# Frequently Asked Questions

**Q: Can I download RE:IN?**

A: We provide RE:IN as a web service, which runs in your browser. This means that you do not have to download anything to use RE:IN.

**Q: Do I need to pay to use RE:IN?**

A: No, the required computational power is provided through Microsoft’s Azure cloud service.

**Q: How do I cite RE:IN?**

A: Our methodology manuscript, *A Method to Identify and Analyze Biological Programs through Automated Reasoning*, is currently under review. Until it is published, please reference our publication doi:10.1126/science.1248882.

**Q: How do I load my own study into RE:IN?**

If you are a first time user, the best place to start is the tutorial that is provided on our webpage. This takes you through how to encode models and constraints into RE:IN, and how to conduct analysis. Example case studies are also provided, which link to our methods publication on this tool and the approach behind it (*A Method to Identify and Analyze Biological Programs through Automated Reasoning*).

**Q: Can RE:IN handle asynchronous update schemes?**

A: Yes. This option is set in the tool in the ‘Options’ tab, where you will find a drop down menu to select either synchronous or asynchronous updates.

**Q: How large a network can RE:IN handle?**

A: This is not a simple question to answer, as it depends on the number and nature of the constraints that you are encoding together with your ABN. If you find that RE:IN takes a significant amount of time to produce a solution, then you contact one of the scientists under the ‘People’ tab on this project page.

**Q: Can I set all interactions to be possible in my Abstract Boolean Network?**

Yes. It is important to bear in mind that not including an interaction as optional is as strong an assumption as including an interaction as definite. Any interaction not set as possible or definite cannot be considered by the solver.

**Q: Can RE:IN handle cycles or oscillations?**

A: Yes, you can specify oscillations simply under synchronous updates by specifying three consecutive states, and cycles can be encoded as for the Yeast cell cycle example we provide.

**Q: How can I identify that a component is not required?**

A: As we outline in our methods publication, it is best to start with the set of critical components that are known to be functionally relevant for the system under investigation. During the analysis, by searching for the set of required and disallowed interactions, should you identify that there are no interactions pointing out of a component – not even possible interactions – then you have confirmed that it is not required to act as a regulator, and can be removed from the analysis (provided there is no biological evidence for its importance).

**Q: Is there a way to define transient states through which the system must pass, without specifying exact timesteps?**

A: Yes, this is possible by capturing different possible instances of these transient states within ‘or’ statements. Similarly, ‘and’ statements can be used. For example:

#Experiment[0] |= $InitialState;

(#Experiment[1] |= $TransientState) or (#Experiment[2] |= $TransientState) or (#Experiment[3] |= $TransientState);

#Experiment[4] |= $FinalState;

**Q: How many timesteps should I use in my constraints?**

Time steps need to be specified, but the values do not need to match precisely with physical time. For example, when specifying that a steady state is reached ‘eventually’, selecting a large final time step also allows such experiments to be reproduced with shorter trajectories, where the final steady state is repeated a number of times. Similarly, when encoding transient states or cycles, the precise step of each event can be omitted by instead specifying a range as above (e.g. the event is satisfied at step 0 or at step 1, etc.).

Within the tool, we enable the user to examine the recurrence diameter for the set of models. In principle, the recurrence diameter of a system provides an over-approximation of the longest trajectories that need to be considered to reproduce any experiment. Under the ‘Tools’ tab on the right hand side of RE:IN, you can either ask RE:IN to calculate the diameter, using the ‘Find Diameter’ button, or instead check whether a maximum timestep is sufficient, by entering a value and clicking ‘Check Diameter’.

**Q: Can I download concrete model solutions for further inspection?**

A: The Boolean representation of a concrete model is provided in the ‘Solution’ tab, under ‘Model’.

**Q: Can population averages be used instead of Boolean values?**

A: Currently RE:IN only supports discrete ON / OFF states for the components in the network.

**Q: Can RE:IN handle multiple discrete levels of activation, e.g. low / medium / high?**

A: Currently, RE:IN only considers ON / OFF states for each component. If you wish to consider multiple levels for a given component, one workaround is to add two nodes to represent the same component, with matching interactions. Then, if both nodes are active, the component is ‘high’, only one means it is ‘medium’ and both off means it is ‘low’.

**Q: Can I include both positive and negative interactions between the same pair of components?**

A: Yes. In general, our set of regulation conditions can cope with the situation in which both a positive and negative interaction exists between two genes. Biologically, a transcription factor can act either as an activator or a repressor for a given target in the presence of different cofactors, but such detailed regulatory mechanisms are abstracted through the use of the regulation conditions we define. If both a positive and negative interaction is included between two genes, the number of possible contexts is reduced. For example, if A activates B, A represses B, A is active and no other regulators of B are active, then the regulation conditions for B will be evaluated in the context where some activators and some repressors are active solely due to A. While this might not capture precisely the intended regulation mechanism, we do not restrict the set of models we consider to include either an activation or a repression between two genes but not both.

**Q: I’m stuck. Who should I contact?**

A: Feel free to get in touch with those under the ‘People’ tab on the project webpage. (We will add to this page if questions are posed that we have not yet included.)