PORT OF
TILBURY
LONDON

PLANNING ACT 2008

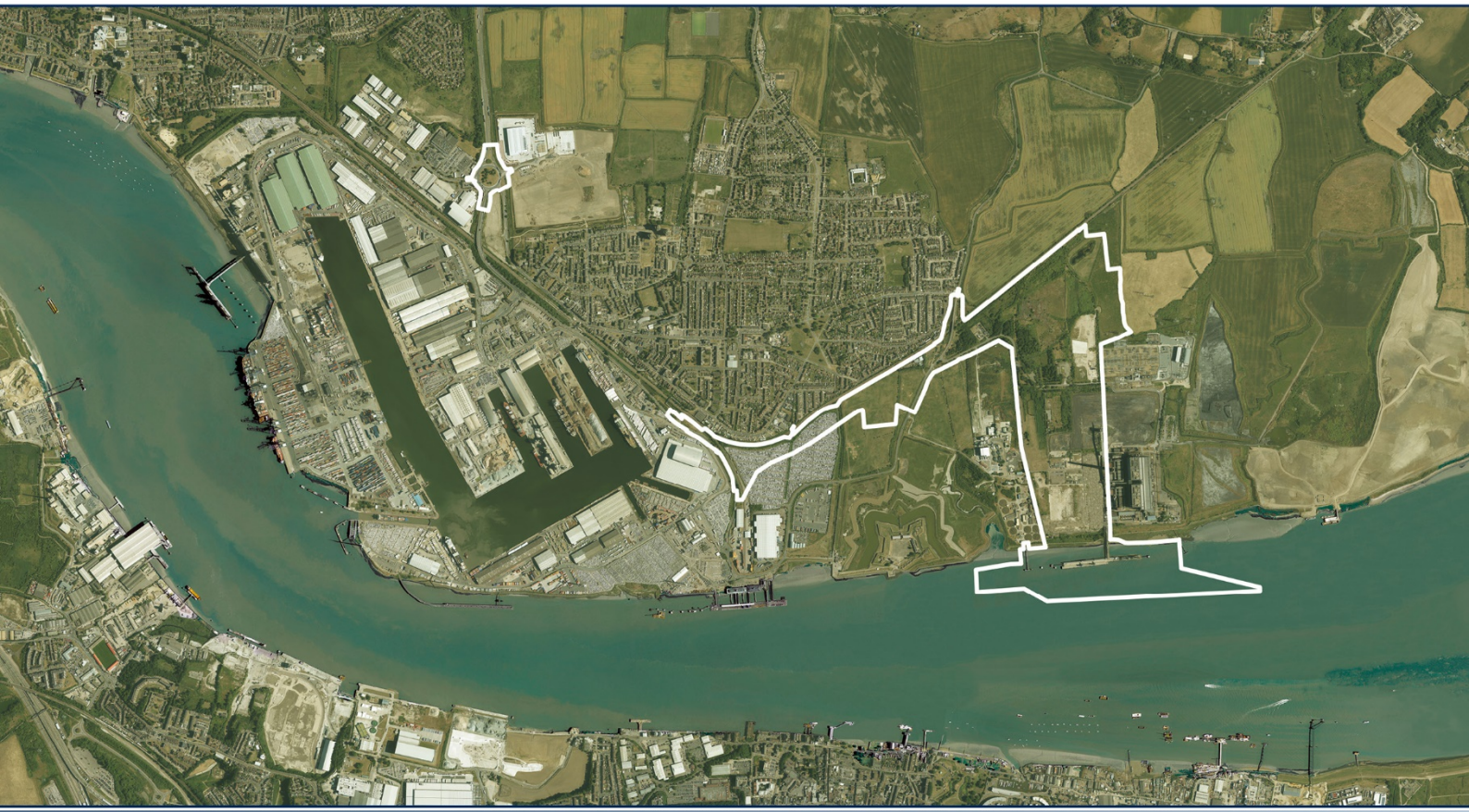
INFRASTRUCTURE PLANNING
(APPLICATIONS: PRESCRIBED FORMS AND PROCEDURE) REGULATIONS 2009

PROPOSED PORT TERMINAL AT FORMER TILBURY POWER STATION

TILBURY2

GREAT CRESTED NEWT EDNA ANALYSIS RESULTS (2016-17)

DOCUMENT REF: APPENDIX 10.G



TILBURY2

DNA Analysis Report - Commercial in Confidence

Customer:	Bioscan UK Ltd
Address:	Little Baldon Oxford OX44 9PU
Contact:	[redacted]
Email:	[redacted]
Tel:	[redacted]
Report date:	20-May-2016
Order Number:	GCN16-0068
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

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CustomerReference	Fera Reference	GCN Detection	GCN Score	Inhibition	Degradation
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
tilbury pond	S16-003104	Inconclusive	0	No	YES
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
tilbury ditch	S16-003107	Negative	0	No	No
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

The results indicate that eDNA for great crested newts was detected in three of the samples and in the remaining samples eDNA was not detected (as detailed in the table above). However, with sample S16-003104 we detected degradation of the internal control. Therefore, due to the risk of any eDNA also being degraded resulting in a false negative, we have issued an inconclusive result. We did note an excess of sediment in this sample which may have contributed to the result. Analysis was conducted in the presence of the following controls: 1) Extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

This test procedure was developed using research funded by the Department of Environment, Food and Rural Affairs, and was performed under the conditions of licensing arrangements with Applied Biosystems and patent rights owned by F. Hoffman-La Roche Ltd.

Issuing officer: Steven Bryce
Tel: 01904 462 324
Email: e-dna@fera.co.uk

DNA Analysis Report - Commercial in Confidence

Customer:	Bioscan UK Ltd
Address:	The Old Parlour Little Baldon Farm, Little Baldon Oxford Oxfordshire OX44 9PU
Contact:	[redacted]
Email:	[redacted]
Tel:	[redacted]
Report date:	21-Jul-2016
Order Number:	GCN16-0317
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	GCN Score	Inhibition	Degradation
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
-	S16-013602	Inconclusive	0	No	YES

The results indicate that eDNA for great crested newts was not detected in any of the samples submitted. However, with sample S16-013602 we detected degradation of the internal control. Therefore, due to the risk of any eDNA also being degraded resulting in a false negative, we have issued an inconclusive result. Analysis was conducted in the presence of the following controls: 1) Extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

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Issuing officer: Steven Bryce
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DNA Analysis Report - Commercial in Confidence

Customer:	Bioscan UK Ltd
Address:	The Old Parlour Little Baldon Farm, Little Baldon Oxford Oxfordshire OX44 9PU
Contact:	[redacted]
Email:	[redacted]
Tel:	[redacted]
Report date:	04-May-2017
Order Number:	GCN17-0365
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

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CustomerReference	Fera Reference	GCN Detection	GCN Score	Inhibition	Degradation
98 KEATS GARDENS	S17-003818	Negative	0	No	No
16 TEEC POND	S17-003819	Inconclusive	0	No	YES
65 CHADWELL CROSS SEWER	S17-003820	Negative	0	No	No
12a GATEHOUSE DITCH	S17-003821	Negative	0	No	No
9 ACCESS LANE DITCH (PINCOCKS	S17-003822	Inconclusive	0	No	YES

The results indicate that eDNA for great crested newts was not detected in any of the samples submitted. However, with samples S17-003819 and S17-003822 we detected PCR inhibitors and degradation of the internal control. Therefore, due to the risk of inhibition of the eDNA assay and any eDNA also being degraded resulting in a false negative, we have issued an inconclusive result. Analysis was conducted in the presence of the following controls: 1) Extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

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Email: e-dna@fera.co.uk

DNA Analysis Report - Commercial in Confidence

Customer:	Bioscan UK Ltd
Address:	The Old Parlour Little Baldon Farm, Little Baldon Oxford Oxfordshire OX44 9PU
Contact:	[redacted]
Email:	[redacted]
Tel:	[redacted]
Report date:	01-Jun-2017
Order Number:	GCN17-0365
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

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The results are defined as follows:

Positive:	DNA from the species was detected.
eDNA Score:	Number of positive replicates from a series of twelve.
Negative:	DNA from the species was not detected; in the case of negative samples the DNA extract is further tested for PCR inhibitors and degradation of the sample.
Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	GCN Score	Inhibition	Degradation
9 ACCESS LANE DITCH (PINCOCKS	S17-003798	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in the sample submitted. Analysis was conducted in the presence of the following controls: 1) Extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

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DNA Analysis Report - Commercial in Confidence

Customer:	Bioscan UK Ltd
Address:	The Old Parlour Little Baldon Farm, Little Baldon Oxford Oxfordshire OX44 9PU
Contact:	[redacted]
Email:	[redacted]
Tel:	[redacted]
Report date:	12-Jun-2017
Order Number:	GCN17-0365
Samples:	Pond Water
Analysis requested:	Detection of Great Crested Newt eDNA from pond water.

Thank you for submitting your samples for analysis with the Fera eDNA testing service. The details of the analysis are as follows:

Method:
The method detects pond occupancy from great crested newts (GCN) using traces of DNA shed into the pond environment (eDNA). The detection of GCN eDNA is carried out using real time PCR to amplify part of the cytochrome 1 gene found in mitochondrial DNA. The method followed is detailed in Biggs J., et al, (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. Appendix 5. Technical advice note for field and laboratory sampling of great crested newt (Triturus cristatus) environmental DNA. Freshwater Habitats Trust, Oxford.

The limits of this method are as follows: 1) the results are based on analyses of the samples supplied by the client and as received by the laboratory, 2) any variation between the characteristics of this sample and a batch will depend on the sampling procedure used. 3) the method is qualitative and therefore the levels given in the score are for information only, they do not constitute the quantification of GCN DNA against a calibration curve, 4) a 'not detected' result does not exclude presence at levels below the limit of detection.

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Inconclusive:	Controls indicate degradation or inhibition of the sample, therefore the lack of detection of GCN DNA is not conclusive evidence for determining the absence of the species in the sample provided.

DNA Analysis Report - Commercial in Confidence

CustomerReference	Fera Reference	GCN Detection	GCN Score	Inhibition	Degradation
16 TEEC POND	S17-003799	Negative	0	No	No

The results indicate that eDNA for great crested newts was not detected in the sample submitted. Analysis was conducted in the presence of the following controls: 1) Extraction blank, 2) appropriate positive and negative PCR controls for each of the TaqMan assays (GCN, Inhibition, and Degradation). All controls performed as expected.

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