Interactomics Protein Arrays and Label-Free Biosensors

Professor Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology Bombay

Module 05
Lecture 26
An Introduction to Cell-free Protein Synthesis

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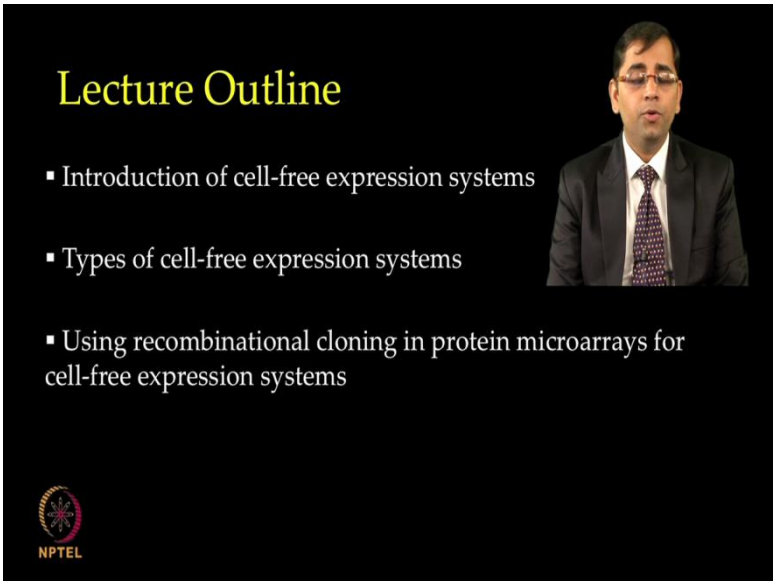

Welcome to MOOC NPTEL course on interactomics. In our previous lecture, we had discussed about recombinational cloning. In today's lecture, we will discuss how recombinational cloning is of use to the protein microarray field.

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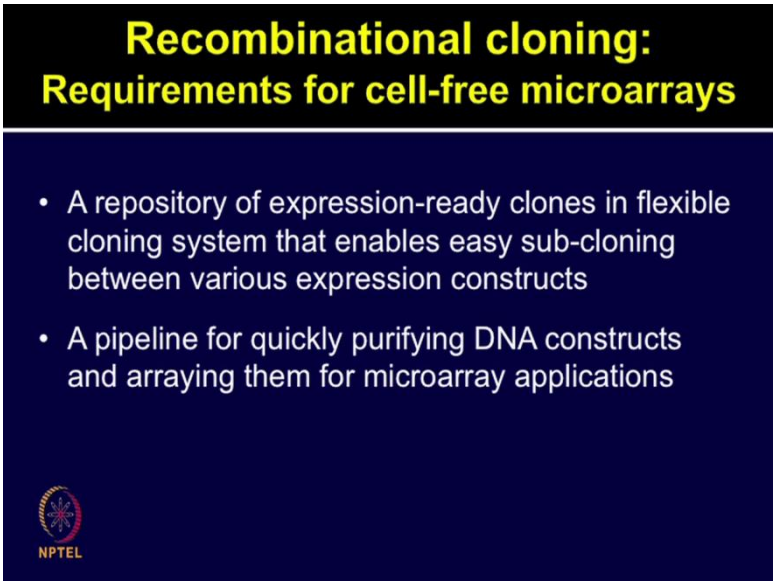

Once, we have done, the recombinational cloning and obtained several master clones, the repository of expression ready clones in a flexible cloning system, it will enable the easy sub-cloning between various expression constructs. A pipeline for quickly purifying DNA constructs and arraying them for the microarray applications can be generated thereafter. But all this requires large number of genes of interest, the c-DNA clones in the right expression vectors, by doing the recombinational cloning one can actually have the flexibility to perform various type of applications including microarray applications.

The method by which one can do this is by using cell-free expression systems. Master clones are important and are required regardless of whether you want to make the protein by cell-based or cell-free method, since microarray require printing of proteins in a high throughput manner. It is very difficult to purify thousands and thousands of proteins and especially when you want to preserve their activity and want to perform functional assays to test using these assays. To circumvent these problems people have started using cell-free protein synthesis method. The cell-free synthesis system makes use of temporary DNA in the form of plasmids or PCR products for direct in-vitro protein synthesis in the presence of a crude cell lysate, which contains all the necessary machinery required for both transcription and translation with essential amino acids, nucleotides, salts and other energy generating factors which are added (())(2:57).

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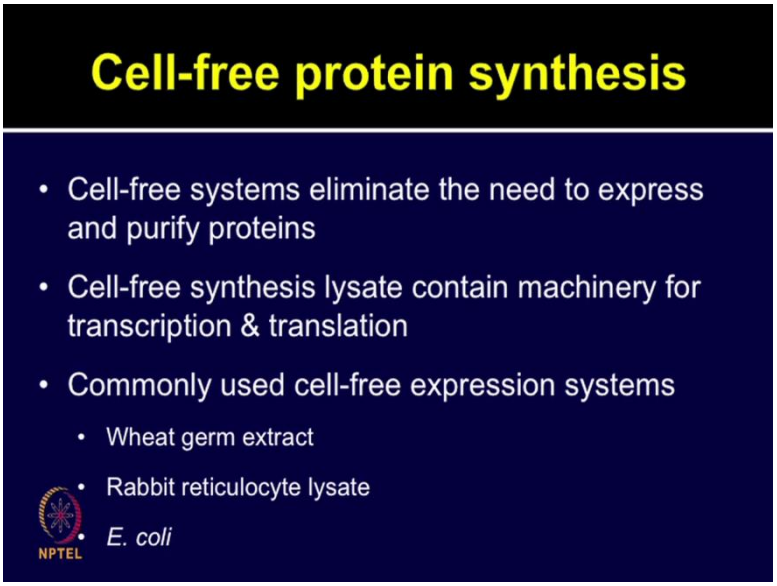

The cell-free synthesis system eliminates the need for protein expression and purification in cell-based system. Various types of DNA templates can be used such as PCR product or plasmids. The cell-free synthesis lysate contains machinery for transcription and translation.

The commonly used cell-free expression systems are E. coli, wheat germ extract and rabbit reticulocyte lysate, others include those obtained from (())(3:41), hybridomass, insect and mammalian cells, one can use any of these cell free protein system but it depends on, the application requirements.

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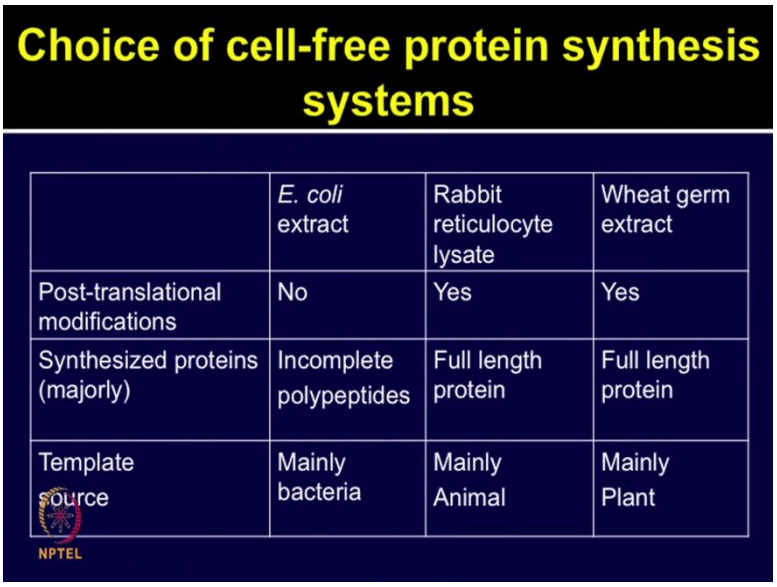

Let us do the comparison of these 3 widely used cell-free protein synthesis system, E. coli extract, rabbit reticulocyte lysate and wheat germ extract. Post-translational modification is not possible by using E. coli extracts, whereas rabbit reticulocyte lysate and wheat germ extracts that are eukaryotic systems can provide, the post-translational modifications. The synthesized proteins majorly are incomplete polypeptides, in case of E. coli extracts whereas in rabbit reticulocyte lysate and wheat germ extract, it is full length protein. The template source in E. coli is mainly from bacteria, in RRL, it is mainly from animals, especially rabbits and in WGE or wheat germ extract, it is mainly from the plants.

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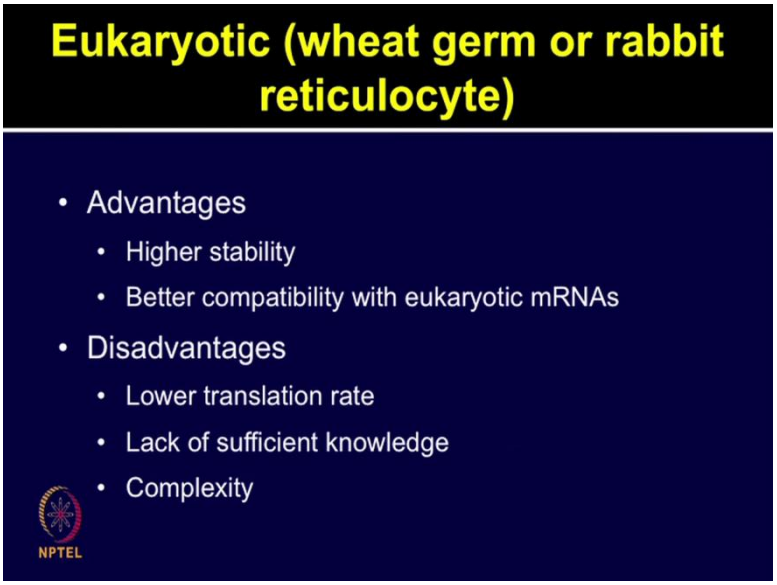

Since, we are talking about proteomics applications for the eukaryotic systems. Let us talk about eukaryotic cell-free expression systems, wheat germ extract or rabbit reticulocyte lysate, their advantages and disadvantages. Advantages of the system include higher stability, so the longer lifetime of the cell-free systems, better compatibility with eukaryotic m-RNAs and synthesis of eukaryotic proteins however, there are certain disadvantages such as lower translation rate, lack of sufficient knowledge to construct effective genetic vectors and the complexity of genetic constructs for the effective protein expression.

So, we will discuss the commonly used cell-free expression system in the following animation. The open nature of cell-free expression system provides various benefits such as an adjustable environment involvement to allow the proper protein folding disulphite bond formation and addition of labeling agents during the translational process which enables easy detection of synthesized protein.

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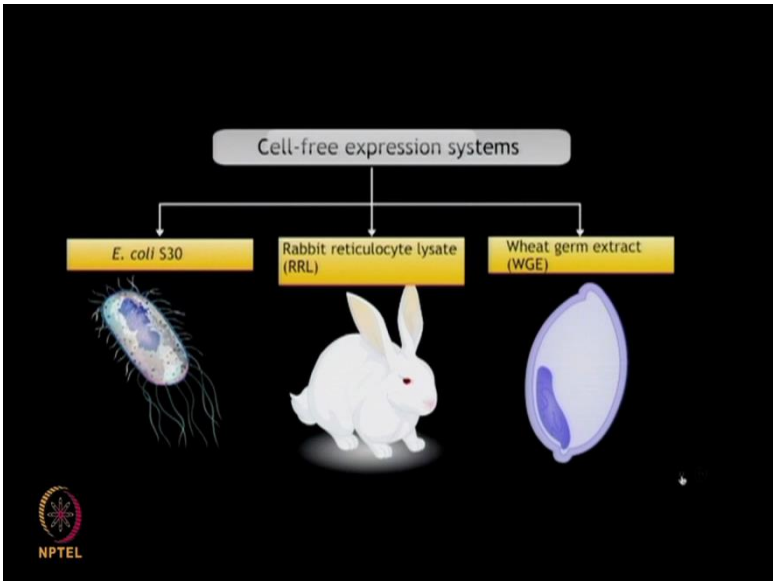

The commonly used cell-free expression systems include E. coli S30, rabbit reticulocyte lysate or RRL and wheat germ extract WGE. E. coli S30, this is a commonly used bacterial expression system that is capable of producing protein yields of around 6 mg per ml. This system however is not capable of carrying out the post-translational modifications or PTMs of proteins due to the absence of required machinery for this process and very often produces incomplete protein chains. DNA templates obtained from bacterial sources are commonly used for this cell-free lysate. Next, we will talk about rabbit reticulocyte lysate or RRL, a mammalian cell-free expression system that also gives protein yield of around 6 mg per ml. This system is more suitable for expression of full length eukaryotic proteins from plant and animal sources that require proper protein folding and post-translational modifications.

Wheat germ extracts or WGE, this is a cell-free expression system that is capable of producing full length proteins with correct folding and PTMs form plants sources. Yields obtained in this system are however slightly lower than the E. coli and RRL based cell-free expression systems.

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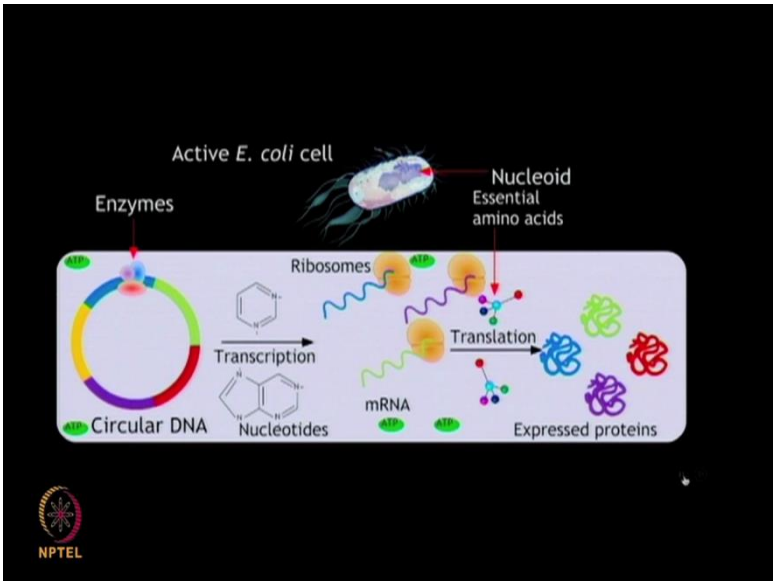

E. coli S30 extract, the actively growing and replicating E. coli cells can be used for extracting cell-free lysates, these cells that are in the process of growth and division are constantly producing proteins and other factors required for various cellular processes. The cofactors and enzymes such as RNA polymerase peptidyl transferase are available in significant quantities due to the cellular process of transcription and translation taking place in the cell. As you can see in the animation, the two step have occurred here, the transcription and translation and the required material have been provided (())(9:37).

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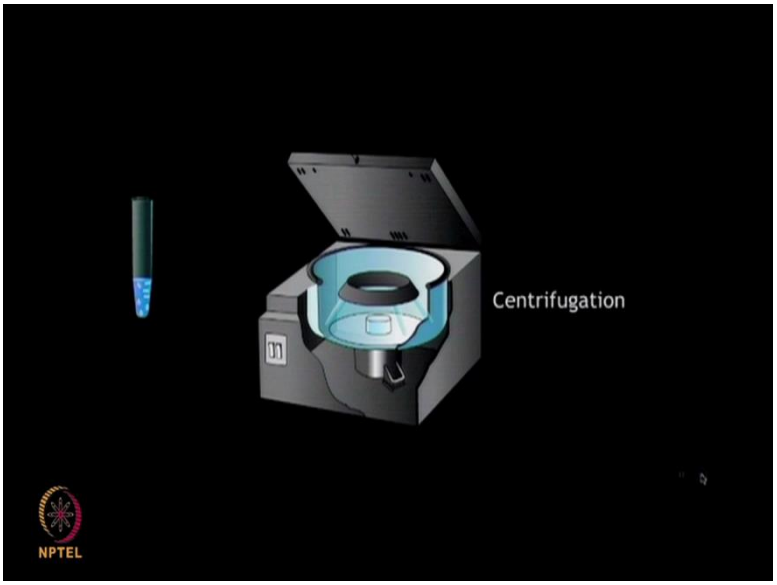

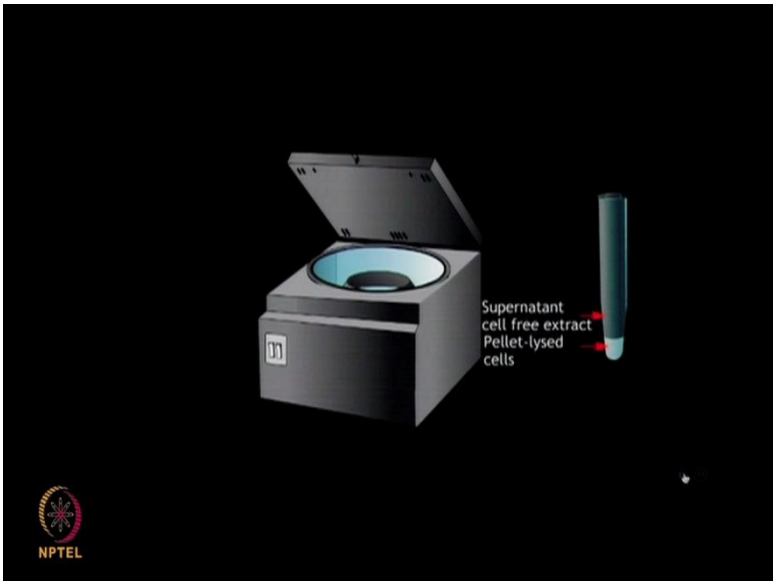

The cells are lies in a suitable buffer and after that they are centrifuged at 30000 Gto collect the supernatant containing the extract or this lysate which is present in the supernatant after the centrifugation step that contains the cell-free extract. So, lysate that will extracted from such actively growing and dividing cells will contain all the required cellular machinery to carry out in-vitro protein synthesis and requires addition of essential amino acids, nucleotides, salts and other energy generating factors.

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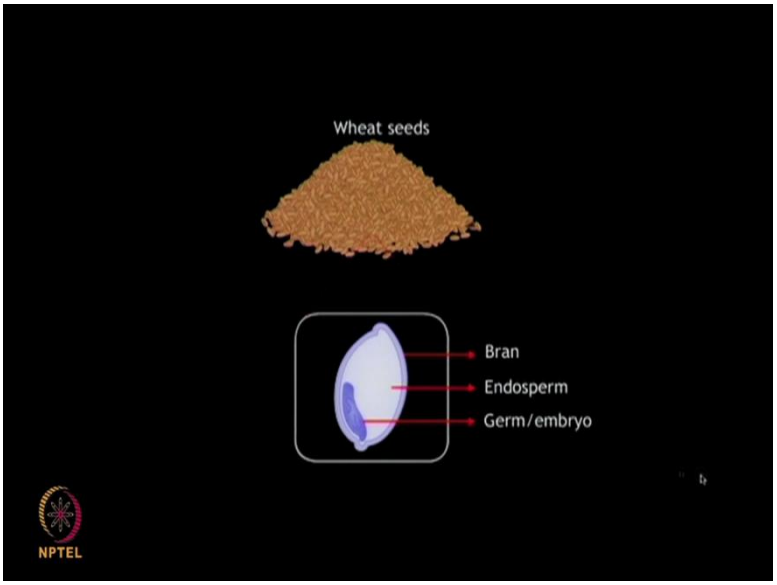

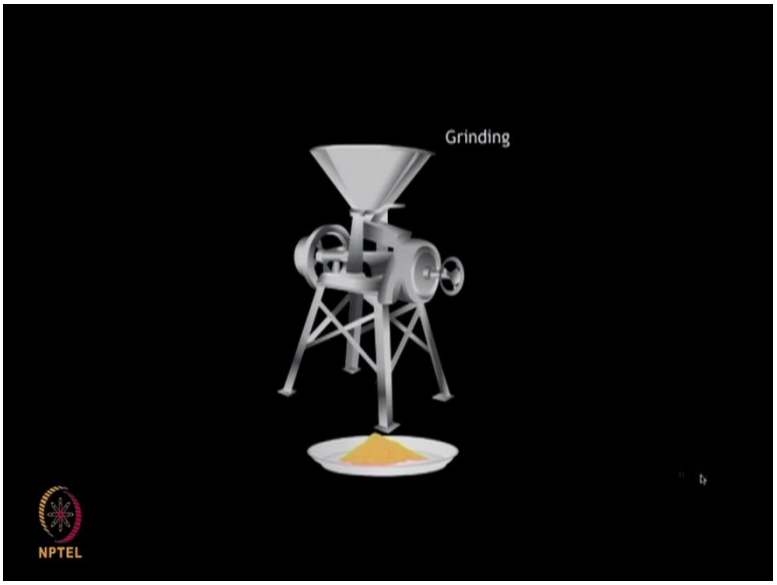

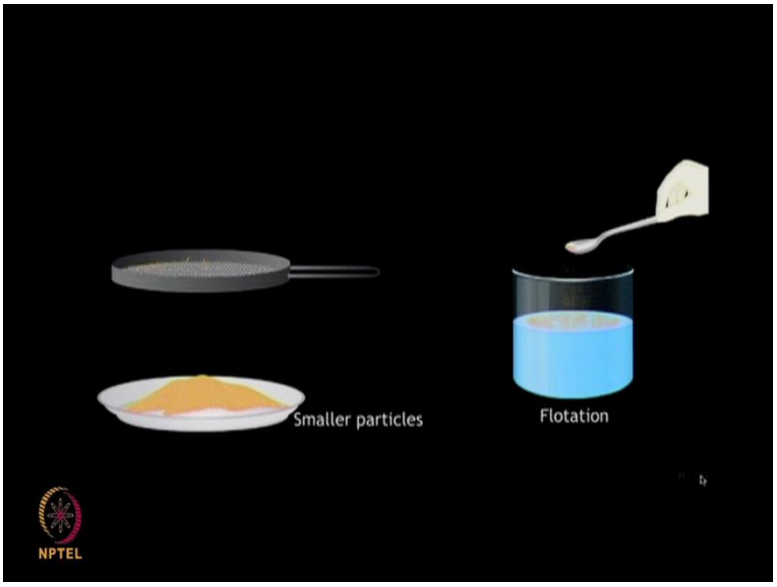

Let us now talk about wheat germ extract or WGE. This is one of the most commonly used eukaryotic cell-free expression system which is obtained from the embryo of wheat seed. The seeds are grinded and then sieve to remove their outer coating fragments. Once, the grinding is finished, the embryos and other small particles are floated in organic solvents such as cyclohexane after these embryos are floated in the solution, the floating embryos are quickly removed and dried to avoid any damage from the organic solvent.

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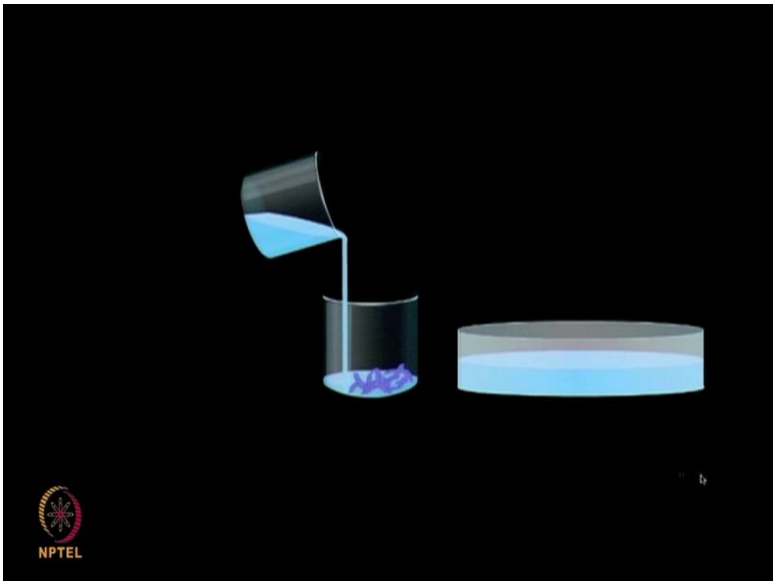

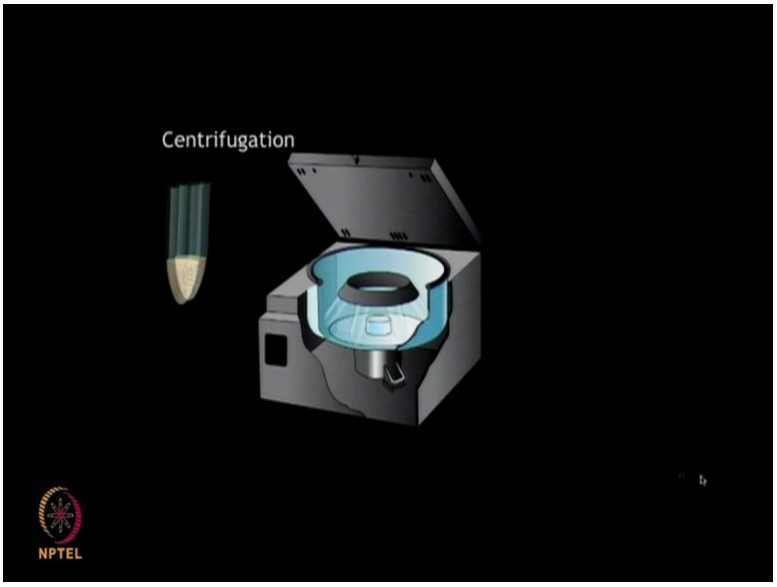

The dried embryos are carefully sorted such that only good embryos without any endosperm coating are selected; one needs to repeat this washing step few times because the endosperm contains certain inhibiters of protein synthesis which must be removed. The selected embryos are washed thoroughly with cold water, after which they are mixed with extraction buffer and again grind it. Now, the solution must be centrifuged at 30000G at 4 degree centigrade which results in the wheat germ extract forming a layer between the top fatty layer fraction and the pellet at the bottom, this you can see in the tube here.

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So, this fraction can then be separated and purified by chromatographic methods to remove any components of the extraction buffer. This cell-free lysate is capable of synthesizing full length eukaryotic proteins which provides yields of around 4 microgram 4 mg per ml. So, what do you need for a successful cell-free protein synthesis reaction?

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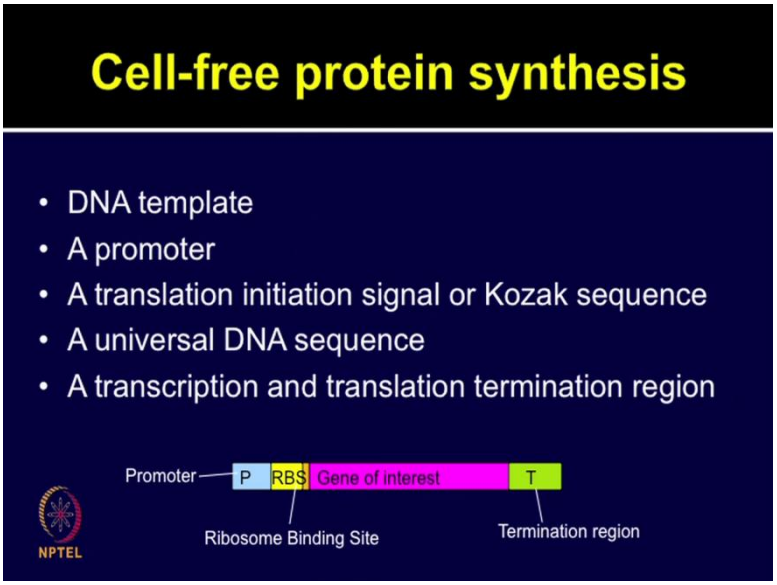

You need certain elements like a DNA template, which may be a plasmid or a PCR fragment, a promoter which could be T7, SP6 or T3, a translation initiation signal, for example, (())(14:13) in case of prokaryotes or Kozak in eukaryotes. A universal DNA sequence for protein initiation and a transcription and translation termination region, all these components are essential for cell-free protein synthesis. The cell-free expression system, allows rapid

conversion of genetic information directly to the functional protein. It facilitates synthesis of several proteins in a single reaction. So, let me show you an animation for in-vitro protein synthesis which will explain you these concepts very easily.

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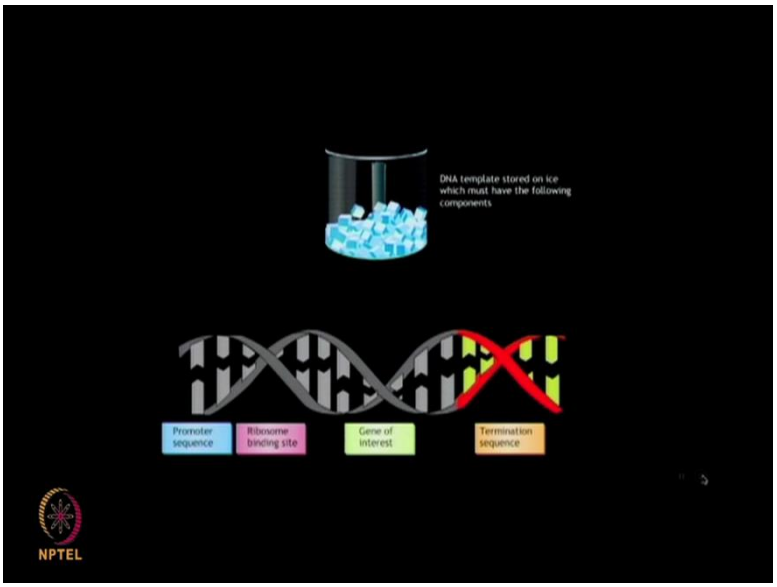

First, the DNA template is thought (())(15:06) and then placed on ice during the preparatory process. For in-vitro protein synthesis to take place, the DNA template must contain the gene coding for the protein of interest. In addition to this, there must be a promoter sequence which can initiate the transcription process, a translation initiation sequence for binding of ribosome as well as a suitable termination sequence to correctly synthesize only the proteins of interest.

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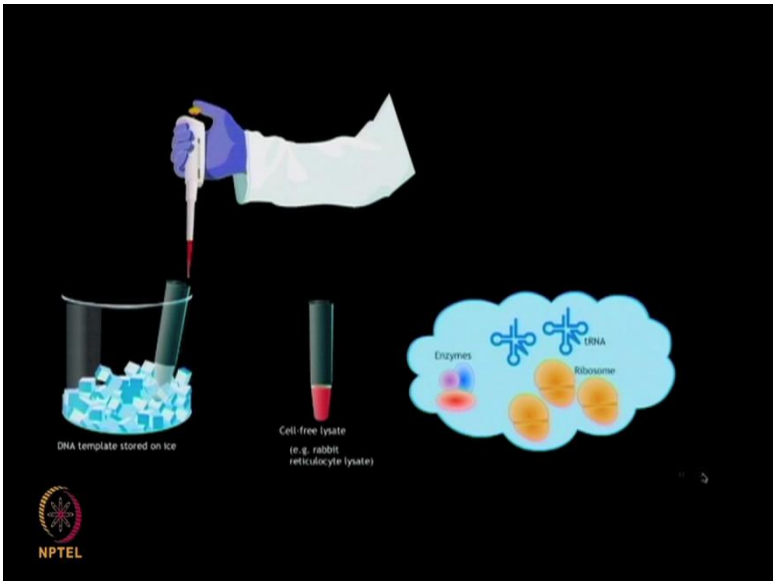

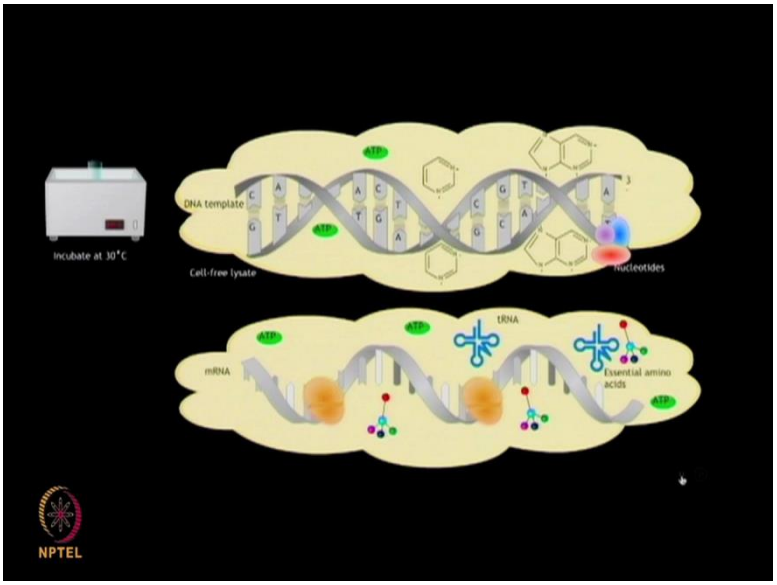

The thought cell-free lysate containing the essential cellular machinery for protein synthesis is added to the DNA template followed by other exogenous factors that are require for the process such as essential amino acids, nucleotides, ATP excetra. All these are done while storing the template on ice to ensure that there is number loss of activity. The tube containing all the required components is then incubated at 30 degree centigrade. The enzymes for transcription bind to the promoter sequences and in the presence of other factors such as ATP and nucleotides, they carry out synthesis of m-RNA transcript. This m-RNA is then translated into the corresponding proteins due to the help of ribosomes, t-RNA, enzymes and other factors which are required for the process.

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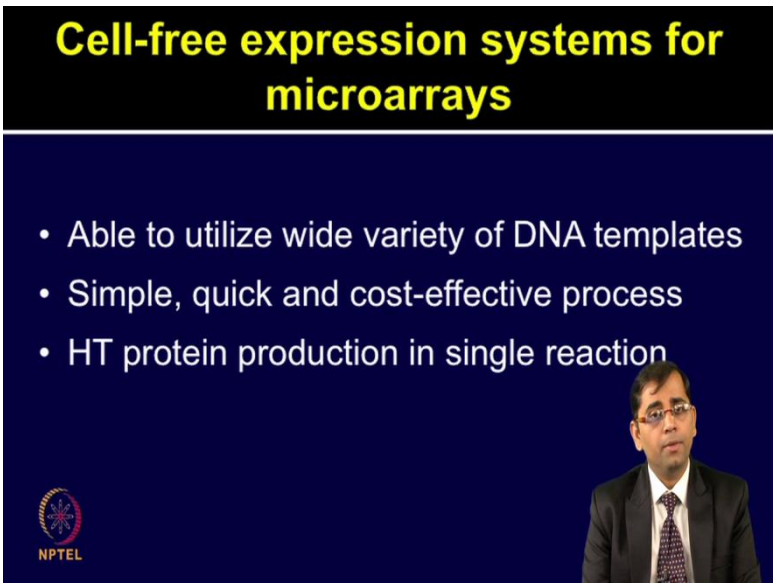

The cell-free expression systems are required for certain types of microarrays but why should it be used, because it has the ability to utilize wide variety of DNA templates, one can make use of all the master clones obtained from the recombinational cloning. Cell-free expression system is a simple, quick and cost-effective process. It provides a high throughput production in a very short time and in a single reaction. So, all of these requirements are very useful for high throughput protein production and for the microarray generation.

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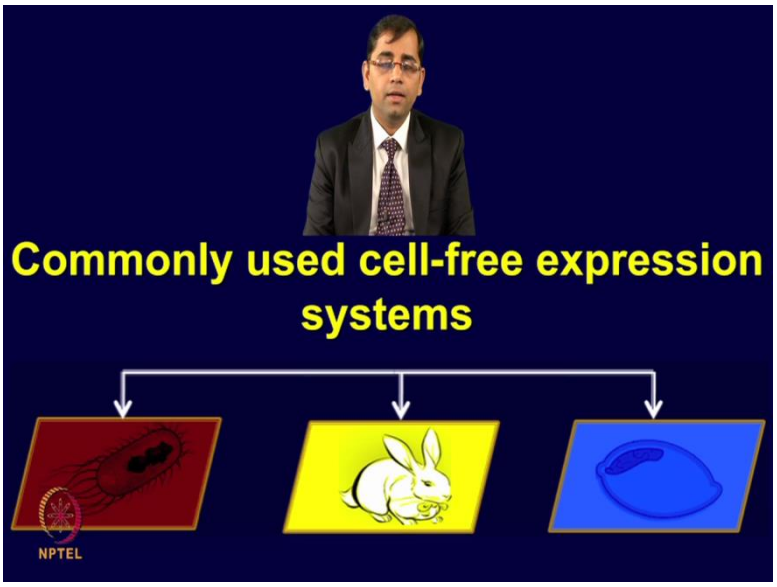

In summary, today we have tried to understand how recombinational cloning techniques can be applied to protein microarrays using cell-free expression systems by easily producing proteins. We discussed about, wheat germ extracts, rabbit reticulocyte lysate and E. coli based systems. There are currently only a handful of commercial alternative to cell-free expression system, products some (()(18:30), thermo fisher and NAB are few among these, which are commercially available in the market. The concept learned today will be very important when we discuss further about protein microarrays and especially the cell-free based protein microarrays and its type in the following lectures, Thank you.

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Summary

- Recombinational cloning is a powerful technique to increase throughput in a protein microarray experiment.
- This method utilizes cell-free expression system (CFES), where the system contains the entire transcription translation machinery to express protein *in-vitro*
- There are various kinds of CFES commercially available where one can choose depending on the type of protein they want to express.
- Thus, by printing a large repertoire of cDNAs on a chip, high-throughput expression can be facilitated using CFES.

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Summary

- Advantages of CFES include higher stability; better compatibility and synthesis of eukaryotic proteins.
- Drawback include lower translation rate, lack of sufficient knowledge to construct effective genetic vectors and the complexity of genetic constructs for the effective protein expression.

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References

- Alberti, S., Gitler, A.D., and Lindquist, S. (2007). A suite of Gateway® cloning vectors for high-throughput genetic analysis in Saccharomyces cerevisiae. Yeast 24, 913–919.
- Xu, R., and Qingshun, L.Q. (2008). Protocol: Streamline cloning of genes into binary vectors in Agrobacterium via the Gateway® TOPO vector system. Plant Methods 4, 4.
- Chandra, H., and Srivastava, S. (2010). Cell-free synthesis-based protein microarrays and their applications. Proteomics 10, 717–730.
- Chandra, H., Reddy, P.J., and Srivastava, S. (2011). Protein microarrays and novel detection platforms. Expert Rev. Proteomics 8, 61–79.
- Gupta, S., Manubhai, K.P., Kulkarni, V., and Srivastava, S. (2016). An overview of innovations and industrial solutions in Protein Microarray Technology. PROTEOMICS 16, 1297–1308.

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References

- Mitchell, P. (2002). A perspective on protein microarrays. Nat. Biotechnol. 20, 225–229.
- Ramachandran, N., Larson, D.N., Stark, P.R.H., Hainsworth, E., and LaBaer, J. (2005). Emerging tools for real-time label-free detection of interactions on functional protein microarrays. FEBS J. 272, 5412–5425.
- Ray, S., Mehta, G., and Srivastava, S. (2010). Label-free detection techniques for protein microarrays: Prospects, merits and challenges. PROTEOMICS 10, 731–748.
- Syed, P., Gupta, S., Choudhary, S., Pandala, N.G., Atak, A., Richharia, A., Kp, M., Zhu, H., Epari, S., Noronha, S.B., et al. (2015). Autoantibody Profiling of Glioma Serum Samples to Identify Biomarkers Using Human Proteome Arrays. Sci. Rep. 5, 13895.
- MacBeath, G. (2002). Protein microarrays and proteomics. Nat. Genet. 32, 526–532.