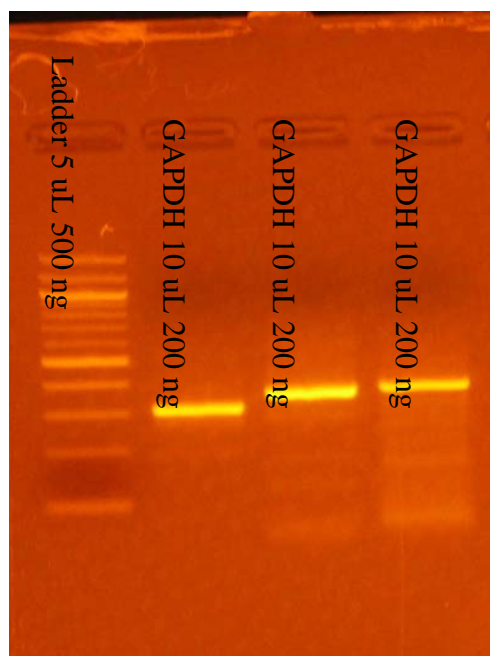


Submitting Amplicons for Sequencing to the University of Florida Interdisciplinary Center for Biotechnology Research

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Materials Required

- 200 ng purified PCR product/reaction at a concentration of at least 10 ng/ μ l (currently we use a Qiagen PCR purification kit)
- 5 μ l of primer/reaction at a concentration of 10 μ M, but 100 μ M is OK (the concentration used for real time PCR).
- Photograph of agarose gel showing sample ID for each lane as well as mass and volume of each sample and identification of DNA ladder (See example below).



Ordering Online

1. Before going into LIMS, you must log into myICBR at <https://login.ufl.edu/idp/Authn/UserPassword> (currently, only Jim and Kyle have login capabilities to do this, find one of them)

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UF Interdisciplinary Center for Biotechnology Research

Log in to myICBR | Log in to dnaTools

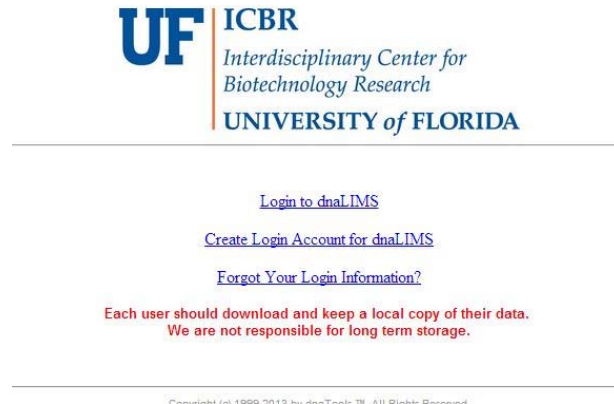
Search ICBR Web

2. Create a myICBR submission ID number (go to https://myicbr.biotech.ufl.edu:449/submission_new.aspx).
3. Go to the DNAlims at <http://dnalims.dnatools.com/>.

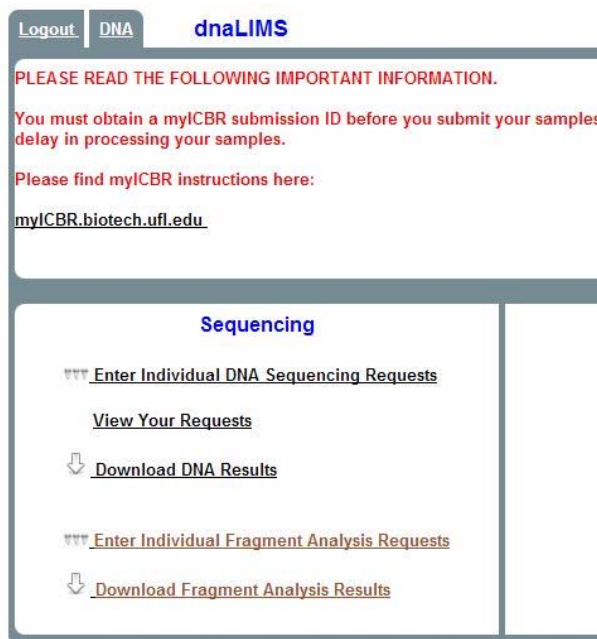
DNAlims website password and username:

Username: see Jim or Kyle

p/w: see Jim or Kyle



4. Click on “enter individual dna sequencing requests”



- Enter the total number of samples you want to sequence (for every gene, you will have 2 samples, one for the forward primer and one for the reverse primer). Once that is entered, click “RS” in the service requested box. Then click submit.

Enter the Number of Reactions to Create Sequencing Requests For:

Select the Sequencing Type.

Service Requested:

Service Type	Service Description
R	Reaction, unedited
RS 24cm	Reaction, up to 550 bases edited
RL 48cm	Reaction, 800-1000 bases edited
L	Load only, unedited
LS 24cm	Load only, up to 500 bases edited
LL 48cm	Load only, 800-1000 bases edited

By clicking the Submit button you indicate your understanding and acceptance of the Project Management Plan.

[Project Management Plan](#)

Clear

Submit

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- Insert your myICBR submission ID in the appropriate box. Leave everything else the same and proceed to enter template information, etc. The template is your gene name. DNA type is PCR. Primer is custom. Do not change the difficulties. Enter in all the other information as it pertains to your sample. The primer name is the gene name with F or R at the end. So, you will need a total of three tubes per gene: 1.) a tube with at least 20 ng/μl and 10 μl of your purified PCR sample 2.) 5 μl of your Forward primer and 3.) 5 μl of your reverse primer.

DNA Sequencing Request Form

myICBR Submission ID:	<input type="text"/>
Principal Investigator:	Peter Hansen
Service Requested:	RS
Print Chromatograms:	no <input type="button" value="v"/>
Primer Design:	<input type="text" value="0"/> Number of Primers to Design
Primer Synthesis:	<input type="text" value="0"/> Number of Primers to Synthesize
Reaction Mix Order:	<input type="text" value="0"/> How much BigDye to order?
Comments:	<input type="text"/>

No Validation response means all required fields are filled in.

Template Learn More			DNA Type [Plasmid or PCR]		Primer Learn More	Difficulties
Name <small>Index Fill Fill 2X Clear</small>	Conc. ng/ul <small>Fill Fill Clear</small>	ug of DNA <small>Fill Clear</small>	Name <small>Fill Clear</small>	Size bp <small>Fill Fill Clear</small>	Name <small>Fill Alternate Clear</small>	Special Request <small>Fill Clear</small>
1	<input type="text"/>	<input type="text"/>	Select <input type="button" value="v"/>	<input type="text"/>	-- Select -- <input type="button" value="v"/>	-- None -- <input type="button" value="v"/>
2	<input type="text"/>	<input type="text"/>	Select <input type="button" value="v"/>	<input type="text"/>	-- Select -- <input type="button" value="v"/>	-- None -- <input type="button" value="v"/>
3	<input type="text"/>	<input type="text"/>	Select <input type="button" value="v"/>	<input type="text"/>	-- Select -- <input type="button" value="v"/>	-- None -- <input type="button" value="v"/>
4	<input type="text"/>	<input type="text"/>	Select <input type="button" value="v"/>	<input type="text"/>	-- Select -- <input type="button" value="v"/>	-- None -- <input type="button" value="v"/>
5	<input type="text"/>	<input type="text"/>	Select <input type="button" value="v"/>	<input type="text"/>	-- Select -- <input type="button" value="v"/>	-- None -- <input type="button" value="v"/>
6	<input type="text"/>	<input type="text"/>	Select <input type="button" value="v"/>	<input type="text"/>	-- Select -- <input type="button" value="v"/>	-- None -- <input type="button" value="v"/>

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- Once this is complete, press submit and print the page that it generates. Print your gel picture with information on it that you made earlier and tape it to this new sheet. Take your samples and this sheet to the ICBR located on the first floor of the Cancer and Genetics Building. Put the samples in the refrigerator located in the receptionist area.

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