

Proteomics Course

LECTURE-28

Microarray related concepts: Recombinational cloning Cell-free expression



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Lecture outline

- Recombinational cloning
- Cell-free protein synthesis system

Recombinational Cloning

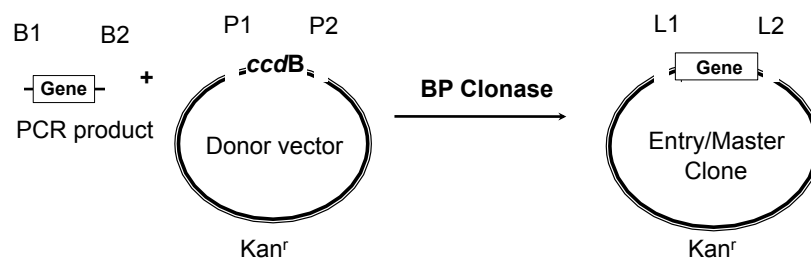
Recombinational cloning

- A novel site-specific recombination technique to transfer DNA sequences
 - universal strategy to move DNA sequence to any vector
- Recombinational cloning is a promising approach for high throughput genomics and proteomics application

Recombinational cloning: terminology

- Expression clone
- Donor vector
- Master clone
- Destination vector

Recombinational cloning: BP Reaction

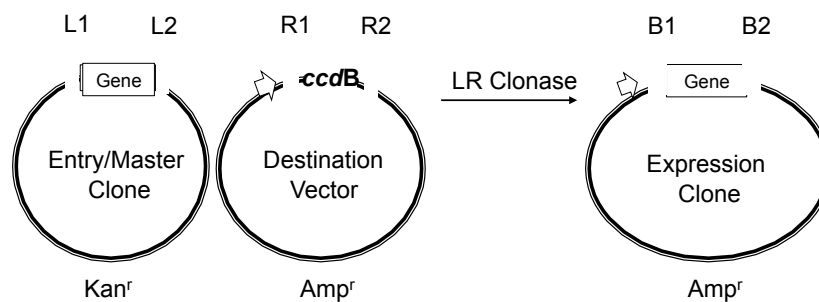


Recombinational cloning: BP Reaction

- Add all the components of the mix
- Mix well pipetting up and down
- Incubate at 25°C for 1-3 hours
- Transformation (Kan plate)

Material/equipment	1 sample
BP reaction buffer	2 μ l
pDONR221 (50 ng/ μ l)	2 μ l
BP clonase	2 μ l
PCR products	4 μ l

Recombinational cloning: LR reaction

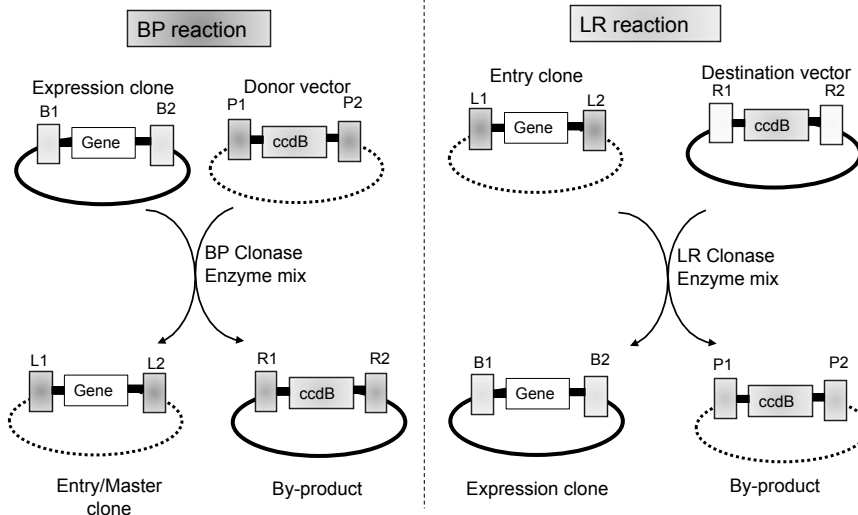


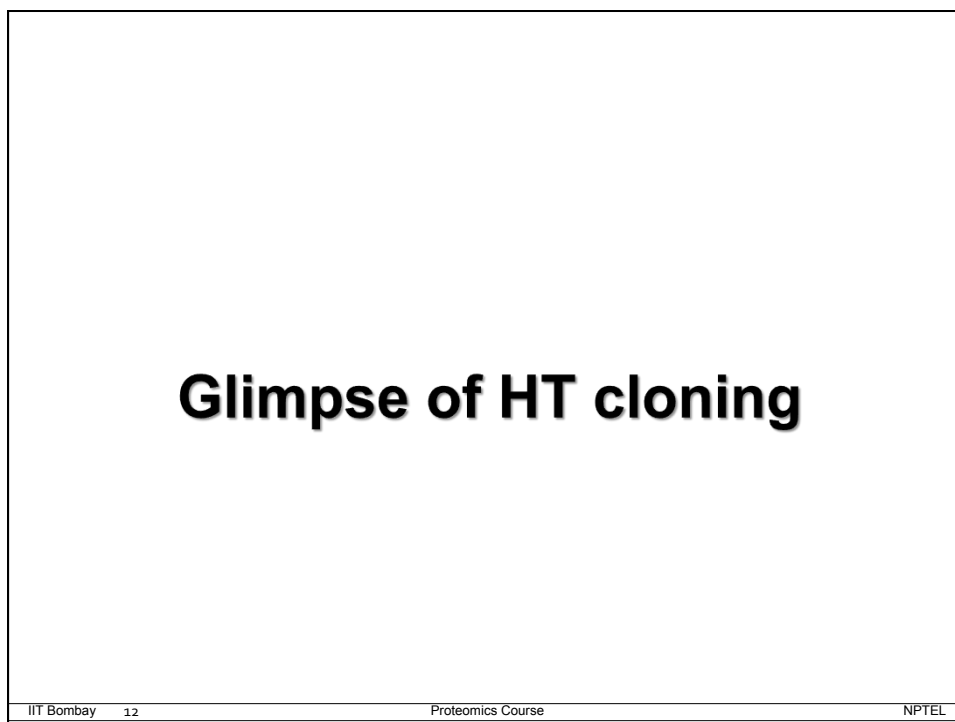
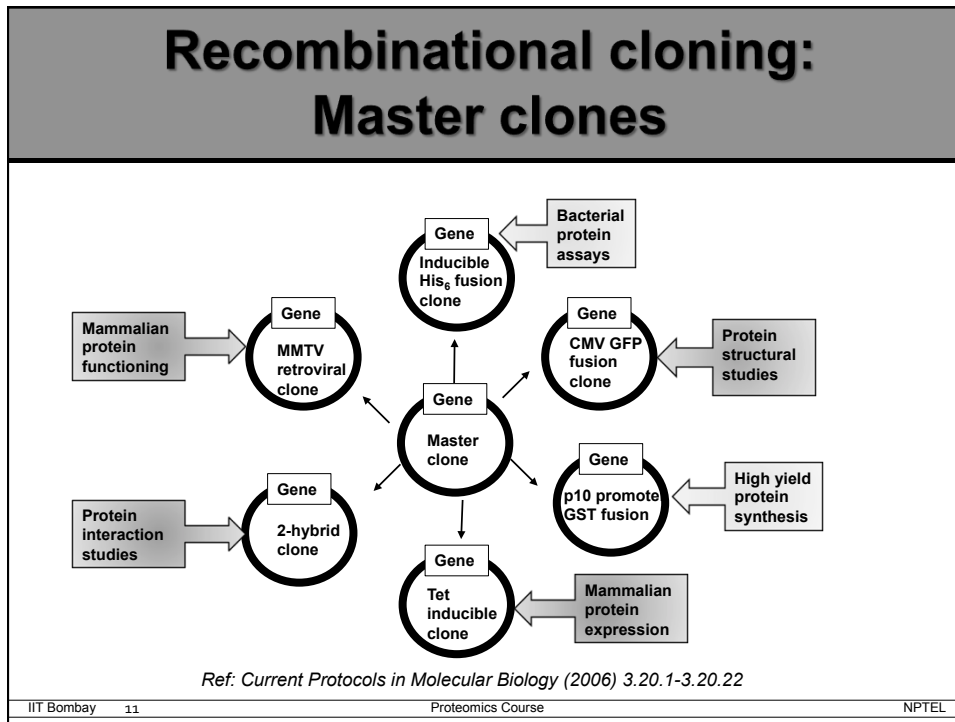
Recombinational cloning: LR reaction

- Add all the components of the mix
- Mix well pipetting up and down
- Incubate at 25°C for 1-3 hours
- Transformation (Amp plate)

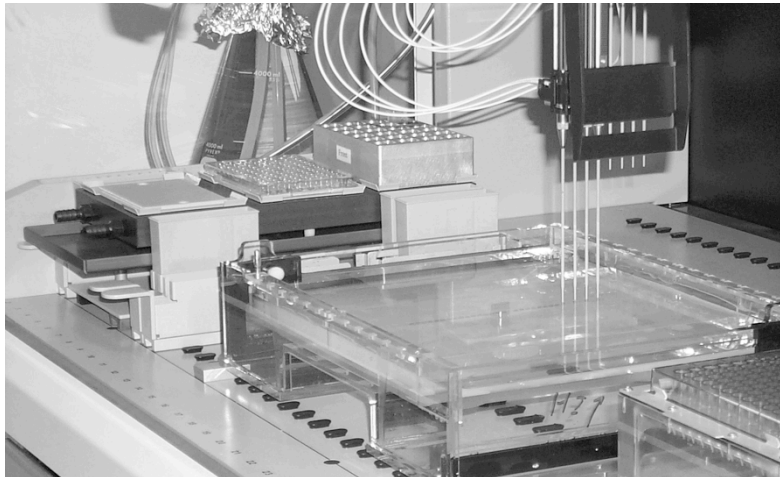
Material/equipment	1 sample
Entry clone DNA (100 ng)	1-7 μ l
Destination vector (150 ng/ μ l)	1 μ l
TE buffer	to 8 μ l
LR Clonase II enzyme mix	2 μ l

Recombinational cloning: Overview





Recombinational cloning: HT - Robot for loading DNA gels



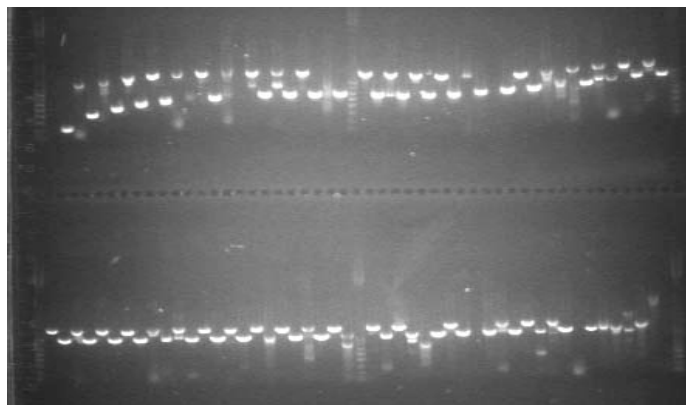
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Recombinational cloning: HT – agarose gel loading



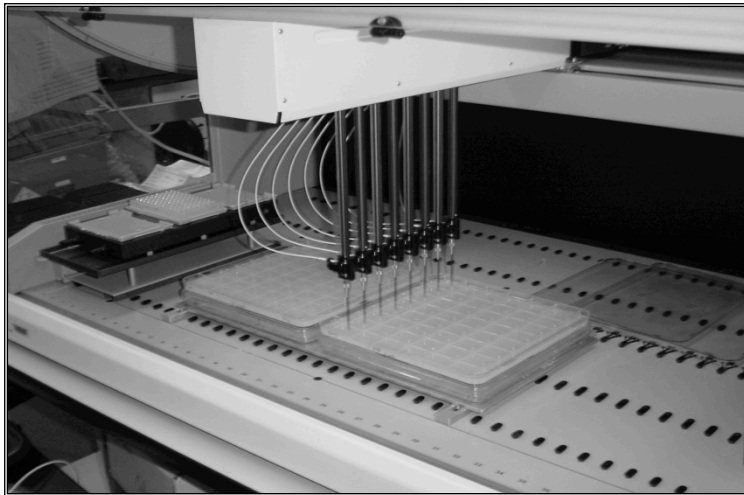
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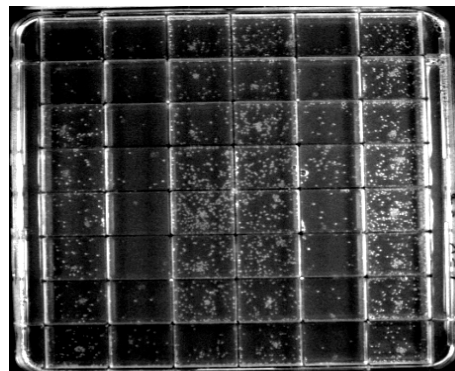
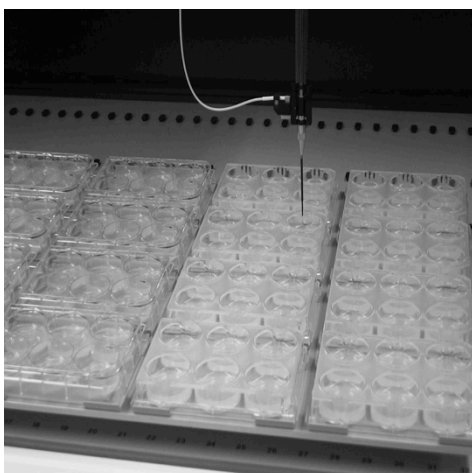
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Recombinational cloning: HT – plating by robot



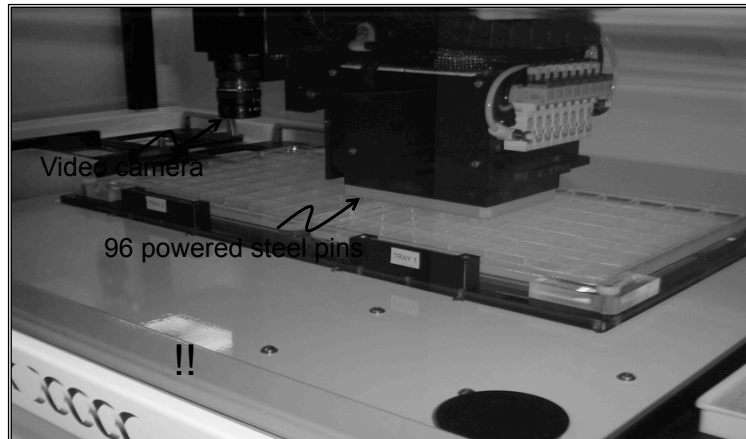
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Recombinational cloning: HT – transformation and plating



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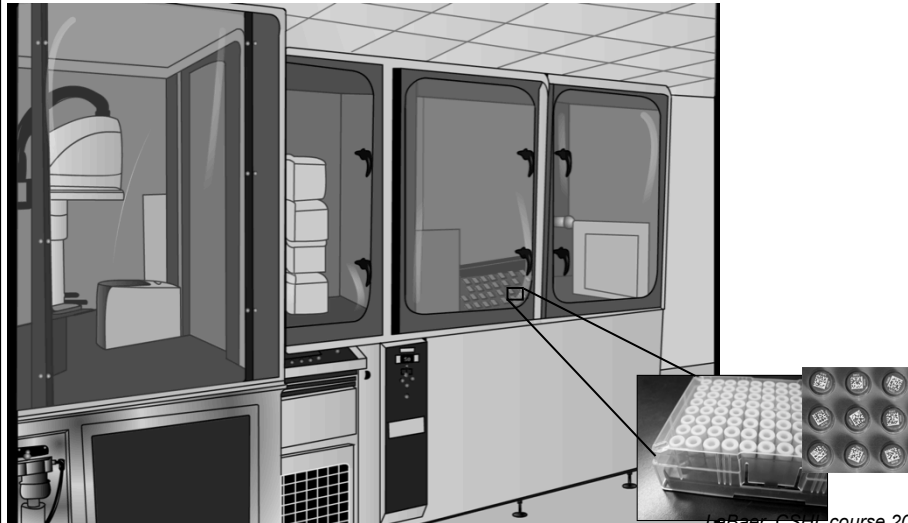
Recombinational cloning: HT – picking bacterial colonies by Robot



Recombinational cloning: HT – Automated DNA Preparation



Recombinational cloning: cDNA clone repositories



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Recombinational cloning: Advantages

- Directional cloning
- Maintains reading frame
- No restriction enzymes
- No ligation
- 1 hour, RT reaction with >99% efficiency
- No re-sequencing
- Compatible for automation

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Recombinational cloning: Requirements for cell-free microarrays

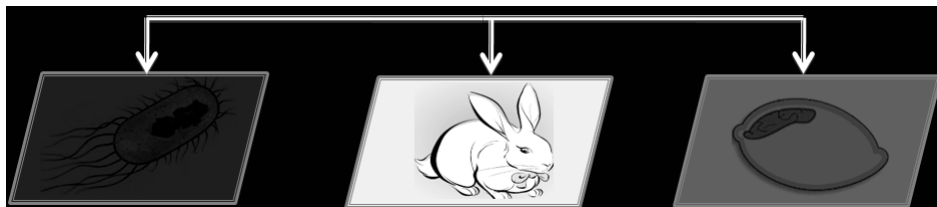
- A repository of expression-ready clones in flexible cloning system that enables easy sub-cloning between various expression constructs
- A pipeline for quickly purifying DNA constructs and arraying them for microarray applications

Cell-free protein synthesis

Cell-free protein synthesis

- Cell-free systems eliminate the need to express and purify proteins
- Cell-free synthesis lysate contain machinery for transcription & translation
- Commonly used cell-free expression systems
 - Wheat germ extract
 - Rabbit reticulocyte lysate
 - *E. coli*

Commonly used cell-free expression systems



Choice of cell-free protein synthesis systems

	<i>E. coli</i> extract	Rabbit reticulocyte lysate	Wheat germ extract
Post-translational modifications	No	Yes	Yes
Synthesized proteins (majorly)	Incomplete polypeptides	Full length protein	Full length protein
Template source	Mainly bacteria	Mainly Animal	Mainly Plant

Spirin, 2004

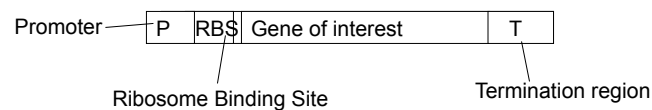
Eukaryotic (wheat germ or rabbit reticulocyte)

- Advantages
 - Higher stability
 - Better compatibility with eukaryotic mRNAs
- Disadvantages
 - Lower translation rate
 - Lack of sufficient knowledge
 - Complexity

Commonly used cell-free expression systems: *Animation*

Cell-free protein synthesis

- DNA template
- A promoter
- A translation initiation signal or Kozak sequence
- A universal DNA sequence
- A transcription and translation termination region



Cell-free expression systems for microarrays

- Able to utilize wide variety of DNA templates
- Simple, quick and cost-effective process
- HT protein production in single reaction

Summary

- Recombinational cloning
 - BP and LR reactions
 - HT cloning
- Cell-free protein synthesis system
 - Wheat germ extract
 - Rabbit reticulocyte lysate
 - *E. coli*

Microarray data analysis challenges

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