



Wigan 
Council

Having reviewed the material, I would be grateful for clarification on several points relating to the Great Crested Newt (GCN) eDNA report supplied. The document includes methodological details but does not contain the specific information required to understand its relevance to the Haigh Hall Miniature Railway.

In accordance with the Freedom of Information Act 2000, please provide:

Exact Sampling Location

The precise location of the GCN eDNA sample referred to in the SureScreen Scientifics report, including one of the following:

- 1a. Ordnance Survey grid reference; or
- 1b. A map or plan indicating the sampling point; or
A written description of the pond or waterbody from which the sample was taken.

OS grid reference: 53.57092 N, 2.60287 W

Attached eDNA report is titled 'walled garden pond' and grid reference in report will confirm location

Purpose and Commissioning of the Survey

1. Confirmation of whether this eDNA sampling was commissioned specifically in connection with the Haigh Hall Miniature Railway restoration works, or whether it forms part of an unrelated project within the Haigh Hall estate or surrounding woodland.

Commissioned in relation to both the railway works and the restoration of the Bothy Yard, which lies further north

Full Test Result

The complete analytical outcome for the GCN sample, including:

- 3a. Whether the result was Positive, Negative, or Inconclusive;
- 3b. The number of positive qPCR replicates (out of 12);
- 3c. Any inhibition or degradation notes recorded by the laboratory;
- 3d. Any cover sheet, result summary, or accompanying interpretation issued by SureScreen Scientifics.

See attached eDNA report for information related to 3a, 3b and 3c. No further documentation was issued by SureScreen Scientifics.

Folio No: 3008-2025
Purchase Order: SCIN-41162
Contact: Wigan Council
Issue Date: 06.08.2025
Received Date: 26.06.2025

GCN Report

Technical Report

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GCN eDNA Analysis

Summary

When great crested newts (GCN), *Triturus cristatus*, inhabit a pond, they continuously release small amounts of their DNA into the environment. By collecting and analyzing water samples, we can detect these small traces of environmental DNA (eDNA) to confirm GCN habitation or establish GCN absence.

Results

Lab ID	Site Name	OS Reference	Degradation Check	Inhibition Check	Result	Positive Replicates
GCN25 8700	Walled Garden Pond	53.57092 N, 2.60287 W	Pass	Pass	Negative	0/12

Matters affecting result: none

Reported by: Amy Bermudez

Approved by: Consuela Sopronyi

Methodology

The samples detailed above have been analyzed for the presence of GCN eDNA following the protocol stated in DEFRA WC1067 'Analytical and methodological development for improved surveillance of the Great Crested Newt, Appendix 5.' (Biggs et al. 2014). Each of the 6 sub-sample tubes are first centrifuged and pooled together into a single sample tube which then undergoes DNA extraction. The extracted sample is then analyzed using real-time PCR (qPCR), which uses species-specific molecular markers to amplify GCN DNA within a sample. These markers are unique to GCN DNA, meaning that there should be no detection of closely related species.

If GCN DNA is present, the DNA is amplified up to a detectable level, resulting in positive species detection. If GCN DNA is not present then amplification does not occur, and a negative result is recorded. Analysis of eDNA requires attention to detail to prevent the risk of contamination. True positive controls, negative controls, and spiked synthetic DNA are included in every analysis and these have to be correct before any result is declared and reported. Stages of the DNA analysis are also conducted in different buildings at our premises for added analytical security.

SureScreen Scientifics Ltd is ISO9001 accredited and participates in Natural England's proficiency testing scheme for GCN eDNA testing.

Interpretation of Results

Sample Integrity Check:	When samples are received in the laboratory, they are inspected for any tube leakage, suitability of sample (not too much mud or weed etc.) and absence of any factors that could potentially lead to inconclusive results. Any samples which fail this test are rejected and eliminated before analysis.
Degradation Check:	Pass/Fail. Analysis of the spiked DNA marker to see if there has been degradation of the kit or sample between the date it was made to the date of analysis. Degradation of the spiked DNA marker may lead indicate a risk of false negative results.
Inhibition Check:	Pass/Fail. The presence of inhibitors within a sample is assessed using a DNA marker. If inhibition is detected, samples are purified and re-analyzed. Inhibitors cannot always be removed, if the inhibition check fails, the sample should be re-collected.
Result:	Presence of GCN eDNA (Positive/Negative/Inconclusive) Positive: GCN DNA was identified within the sample, indicative of GCN presence within the sampling location at the time the sample was taken or within the recent past at the sampling location. Positive Replicates: Number of positive qPCR replicates out of a series of 12. If one or more of these are found to be positive the pond is declared positive for GCN presence. It may be assumed that small fractions of positive analyses suggest low level presence, but this cannot currently be used for population studies. In accordance with the WC1067 Natural England protocol, even a score of 1/12 is declared positive. 0/12 indicates negative GCN presence. Negative: GCN eDNA was not detected or is below the threshold detection level and the test result should be considered as evidence of GCN absence, however, does not exclude the potential for GCN presence below the limit of detection. Inconclusive: Controls indicate inhibition or degradation of the sample, resulting in the inability to provide conclusive evidence for GCN presence or absence.