Cobra and Lancaster partnership helps unravel new analytical tool for DNA topology

An increased demand for plasmid DNA in the biopharmaceutical sector — for example, for use in gene therapies — necessitates the use of techniques to analyse the tertiary structure of the DNA, yet current methods are invasive and require a high level of sample preparation.

A business interaction voucher from BioProNET has enabled Lorna Ashton from Lancaster University to work with Cobra Biologics to assess a novel method for determining the topology of plasmid DNA.

The project used Ramen spectroscopy; a method for monitoring physiochemical properties of molecules, in which the scattering of light caused by molecular vibrations gives a unique fingerprint of that molecule. It has the the advantages of being non-invasive and providing almost real-time information on molecules.

"Raman spectroscopy is sensitive to changes in DNA and RNA structure but is underused in biopharmaceutical analytical R&D", explains Lorna.

The business interaction voucher enabled Cobra to explore an alternative to current analytical methods by working with Lorna, who has extensive experience of Ramen spectroscopy, while at the same time allowing Lorna to access otherwise unavailable plasmid DNA samples.

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Cobra provided DNA samples in three topological isoforms — supercoiled, nicked (open circle) and linearised forms — that were verified using two current analytical methods (agarose gel electrophoresis and free-solution capillary electrophoresis) at Cobra.

Then, after method optimization, Lorna determined Raman spectra for each of the isoforms of the plasmid DNA. Next, data processing and statistical analysis were performed to assess any clustering of samples with different topologies.

"The acquired Raman spectra revealed different spectral features arising from the supercoiled, open circle and linearized topologies", says Lorna. "This indicates that Raman spectroscopy can be used to distinguish the different isoforms."

However, within the duration of the project it was not possible to assess if Raman spectroscopy could provide quantitative data on the relative amounts of each of the topologies in a sample. Although further work is required to move the project forward, Daniel Smith from Cobra notes that the project has provided "encouraging preliminary data, which that will support continuation of the project in a collaborative manner".