How does it work

We received questions from several researchers using nematodes : ‘how does it work’ and ‘how is it different from what we use today’ ?

Let’s start with a quick overview and some observations from our side on what methods are available today to synchronize nematodes without using chemicals. As the objective is NOT to use any chemicals, we will not cover ‘bleaching’ in order to synchronize nematodes, besides that it is inherently unpredictable and its accuracy is far from perfect, let alone to cause ‘Transgenerational Effect’ effects from the bleaching chemicals. This leaves two other methods, not using ‘bleaching / chemicals’ to be considered:

Sedimentation:

The ‘sedimentation’ protocol is a ‘gravity’ based method of cleaning and semi-staging nematodes based on the relative weight / size of the organisms. The method requires a setup with a 3 step gradient of layers of sucrose solutions, each with a different density in order to separate the nematodes. Avoiding mixing the different solutions requires some skills and practice. As it’s not an ‘exact’ method the end-results are by definition not predictable. Using sucrose, this protocol is certainly not without its downside as the nematodes are exposed to high levels of ‘sucrose’ that will to a certain extend influence / interfere with the results. The question remains as to what extend sucrose is considered an ‘environmental influence’ contributing to ‘Transgenerational Effect’.

Sieving: (selecting on size)

The working principle of using sieves (mesh filters) is based on the assumption that there is a direct correlation between size and age in order to select a sub population. There are many issues with this assumption as external factors such as NGM versus Liquid cultures, sufficient feedstock, food arrest, different strains, temperature, etc less or more influence the development and therefor the physical dimensions of the nematodes. This method is far from accurate and there is no known ‘t=0’ reference to the exact time the L1’s hatched. It’s at best a ‘relative’ size sorting, where size is in fact the only ‘attribute’ of selecting a sub culture. Yes, there is a correlation between size and age, however at best ‘relative’ and for sure not ‘absolute’ in reference to the ‘t=0’ being the time of L1 hatching. Example: when food arrest is applied, there is almost no physical difference between a ~16 hours old L1 and one that only just hatched.

Over time, different systems using the ‘sieving principle’ have been invented / developed using mesh-membranes or microfluidics devices that have obstacles such as micro-pillars, micro-channels, etc to block the passage of nematodes above a certain size. In some cases, there are devices being promoted today that in fact need fluidic pressure (!) to push through the nematodes in these type of contraptions with an ‘advertised’ synchronization of approximately 80% !?. Not to mention ‘mechanical’ induced physical damage or stress …

Unless somebody proves us wrong, there are today NO known systems based on the principle of ‘sieving’ that have factually (!) demonstrated to be able to accurately (> 99%) select a L1 sub-population of nematodes with repeatable / predictable results. The ‘sieving principle‘ by itself simply does not allow this to happen.

Facing the challenges of ‘NO CHEMICALS’ being allowed in the process, plus the need for an ‘absolute’ Accuracy, Predictable, Repeatability and Ease of Use, a different working principle was needed for synchronizing nematodes in both high and low volume applications.

Early 2015 an innovative new working principle was invented, being able to synchronize nematodes, without the use of chemicals, with an absolute working principle, assuring accurate, repeatable results and not the least of all very ease of use. In addition its scalable in case very large volumes are required. Sounds almost too good to be true, right?

So how does it work ?

The working principle of the NemaSync C. elegans Synchronizer (CES) uses a 2 step process:

In the first step we ‘wash out’ all the debris and anything smaller than adults and eggs. After the ‘wash’, we proceed with the second step being the actual ‘harvest step’ / ‘synchronization step’. Adults and eggs are transferred after the washing step to the ‘Harvest’ filter. In this second stage, adults and egg’s cannot pass through, however as soon as the eggs hatch, the L1's will transfer through the filter with little or no delay. So you have L1’s where the ‘t=0’ is known with an error margin of less than a minute and depending on your total ‘harvest time window’ it will decide the ‘time spread’ between the first L1 and the last one L1 you harvest.

That’s all! Sounds simple and so it is!

For us (the NemaSync team) the real challenge and cost turned out to be the design and manufacturability of the micro filters as from the almost 200k elongated apertures on a 80mm diameter filter not a single aperture is allowed to have a defect or deviate from a critical set of dimensions (ZERO defect is required as 99.999% is not good enough). Making these high precision micro-filters requires similar production and clean room technology as seen in today’s semiconductor industry.

It took us more than 4 years to solve the many challenges we faced, however today we have a system that not only works guaranteed ‘as advertised’, however it can now also be scalable manufactured.

Overcoming all the setbacks & challenges we faced, we were very excited and proud to introduced the first ‘production’ version of the ‘CES’ - C. elegans Synchronizer at the 2018 EMBO convention in Barcelona with multiple live demonstrations showing the unprecedented results for everybody to witness. The 2018 version of the CES (v1) was still rather large and heavy on ‘glass and metal’ and in the meantime a much smaller and simpler version has been introduced at the WORM#19 conference in LA, the ‘CES v2’. Not just much smaller, but also at 8x lower in cost.

We hope that this somewhat long newsletters gives you some insight in the development and working of the truly unique ‘CES’ and to a certain extend the answer to the questions of ‘how does it work’ and why it is different from any other synchronization protocol we have seen so far.

Please feel free to visit the www.nemasync.com website for more information, including a short demo video of the CES protocol. Please do not hesitate to contact us if you have any questions or if you are interested in the CES itself.