 bp to 1 kb.

### Kit Contents : ▶

- ▶ Primer-1
- ▶ Primer-2
- ▶ Control DNA
- ▶ StepUp™ 100 bp DNA Ladder
- ▶ 10X Assay Buffer
- ▶ Taq DNA Polymerase
- ▶ 10 mM dNTP
- ▶ 2.5X Gel Loading Buffer
- ▶ Nuclease Free Water
- ▶ Solution A
- ▶ Solution B
- ▶ Solution C
- ▶ 1.5 ml Vials
- ▶ Scraper
- ▶ Agarose
- ▶ 50X TAE
- ▶ PCR Tubes
- ▶ Mineral Oil



### Ordering Information:

Cat. No	PI No.	Product Description
6110100021730	KT101B	GeNei™ PCR Application Teaching Kit, 20 expts.

## GeNei™ Genotyping Analysis Teaching Kit

### Description : ▶

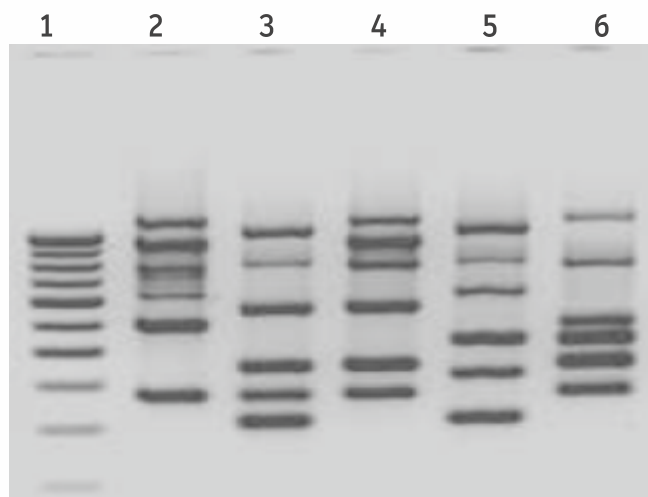
Genotyping is the process of determining differences in the genetic makeup (genotype) of an individual by examining the individual's DNA sequence using biological assays and comparing it to another individual's DNA sequence or a reference sequence. This can be done using methods like Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic Detection (RAPD), Amplified Fragment Length Polymorphism Detection (AFLPD), PCR etc. Microsatellites, also known as Simple Sequence Repeats (SSRs) or Short Tandem Repeats (STRs) are repeating sequences of 2-6 base pairs of DNA. They are used as molecular markers in genetics, for kinship, population and other studies. Microsatellite can be amplified for identification by the Polymerase Chain Reaction (PCR) process, using the unique sequences of flanking regions as primers, thus permitting the amplification of various alleles. Multiplex PCR is a variant of PCR which enables simultaneous amplification of many targets of interest in one reaction by using more than one pair of primers. This method was first described by Chamberlain et. al. in 1988 and is now being widely used in many areas of DNA testing like deletion analysis, mutations and polymorphisms etc. Typically it is used for genotyping applications where simultaneous analysis of multiple markers is required or for detection of pathogens or genetically modified organism (GMO) or for microsatellite analyses.

This kit demonstrates the use of multiplex PCR in context to parentage determination. Two different parent DNA samples (mother - 2 nos and father - 2

nos) and one sample of child are supplied along with a set of primer mix containing primers required for multiplex PCR. Students will carry out PCR amplification of the DNA templates using the primer mixes provided, and analyze the amplified products on agarose gel to determine the parentage of the child.

**Kit Contents : ▶**

- ▶ Template DNA
- ▶ Forward Primer Mix
- ▶ Reverse Primer Mix
- ▶ 10X Assay Buffer
- ▶ 10mM dNTP Mix
- ▶ Taq DNA Polymerase (1 U/μl)
- ▶ StepUp™ 100 bp DNA Ladder (Ready to use)
- ▶ 2.5X Gel Loading Buffer
- ▶ Nuclease Free Water
- ▶ Mineral Oil
- ▶ Agarose
- ▶ 50X TAE
- ▶ PCR Vial



**Fig:** Analysis of amplified products of Multiplex PCR of Parents and Child on a 2% agarose gel

Lane 1 : StepUp™ 100 bp DNA Ladder    Lane 4 : Child  
 Lane 2 : Mother 1    Lane 5 : Mother 2  
 Lane 3 : Father 1    Lane 6 : Father 2

Analysis of amplified products of Multiplex PCR of Parents and Child on a 2% agarose gel.

All the bands present in the child are present either in the father 1 or in the mother 1 (that is F1 and M1 respectively). Hence it can be concluded that couple #1 i.e. M1 and F1 are the real parents of the child.

All the 4 varieties of rice DNA have generated a signature RAPD profile with minor variations within themselves. The study establishes the robustness of RAPD technique that can distinguish genomes even with minor variations. This variation is seen as a difference in either intensities or number (presence or absence) of DNA bands.

**Ordering Information:**

Cat. No	PI No.	Product Description
6110200011730	KT102A	GeNei™ Genotyping Analysis Teaching Kit, 5 expts.

**GeNei™ Student RT PCR Teaching Kit**

**Description : ▶**

Reverse Transcription Polymerase Chain Reaction (RT-PCR) is a modification of polymerase chain reaction for amplifying data contained in RNA. The RNA strand (mRNA) is first reverse transcribed into its DNA complement or complementary DNA (cDNA), a reaction catalyzed by reverse transcriptase. Reverse transcriptase is a common name for the enzyme that functions as RNA Dependant DNA Polymerase. This is encoded by retroviruses which copy viral RNA into DNA prior to its integration into host cells. Thereafter the cDNA is amplified by PCR using thermo stable Taq DNA Polymerase. RT PCR can be done using either total RNA or purified mRNA as the starting material. There are three ways to prime mRNA for cDNA synthesis: either by using Gene specific primers or using Oligo dT or using Random hexamer. In the first method a 3' gene specific primer is annealed to the mRNA and extended with reverse transcriptase. In the second and third method entire population of mRNA molecules are first converted to cDNA, two gene specific primers are then added in the PCR step for amplification. For the eukaryotic mRNA all three methods of priming can be used whereas for prokaryotic mRNA only the gene specific primer and random hexamer can be utilized. The reaction also requires assay buffer, Dithiothreitol (DTT) a reducing agent, deoxyribonucleotides (dNTPs) for

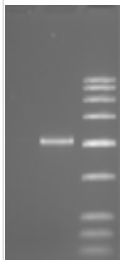
new strand synthesis, RNase inhibitor (RNasin) which inhibits RNase and Moloney murine leukemia virus Reverse transcriptase (MMuLV RT).

The kit provides enough reagents to perform 5 sets of experiments; each set of experiment includes 2 positive reactions and one negative control. Using this kit, the students will perform a cDNA synthesis reaction from Total RNA of Mouse Liver. In the PCR reaction, the cDNA is used as template and GD3PH a house keeping gene is amplified using specific primers. The reactions are carried out in two steps. Step I: Total RNA is taken in a vial and primed with the oligo dT followed by addition of RT enzyme and RT mix and incubated at 37°C to generate cDNA. Step II: cDNA synthesized is taken as template for PCR to amplify the GD3PH gene (~1000 bp fragment) using gene specific primers provided. A negative control experiment will be performed for every 2 positive reactions. For the negative control reaction the students will carryout step I and step II without the addition of Reverse transcriptase (MMuLV RT). The amplified products are analyzed on a 1.5% agarose gel, along will a Low Range DNA Ruler

### Kit Contents : ▶

- ▶ Total RNA
- ▶ Oligo dT Primer
- ▶ Rnasin
- ▶ M-MuLV Reverse Transcriptase
- ▶ RT Mix
- ▶ PCR Mix
- ▶ Taq DNA Polymerase
- ▶ Low Range DNA Ruler (Ready to use)
- ▶ Nuclease Free Water
- ▶ Gel Loading Buffer
- ▶ Mineral Oil
- ▶ Agarose
- ▶ 50X TAE
- ▶ RT PCR Tubes

1 2 3



1.5% Agarose gel showing amplification of GD3PH gene from cDNA using this kit.

**Lane 1** : Negative Control  
**Lane 2** : Positive Control  
**Lane 3** : Low Range DNA Ruler

### Ordering Information:

Cat. No	PI No.	Product Description
6113600011730	KT136A	GeNei™ Student RT PCR Teaching Kit, 4 reactions each, 5 expts.

## DNA FINGER PRINTING TECHNIQUES

### GeNei™ DNA Fingerprinting Teaching Kit (RFLP)

#### Description : ▶

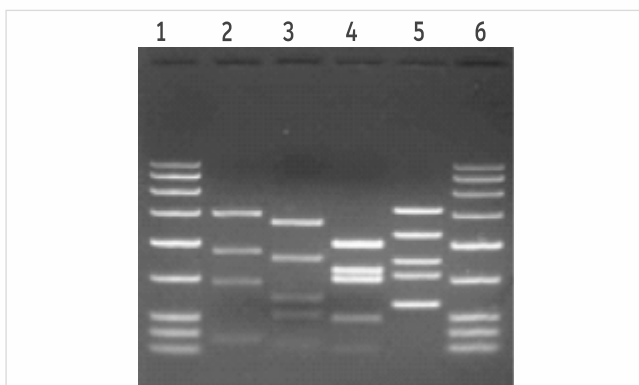
DNA Fingerprinting is a technique that is used to identify No two individual have identical DNA so this procedure can be used to identify if a sample DNA come from a particular individual. The technique requires that the DNA be cutup into small fragments. Restriction enzymes (REs) are used to perform this digestion. The technique takes advantage of the polymorphism in the genetic codes of individuals which result in variations in phenotype. RFLP Methodology involves cutting a particular region of DNA with known variability, with REs, then separating the DNA fragments by Agarose Gel Electrophoresis (AGE) and determining the number of fragments and their relative sizes. RFLP is one technique used by forensic scientists in DNA finger printing. It is also used for tracing ancestry, to study evolution and migration of wildlife and detection and diagnosis of certain diseases

The kit is designed to identify the bacterial isolate that has acquired a Multiple Drug Resistant (MDR) plasmid using RFLP method. Five plasmids isolated from individual isolates (one Control and four Test Samples) are supplied. Following restriction digestion and resolution of fragments on agarose gel, students will compare the DNA band pattern of four Test Samples with that of the Control. On analyzing the results, they will identify the isolate that has acquired the MDR plasmid.



## Kit Contents : ▶

- ▶ Restriction Enzyme: Ssp I
- ▶ 10X Assay Buffer
- ▶ Control DNA
- ▶ Test Samples (1, 2, 3, 4) (each)
- ▶ Low Range DNA Ruler (Ready to use)
- ▶ Gel Loading Buffer (2.5 X)
- ▶ Agarose
- ▶ 50X TAE
- ▶ 1.5 ml vials



DNA Fingerprint from Test plasmid DNA sample

**Lanes 2-5** : Test Plasmid DNA digested with restriction nzymes

**Lanes 1&6** : Low Range DNA Ruler

The Test plasmid DNA that has similar restriction profile as that of MDR Control plasmid DNA, would impart multiple drug resistance to the host. The restriction enzyme recognizes and cuts only a particular base sequence unique to it. Any mutation in this unique sequence, would mean that there is a loss of a site, hence giving rise to different band pattern. It is this specificity that helps in achieving reproducible restriction profile on digestion of a DNA sample.

## Ordering Information:

Cat. No	PI No.	Product Description
6109400011730	KT94A	GeNei™ DNA Fingerprinting Teaching Kit, 5 expts. (RFLP)
6109400021730	KT94B	GeNei™ DNA Fingerprinting Teaching Kit 25 expts. (RFLP)

## GeNei™ DNA Fingerprinting Teaching Kit (RAPD)

### Description : ▶

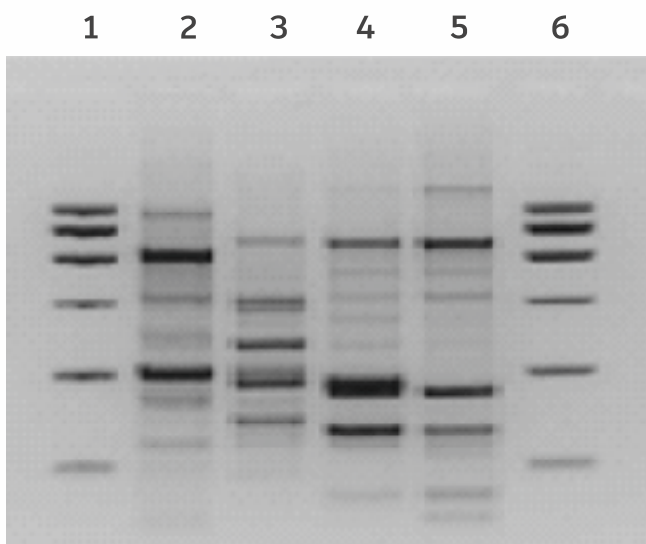
A DNA fingerprint can be called a genetic photograph of an individual. RAPD is a very general method for obtaining a molecular fingerprint of a species or strain. Low stringency PCR amplification of genomic DNA using a single short primer (10-22mer) of arbitrary sequence is used to generate a set of fragments that is characteristic of the variety from which the DNA was prepared. Each fragment in the profile is the result of hybridization and extension of a primer on opposite DNA strands in the appropriate orientation and separated by such a distance allowing efficient PCR. This random primer can bind under appropriate conditions to a number of partially or perfectly complementary sequences at unknown locations in the genome of an organism. If the binding sites occur in a spacing and orientation that allows amplification of DNA fragments, fingerprinting patterns are produced. By performing similar experiments with different primers and many crop/varieties, quantitative data can be derived which can be then used to prepare dendrograms for taxonomic studies. A particular band can also be a "Mendelian" trait of an organism and can be used as a molecular marker to study the segregation of other traits of economic significance.

The kit demonstrates the use of RAPD technique in context of bacterial strain typing / identification. Genomic DNA from four different strains is supplied as reference along with the Test Genomic DNA. Identification of the Test Genomic DNA is done by comparing its pattern with the reference genomic DNA provided. Students will carry out RAPD PCR using random primer, analyze on agarose gel and identify the strain.

### Kit Contents : ▶

- ▶ *Serratia marcescens* Genomic DNA
- ▶ *Bacillus subtilis* Genomic
- ▶ *E.coli* B Genomic

- ▶ E.coli K12 Genomic DNA
- ▶ Test Genomic DNA
- ▶ Random Primer
- ▶ Low Range DNA Ruler (Ready to use)
- ▶ Taq DNA Polymerase
- ▶ 10X Assay Buffer
- ▶ 10 mM dNTP Mix
- ▶ 2.5X Gel Loading Buffer
- ▶ Nuclease Free Water
- ▶ Mineral Oil
- ▶ Agarose
- ▶ 50X TAE
- ▶ PCR vials



Analysis of DNA fingerprints (by RAPD) of Test DNA and 4 Control Genomic DNAs on a 2% Agarose gel.

- Lane 1 & 6 : Low Range DNA Ruler
- Lane 2 : RAPD Pattern of *Serratia marcescens*
- Lane 3 : RAPD Pattern of *Bacillus subtilis*
- Lane 4 : RAPD Pattern of *E. coli B*
- Lane 5 : RAPD Pattern of *E. coli K12*

## Ordering Information:

Cat. No	PI No.	Product Description
6109500011730	KT95A	GeNei™ DNA Fingerprinting Teaching Kit, 5 expts. (RAPD)
6109500021730	KT95B	GeNei™ DNA Fingerprinting Teaching Kit, 25 expts. (RAPD)

## GeNei™ Single Nucleotide Polymorphism (SNP) Teaching kit

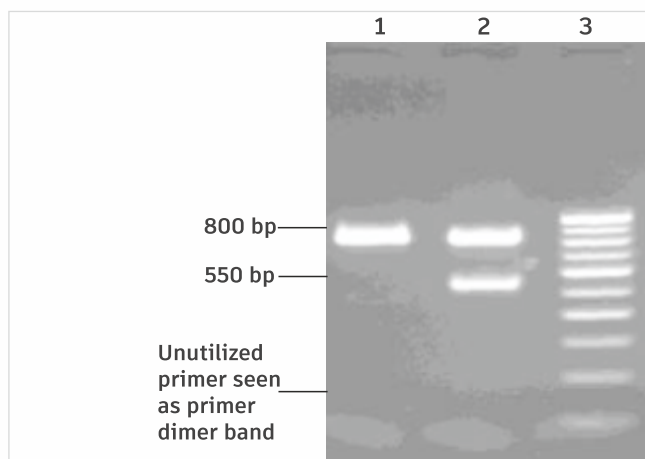
### Description :▶

Single Nucleotide Polymorphism or SNPs (pronounced “snips”) are DNA sequence variations changes that can occur within an individual's DNA sequence. The Genetic code is specified by the four nucleotides A (Adenine), G (Guanine), C (Cytosine) and T (Thymine). SNP occurs when a single nucleotide (A, T, G or C) in the DNA sequence is altered. An example for SNP is AAGGTTA is altered to ATGGTTA where the second nucleotide (A) in the first snippet is replaced by T in the second snippet. PCR based SNP detection facilitates scientific research in variety of fields ranging from population genetics and evolutionary biology to large-scale disease and drug associated studies. In this technique selected regions of a DNA sequence from multiple individuals sharing a common trait are compared, The PCR includes 3 primers, one specific for the SNP region and one specific for a consensus region. One of the primers (forward or reverse) is common to both normal and SNP type DNA. The consensus primers will amplify the consensus region (an 800 bp fragment) in both normal and SNP type DNA, while the SNP specific primers will amplify a 550 bp fragment from the SNP type DNA and not normal DNA (refer fig. 1 and fig. 2). This is because the SNP specific primer will bind to normal DNA inadequately forming mismatch at the 3' end whereas in the SNP type DNA it makes a perfect match.

Two different DNA samples (normal and SNP type) are provided along with a primer mix containing 3 different primers required for PCR. Primers specific to the SNP will amplify only if the SNP is present but the consensus primers will amplify both in the normal as well as the SNP type DNA. Thus, the PCR product from the SNP type DNA will show 2 bands (800bp and 550bp) whereas the PCR product from the normal DNA will show only one band (800 bp Refer Fig 3). Students will carry out PCR using the 2 templates and the primer mix, analyze the PCR products on agarose gel and determine which DNA has the SNP.

**Kit Contents : ▶**

- ▶ Template DNA 1
- ▶ Template DNA 2
- ▶ Primer Mix
- ▶ 10X Assay Buffer
- ▶ 10 mM dNTP Mix
- ▶ StepUp™ 100 bp DNA Ladder (Ready to use)
- ▶ Taq DNA Polymerase (3 U/μl)
- ▶ 2.5X Gel Loading Buffer
- ▶ Nuclease Free Water
- ▶ Mineral Oil
- ▶ Agarose
- ▶ 50X TAE
- ▶ RT PCR vials



**Fig 3 :** Analysis of amplified products from Normal and SNP DNA on 2% Agarose Gel.

- Lane 1 :** Normal DNA
- Lane 2 :** SNA DNA
- Lane 3 :** StepUp™ 100 bp DNA Ladder

Analysis of amplified products from Normal and SNP DNA on a 2% Agarose Gel.

Amplification using SNP type DNA gives 2 bands of 800bp and 550bp whereas amplification using normal DNA gives only one band of 800 bp. Hence it can be concluded that DNA Template 1 is normal and DNA Template 2 is of SNP type.

**Ordering Information:**

Cat. No	PI No.	Product Description
6109900011730	KT99A	GeNei™ Single Nucleotide Polymorphism (SNP)Teaching kit, 5 expts.

**GeNei™  
AFLP Teaching Kit**

**Description : ▶**

All DNA fingerprinting methods study patterns associated with genetic markers; however, individual techniques differ in terms of the number and type of genetic markers examined. For example, some approaches allow the examination of a marker at a single locus (called single-locus markers), whereas others allow the simultaneous investigation of multiple loci (called multi-locus markers). Some approaches focus on co-dominant markers, which provide information about both alleles present at a given locus. In contrast, other techniques are concerned with dominant markers, which only report the presence or absence of a given allele and cannot provide information about whether an individual is homozygous for that allele. The oldest method used in DNA fingerprinting studies is restriction fragment length polymorphism (RFLP) analysis. A second DNA fingerprinting technique focuses on microsatellite regions of the genome that contain simple sequence repeats (SSRs), which are short stretches of two to six nucleotides that are repeated multiple times. Yet another approach to DNA fingerprinting, called random amplified polymorphic DNA (RAPD) analysis, uses PCR primer sets designed to randomly amplify DNA fragments scattered throughout the genome. Researchers are now increasingly turning to AFLP-PCR in their DNA fingerprinting efforts. AFLP-PCR uses many of the same steps as RFLP, SSR, and RAPD, however, this method includes additional steps that permit high-resolution interrogation of the entire genome, and it yields highly specific, reproducible genotypic data.

This technique involves five major steps, as described in the following sections.

**Step 1:** Preparing the AFLP Template and Restriction of the Genomic DNA AFLP analysis starts with the isolation and purification of a sample of genomic DNA. This is digested with a pair of restriction enzymes, often Mse I / Taq I and EcoR I. Mse I recognizes 5'-TTAA-3' and cleaves after the first

5'-T, Taq I recognizes 5'-TCGA-3' and cleaves after 5'-T, whereas EcoR I recognizes 5'-GAATTC-3' and cleaves after the 5'-G. Mse I and EcoR I generate DNA fragments with 5' overhangs (5'-TA-3' and 5'-AATT-3', respectively) that are distinct from each other and are non-complementary. Three types of restriction fragments are generated: One with EcoR I cut at both ends, one with EcoR I cut at one end, and Taq I cut with other end and one with Taq I cuts at both ends.

**Step 2:** Ligation Reaction with Restriction Fragments and Adaptors Double-stranded adapters consist of a core sequence and an enzyme-specific sequence. They are specific for either the EcoRI site or the Taq I site. Ligation of the adapter to the restricted DNA alters the restriction site to prevent a second restriction from taking place after ligation has occurred.

**Step 3:** Pre-Selective Amplification: 25 Primers used in this step consist of a core sequence, an enzyme specific sequence and a selective single-base extension at the 3'-end. Sequences of the adapters and restriction sites serve as primer binding sites for the "pre selective PCR amplification." Each pre-selective primer has a "selective" nucleotide that will recognize the subset of restriction fragments having the matching nucleotide downstream from the restriction site. The primary products of the pre-selective PCR are those fragments having one Taq I cut and one EcoR I cut and having the matching internal nucleotide.

Example of an EcoRI Adapter sequence is as follows: 5'-ATG TTA GAG TGC GTA CCA ATT C-3': Core Sequence: ATG TTA GAG TGC GTA CC Enzyme Specific sequence: A ATT Selective single-base extension: C

**Step 4:** Selective Amplification The selective amplification step consists of an identical sequence to the pre-selection primers plus two additional selective nucleotides at the 3'-end (i.e., a total of three selective nucleotides). These two additional nucleotides can be any of the 16 possible combinations of the four nucleotides. From the huge number of fragments generated by the two restriction enzymes, only that subset of fragments having matching nucleotides at all three positions will be amplified at this stage (50-200 fragments).

This step reduces the complexity of the PCR product mixture by 256-fold. Different primer combinations will generate different sets of fragments. Preliminary screening is used to choose primer pairs that generate suitable levels of variation for the taxa being studied.

**Step 5:** Scoring AFLP Products: AFLP-PCR products can be separated and scored with a variety of techniques, ranging from simple agarose gel electrophoresis to automated genotyping. Polyacrylamide gel electrophoresis provides maximum resolution of AFLP banding patterns to the level of single nucleotide length differences, whereas fragment length differences of less than 10 nucleotides are difficult to score on agarose gels. Although agarose gels provide the least resolution, they are user friendly, inexpensive and require minimal equipment.

## Kit Contents : ▶

- ▶ Yeast Genomic DNA
- ▶ EcoRI
- ▶ Taq I
- ▶ Assay Buffer E
- ▶ Adapter – EcoR I
- ▶ Adapter – Taq I
- ▶ Ligase Buffer
- ▶ Instant Ligase
- ▶ Primer 1
- ▶ Primer 2
- ▶ Primer 3
- ▶ Primer 4
- ▶ Primer 5
- ▶ Primer 6
- ▶ dNTP mix
- ▶ Taq DNA Polymerase
- ▶ Taq DNA Polymerase 10X Assay Buffer
- ▶ 2.5X Gel Loading Buffer
- ▶ Nuclease Free Water
- ▶ 20bp DNA Ruler (Ready to use)
- ▶ 30% Acrylamide mix
- ▶ 10X TBE
- ▶ Ammonium persulphate (APS)
- ▶ TEMED
- ▶ Mineral Oil
- ▶ PCR Tubes

## Ordering Information:

Cat. No	PI No.	Product Description
6114700011730	KT147A	GeNei™ AFLP Teaching Kit, 5 expts.

## BLOTTING TECHNIQUES

### GeNei™ Southern Hybridization Teaching Kit

#### Description : ▶

**Principle:** Southern Hybridization technique involves transfer of DNA fragments separated in electrophoretic gels to nylon membrane for detection of specific base sequences using complementary probes. Prof. E. M. Southern developed this technique in 1975, hence referred to as Southern transfer. Southern blots are used to identify and quantitate specific DNA sequences, in analysis of genome organization and expression, in the study of genetic diseases, in DNA fingerprinting and analysis of PCR products. In this technique, DNA molecules are size fractionated on agarose gel and transferred to a nitrocellulose or nylon membrane by capillary or electrophoretic transfer. The DNA is immobilized on the membrane by UV crosslinking or by baking at 80°C. The membrane is washed, prehybridized and then hybridized with a biotin labeled probe. After hybridization, the unbound probe is removed by washing the membrane. The membrane is then incubated with a protein block to reduce non-specific interaction. The bound (hybridized) probe is detected by incubating the membrane with streptavidin enzyme conjugate and finally developed with the substrate solution until sufficient colour (blue) develops.

The kit demonstrates the technique of Southern Hybridization. The students will carry out agarose gel electrophoresis of the DNA Marker supplied, transfer the DNA bands electrophoretically onto positively charged nylon membrane, hybridize with

biotin labeled probe specific to one of the DNA bands in the DNA Marker and detect the hybridized DNA by adding streptavidin-HRP (Horse radish peroxidase) conjugate. The enzyme conjugate is detected by the addition of the substrate TMB/H<sub>2</sub>O<sub>2</sub> (Tetramethyl benzidine H<sub>2</sub>O<sub>2</sub> substrate) that reacts with HRP to give a blue coloured DNA band on the nylon membrane.

#### Kit Contents : ▶

- ▶ DNA Marker (Ready to use)
- ▶ Biotinylated Probe
- ▶ Prehybridization Buffer
- ▶ Hybridization Buffer
- ▶ 2X Wash Buffers (A, B, C and D) each
- ▶ Blocking Buffer
- ▶ Streptavidin HRP Conjugate
- ▶ Conjugate Dilution Buffer
- ▶ 10X Substrate
- ▶ 10X Electrotransfer Buffer
- ▶ Blocking Powder
- ▶ Tween-20
- ▶ Agarose
- ▶ 50X TAE
- ▶ Filter Paper
- ▶ Nylon Membrane
- ▶ Petridish

#### Observation:

Observe for a single blue band on the nylon membrane.

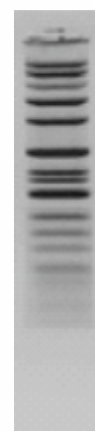


Fig. 1



Fig. 2

**Fig. 1 :** Electrophoretic separation of bands in the DNA Marker on a 1.0% agarose gel before transfer.

**Fig. 2 :** A single blue band on the nylon membrane after electroblotting, hybridization and detection.

In this non-isotopic detection technique, a Biotinylated Probe (sequence complementary to the target DNA) is used. The probe binds to the complementary sequences of the DNA Marker. This hybridized complex (probe-target complex) is detected by Streptavidin-HRP Conjugate, an enzyme that binds to biotin molecule of the probe. On addition of the Substrate solution, the enzyme reacts with the substrate to form the blue coloured precipitate which is seen as a band as shown in

**figure 2.** The probe-target complex is seen as the major blue band however 1 or 2 very less intense blue bands may be seen due to random nonspecific annealing of the probe to the DNA bound on the membrane.

### Ordering Information:

Cat. No	PI No.	Product Description
6109600011730	KT96A	GeNei™ Southern Hybridization Teaching Kit, 5 expts.
6109600031730	KT96	GeNei™ Southern Hybridization Teaching Kit with ETS5, 5 expts.

## GeNei™ Northern Blotting Teaching Kit

### Description : ▶

Northern blotting is a fundamental technique used in the detection and quantitation of specific mRNA in a given population of cells. It is a variation of the Southern blotting technique developed by Southern et al, in 1975. This technique was developed by Arwine and his co-workers and involved blot transfer of RNA onto chemically reactive paper where it binds covalently. Northern blotting differs from Southern Blotting in that the RNA can be probed using either a RNA probe or DNA probe, also referred to as RNA-DNA/RNA-RNA hybridization, whereas Southern blotting refers only to DNA-DNA hybridization.

Northern Blotting technique involves the following steps:

1. RNA isolation (total or poly(A) RNA)
2. Probe generation
3. Denaturing agarose gel electrophoresis
4. Transfer to solid support and immobilization
5. Prehybridization and hybridization with probe
6. Washing
7. Detection

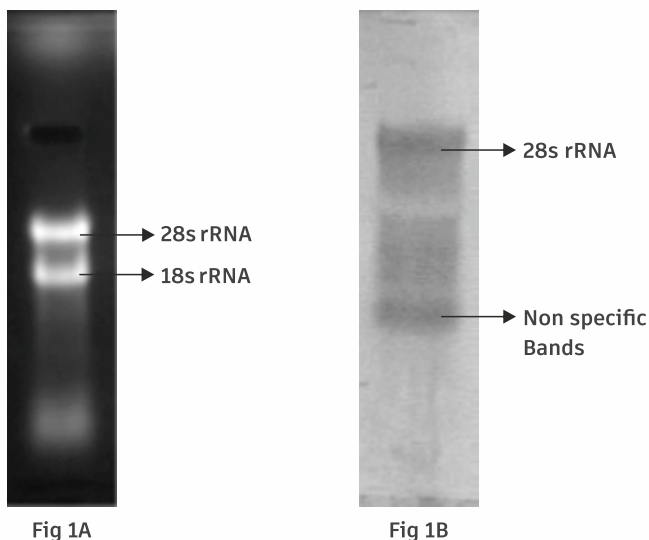
Using this kit, students will electrophorese Total RNA samples on denaturing agarose gel and transfer the RNA onto nylon membrane by upward capillary action. Following UV fixation and baking, 28s Ribosomal RNA (rRNA) of the Total RNA sample will be probed with biotin labeled DNA probe in the hybridization step. Low stringency and high stringency washes will be carried out to remove un-hybridized probe and non-specifically hybridized probe. 28s rRNA will then be detected by adding a conjugate which is Streptavidin labeled with alkaline phosphatase (ALP). Finally the substrate (BCIP/NBT) is added which when reacted upon by the enzyme ALP will result in a purple color band on the membrane, corresponding to the position of the 28s rRNA on the membrane.

### Kit Contents : ▶

- ♦ Biotinylated Probe
- ♦ Total RNA
- ♦ RNA Sample Buffer
- ♦ Gel Loading Buffer
- ♦ Hybridization Butter
- ♦ Hybrizaion Buffer
- ♦ Streptavidin ALP Conjugate
- ♦ Dilution Buffer
- ♦ Substate
- ♦ Low Stringency Wash Buffer
- ♦ High Stringency Wash Buffer
- ♦ 10X Electrophoresis Buffer
- ♦ Blocking Power
- ♦ Agarose
- ♦ Wicks
- ♦ Nylon Membrane
- ♦ Filter Paper
- ♦ Petridish
- ♦ Tween - 20
- ♦ Formaldehyde
- ♦ Transfer Buffer
- ♦ Blotting She

MICROBIAL GENETICS

GeNei™ Bacterial Conjugation Teaching Kit



**Fig 1A** : Total RNA on 1% denaturing agarose gel  
**Fig 1B** : Nylon membrane after hybridization & detection

Northern Blotting is a hybridization technique used to probe the gene of interest. In this non-isotopic method of detection, a Biotinylated DNA probe is used which binds to the 28s rRNA due to sequence complementarity. The DNA-RNA (Probe Target) complex is detected by Streptavidin-ALP Conjugate, an enzyme that binds to the Biotin molecule of the probe. On addition of Substrate solution, the enzyme reacts with its substrate to form a purple colored precipitate which appears as a purple band on the membrane.

- ◆ Identify RNA-Seq data by confirming the presence and expression levels of specific RNA transcripts.
- ◆ Cell and Tissue-Specific Expression Studies: Northern blotting is applied to study cell and tissue-specific expression patterns of RNA molecules, helping to characterize gene expression in different biological contexts.
- ◆ Investigation of RNA Modifications: Northern blotting can be adapted to study RNA modifications, such as methylation or pseudouridylation, by detecting changes in mobility or hybridization patterns.

Ordering Information:

Cat. No	PI No.	Product Description
6113800011730	KT138A	GeNei™ Northern Blotting Teaching Kit, 5 expts.

Description :▶

Transfer of genetic material from one bacterial strain (donor) to another strain (recipient) is a common event that occurs in nature with the objective of mixing the gene pool, in otherwise asexually reproducing organisms. DNA transfer among bacteria is mediated in three ways viz., transfection, transduction, and conjugation. Conjugation is the most widespread process of transferring genetic material from one bacterial cell to another. It is a process in which unidirectional transfer of DNA is mediated by conjugal plasmids or conjugal transposons requiring cell-to-cell contact. The process of bacterial conjugation was discovered by Lederberg and Tatum in 1946. Conjugation is best understood by considering properties of the 'F' factor, which is a small circular plasmid DNA that can replicate autonomously in the cell or can integrate into the host chromosome and thus transfer host chromosomal markers. When individual cells with an integrated 'F' are isolated and allowed to form pure colonies, the resulting strain can transfer chromosomal markers at very high frequency and are termed Hfr strains.

This kit demonstrates:

- ◆ Two E. coli strains are provided, A and B referred to as parental strains. Strain A carries an antibiotic resistance gene in the 'F' plasmid coding for Tetracycline, while Strain B is devoid of any 'F' factor but carries Streptomycin resistance gene in its chromosome.
- ◆ Both the strains will grow on medium containing the respective antibiotics to which they are resistant. On conjugating the two parental strains, the resulting bacteria will be resistant to both the antibiotics, i.e., the "conjugated bacteria" when plated on a medium containing both Tetracycline and Streptomycin, will survive suggesting that gene transfer has taken place from Strain A to Strain B.

- ◆ The parental strains when plated on the same medium do not survive as they are sensitive to either one of the antibiotics.

## Kit Contents : ▶

- ▶ *E. coli* strain(Donor) (Lyophilised Vial A)
- ▶ *E. coli* strain (Recipient) (Lyophilised Vial B)
- ▶ Streptomycin
- ▶ Tetracycline
- ▶ Agar
- ▶ LB Broth
- ▶ Instruction Manual.

## Ordering Information:

Cat. No	PI No.	Product Description
6104500011730	KT45	GeNei™ Bacterial Conjugation Teaching Kit, 5 expts.
6104500021730	KT45A	GeNei™ Bacterial Conjugation Teaching Kit, 20 expts.

## GeNei™ Plasmid Curing Teaching Kit

## Description : ▶

Curing is the process of removing plasmids from the bacterial cells, as a result of which the bacterium will no longer have the genetic advantages contained on the plasmid. This can be achieved by the use of compounds, which will act as DNA intercalating agent and interfere with DNA replication and consequently result in the elimination of plasmid. Curing can be achieved with high copy number plasmids by growing cells for many generations without a selective agent (e.g antibiotic). Curing can be accelerated with the addition of curing agents, at concentrations that will not inhibit or kill the host organisms. Large category of plasmids carry genes which render bacteria resistant to chemotherapeutic drugs. Plasmids can carry up to ten different resistance genes. Major epidemics

with high morbidity and mortality have been caused by plasmid carrying multiresistant pathogens. Efforts have been made in numerous laboratories to discover and develop chemical compounds which will eliminate plasmid from the host bacteria. The most prominent category of plasmid eliminating compounds are those which bind to plasmid DNA by intercalation and inhibit the conjugational replication of this DNA. Among such substances are phenanthridines such as Ethidium and Propidium salts, antibiotics such as mitomycin, Rifampicin etc. Clearly, the elimination of plasmids from their host bacteria emerges as a current topic in chemotherapy research.

Using this kit, students will cure the plasmid carrying Green Fluorescent Proteins (GFP) gene from *E. coli* strain using a curing agent. The presence of ampicillin resistance gene in the plasmid will help in selecting cured and non cured cells. Students will revive the strain and grow the *E. coli* for seven generations in the growth medium along with the curing agent, followed by plating on selective medium to identify the cured cells.

## Kit Contents : ▶

- ▶ Ampicillin
- ▶ Host (Lyophilized)
- ▶ Curing Agent
- ▶ LB broth
- ▶ Agar
- ▶ 1.5 ml vials

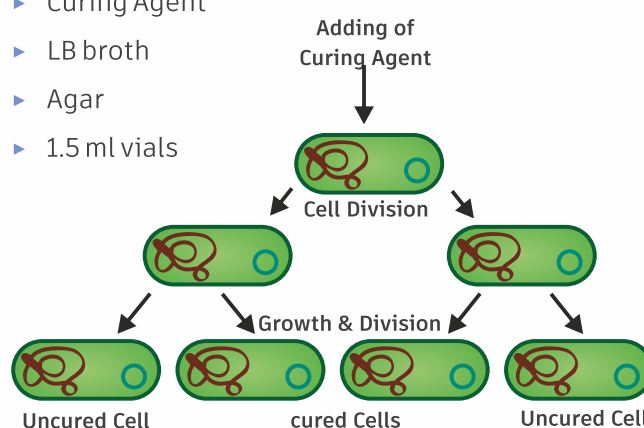


Fig : Diagrammatic representation of the Process of Plasmid Curing

## Ordering Information:

Cat. No	PI No.	Product Description
6119000021730	KT190	GeNei™ Plasmid Curing Teaching Kit, 5 expts.



## GeNei™ Bacterial Transduction Teaching Kit

### Description : ▶

may then be integrated into the recipient's nucleoid by various mechanisms and it confers a new property to the recipient cell like antibiotic resistance or production of some amino acids etc Natural mechanism of genetic recombination in bacteria include

- ◆ Transformation
- ◆ Transduction
- ◆ Conjugation

Transduction is the transfer of fragments of DNA from one bacterium to another bacterium by a bacteriophage. This genetic transfer occurs in both Gram-positive and Gram-negative bacteria. Transduction is observed with temperate bacteriophage (those that can form prophages) A prophage is a bacterial virus that has integrated its DNA into the DNA of a bacterial cell. This process of integration of viral DNA into bacterial DNA is called lysogenization.

In this kit, an E.coli phage is supplied, which exhibits both lytic and lysogenic life cycle. Three E.coli strains (Donor, Recipient and Susceptible host) are provided in the lyophilized forms. This kit simulates the process of transduction: i.e. genetic transfer of antibiotic resistant gene from one E.coli (Donor) strain to another (Recipient) through the bacteriophage. The donor strain is resistant to chloramphenicol and the recipient is sensitive to this antibiotic but resistant to ampicillin. Upon phage infection, phage DNA enters the donor cell and integrates into the bacterial chromosome. On temperature induction, the phage DNA is excised from the bacterial chromosome, the phage replicates and the host cell lyses releasing mature phage particles. These phage particles are then taken up for infecting the recipient strain (chloramphenicol sensitive) where lysogenization occurs. The screening of the transductants is based on the antibiotic selection marker. The recipient

strain which will acquire the chloramphenicol resistant gene from the phage will survive on LB chloramphenicol plate, thus indicating the transfer of the gene from donor to recipient through a bacteriophage. To confirm the presence of the phage in lysogenized recipient strain, the phage is induced and titrated against the given susceptible host.

### Kit Contents : ▶

- ◆ Donor strain
- ◆ Recipient strain
- ◆ Susceptible host
- ◆ Phage lysate
- ◆ 0.1M CaCl<sub>2</sub>
- ◆ MgSO<sub>4</sub>
- ◆ Chloramphenicol
- ◆ 20% Maltose
- ◆ Ampicillin
- ◆ LB broth
- ◆ Agar
- ◆ 1.5 ml vials

### Ordering Information:

Cat. No	PI No.	Product Description
6112300011730	KT123	GeNei™ Bacterial Transduction Teaching Kit, 5 expts.

## GeNei™ Bacterial Transposons Teaching Kit

### Description : ▶

Transposons are 'Transposable elements' which are able to move from one place to another within a cell's genome. Sometimes a copy is made and the entire copy moves. This kind of insertion requires target DNA sequences. In the process they may cause mutations, increase or decrease in the amount of DNA in the genome, promote gene rearrangements, regulate gene expression or induce chromosome breaks or rearrangements. These mobile segments of DNA are sometimes

referred to as 'Jumping genes'. The first Transposable elements (TEs) were discovered in maize (*Zea mays*), by Barbara McClintock in 1948, for which she was awarded a Nobel Prize in 1983. She noticed insertions, deletions, and translocations, caused by these elements which lead to a change in the color of corn kernel. There are two distinct types of transposons: DNA Transposons, consisting only of DNA that moves directly from one place to place and Retrotransposons, which first transcribe DNA into RNA and then use Reverse Transcriptase to make a DNA copy of the RNA to insert in a new location. In bacteria, transposons can jump from chromosomal DNA to plasmid DNA and back. Usually, these are additional gene that serves a purpose other than transposition, which has resistance to antibiotics. This class of bacterial transposons is associated with the Tn family when the transposable elements lack an additional gene, they are called insertion sequence.

Using this kit, students can learn the process of transposition occurring naturally between two strains. Two *E.coli* strains are provided of which Strain A is resistant to Ampicillin and Chloramphenicol. Then Ampicillin Resistance Marker is present on a transposable plasmid. Strain B is resistant to streptomycin. When these two strains are grown together in appropriate conditions, Ampicillin resistance gene from one strain will get transferred to the other making it resistant to ampicillin along with streptomycin. This can be observed by the growth of the recipient on ampicillin plate and also by colony morphology. To confirm the transfer of the ampicillin resistance gene, DNA miniprep (Plasmid Isolation) is carried out.

### Kit Contents : ▶

- ▶ *E.coli* Strain A
- ▶ *E.coli* Strain B
- ▶ Chloramphenicol
- ▶ Ampicillin
- ▶ Streptomycin
- ▶ Solution I
- ▶ Solution II
- ▶ Solution III
- ▶ Solution IV

- ▶ 2.5X Gel Loading Buffer
- ▶ RNase A
- ▶ 1X TE
- ▶ Agarose
- ▶ 50X TAE
- ▶ LB Broth
- ▶ Agar
- ▶ 1.5 ml Vials

### Ordering Information:

Cat. No	PI No.	Product Description
6112400011730	KT124A	GeNei™ Bacterial Transposons Teaching Kit, 5 expts.

## GeNei™ Phage Titration Teaching Kit

### Description : ▶

Viruses that infect bacteria are referred to as bacteriophages, meaning bacteria eaters. These were first identified and described by Frederick Twort and Felix D' Herelle in 1917. Over the years, phages have become indispensable molecular tools in genetic engineering and related areas. Structurally three kinds of phages are recognized Icosahedral head (tail less), Icosahedral head with tail and Filamentous phage. The phage genome can be either circular or linear, single or double stranded DNA or linear RNA with one or more proteins. The proteins form a capsid around the nucleic acid and protect them from host nucleases. Bacteriophages remain in a state of dormancy in the environment and do not express any genes during this state and essentially persist until they come in contact with a susceptible host cell. On entering bacteria, the phage genome either integrates with the host genome (lysogeny) or independently produces prophages and lyse the host (lytic infection), resulting in death of the host cell and release of progeny phage particles. Each phage adsorbs to one cell and initiates an infection resulting in the release of about 100 phages/cell, these viruses infect the surrounding bacteria thereby releasing more virus particles. Due to successive rounds of infection, a

spreading zone of lysis results in a clear area against a turbid background (due to growth of host bacteria). This clearing is referred to as plaque. The number of plaques formed is equivalent to the number of phage particles. Phage capable of only lytic growth is called virulent.

In this kit, an E. coli lambda phage is supplied, which is a Temperate phage. The DNA of λ phage is linear double stranded having 48,502 base pairs. The 5' terminus of each strand, has a 12 nucleotide single stranded extension, complementary to each other, called the cohesive (cos) ends. The phage recognizes specific receptor on the surface of the host cell i.e., Maltose Binding Protein for the purpose of adsorption. Hence, the host E.coli is grown in a medium containing Maltose and Magnesium which further facilitates the process of adsorption. On entering the host cell, the DNA circularizes due to base pairing between cohesive ends of the DNA. This serves as a template for transcription of phage genes during early stages of infection. The phage DNA then replicates by rolling circle mechanism, synthesizes new capsid proteins, tail fiber protein and packages its DNA into capsids. Once assembled, prophage then brings about lysis of host cells and releases new infectious virus particles. Using this kit, students will prepare host plating cells, infect it with the phage lysate supplied and estimate the phage titre value.

**Kit Contents : ▶**

- ◆ Host 1
- ◆ Phage Lysate
- ◆ 20% Maltose (sterile)
- ◆ SM Buffer
- ◆ LB Broth
- ◆ Agarose
- ◆ Mineral Oil
- ◆ 1.5 ml vials

**Ordering Information:**

Cat. No	PI No.	Product Description
6100500011730	KT05	GeNei™ Phage Titration Teaching Kit, 5 expts
6100500021730	KT05A	GeNei™ Phage Titration Teaching Kit, 20 expts. .

**BASIC MICROBIOLOGY TECHNIQUES**

**GeNei™ Bacterial Growth Curve Teaching Kit**

**Description : ▶**

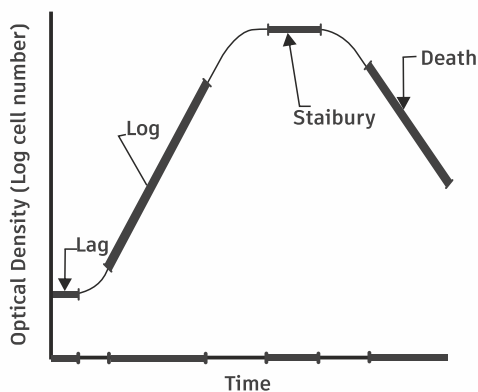
Bacterial growth usually refers to reproduction, increase in cell population number, size or both. Population growth is studied by analyzing the Growth Curve of a microbial culture. A wide variety of techniques can be used to study microbial growth, like changes in the total cell number, in a population of viable microorganisms, cell mass or dry weight determination, direct counting of cells under light microscope and turbidity measurements. Turbidity (cloudiness) of a broth culture relates to increase in cell number. Determination of bacterial growth involves inoculation of a sterile broth medium with bacteria and incubation of the culture under optimum conditions like temperature, pH, and oxygen. Since no fresh medium is provided to the bacteria during incubation, nutrient levels decline and concentration of waste increases. When the growth of microorganism reproducing by Binary Fission is plotted as the logarithm of cell number versus incubation time, the resulting curve is composed of 4 distinct phases. These are:

- ◆ Lag phase
- ◆ Log phase
- ◆ Stationary phase and
- ◆ Death phase

During the Lag Phase, there is no increase in cell number. The bacteria are preparing for reproduction, synthesizing DNA and various enzymes required for cell division. During the Log Phase of growth (so named because the logarithm of the bacterial biomass increases linearly with time), bacterial reproduction occurs at a maximal rate for the specific set of growth conditions. This growth phase is also called the Exponential Growth

Phase because the number of cells increase as an exponential function of  $2n$ . During active bacterial growth, the size of the microbial population is doubling. The time required to achieve a doubling of the population size, known as Doubling Time or Generation Time, is the unit of measure of the microbial growth rate. Stationary Growth Phase is reached when there is no fresh nutrient available and there is no further net increase in bacterial cell number. The transition between the exponential and stationary phases involves a period of unbalanced growth during which the various cellular components are synthesized at unequal rates. During the Stationary Phase, the growth rate is equivalent to death rate. Death Phase represents the result of the inability of bacteria to carry out further reproduction.

In this kit E.coli strain and Luria Bertini (LB) media are supplied. Growth Curve of this strain is studied by measuring the optical density using a Spectrophotometer at regular time intervals of its growth and there after plotting a graph of time versus optical density.



**Fig 1 :** A typical Bacterial Growth Curve, showing the four typical phases of growth.

### Kit Contents : ▶

- ▶ E.coli Strain
- ▶ LB Broth
- ▶ Agar

### Ordering Information:

Cat. No	PI No.	Product Description
6104600011730	KT46	GeNei™ Bacterial Growth Curve Teaching Kit, 5 expts.

## GeNei™ Bacterial Antibiotic Sensitivity Teaching Kit

### Description : ▶

The principle of antibiotic sensitivity test is based on the Bauer-Kirby Disc diffusion method. It is a standard qualitative test wherein the bacterial culture is spread onto the surface of Mueller-Hinton agar, followed by addition of antibiotic impregnated discs to the agar surface. The antibiotic diffuses through the agar to form a concentration gradient. This concentration gradient influences the growth of the bacterial strain. If an organism is susceptible to an antibiotic, a clear zone appears around the disc where the growth has been inhibited called the zone of inhibition. If resistant, no clear zone of inhibition appears. The diameter of the zone of inhibition surrounding the antibiotic disc is measured to determine whether the microorganism is sensitive (S), intermediately sensitive (I) or resistant (R) to a particular antibiotic. The size of zone of inhibition depends on:

- The rate of diffusion of the antibiotic through agar and
- The concentration of the antibiotic disc

Hence, by determining the susceptibility of a pathogen, clinicians can select the most appropriate agent for treating the disease. The test also helps in studying microbial strains.

Using this kit, students will perform antibiotic sensitivity tests for three different strains E. coli, P. aeruginosa and S. aureus. Five different antibiotic discs- Chloramphenicol, Tetracycline, Kanamycin, Gentamycin and Vancomycin are provided against which the sensitivity of each of the strains will be tested. By measuring the diameter of zone of inhibition, students will determine the sensitivity of each of the strains against the five antibiotics using the zone size interpretative chart.

### Kit Contents : ▶

- ▶ Antibiotic discs
  - Chloramphenicol
  - Tetracycline
  - Kanamycin

- Gentamycin
- Vancomycin
- ▶ Lyophilized Strains
  - Escherichia coli
  - Staphylococcus aureus
  - Pseudomonas aeruginosa
- ▶ Cotton Swab
- ▶ Mueller- Hinton media
- ▶ Agar

## Ordering Information:

Cat. No	PI No.	Product Description
6106800011730	KT68	GeNei™ Bacterial Antibiotic Sensitivity Teaching Kit, 5 expts.

## GeNei™ Isolation and Identification of Soil Bacteria Teaching Kit

### Description : ▶

There are many methods of isolating bacteria from soil. Among them the most commonly used method is by serial dilution. In this method, soil is suspended in sterile water and serially diluted, thus decreasing the number of organisms in each dilution. This helps in isolating organisms in pure form when plated on agar surface. The serial dilution technique also helps in determining the number of organisms that may be present in a given soil sample and this in turn helps in determining the quality of the soil sample. Bacteria are identified and classified based on morphological, physiological, metabolic, serological bacteria phage typing, ecological, genetic, biochemical tests, staining and molecular characteristics. The kit focuses on the identification of the isolated bacteria by colony morphology and microscopic observation post Gram staining.

The colony morphology identification includes the following:

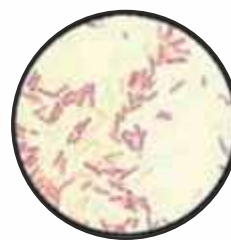
Form: Punctiform, Circular, Filamentous, Irregular, Rhizoid, Spindle

- ◆ Elevation: Flat, Raised, Convex, Pulvinate, Umbonate Margin: Entire, Undulate, Lobate, Erode, Filamentous, Curled
- ◆ Colour: Golden, Yellow, Red, Creamish, White
- ◆ Density: Opaque, Translucent
- ◆ Consistency: Rough, Smooth, Mucoïd, Butyrous, Powdery

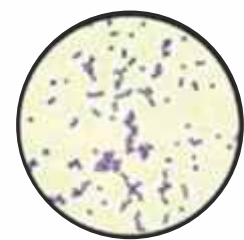
The kit contains medium for isolating bacteria, Gram staining reagents for identification of the isolated bacteria and 5 reference strains for easy identification. It enables the students to learn techniques like spread plate and streak plate method, serial dilution and preparation of medium

### Kit Contents : ▶

- ▶ Strains:
  - Serratia marcescens
  - Bacillus subtilis
  - Micrococcus luteus
  - Staphylococcus aureus
  - Nocardia sp.
- ▶ LB broth
- ▶ Agar
- ▶ Crystal violet stain
- ▶ Safranin stain
- ▶ Gram's iodine
- ▶ Decolourizing solution
- ▶ Glass spreader
- ▶ Glass slides



Gram Negative rods



Gram Positive Cocci (in cluster & in pairs)

### Gram Staining of Bacteria

## Ordering Information:

Cat. No	PI No.	Product Description
6109000011730	KT90	GeNei™ Isolation and Identification of Soil Bacteria Teaching Kit, 5 expts.