**Dr. XXXX, PhD — Budget Justification**

# Personnel

**Dr. XXXXX, PhD, *Project Leader.*** Dr. Naylor will commit a total of 6 calendar months to this project as Project Leader; salary support is requested. As Project Leader, Dr. XXX will oversee all research aspects of this project and will devote 3.5 calendar months during the academic year and 2.5 calendar months during the summer. A 3% increase in salary is requested for Years 2–5. Fringe benefits are calculated at 31% of salary.

**To Be Named, *Undergraduate students***. Four (Years 1–4) and five (Year 5) undergraduate students will commit 100% effort during summer months (equivalent to 10 weeks). The undergraduate students will conduct experiments, evaluate the experimental data, and design follow-up experiments, under the supervision of the Project Leader. In all years, benefits are calculated at 7.65% (FICA) of salaries.

# Consultants

Not applicable

# Equipment

**In Year 1**, $8,500 is requested to purchase equipment. We will purchase a laminar flow hood to eliminate serious problems in culturing *D. discoideum*, which can result in lost time and resources due to contaminated cultures. We will also purchase a thermocycler for using PCR to create clones and knockout strains.

**In Year 2**, $7,000 is requested to purchase electroporator, which is necessary to transform cells with expression vectors or gene deletion vectors to create *D. discoideum* strains necessary for Aims 1 and 2.

# Supplies

Funds in the amount of $51,634 are requested to purchase necessary supplies to complete the experiments described in Specific Aims 1 and 2, summarized in the table below.

* Year 1: $8,398 to purchase microscopy, culturing, cloning, transformation, and consumable supplies, as well as small equipment such as a pH meter and stir plate
* Year 2: $10,075 to purchase microscopy, culturing, cloning, transformation, and consumable supplies
* Year 3: $14,266 to purchase microscopy, culturing, cloning, transformation, co-immunoprecipitation, and consumable supplies
* Year 4: $12,919 to purchase microscopy, culturing, co-immunoprecipitation, and consumable supplies
* Year 5: $11,998 to purchase microscopy, culturing, co-immunoprecipitation, and consumable supplies

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| Supply Category | Task | Year 1 | | Year 2 | | Year 3 | | Year 4 | | Year 5 | |
| Microscopy | Refine visualization assay |  |  |  |  |  |  |  |  |  |  |
| Cloning and transformation | Create Δ*fszA* |  |  |  |  |  |  |  |  |  |  |
| Microscopy | Quantify fission events |  |  |  |  |  |  |  |  |  |  |
| Cloning and transformation | Clone FszA and create strain |  |  |  |  |  |  |  |  |  |  |
| Microscopy | Observe dynamics |  |  |  |  |  |  |  |  |  |  |
| Co-immunoprecipitation microscopy | Co-immunoprecipitation and interaction confirmation |  |  |  |  |  |  |  |  |  |  |
| Antibody production | Purify FszA protein |  |  |  |  |  |  |  |  |  |  |
| Antibody production | Create and screen for antibodies |  |  |  |  |  |  |  |  |  |  |

# Travel

Travel funds in the amount of $8,500 are requested for the Project Leader to attend the National IDeA conference in Washington, DC, in Years 1, 3, and 5 and for a student to travel in Years 1 and 5 (5 trips x $1,700/person).

Travel funds are requested for two trips for the Project Leader and one student to travel to the Regional IDeA conferences in Years 2 and 4 (2 trips x $1,000/person x 2 people = $4,000).

For the Project Leader and two students to attend annual meetings of American Society of Cell Biology (ASCB) or American Society of Microbiology (ASM), funds in the amount of $12,500 are requested (5 trips x $1,500/project leader and 5 trips x $500/student x 2 students); the conference will be chosen based on conference agenda, our research to present, and interests of the students. For example, a current student is interested in pursuing a PhD in microbiology, so we would choose to go to the ASM conference this year.

In addition, $250 ($50/year x 5 years) is requested for mileage and parking to visit the mentor’s laboratory at the University of Arkansas for Medical Sciences in Little Rock, AR.

# Patient Care Costs

Not applicable

# Alterations & Renovations

Not applicable

# Other Expenses

**Use of confocal microscope.** Funding is requested to cover the cost of using the confocal microscope. In lieu of paying hourly use fees, we will pay a portion of the annual service contract for Years 1–4 ($6,667). Our use of the confocal microscope will be greatly diminished in Year 5, so we will contribute $500 to the service contract. An alternative to paying the service contract would be to purchase necessary software such as deconvolution software. Preliminary data suggest that, due to low signal in this experimental system, we may need to increase sensitivity by opening the pinhole and using deconvolution software to treat our laser confocal microscope as a wide-field scope. Before purchasing deconvolution software, however, we will first explore all alternatives to improve image quality, including working with Zeiss and the INBRE-supported Digital Microscopy Facility.

**Antibody production.** Ultimately these experiments will require the use of antibodies directed to FszA. If it becomes unfeasible to create these first and then perform the experiments, the work will be done simultaneously using TAP or FLAG Tags for protein pull down assays rather than co-immunoprecipitations. For production of protein and antibodies, we request $500 in Years 1–2 for cloning and protein purification, $2,000 in Year 3 to increase the yield of protein and begin antibody production, and $1,000 in Year 4 to finish antibody production and testing (total requested amount is $4,000).

**Equipment maintenance.** Funding in the amount of $200 per year is requested for annual equipment maintenance to repair, replace, and maintain equipment that will be used during this funding period.

**Publication costs.** In Years 4 and 5, $500 is requested for publication costs.

**Mass spectrometry.** In Year 5, $5,000 is requested for the mass spectrometry experiments described in Aim 2 (to be conducted in the INBRE-supported Proteomics Facility at UAMS). The current cost of mass spectrometry is $2,500 per experiment, which includes experimental and control reactions. To repeat the experiment at least once, $5,000 is requested.

# Consortium/Contractual Costs

Not applicable