Computational Systems Biology
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Lecture - 63
Perturbations to Metabolic Networks: Deletions

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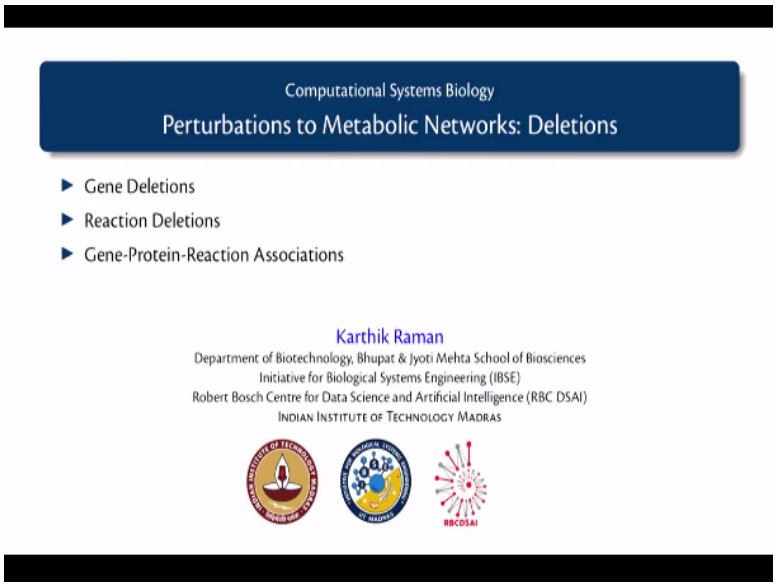

So from this video onwards we will start looking at perturbations to metabolic networks wherein we will particularly start focusing on gene deletions or reaction deletions and in this video we will focus also on gene-protein-reaction associations which is central to our understanding of you know what happens when you remove a gene or a reaction from the model right.

So there is a relationship between genes and reactions, a many to many relationships in fact so when you remove a particular set of genes, there is a particular set of reactions that go out and so on.

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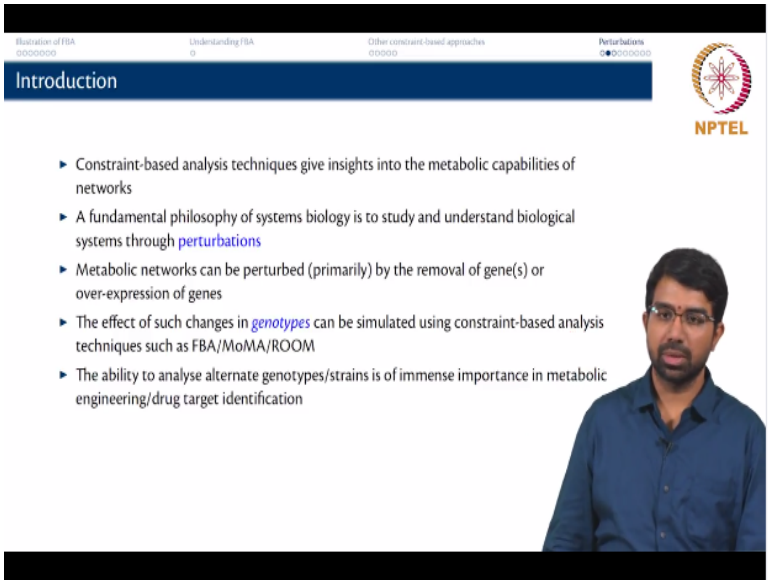

So if you recall the whole premise of systems biology or the whole very important aspect of systems biology is to be able to study perturbations, knock something out, add something in, see what is the effect right and metabolic networks can be perturb primarily by removal of genes or over expression of genes or reactions as well right and the effect of such changes can be studied using constraint-based techniques such as FBA, MoMA, ROOM.

So ROOM was the name of the other method right, it was regulatory on-off minimization. So the ability to analyze alternate genotypes or strains of great importance in metabolic engineering and drug target identification. So this is a very commonly used technique wherein we try to see how to reroute right.

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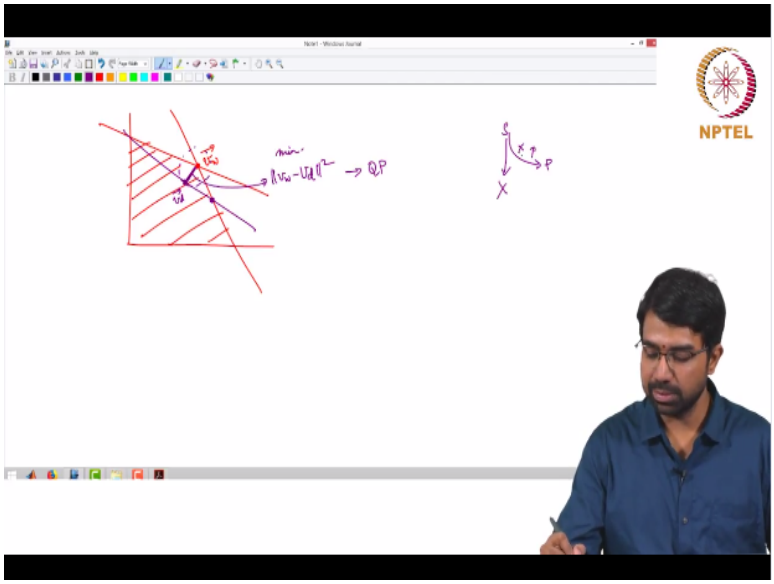

I did mention it in a few classes earlier. This is what the cell is trying to do and you want to reroute it towards this. So can you delete some genes, increase some genes.

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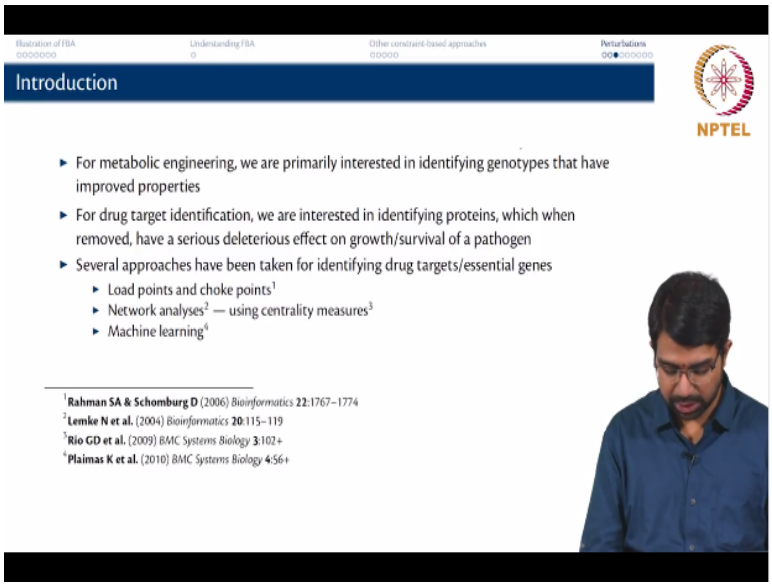

So for metabolic engineering, we are primarily interested in identifying genotypes that have improved properties whereas for drug target identification we want the opposite, you want to identify phenotypes that are lethal. What genes do I hit in the pathogenic organism so that I stop it from doing and there are many approaches that have been takes for identifying these kinds of essential genes.

So there are some basic network topology measures which look at load points and choke points and so on. There are again centrality measures that have been used and machine learning with the most effective or the more reliable methods involve flux balance analysis because flux balance when you have a good model can give you very robust predictions.

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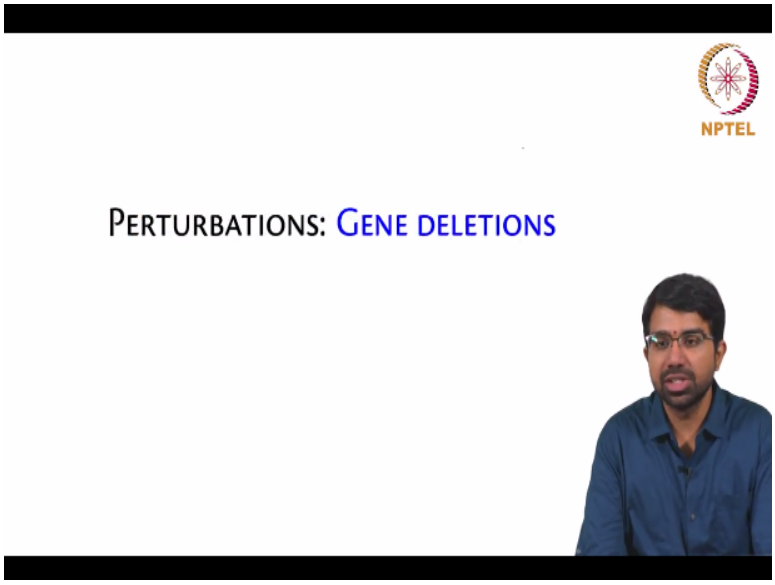

So let us look at gene deletions.

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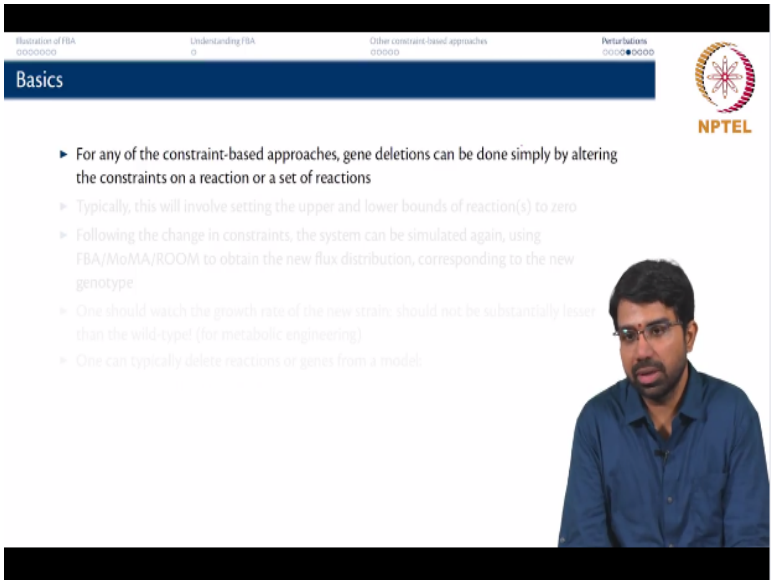

For any of the constraint-based approach gene deletions can be simply done by altering the constraints on a set of reactions.

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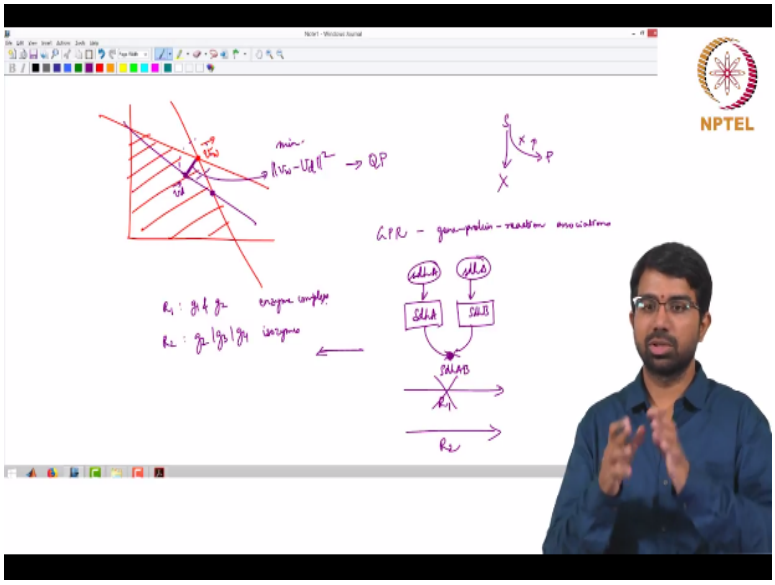

So at this point it is important to recall what is a GPR? We did this some classes ago, gene-protein-reaction associations right. Succinate dehydrogenase A and a gene succinate dehydrogenase is B which codes for a protein SdhA, SdhB and maybe they complex together to form a protein SdhAB which catalyzes reaction 1 and reaction 2 so which means if you now remove SdhA reaction 1 with be turned off, you remove SdhB reaction 2 will be turned off.

And in fact there is no way to turn off reaction so I should draw it may be like this. There is no way to turn off reaction 1 alone right because to turn off reaction 1 you will need to remove SdhA which will automatically turn off reaction 2 right. So this can translate to a set of Boolean rules. So you can say R1 is g1 and g2 right or let me just use the and symbol or R2 is g2 or g3 or g4 right.

So these are like isozymes and this is like an enzyme complex **"Professor - student conversation starts."** Oh yeah so isozymes are enzymes that are equivalent right so they may be able to substitute for one another but they may be different. We will just come back to that. So they may not be perfectly equals right, so isozyme 1 might do the reaction very well, isozyme 2 might be able to do the reaction to 80% of that capability.

So it might end up that the organism grow slower when you delete isozyme 1 but when you delete isozymes 2 there is no effect on the organism. **"Professor - student conversation ends."**

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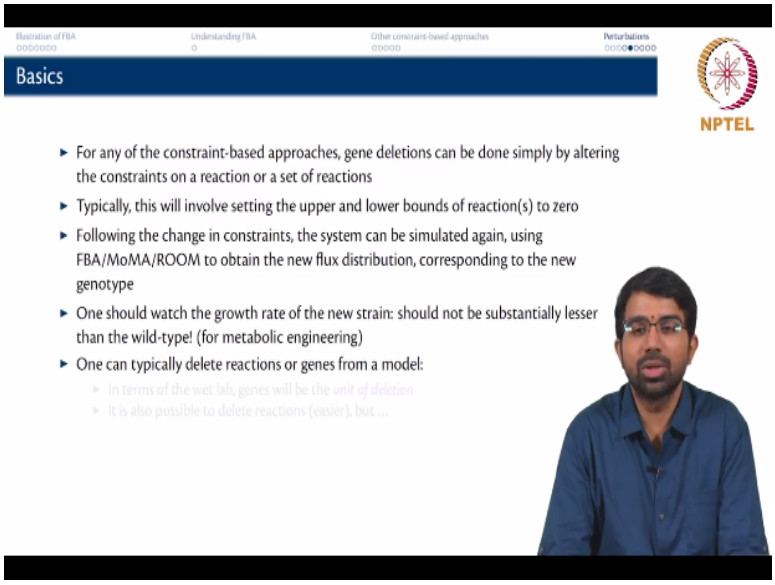

Once again what does it mean to delete a reaction? We said v5=0 is what we said but how do you put that as a constraint. Easiest way to do it to set lower bound and upper bound as 0, you can add another column to the stoichiometric matrix if you want right where you just have v5 alone right and then say that that is equal to 0 but it makes much easier to basically add, change the lower bound and upper bound vectors.

So typically you change the lower and upper bounds to 0. Following this change in constraints, you can resimulate the system using any algorithm of your choice FBA, MoMA, ROOM whatever corresponding to that altered genotype, the perturbed genotype but you still watch out for the growth of the new strain especially in case of metabolic engineering. The growth rate really drops even though you get enough product, it may not be sufficient right.

So you can either delete reactions or genes from a model right. So what is the difference between the two? **"Professor - student conversation starts."** So it finally has to result in the reaction deletion right but when you delete reactions and all you may be doing some unacceptable deletions as I showed in the previous example you could not have deleted R1 alone right. **"Professor - student conversation ends."**

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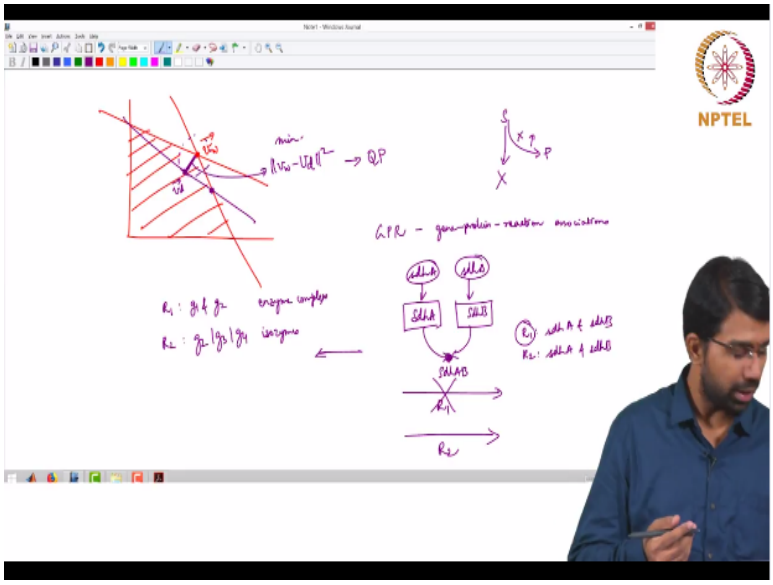

There was no way to delete R1 alone, so if you wanted to delete R1 you have to remove one of these which means R2 to go out automatically. So this simulation is not biologically relevant in some sense but the reaction still remains the unit of the model right. The unit in the model which you can delete still remains the reaction there is no meaning in removing a gene from the reaction from the model.

When you say you remove a gene from a model, it means that you are removing all the corresponding reactions out of the model.

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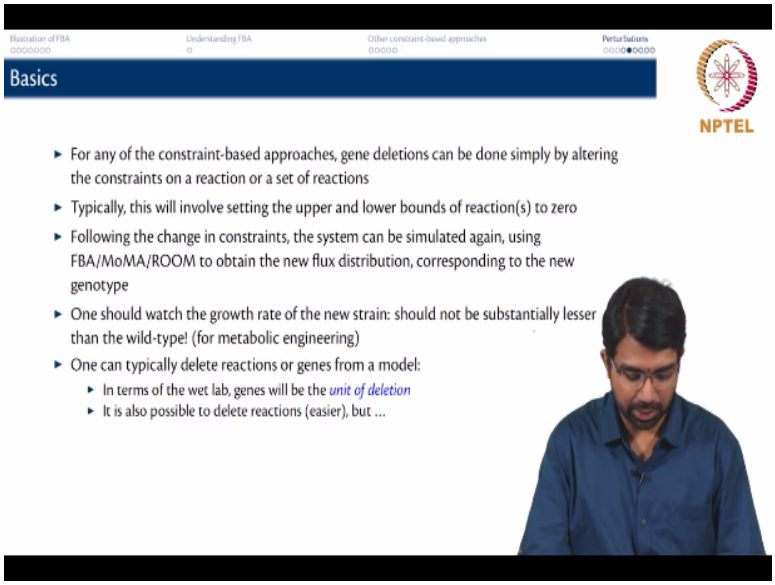

In terms of the wet lab, the genes are the unit of deletion. You cannot knockout a reaction in the wet lab. You will be able to knockout a gene using SIR or any of those techniques right.

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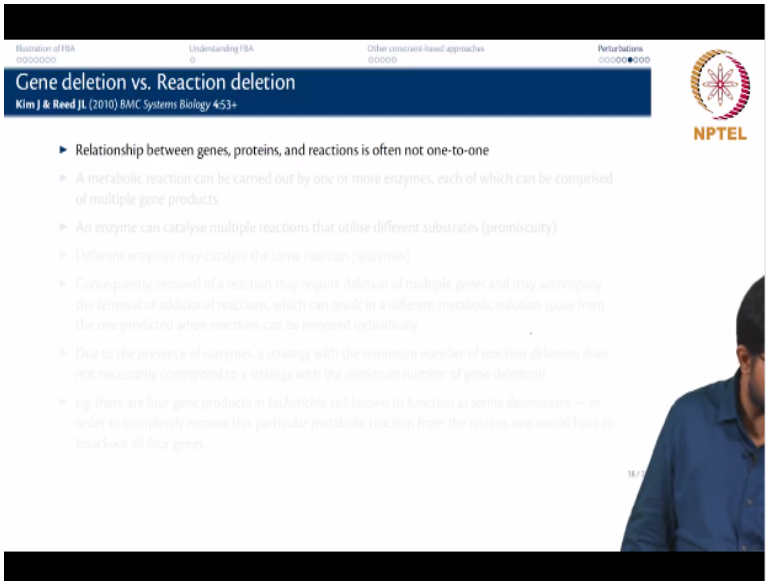

So it is also possible to delete reactions but you have to be careful because of these complex gene-protein-reaction relationships. So this relationship between genes, proteins and reactions is often not one-to-one so it can be even more complex.

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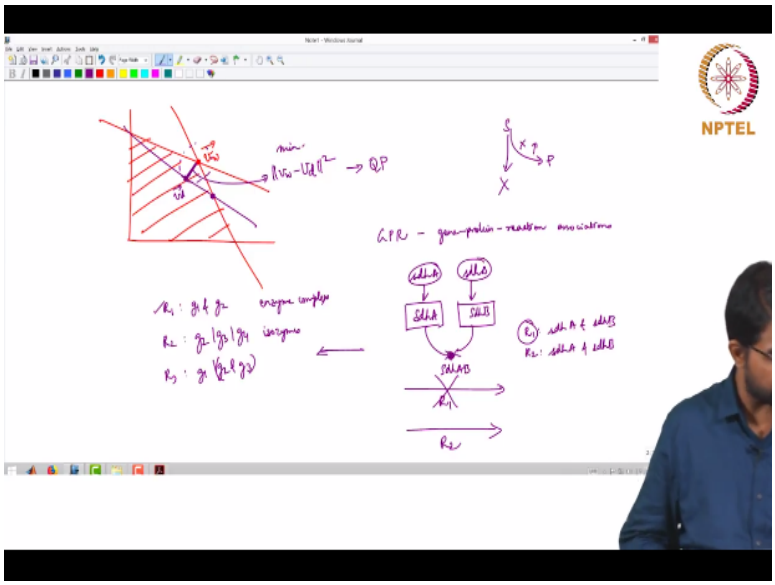

So we just had a simple example here. You already see this is one to many right so you can have another R3 which is g1 or g2. So when you touch g1 or when you touch g2 here, all these reactions are going to change. In fact, only this but you will have to see what are the effect on all the reactions where g2 is involved. It could be something like so you can have very complex gene-protein-reaction associations.

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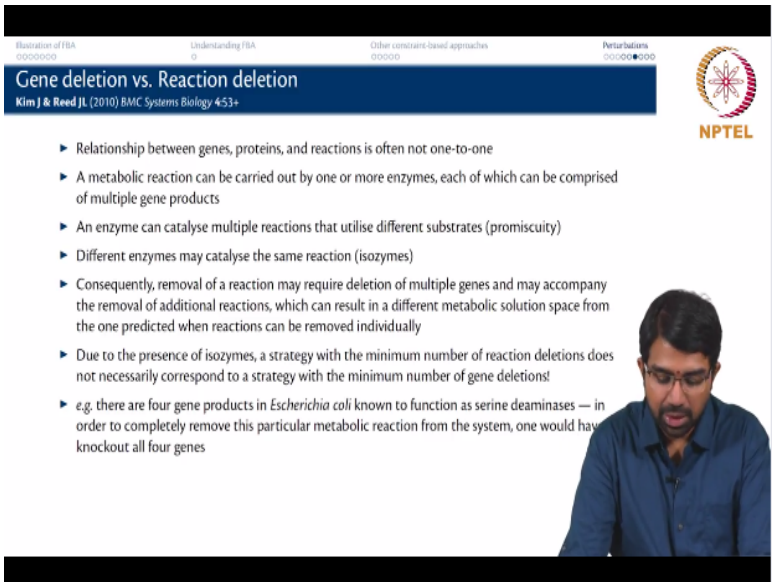

So metabolic reaction can be carried out by one or more enzymes, so it could be a multienzyme complex that is carrying out a complex series of reactions. So the same enzyme carries out 20 reactions, it is quite possible right. It could be a polymerase or fatty acid synthase. An enzyme can catalyze multiple reactions that utilize different substrates promiscuous enzymes.

Different enzymes may catalyze the same reaction your isozymes. So consequently the removal of a reaction may require deletion of multiple genes and may accompany the removal of additional reactions as well right which can result in a very different metabolic solution space from what you do when you just remove one reaction at a time. So when you remove g2 here it takes away R1 and R3 right.

So it has effect on several reactions at the same time. So then again due to the presence of isozymes the strategy corresponding to minimum number of reaction deletions does not correspond to a strategy with a minimum number of gene deletions simply because there are 4 genes in E. coli that can function as serine deaminases.

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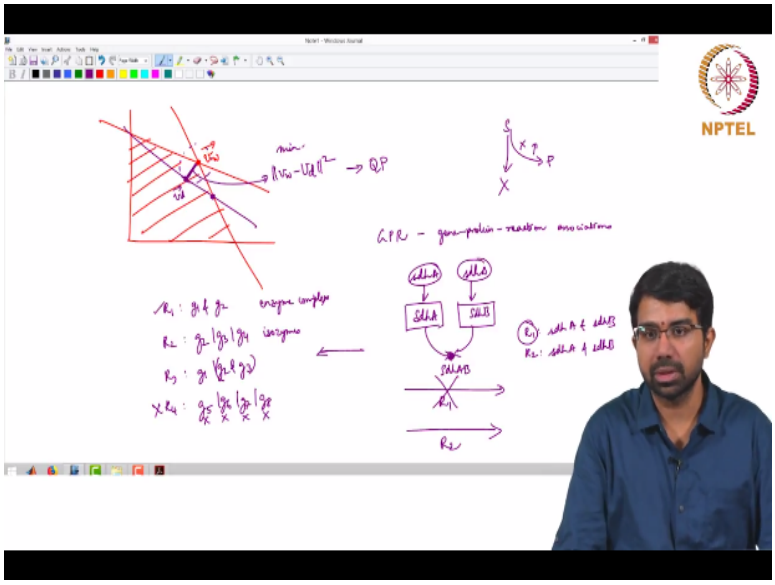

So to completely remove that reaction that reaction is something like R4 is g5 or g6 or g7 or g8, so to remove R4 you have to knockout all these 4, this might be quite a challenge experimentally to knockout 4 enzymes.

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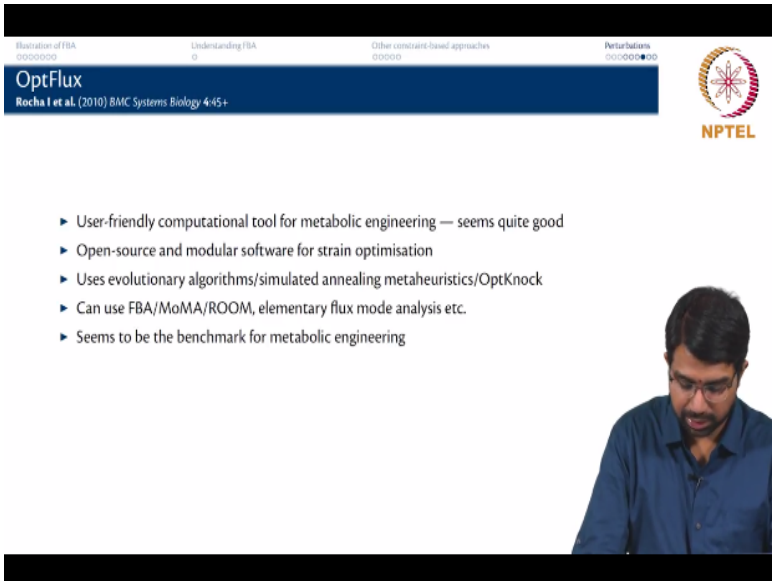

So there are some very nice tools that are useful for these kinds of studies. So there is OptFlux which is a very user friendly computational tool for metabolic engineering. It is an open source in modular software for strain optimization. It also uses simulated annealing and evolutionary algorithms to optimize the genotype. It basically tries to do a search in genotype space, the optimization is essentially on the genotype.

Can I find the best genotype that has the optimal genotype that has my interesting phenotype and it can use FBA, MoMA, ROOM and there are other tools. We will study about elementary fluxes later on and it seems to be the benchmark for metabolic engineering.

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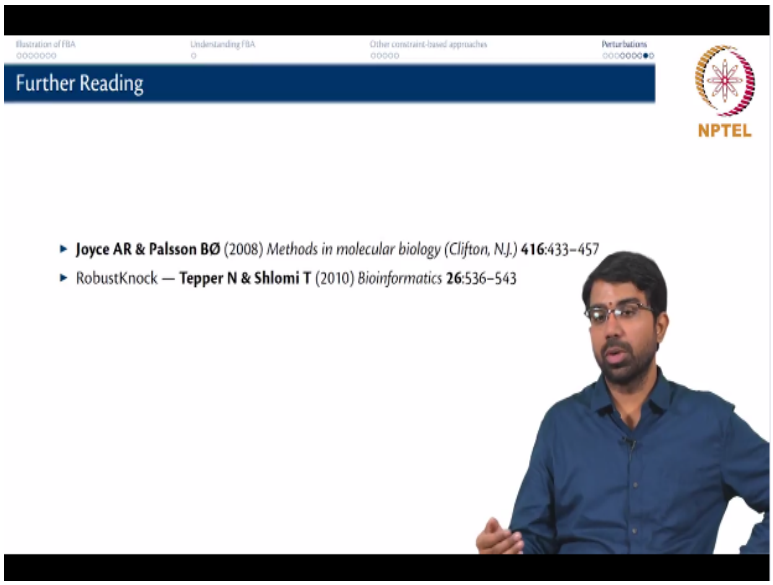

And these are two very good papers which I think you should take a look at. RobustKnock is another tool and the first method in molecular biology chapter essentially talks a lot about the protocol for doing gene deletion. You said this to 0 then you observe this, what does it mean, when you will get a true position true negative, we will try to discuss some of these in the coming class.

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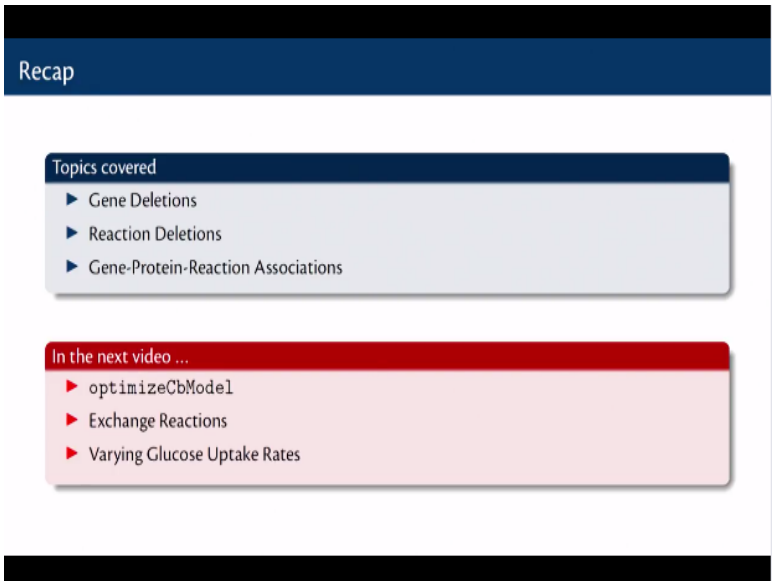

So in this video, we had an overview of how we perform gene deletions or reaction deletions, how do we set up that as an optimization problem and we also understood that you know

there is a corresponding between genes and reactions which is usually captured by other gene-protein-reaction associations and this is important to know what genes can be deleted or what genes need to be deleted when you want to turn off a reaction and vice versa.

In the next video, we will do a lab wherein we look at this all important function or you know I could call it even the FBA function called optimizeCbModel, it performs both FBA and I think even MoMA and we will also understand the concept of exchange reactions and how do you find them out from a model because if you want to change the medium of growth for a model and so on how do we go about doing it and will also do a simple experiment wherein we vary glucose uptake rates and study what is the effect on growth rate of the organism.