

**Molecular studies with *Entamoeba histolytica*-  
the causative agent of Human Amoebiasis**

**Sudha Bhattacharya  
Jawaharlal Nehru University  
New Delhi**

# Science as a career

- Explore the unknown mechanisms that govern us.
- The challenge of discovering nature is highly stimulating.
- Service to humanity

→ **Imparting knowledge to future generations**

→ **Producing useful products through technology**

- If practiced correctly, science makes you a better human being.

# Career options

- Teaching alone
- Research alone
- Teaching+Research
- Technology

→ **Medicine**

→ **Engineering**

→ **Agriculture**

→ **Biotechnology**

→ **Other emerging technology**

# Pluses and minuses of a science career

## ● Minuses

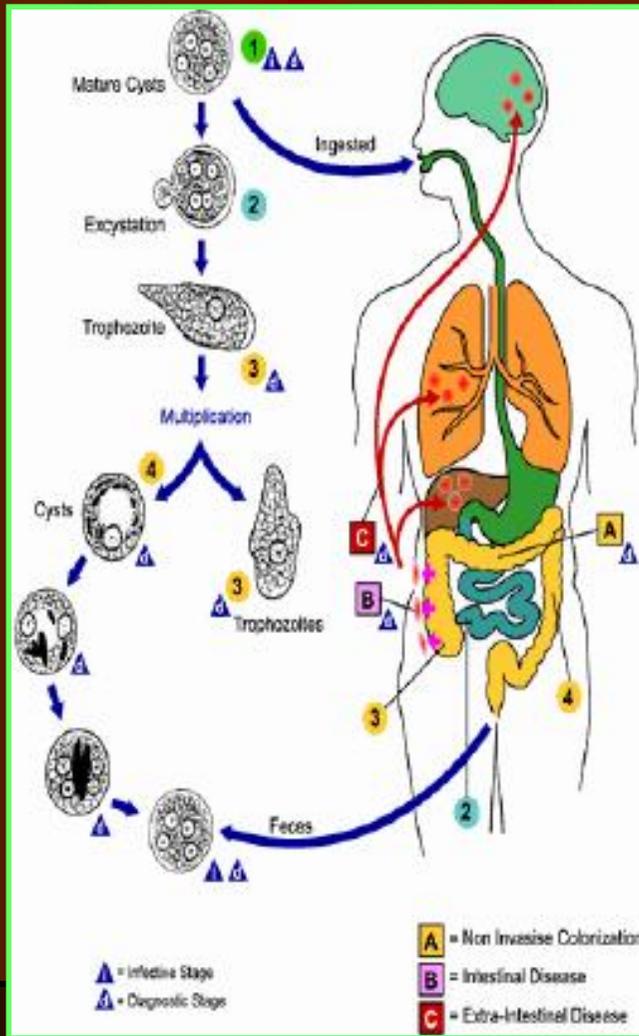
- Many years of study
- Salaries relatively low

## ● Pluses

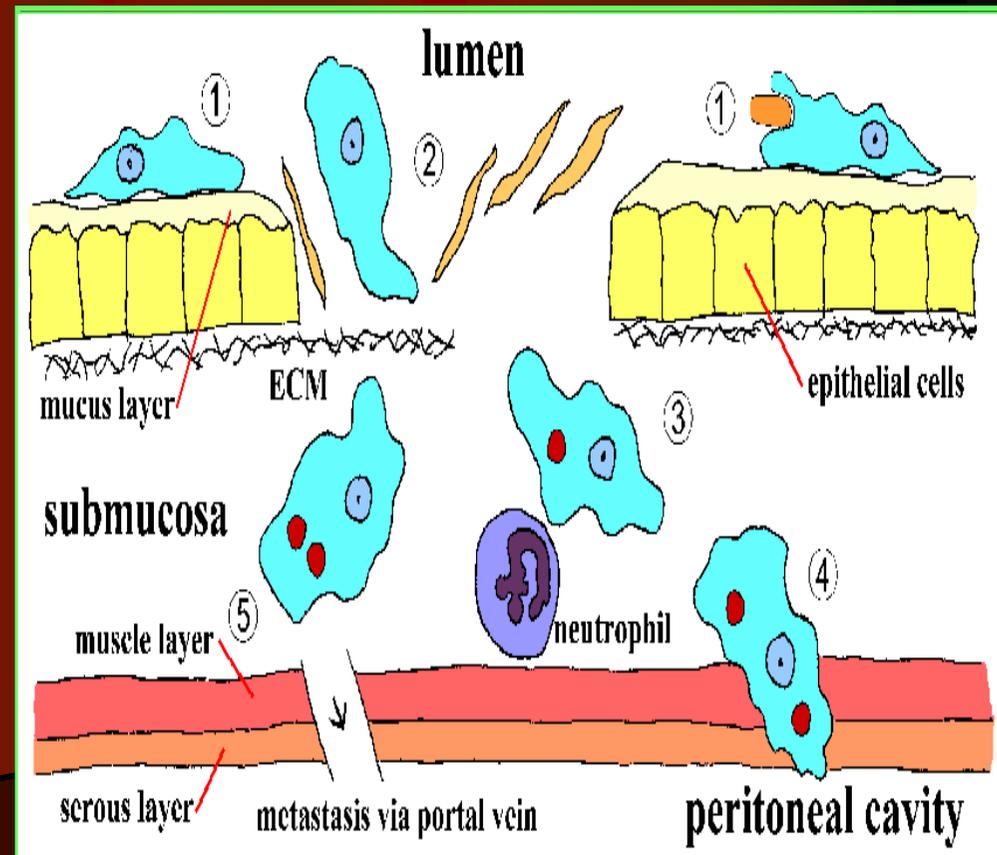
- Job satisfaction
- Flexi time possible
- Held in regard by society

# Life cycle of Entamoeba histolytica

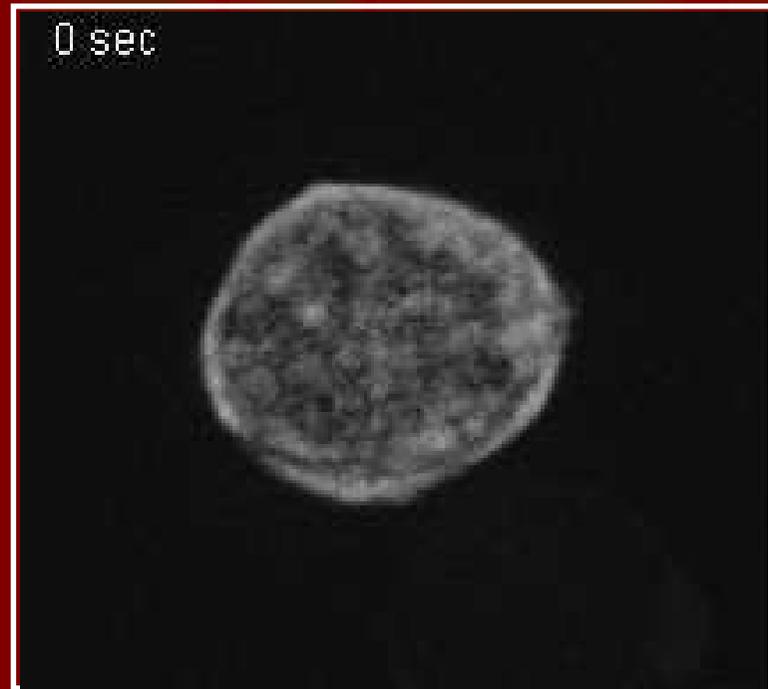
## Life Cycle



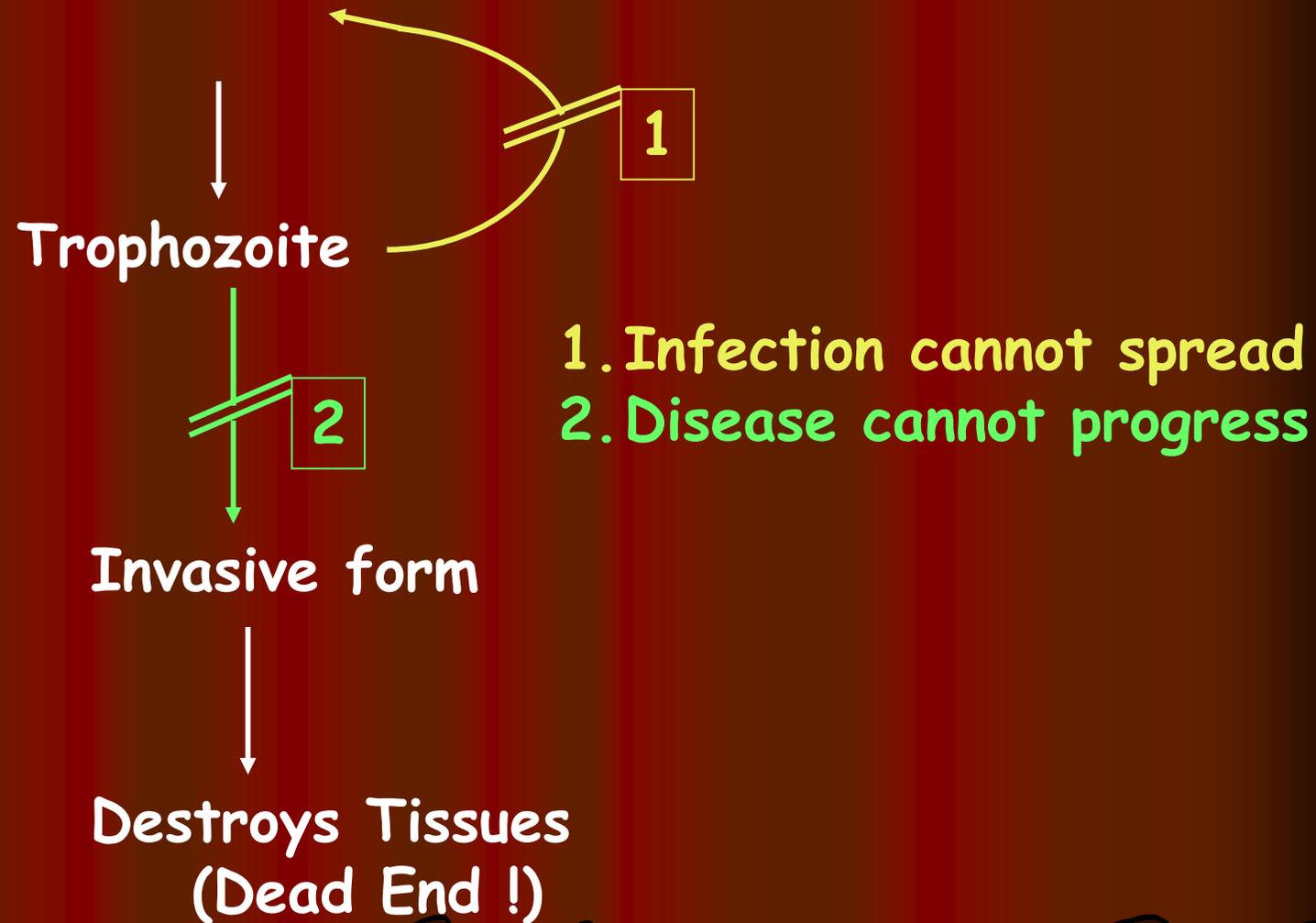
## Pathogenesis of Invasive Amoebiasis



# Movie representing a motile amoeba expressing GFP-CaBP1



# Amoebiasis: How to intervene

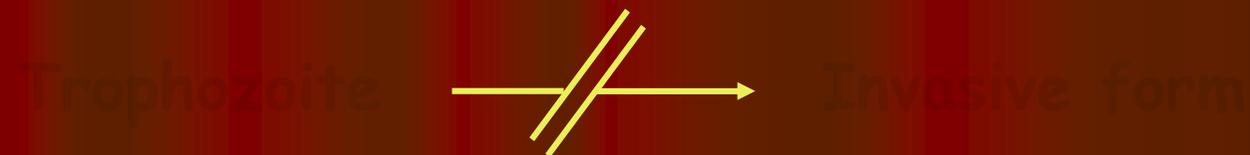


# Amoebiasis: Intervention

## 1. Infection cannot spread



## 2. Disease cannot progress



*E. histolytica* and *E. dispar* are sibling species



*E. histolytica*

*E. dispar*



amoebiasis

nonpathogen



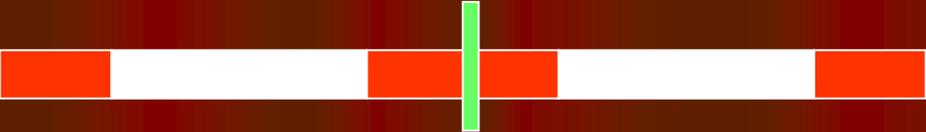
A major difference between  
*E. histolytica* & *E. dispar*

# Transposons

{mobile genetic elements}

Code for the enzymes needed for their movement.

e.g. Endonuclease, Reverse transcriptase

**Into a gene**  **Gene inactivation**

**Near a gene**  **Modify gene expression**

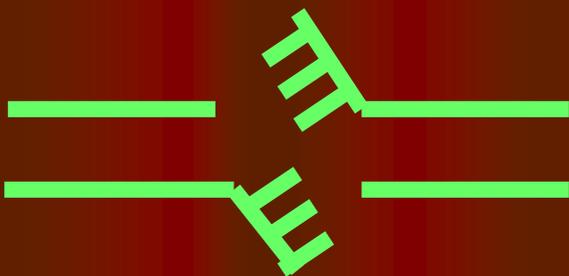
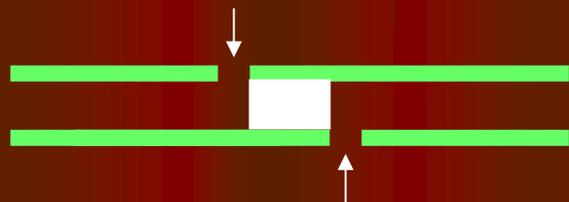
**Away from gene**  **DNA rearrangement**

# Inserting into a new site involves cutting and joining of two DNAs

Target DNA



TARGET SITE



Seperation of DNA strand

TSD



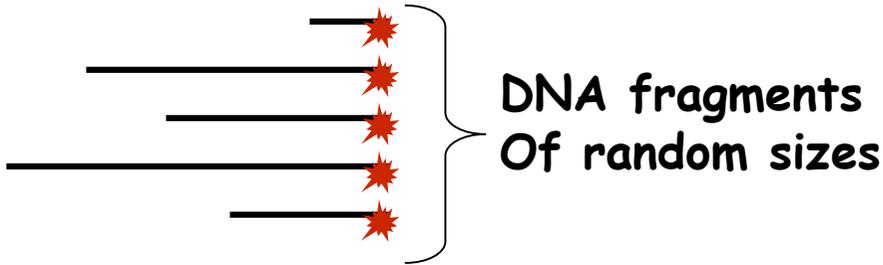
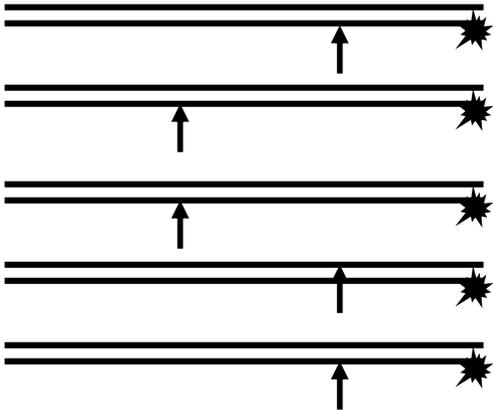
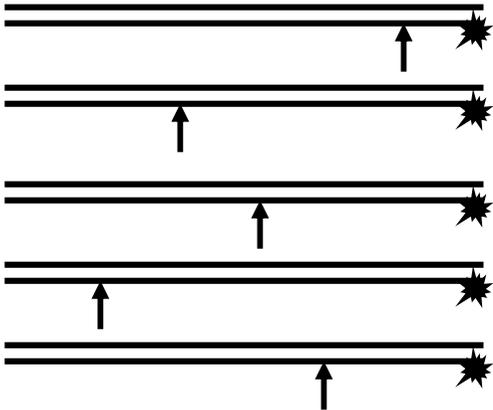
TSD

Transposon joined to target site

Transposon

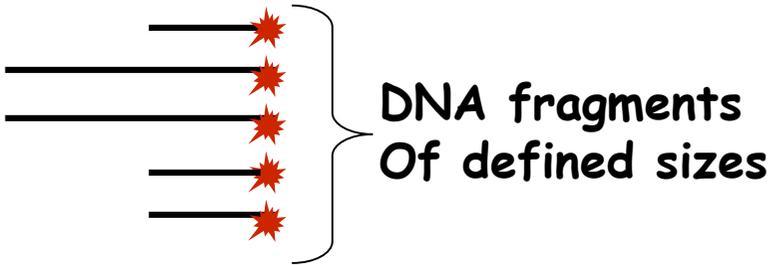


# Target site specificity of *E. histolytica* EN



DNA fragments  
Of random sizes

Non specific



DNA fragments  
Of defined sizes

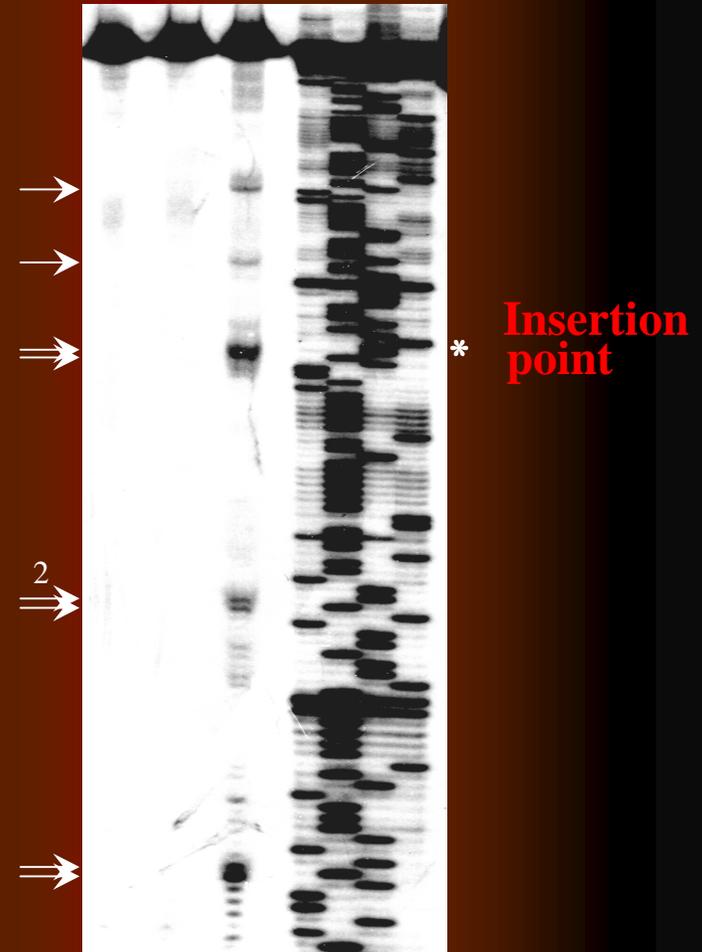
Specific

# Nicking profile of EN on the 174 bp substrate

Bottom strand labelled 174bp substrate



Transposon  
insertion point



- Endonuclease nicks at three hot spots
- The transposon inserts at hot spot # 3

Is the *E. dispar* Endonuclease different from  
*E. histolytica*?

# Sequence Comparison between Eh and Ed endonuclease

- EhLINE1ORF (64)ME(1)N NRWIMSGS(10)N(3)I (1) DL(5)I (1)R(36)H(1)E(100)MG(1)V R------(10)M(3)C(1)YY(5)F(1)G(36)Q(1)A(
- EdLINE1ORF (64)ME(1)N NRWIMSGS(10)N(3)I (1) DL(5)I (1)R(36)H(1)E(100)MG(1)V R------(10)M(3)C(1)YY(5)F(1)G(36)Q(1)A(
- EhLINE1ORF 1)G(5)L(23)R(13)K(41)K(53) I (61)R(5)C(6)N(24)- (48)T
- EdLINE1ORF 1)E(5)Q(23)N(13)N(41)N(53)S(61)N(5)S(6)K(24)Q(48)N
- EhLINE1ORF (19)N(36)YMDD(12)I (28)K(36)T(16)S(3)R(56)E(71)K(23)
- EdLINE1ORF (19)K(36)YMDD(12)T(28)N(36)I(16)C(3)S(56)N(71)N(23)
- EhLINE1ORF C(2)I (19)K(24)C(15)CPLCKDKIAT VEHILLSCIC (41)K(16)Q
- EdLINE1ORF S(2)T(19)N(24)S(15)CPLCKDKIAT VEHILLSCIG (41)N(16)L
- EhLINE1ORF (4)PD(14)D(50)K(2)N(12)E(26)S(3) LEEDEDE(14)R(2)A(4)T
- EdLINE1ORF (4)PD(14)D(50)A(2)I (12)T(26)V(3)Q--DEDE (14)T(2)T(4)T

Only a subset of Endonuclease-  
recognition sites are utilized for  
Transposon insertion

Genic

Intergenic

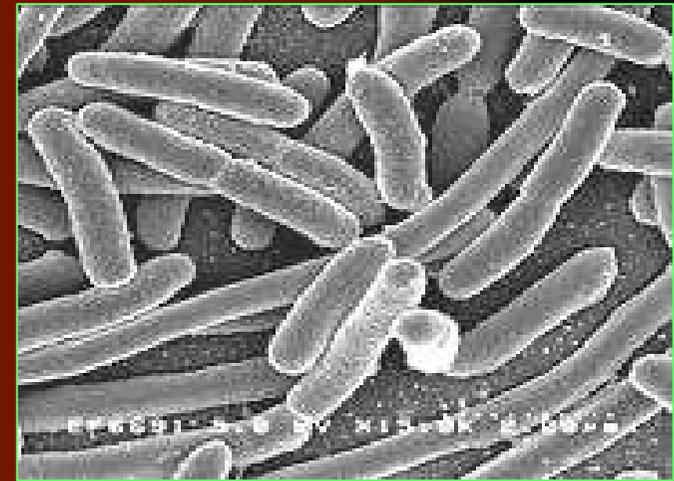
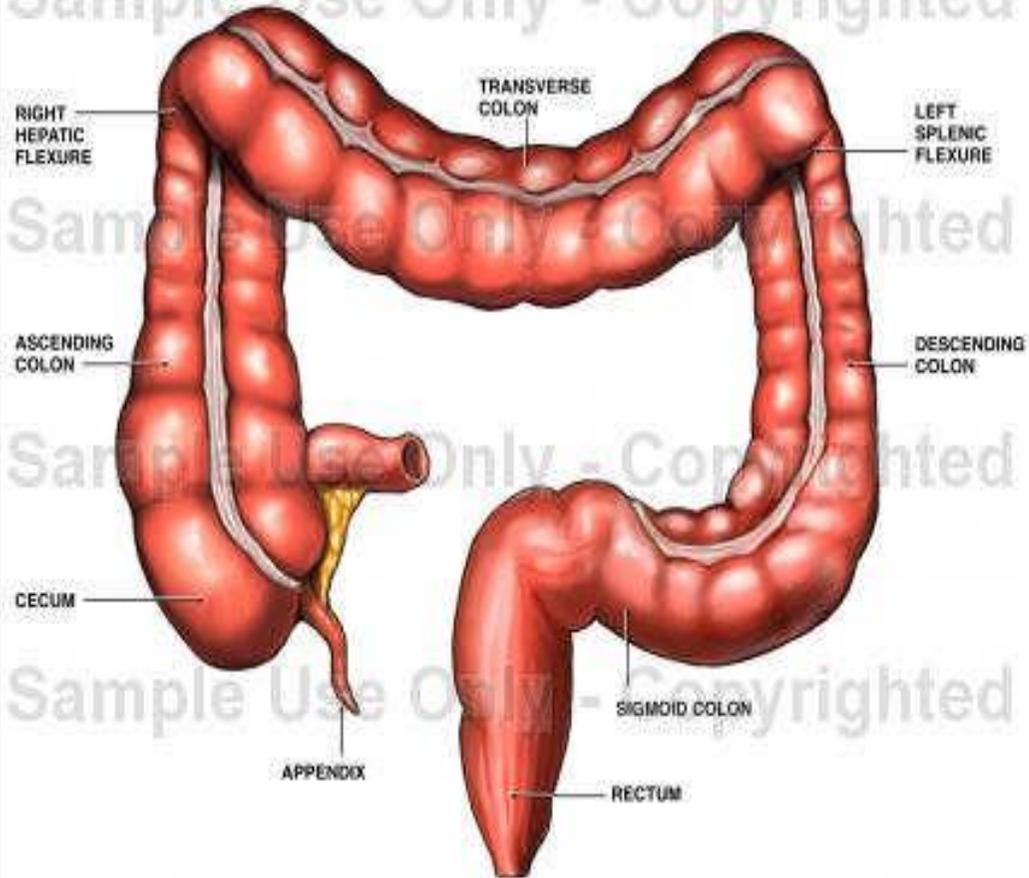
**No Transposon yet found in genic regions.**

# Understanding Transposon Insertion

- 1. Derive the rules which govern choice of target site**
- 2. Predict future transposition events**

# HUMAN GUT FLORA

Anatomy of the Large Intestine



# Continued....

- Human body is composed of  $\sim 1 \times 10^{13}$  cells.
- Human gut contains  $1 \times 10^{14}$  microorganisms!!
- About 1,000 different bacterial species reside in the gut.
- Gut flora does useful functions e.g. :
  - - produces vitamins (biotin, vit K )
  - - ferments unused carbohydrates
  - - Prevents growth of harmful species

## Continued....

- Gut flora influences digestion of fatty acids- which is linked to obesity.

OBESE mice & human – Bacteroidetes ↓  
Firmicutes ↑

(reversed in lean)

# Gut flora and Amoebiasis.....

Does the gut flora change in  
Amoebiasis patients ?

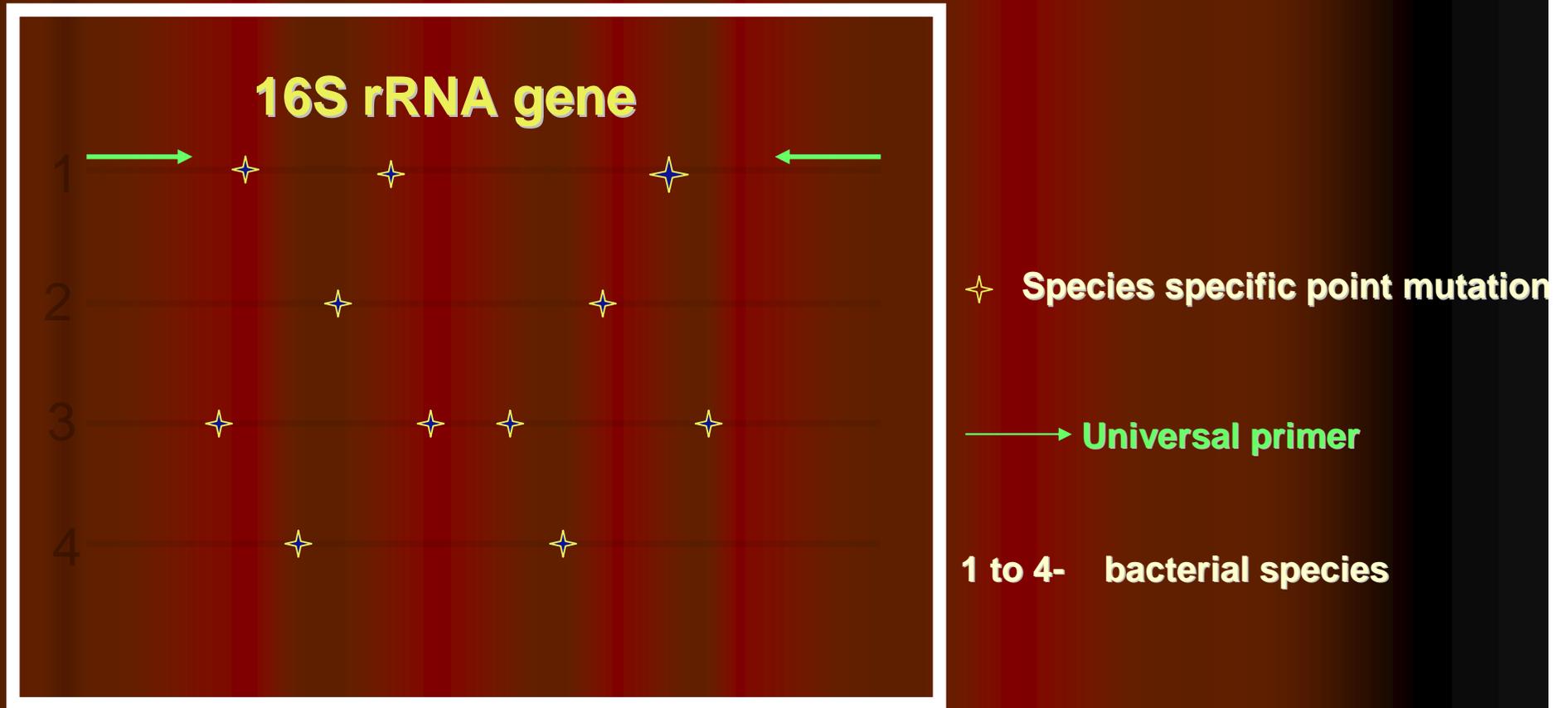
# Gut flora and Amoebiasis.....II

Identification of bacteria by PCR  
amplification of 16S ribosomal RNA gene

## Why 16S rRNA gene ?

- universally present
- highly conserved
- universal primer available
- species-specific point mutations

# Identification of bacteria by PCR



# Gut flora and Amoebiasis.....III

Results with patients :

**Stool samples screened from:**

1. Amoebic liver abscess patients.
2. Asymptomatic individuals (Eh+ve).
3. Asymptomatic individuals (Eh-ve).

## Continued....

- Lactobacillus was low in ALA patients and Eh+ve individuals.
- Bifidobacterium, Bacteroides and Clostridium were low in ALA patients, but this was due to Metronidazole anti amoebic drug.
- No change in Peptostreptococcus, Ruminococcus, Campylobacteria and Peptococcus.

# Bacteria in pus from Amoebic Liver Abscess patients

- So far it was believed that pus is sterile (from microscopy & culturing)
  - Out of 35 ALA patients examined,
    - 25 had Peptostreptococcus
    - 5 had Bacteroides.
- Both anaerobes

**Do bacteria aggravate the outcome of ALA?**

**Can antibiotics specific for these bacteria help patients who respond poorly to metronidazole?**

J.N.U.

