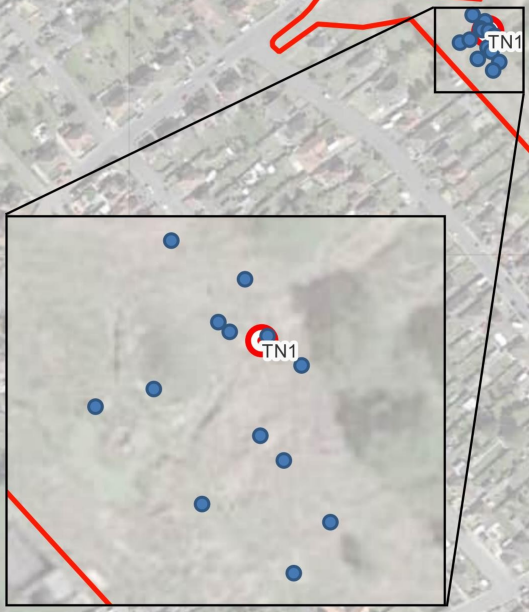


**Key**

- Site Boundary
- Reptile Mat
- ⊙ Target Notes

TN1: Nest of tiny baby voles  
 TN2: Farmer cut edges, reptile mats removed



<b>RammSanderson</b>		
Title: Reptile Survey Plan		
Project: Newark Road, Sutton in Ashfield		
Client: Hallam Land Management Limited		
Date: 31/10/2022	Fig:	Author: LG
A4 Scale: 1:4000	ID: RSE_6136_REP_1022_V1R1	



# GREAT CRESTED NEWT **DETECTION RESULTS**

**Company:** RammSanderson Ecology

**Order number:** SO00432

**Project code:** 6136\_Newark Road, Sutton-in-Ashfield

**Date of Report:** 7 July 2022

**Number of samples:** 1

Thank you for sending your sample(s) for analysis by NatureMetrics. Your sample(s) have been processed in accordance with the protocol set out in Appendix 5 of Biggs et al. (2014).

## Summary of the results

Results indicate GCN presence in 'Ditch 1'.

The extraction blank control and the qPCR negative controls were negative, and the qPCR positive controls and their replicates amplified as expected.

Results are based on the samples as supplied by the client to the laboratory. Incorrect sampling methodology may affect the results. Note that a negative result does not preclude the presence of GCN at a level below the **limits of detection**.

## Methods

**eDNA** was precipitated via centrifugation at 14,000 x g and then extracted using Qiagen DNeasy Blood and Tissue extraction kits. **qPCR** amplification was carried out in 12 replicates per sample, using GCN specific **primers** and **probe** (developed by Thomsen et al. (2012) and adopted by Biggs et al. (2014)), in the presence of **extraction negative controls**, **qPCR positive controls**, and **qPCR negative controls**. A score is given for the number of positive replicates out of 12.

The **qPCR** method follows the recommendations set out by NatureMetrics for Natural England in the qPCR validation project and helps improve the reliability of the interpretation of the data. Results from the GCN assay are considered to have a **high** confidence rating according to our **Validation Scale** (Harper et al. 2021).

The quality control methods exceed the requirements outlined in Appendix 5 of Biggs et al. (2014). These consist of the use of **kit blanks**, additional **extraction negative controls** and **qPCR negative controls**, and **qPCR positive controls**. The qPCR positive controls are standards of known concentration amplified in triplicate to generate **limit of detection** and give confidence to any weak and late amplifications.