

SERVICES & FACILITIES ANNUAL REPORT - FY April 2016 to March 2017

SERVICE NERC Biomolecular Analysis Facility, NBAF	FUNDING PAYG & Block	AGREEMENT F14/G6/48 (NBAF-B: R8/H10/61)	ESTABLISHED as S&F 1998 (NBAF-S) 2005 (NBAF-E & NBAF-L) 2009 (NBAF-B)	TERM N/A
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TYPE OF SERVICE PROVIDED:

NBAF provides fully-supported access to a wide range of advanced molecular genetic, genomic, metabolomic and bioinformatic technologies to the UK environmental science community in order to address ecological or evolutionary questions. Service is provided at four nodes: Birmingham (NBAF-B: metabolomics), Edinburgh (NBAF-E: sequencing and bioinformatics), Liverpool (NBAF-L: microarraying, sequencing and bioinformatics) and Sheffield (NBAF-S: genotyping and population genetics). Access to the Facility is organised centrally through competitive peer-reviewed proposals that are assessed by the independent members of the Steering Committee (application form at <http://www.nbaf.nerc.ac.uk>) to ensure that (i) only the best science is supported, (ii) access to more than one node is coordinated, and (iii) projects are followed through to dissemination of the results. Each node is embedded in a well-equipped and vigorous research environment that, together with continuing developments in equipment and training, makes 'state-of-the-art' services available to the NERC community. NBAF provides access to high-level capability, and the associated training, that are rarely available elsewhere. NBAF-S, and to a limited extent NBAF-B, are equipped to train and supervise researchers (mostly PhD students) in undertaking their own analyses at the bench. NBAF-B supports metabolomic analyses using both mass spectrometry and NMR methods. At NBAF-S most studies require the genotyping of microsatellites or single nucleotide polymorphisms (SNPs), or metabarcoding methods. Samples are prepared and analysed in large-scale studies using ABI or Illumina sequencer, SNP typing or qPCR platforms. NBAF-E supports sequencing applications using ABI 3730 instruments for capillary sequencing and next-generation HiSeqX, HiSeq 4000, HiSeq 2500 and MiSeq instruments for whole-genome, transcriptome and reduced-representation sequencing, including targeted resequencing, amplicon sequencing and genotyping-by-sequencing, at any scale (from viruses to polyploid animals and plants). NBAF-L offers sequencing, gene expression and bioinformatic services, particularly for environmental diversity through amplicon sequencing on long-read platforms, targeted resequencing of exons and reduced genomic regions, and gene expression analysis on microarray and short-read (Illumina and Agilent) platforms. Building on dedicated bioinformatic expertise, all NBAF nodes provide integrated experimental design and assay services; NBAF-L also offers statistical and network-based interpretation of results. NBAF collectively provides a wide menu of training courses in genomics and metabolomics. NBAF also offers project-focussed wet lab and bioinformatics training. Access to NBAF-E and NBAF-L is PAYG.

ANNUAL TARGETS AND PROGRESS TOWARDS THEM

Capacity is defined by the availability of staff time, and all four nodes make >85% of funded staff time available to users, with any remainder allocated to R&D. Due to staffing shortages, NBAF-B completed all projects in 2016-17 except for one existing project and three new projects that have been delayed until recruitment is completed. Almost all projects at the other nodes were accommodated according to the agreed schedule, with most slippage arising from delays in the arrival of users or their samples.

SCORES AT LAST REVIEW (each out of 5)			Date of Last Review:	
Need	Uniqueness	Quality of Service	Quality of Science & Training	Average
5	4.5	5	5	4.88

CAPACITY of HOST ENTITY FUNDED by S&F %	Staff (grade, fte): NBAF-S: DA Dawson (G7 100%), G Horsburgh (G7 100%), H Hipperson (G7 100%), C Pagnier (G5 80%), R Tucker (G6 20%); NBAF-B: U Sommer (G7 100%; left 31 March 2017), C Heude (G7 100%; left 31 Dec 2016), J Engel (G7 100%; left 31 Dec 2016); NBAF-E: K Gharbi (UOE8 30%), U Trivedi (UOE7 50%), S Wardlow (UOE5 100%), M Arnot (UOE06 20%); NBAF-L: Y Fang (Res 7, 75%), K Jackson (Res 9, 20%), L Parsons (Cler 5 30%), R Gregory (Res 7 20%), J Kenny (Res 8 20%)	Next Review (March)	Contract Ends (31 March)
		TBC	2018

FINANCIAL DETAILS: CURRENT FY

Total Resource Allocation £1900.38k	Unit Cost £k			Capital Expend £4.94k	Income £k	FCC £2027.77k
	Unit 1	Unit 2	Unit 3			
		Variable				

FINANCIAL COMMITMENT (by year until end of current agreement) £k

2015-16	2016-17			
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STEERING COMMITTEE	Independent Members	Meetings per annum	Other S&F Overseen
NBAFSC	Chair +7	1-2	None (one SC for 4 nodes)

APPLICATIONS: DISTRIBUTION OF GRADES (current FY — 2016/17)													
	10	9	8	7	6	5	4	3	2	1	0	R*	Pilot
NERC Grant projects*	1	9	5	0	0	0	0	0	0	0	0	0	0
Other academic	0	0	2	3	3	0	0	0	0	0	0	1	27
Students	0	1	9	8	6	1	0	0	0	0	0	2	17
TOTAL	1	10	16	11	9	1	0	0	0	0	0	3	44

APPLICATIONS: DISTRIBUTION OF GRADES (per annum average previous 3 financial years — 2013/2014, 2014/2015 & 2015/2016)													
	10	9	8	7	6	5	4	3	2	1	0	R*	Pilot
NERC Grant projects*	1.7	5.7	11.7	4.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other academic	0	0.7	2	3.3	1.3	0.0	0.0	0.0	0.0	0.0	0.3	0.0	21.3
Students	0	2.3	2.3	3.3	1.7	0.7	0.3	0.0	0.0	0.0	0.0	0.7	11.3
TOTAL	1.7	8.7	15.7	10.9	3.3	0.7	0.3	0.0	0.0	0.0	0.3	0.7	32.6

PROJECTS COMPLETED (current FY – 2016/17)												
	10	9	8 (α4)	7	6 (α3)	5 (α2)	4	3 (α1)	2	1 (β)	0	Pilot
NERC Grant projects*	2	15	17	3	1	0	0	0	0	0	0	0
Other Academic	0	3	10	7	0	0	0	0	0	0	0	8
Students	0	5	13	12	11	0	0	0	0	0	0	4

Project Funding Type (current FY – 2016/17) (select one category for each project)												
Grand Total	Infrastructure						PAYG					
	Supplement to NERC Grant *		PhD Students NER C	Other	NERC Centre	Other	NERC Grant*	PhD Students NER C	Other	NERC Centre	Other	
99	3.5		14.5	21	2	10	34.5	1.5	4	0	8	

Project Funding Type (per annum average previous 3 financial years - 2013/2014, 2014/2015 & 2015/2016)												
Grand Total	Infrastructure						PAYG					
	Supplement to NERC Grant *		PhD Students NER C	Other	NERC Centre	Other	NERC Grant*	PhD Student NER C	Other	NERC Centre	Other	
143.3	10		10.7	19.5	2.7	6.8	61	4.3	9.8	1	17.5	

User type (current FY – 2016/17) (include each person named on application form)				
Academic	NERC Centre	NERC Fellows	PhD Students	Commercial
189	44	6	7	2

User type (per annum average previous 3 financial years - 2013/2014, 2014/2015 & 2015/2016)				
Academic	NERC Centre	NERC Fellows	PhD Students	Commercial
278	12	19	55	1

OUTPUT & PERFORMANCE MEASURES (current year)											
Publications (by science area & type) (calendar year 2016)											
SBA	ES	MS	AS	TFS	EO	Polar	Grand Total	Refereed	Non-Ref/ Conf Proc	PhD Theses	
0	0	16	0	83.5	0	3.5	107	79	16	12	

Distribution of Projects (by science areas) (FY 2016/17)							
Grand Total	SBA	ES	MS	AS	TFS	EO	Polar
99	0	3.5	18	0	76	0	2.5

OUTPUT & PERFORMANCE MEASURES (per annum average previous 3 years)											
Publications (by science area & type) (Calendar years 2013, 2014 & 2015)											
SBA	ES	MS	AS	TFS	EO	Polar	Grand Total	Refereed	Non-Ref/ Conf Proc	PhD Theses	
0	0.3	23.7	0	94.3	0	1.3	119.7	76.3	21	22.3	

Distribution of Projects (by science areas) (FY 2013/2014, 2014/2015 & 2015/2016)							
Grand Total	SBA	ES	MS	AS	TFS	EO	Polar
151.2	1	3.3	29.7	1.4	107.4	0	9.4

Distribution of Projects by NERC strategic priority (current FY 2016/17)							
Grand Total	Climate System	Biodiversity	Earth System Science	Sustainable Use of Natural Resources	Natural Hazards	Environment, Pollution & Human Health	Technologies
99	7.5	75	0.7	8	4.8	2.7	0

OVERVIEW & ACTIVITIES IN FINANCIAL YEAR (2016/17):

2016-17 saw a 60% increase in applications from last financial year with nine applications more than the last three-year average; publications have also remained high with three publications higher than the last three-year average.

Despite losing 66% of its staff in December 2016, NBAF-B has managed to complete all but one of its existing metabolomics projects, and has now found a solution to completing this final project. In addition several manuscripts have been written and published. Three new projects are on hold. Training continues to grow through the Birmingham Metabolomics Training Centre (BMTC), via delivery of several introductory and advanced courses. The BMTC, in partnership with NBAF-B, has been formally endorsed as a training platform with the ELIXIR-UK node.

NBAF-E is delivered from within Edinburgh Genomics, a large facility with a decade of experience of “next generation” sequencing that supports UK and international genome science in humans, agricultural animals and evolution/ecology. The facility now has the largest capacity of any open-access genomics facility in the UK, and NBAF users have access to a wide range of the most advanced sequencing technologies. The support offered extends from experimental design through to assistance with publication, and includes long experience with “difficult” – especially low quantity – sample sets. Edinburgh Genomics runs six Illumina HiSeqX instruments, designed to deliver medium to high coverage of whole genomes, and these are available to NERC science at very favourable costs. In addition, Edinburgh Genomics has Illumina HiSeq 4000 platform – for RNA-Seq and other counting-by-sequencing applications – and an Illumina HiSeq2500 – used for long-read genomic data and for high-volume amplicon sequencing. The facility recently installed a PacBio Sequel, one of the new generation of single-molecule, long-read sequencers, and this is available for both genome and transcriptome sequencing. NBAF-E also has access to the lower-throughput Illumina MiSeq and to high-volume Sanger sequencing. Larger projects are delivered using dedicated library preparation robotics, which are particularly used for RNA-Seq, double-digest RAD-Seq and multiplexed targeted selection sequencing. Edinburgh Genomics has a dedicated training strand, accessible to NERC science, and ran over 25 short courses in various aspects of genomics and bioinformatics technologies, reaching over 420 scientists.

NBAF-L purchased a 10X Chromium system, which allows barcoded droplet library preparation. Applications relevant to NERC science include the cost-efficient generation of de novo assembly of non-model genomes of >1Gbp. This is already being used to support major grants in NERC highlight topics. Other applications include gene profile of individual cells within populations to identify subsets of cells with different activity. NBAF-L also bought a PacBio Sequel long-read sequencer, which allows NBAF to retain its cutting edge in this area. Applications include de novo assembly of non-model genomes, resolution of transposable element evolution, phylogenetic analysis of viral diversity and analysis of transcriptional splicing variation. NBAF-L ran short courses in gene analysis and metagenomic analysis of communities, which were well received and over-subscribed.

NBAF-S installed a major new qPCR platform, a high-throughput QuantStudio 12k Flex Real-Time PCR system. This system is being used to support high-throughput telomere analyses, in particular, and provides an efficient alternative to the node’s LGC KASP system for some configurations of SNP typing. The system has also proved invaluable for precise sample quantification when preparing libraries for multiplexed high-throughput sequencing. NBAF-S ran 6 courses this year on metabarcoding and on data analysis using the HPC grid at Sheffield. NBAF-S’s population genomics course was again highly over-subscribed, with 146 applicants applying for the 35 available places, and was again very positively received by those who could be accommodated.

NBAF resumed the Pilot Grants Competition in October 2016 and there was a high degree of competition, enabling 11 innovative, high-quality projects to be funded; this enabled new users, in particular (including PhD projects), to gain access to NBAF-E and NBAF-L, which are otherwise only accessible via PAYG.

SCIENCE HIGHLIGHTS. To focus on economic and societal impacts and benefits where possible:

Biodiversity in marine invertebrate responses to acute warming revealed by a comparative multi-omics approach (Clark *et al* 2017 *Global Change Biology* **23**, 318–330; JIF: 8.4)

Understanding and predicting how ecosystems respond to climate change is incredibly complex, not least because of the huge range of different species within the marine environment. An important question to ask is which species, and how many different species, should we use to monitor an ecosystem under change? In a project led by Dr Melody Clark at BAS in collaboration with Professor Mark Viant and NBAF-B, the individual stress responses of six marine invertebrates from the Antarctic Peninsula to the same level of acute warming were identified. These measurements included metabolomics (at NBAF-Birmingham) and gene expression and revealed that the six species – sea lemon, sea cucumber, clam, brachiopod, bivalve mollusc and a crustacean – had quite differing responses. The implications are that in order to better understand how ecosystems are responding to climate change we need to consider a range of species, not just one or two. This study demonstrated the power of combining two state-of-the-art omics technologies to reveal some fascinating insights into the biology of Antarctic species.



Dispersal in the sub-Antarctic: king penguins show remarkably little population genetic differentiation across their range (Clucas *et al* 2016 *BMC Evolutionary Biology* **16**, 211; JIF: 3.4)

In this paper, Clucas and colleagues show a remarkable connectivity between king penguin populations in the Southern Ocean. King penguins are long-lived and were expected to have a strong population structure: colonies can be many thousands of kilometres distant from each other, and adults tend to return to the same colony to breed each year. This would make each colony a genetically unique component of the species, and this in turn leads to strong predictions concerning the species' resilience to climatic change. Surprisingly, Clucas and colleagues found that colonies were strongly genetically

connected, and that physical distance did not predict colony relatedness. Indeed, a new colony on the Falkland Islands appears to have been seeded from the Crozet Islands, 7500 km away. These results suggest large-scale interconnectedness of ecological systems in the Southern Ocean, and have significant implications for management and conservation across the Antarctic.

NBAF-Edinburgh generated the genomic variation data using RAD-Seq, a technique pioneered in NBAF, and provided bioinformatics support.

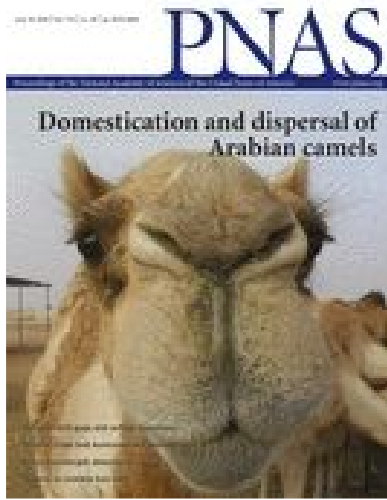
The industrial melanism mutation in British peppered moths is a transposable element (Van't Hof *et al* 2016 *Nature* **534**, 102–105; JIF: 38.1)

A textbook example of evolution that supported Darwin's theory was the spread of a melanic (black) mutation in peppered moths that matched the trees that became soot-darkened during the industrial revolution. Following the Clean Air Act, the lighter form returned. NBAF-Liverpool supported a landmark study by Van't Hof *et al.* that discovered that this mutation was due to a transposon jumping into a gene, and examined its spread in historical samples. This work resulted in a paper in *Nature* and widespread coverage in the press for NERC. Photo credit Ilik Saccheri.



Ancient and modern DNA reveal dynamics of domestication and cross-continental dispersal of the dromedary (Almathen *et al* 2016 *Proceedings of the National Academy of Sciences of the USA* **113**, 6707-6712; JIF: 9.4)

Dromedaries have been fundamental to the development of human societies in arid landscapes and for long-distance trade across hostile hot terrains for 3,000 y. Today they continue to be an important livestock resource in marginal agro-ecological zones. However, the history of dromedary domestication and the influence of ancient trading networks on their genetic structure have remained elusive. This study, which was supported by NBAF-Sheffield, combined nuclear



microsatellite and mitochondrial genotype information from 1,083 extant animals collected across the species' range with ancient DNA sequences of wild and early-domesticated dromedary samples. There was little phylogeographic signal in the modern population, indicative of extensive gene flow across all regions except East Africa, where dromedary populations have remained relatively isolated. In agreement with archaeological findings, wild dromedaries from the southeast Arabian Peninsula were identified to be among the founders of the domestic dromedary gene pool. Approximate Bayesian computation further supported the “restocking from the wild” hypothesis, in which an initial domestication was followed by introgression from individuals from wild, now-extinct populations. Compared to other livestock, which show a long history of gene flow from their wild ancestors, the domesticated population had high initial genetic diversity relative to the native wild ancestors on the Arabian Peninsula and there was only a brief coexistence of early-domesticated and wild individuals. This study also demonstrated the potential to retrieve ancient DNA sequences from osseous remains excavated in hot and dry desert environments. DNA preparation and microsatellite genotyping were performed at NBAF-Sheffield.

FUTURE DEVELOPMENTS/STRATEGIC FORWARD LOOK

Metabolomics: Applications of metabolomics in the environmental sciences are continuing to grow, benefitting from the capabilities of this technology to provide deep mechanistic insights into biochemical processes, both natural and those perturbed by environmental stress. As the diversity of applications increases, so does the diversity of the sample types and the analytical demands on our metabolomics platforms. One arguably obvious trend is the increasing need to improve analytical sensitivity in metabolomics, which is one area of research and development at Birmingham. In collaboration with our technology partner Thermo Scientific, NBAF-B will explore microflow and/or nanoLC-MS methodologies. Other projects have placed new demands on NBAF-B's capabilities for the preparation of samples for metabolomics, which to date has involved manual pipetting. Working with another of our technology partners, Beckman Coulter, we are in the process of developing standard operating protocols for the preparation of both tissue samples and biofluids. The former is the considerably more challenging, with the need to integrate liquid handling, tissue homogenisation, centrifugation and vortexing into a fully automated workflow. Validating such a workflow is an objective of NBAF-B in the coming year. Considering the bioinformatics workflows used in the facility, one longer term ambition has been to deploy arguably complex algorithms written in Matlab and R into a much more user friendly software package that is more accessible to environmental biologists. As such we are continuing to 'wrap' our scripts into Galaxy workflows, building on our earlier work (Galaxy-M) and those of colleagues in France (Workflow4Metabolomics). Our aim is to implement these user friendly workflows into the training courses run in partnership with NBAF-B. Training remains an essential component of the services we provide to the NERC community, and it is our intention to grow the training opportunities available to environment scientists in the coming year.

Long-read sequencing: Genomics technology development in the last few years has focussed on delivering more short-read data cheaper, and also on ways of generating very long (>10 kb) reads. These have utility in genome assembly, rearrangement variant detection, metagenomics analyses and transcript reconstruction. Long-read data are of particular interest to many in the NERC science area. NBAF-L has replaced its PacBio long-read sequencer with the newer PacBio Sequel, which offers 5x the data generation at a similar cost. Edinburgh Genomics, the home of NBAF-E, has now installed a Sequel instrument that is also available to NERC users. These new platforms, coupled with new assembly algorithms, offer the potential for high-quality *de novo* sequencing of genomes for most species. This is a significant boon to the environmental genomics community, who tend to work on 'non-model' species, where important biological questions can be answered within an ecological or evolutionary context. Both NBAF-E and NBAF-L continue to work with research colleagues assessing the Oxford Nanopore MinION system, a long-read platform that has benefits of size and adaptability, particularly to detection of non-canonical bases (such as methylations). This technology is maturing rapidly, and has recently been released for commercial delivery. Additional long-range sequencing technologies that leverage short-read sequencers, based on single-molecule libraries and three-dimensional DNA conformation to deliver kilobase to hundreds of kilobase contiguity, are maturing and the facilities are exploring their utility for NERC science.

Gene expression profiling and chromatin modification assessment: Expression profiling and various methods of assessment of genome or chromatin modification can be achieved by using the ultrahigh-throughput sequencers as “counting instruments”. For all the various approaches to differential expression, differential epigenetic modification and

differential binding of regulatory factors to DNA and RNA, the new HiSeq4000 sequencers at NBAF-E and -L provide a step change in value for money. The facilities will continue to develop new standard assays for single-cell transcriptome and chromatin immunoprecipitation assays, for whole-genome DNA methylation sequencing and related approaches. In particular, we are interested in performing several assays on the same samples from the same organisms and tissues, requiring development of nanogram scale techniques for library preparation. NBAF-L has installed a 10X Chromium system which can allow RNAseq data to be generated and traced to individual members of a cell population using droplet technology. The bioinformatic toolkit for expression and chromatin mark analyses continues to develop rapidly, and NBAF-E, in particular, has been collaborating to develop best-practice methods for analysis, and exploring the parameters of replicate numbers and other experimental design parameters that will maximise access to biological insight.

eDNA and metabarcoding: NBAF-S has a long history of using mitochondrial barcoding to detect and identify parasites (such as malaria strains) and analyse diets (e.g. moth predation by bats, used to describe habitat use). The advent of NGS, especially longer reads on the MiSeq, has made these kinds of studies ever more feasible. NBAF-S has expanded the use of eDNA and metabarcoding techniques rapidly over the last year and has supported several projects in this area. The projects have included testing for parasites in an endangered bird, monitoring the presence of the same parasites in water sources, the description of diet from faeces in a range of species, an attempt to detect genetic variation as well as species presence in freshwater taxa, and detecting and tracking individual animals via their faeces. This area of work is now having significant impact in a wide range of ecological studies and significant further activity is envisaged over the next year.

Population genomics: At NBAF-E, installation of the HiSeqX and its extension to non-human genome sequencing and resequencing brings true population genomics within the reach of NERC science. The HiSeq4000 also offers radically reduced per-gigabase sequencing costs for lower-coverage and reduced-representation sequencing. We are continuing to develop new applications on these exciting new instruments. NBAF-S has now transferred most of its sequencing needs to Illumina platforms, especially the MiSeq as it provides read-lengths of up to 600 bp, so matching the read-length on the ABI platforms used previously. All the NBAF-S genotyping development work is currently conducted on the MiSeq platform, and the use of multiplexed tags within single MiSeq sequencing libraries for microsatellite analyses is currently being trialled. We anticipate that all new NBAF-S projects and genotyping (except SNP – on the LGC KASP system – and telomere analysis – on our new high-throughput QuantStudio 12k Flex Real-Time PCR system) will be MiSeq-based within the coming year.

Pilot grants scheme: The Scheme will be funded again in 2017–18. It is the only means through which non-grant holders are able to access the Edinburgh and Liverpool nodes, and a key avenue to both educating our research community and trialling novel and potentially disruptive technological advances. The cost is relatively small, so that it represents, as before, excellent value for money.