

# Idaho Incubation Fund Program

## Final Report Form

**Proposal No.** IF14-012  
**Name:** Patrick J Hrdlicka  
**Name of Institution:** University of Idaho  
**Project Title:** Production of gender-sorted sperm

### Information to be reported in your final report is as follows:

1. Provide a summary of overall project accomplishments to include goals/milestones met, any barriers encountered, and how the barriers were overcome:

A systematic screening of different DNA-targeting Invaders, revealed several promising candidates for detection of gender-specific chromosomal DNA targets in fixed and live sperm cells from bovine (Invader probes specifically fluoresce upon binding to Y- but not X-chromosomes). Systematic chemical perturbations of these promising leads were synthesized and evaluated. Interestingly, the attached fluorophore seems to have a marked influence on cellular compartmentalization and nuclear uptake efficiency. A scientist from our industry partner, Minitube of America (now MOFA Global) visited our laboratory to explore this fact in greater detail. Moreover, a number of nanoparticle-linked Invaders were prepared to facilitate nuclear uptake and binding to chromosomal DNA.

In parallel with these efforts, we develop optimized Invader chemistries (synthesis of new building blocks; preliminary biophysical characterization). The optimized chemistries will be able to seamlessly enter the production pipeline for improved detection sensitivity.

2. Describe the current state of the technology and related product/service:

Our industry partner has been satisfied with the provided Invader chemistries, and has been working on optimizing a microfluidic flow cytometry separation technique. This will allow us to produce gender-sorted sperm. MOFA Global is currently working on scaling up this separation technology in order to be able to produce the quantities of gender-sorted sperm necessary for market entry.

3. List the number of faculty and student participants as a result of funding:

Patrick Hrdlicka (faculty)

Dale C. Guenther (Ph.D.-student; fully funded)

Saswata Karmakar (Ph.D.-student; partially funded)

Brooke Anderson (Ph.D.-student; partially funded)

Jianqin Liu (Visiting Scientist from our industry partner; self-funded)

4. What are the potential economic benefits:

The project's most immediate economic impact will be revenue generation as outlined in the exclusive licensing agreement that has already been executed between UI, Dr. Hrdlicka and our industry partner, MOFA Global. Minitube estimates that the annual retail revenue from sales of gender-sorted sperm will exceed \$480 mill/year. We are having a dialog with MOFA Global, whether large-scale production of Invaders best would be carried out in a Moscow-based start-up company (housed in the UI Moscow Campus Incubator) or by a third party.

5. Description of future plans for project continuation or expansion:

Chemical optimization of the Invader probes will continue in the Hrdlicka laboratory. Identification of new diagnostic targets will be greatly aided by the recent acquisition of an invert fluorescence microscope (\$81,100; Higher Education Research Council, Idaho State Board of Education. Acquisition of a fluorescence microscope to support development of DNA diagnostics for use in the animal reproduction industry).

We continue our close collaboration with MOFA Global (Hrdlicka is Scientific Advisor).

6. Please provide a final expenditure report (attached) and include any comments here:

In order to further accelerate the project, I partially supported two additional research assistants (who synthesized the necessary Invader building blocks for this study). I accomplished this by waiving my personal summer salary and fringe, and by reallocating funds from the materials and supplies section (less expensive than anticipated) toward student salary and tuition. I and the main student on this project, were supported to present results at an international conference in US, which provided an excellent opportunity to further explore business relationships (as a direct result, a collaboration with ISIS Pharmaceuticals, a world leader in the development and discovery of oligonucleotide therapeutics, has been initiated).

7. List invention disclosures, patent, copyright and PVP applications filed, technology licenses/options signed, start-up businesses created, and industry involvement:

Two PCT applications have been filed to protect the Invader technology and its application in animal reproductive technology.

B. Didion, P. J. Hrdlicka and J. Versteegen. Gender-specific identification of sperm cells and embryos using locked nucleic acid probes for gender-specific sequences. 2013, PCT WO 2013103713A1.

P. J. Hrdlicka. Embodiments of a probe and method for targeting nucleic acids. 2013, PCT WO 2013013068A2.

8. Any other pertinent information:

Funding from this grant was acknowledged in two international peer-reviewed publications:

S. Karmakar, A. S. Madsen, D. C. Guenther, B. C. Gibbons and P. J. Hrdlicka. Recognition of double-stranded DNA using energetically activated duplexes with interstrand zippers of 1-, 2- or 4-pyrenyl-functionalized O<sup>2'</sup>-alkylated RNA monomers. *Org. Biomol. Chem.*, 2014, DOI: 10.1039/c4ob01183j

S. Karmakar, D. C. Guenther and P. J. Hrdlicka. Recognition of mixed-sequence DNA duplexes: Design guidelines for Invaders based on 2'-O-(pyren-1-yl)methyl-RNA monomers. *J. Org. Chem.*, 2013, 78, 12040-12048.

**FINAL EXPENDITURE REPORT**

<b>A. FACULTY AND STAFF</b>		
Name/Title	\$ Amount Requested	Actual \$ Spent
Patrick Hrdlicka	\$3,800.00	\$0
<b>B. VISITING PROFESSORS</b>		
Name/Title	\$ Amount Requested	Actual \$ Spent
<b>C. POST DOCTORAL ASSOCIATES/OTHER PROFESSIONALS</b>		
Name/Title	\$ Amount Requested	Actual \$ Spent
<b>D. GRADUATE/UNDERGRADUATE STUDENTS</b>		
Name/Title	\$ Amount Requested	Actual \$ Spent
Dale C. Guenther	\$20,000.00	\$17,173.80
Saswata Karmakar	\$0	\$6,211.80
Brooke Anderson	\$0	\$2,703.96
<b>E. FRINGE BENEFITS</b>		
Rate of Fringe (%)	\$ Amount Requested	Actual \$ Spent
PI (Hrdlicka)	\$1,400.00	\$0
Grad students (Guenther/Anderson/Karmakar)	\$600.00	\$1,135.02
<b>PERSONNEL SUBTOTAL:</b>	\$25,800.00	\$27,224.58
<b>F. EQUIPMENT: (List each item with a cost in excess of \$1000)</b>		
Item/Description	\$ Amount Requested	Actual \$ Spent
1.		
2.		
3.		
4.		
<b>EQUIPMENT SUBTOTAL:</b>		
<b>G. TRAVEL</b>		
Description	\$ Amount Requested	Actual \$ Spent
1. Travel to industry partner	\$800.00	\$0
2. Conference travel	\$0	\$ 2,628.40
3		
<b>TRAVEL SUBTOTAL:</b>	\$800	\$2,628.40

<b>H. PARTICIPANT SUPPORT COSTS:</b>			
Description		\$ Amount Requested	Actual \$ Spent
1.			
2.			
3.			
<b>PARTICIPANT SUPPORT COSTS SUBTOTAL:</b>			
<b>I. OTHER DIRECT COSTS:</b>			
Description		\$ Amount Requested	Actual \$ Spent
1. Materials and Supplies		\$15,400.00	\$8,768.02
2. Tuition and Fees		\$8,000.00	\$11,379.00
3.			
<b>OTHER DIRECT COSTS SUBTOTAL:</b>		\$23,400.00	\$20,147.02
<b>TOTAL COSTS (Add Subtotals):</b>		\$50,000	\$50,000.00
<b>TOTAL AMOUNT REQUESTED:</b>			<b>\$50,000</b>
<b>TOTAL AMOUNT SPENT:</b>			<b>\$50,000</b>