

Justification of Resources

Staff - directly incurred posts

As of the conditions by the UKRI Future Leader fellowship tempered salary contribution, the grant costs **£208,766.60** to Benjamin Werner's salary (main applicant) over 4 years. Benjamin Werner is employed on a lecturer contract grade 6 spinal point 42 at the Barts Cancer Institute, reflecting his current career stage. The Barts Cancer Institute fully commits to the tempered salary contributions and covers the remaining costs to the main applicant's salary. Salary for one Post-doctoral fellow: **£185,112.10**. This post doc will work on the theoretical aspects of the grant application, including but not exclusively on the stochastic dynamics of ecDNA for different fitness models using stochastic processes and probability theory, implement stochastic computer simulations and Bayesian inference schemes. The post doc will further travel overseas at least once a year to engage in exchanges and discussions with our collaborators as well as attending international conferences to report on the progress. Funding for the full first 4 years is required and fully justified given the complexity and scale of the grant. Salary for one scientific officer: **£185,112.10**. The scientific officer will be implementing the experimental part of the application. The officer will establish the fitness assays for different cell line experiments with different ecDNA amplifications, single cell sorting, whole genome and ATAC-seq sequencing and FISH image. An integral part is the training of the officer in new techniques within the institute and in the labs of our collaborators. The officer is expected to travel on average twice per year overseas for training and research purposes. In addition, the officer will be trained in data analysis and participate in experimental design, planning and data modelling. The aim is to bring the expertise in ecDNA manipulation to the Barts Cancer Institute and employ the officer beyond the initial 4 years of funding. The length and amount of the salary is necessary to ensure the hire of a sufficiently skilled candidate.

Staff – directly allocated posts

In accordance with the UKRI requirements, Benjamin Werner's salary has been costed for 32h/week. Accordingly, Benjamin Werner will spend the majority of his time developing the project. Benjamin Werner will be involved and responsible for all aspects outlined in the proposal. Benjamin Werner will spend the remaining time on teaching, personal development, administrative responsibilities and other collaborative scientific projects within and across institutes. Both the theoretical post doc and the experimental scientific officer will spend 100% of the time on the development and fulfilment of their responsibilities as outlined in the proposal. This time involves opportunities for personal development. No time for any of the international collaborators has been costed in the budget.

Travel and subsistence

The grant application includes substantial collaboration with international research groups (Paul Mischel in San Diego US, Peter Scacherie in Cleveland US and Anton Henssen in Berlin Germany). Extensive travelling will be essential. We have costed on average 2 extended overseas travels per year for the scientific officer for practical experimental training and exchange. Further I and the theoretical post doc will travel once per year overseas for scientific discussion. Flights, accommodation and other costs have been determined to approx. **£1000** per trip amounting to **£16000** to cover international collaborations over 4 years. In addition, the theoretical post doc and the scientific officer and I will each attend at least one international conference per year. Flights, accommodation + conference fees have been costed to **£1200**

per conference per person. In total we request **£30400** for travelling and subsistence's for 4 years.

Other directly incurred costs

Each beneficiary (1 scientific officer, 1 post doc, main applicant) will require computer equipment, in total 3 laptops, 3 external screens, keyboards, external hard disks and other smaller items like adapters, mouse etc., costed to in total to **£8000**. Given the high computational requirements of simulations and data analysis + the necessity to scientifically work on frequent overseas trips necessitates these expanses. We also request a Mathematica licence for 4 years for the post doc and the scientific officer, given possible academic licences this will amount to **£1600** for 4 years. Stochastic simulations, data analysis and storage will require high performance computation on the QMUL internal cluster, estimated to be **£5000** per year. Experimental consumables and service costs are broken down below. This consists of **£4000** for the use and chemicals of the CellenOne (available at the Centre for Cancer Genomics and Computational Biology at Barts) single cell dispensing, **£10.000** to run fitness assays of cell lines containing ecDNA + targeted treatment with for example anti-EGFR inhibitor Erlotinib or CDK4 inhibitors. We plan to run fitness assays on 7 different cell lines (in collaboration with Paul Mischel, Peter Scacherie & Anton Henssen). Experiments need to run in triplicates for high and low ecDNA copy number as well as untreated controls for each, summing to $9 \times 8 = 72$ replicates. Low coverage bulk whole genome sequencing will be used on all 72 replicates at £165 per sample (£15 library preparation per sample and £150 sequencing) to measure average ecDNA copy number amplification per replicate, estimated cost **£11800**. High resolution single cell ATAC-seq for pooled cells of each triplicate will result in $72 \times £500 = £36.000$. Publications have been costed to £7500, with a charge of £1500 per paper. Bench fees are costed to £2000 per year per person, covering general lab usable and IT support, in total £16.000 over 4 years. All directly incurred costs total **£121.000**.

Other directly allocated costs

Estate and other indirect costs do not need to be justified by scientific institutions as of the grant application requirements. The grant office of Queen Mary university and the Barts Cancer Institute have costed **£115,322.00** for Estate costs, **£12,788.00** for Infrastructure technician cost and **£483,726.80** for Indirect costs.

Grant total incurred costs

All incurred costs sum to a total of **£1,342,827.60** for 4 years.