Submitting Samples for Sequencing at Segolip Unit

Purpose

To help the customer submit DNA samples to the SegoliP Unit for sequencing

SegoliP Unit accepts plasmid and PCR-generated templates for sequencing. *One of the most important factors in automated DNA sequencing is the quality of the template.*

Before submission of DNA samples for sequencing, the client should:

1. Ensure that the plasmid supplied is of sufficient quality and quantity. *The quality of the template DNA is a very important determinant of the quality of the sequencing data.*
2. Verify the presence and size of the DNA insert before submitting a plasmid for sequencing.
3. Ensure that the PCR product supplied is of sufficient quality and quantity. *The quality of the template DNA is a very important determinant of the quality of the sequencing data.*
4. Ensure that any primer supplied is of sufficient quality and quantity. *The quality of the primer is a very important determinant of the quality of the sequencing data.*

Plasmid DNA templates

1. The SegoliP Unit recommends the following kits for purification of high-quality plasmid DNA for automated sequencing: The Qiagen Plasmid DNA Kits and the Promega Wizard DNA Purification Systems. *If you choose to purify your plasmid using other methods, the SegoliP Unit recommends that you submit one or two samples for sequencing before sequencing a large number.*
2. Please note that all templates must be in water, **NOT** TE buffer.
3. For plasmid sequencing, submit 10 µl of each template at a concentration of 100ng/µl. *Before submitting a plasmid DNA template for sequencing it is essential that you check for the presence and the size of the insert.*
4. Run 1µl of the plasmid on an agarose gel next to a DNA standard of known amount and submit a copy of the gel image to SegoliP Unit with your samples.
5. If possible, also provide an A_{260}/A_{280} nm spectrophotometer reading. *(The A_{260}/A_{280} ratio should be 1.7 to 1.9. Smaller ratios usually indicate contamination by protein or organic reagents.)*
6. Samples should be submitted in 96-well plates.
   - Aliquot 10µl of your sample in each well. The well number must correspond to that written on the SRI form (e.g., well A01). The plate must be clearly labeled with the Name of the Researcher and the Date as indicated on the SRI Form.

PCR product templates

1. SegoliP Unit recommends the following kits for purification of high-quality PCR products for automated sequencing: The Qiagen QIAquick PCR Purification Systems and the

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Promega Wizard PCR Clean-Up Systems. *If you choose to purify your PCR product using other methods, the Unit recommends that you submit one or two samples for sequencing before sequencing a large number.*

2. Please note that all templates ***MUST*** be in water, ***NOT*** TE buffer.
3. **For PCR product sequencing, submit 10 µl of each template at a concentration of 50ng/µl.**
4. Run 1µl of the PCR products on an agarose gel next to a DNA standard of known amount and submit a copy of the gel image to the SegoliP Unit with your samples.
5. If possible, also provide an A_{260}/A_{280} nm spectrophotometer reading. *(The A_{260}/A_{280} ratio should be 1.7 to 1.9. Smaller ratios usually indicate contamination by protein or organic chemicals.)*
6. Samples should be submitted in 96-well plates.
   - Aliquot 10µl of your sample in each well. The well number must correspond to that written on the SRI form (e.g., well A01). The plate must be clearly labeled with the Name of the Researcher and the Date as indicated on the SRI Form.

Primers

1. M13 forward and M13 reverse primers are available at the SegoliP Unit. Clients are not required to supply them unless they have a modified version of the sequences. Please view our M13 sequences appended in the last page of this procedure.
2. Other sequencing reactions primers should be supplied by the customer. The primers should be at a concentration of 10pmol/µl, minimum 10µl, but sufficient for the number of sequencing reactions requested (5µl for each additional reaction required). Aliquot your primer in a 0.5ml or 1.5ml microcentrifuge tube labeled with the name of the primer corresponding to the SRI Form.

Submitting the templates and primers to the SegoliP Unit

1. Download the SRI form from the Segolip website. Populate the form with your sample information and upload the request to the website.
2. Samples: **DNA sample** Plates must be sealed with an adhesive film or a full plate cover and be clearly labeled with the Name of the Researcher and the Date as indicated on the SRI Form.
3. Place your samples in the fridge labeled “Customer Samples” located in the SegoliP Unit, Lab 4 Bay A.
4. **External customers:** Send the DNA samples and primers for sequencing via a Courier service of choice. Strictly seal your plate with strip caps to avoid cross contamination. Primers in tubes should be firmly sealed and wrapped in parafilm. Please send all details of the consignment (including the courier’s tracking number) to SegoliLab@cgiar.org.

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5. **Samples are to be shipped to the following address:**

   The Segolip Unit,

   International Livestock Research Institute (ILRI)

   P.O. Box, 30709-00100, Nairobi. Kenya.

   Tel: +254 722402608 | Tel: +254 20 422 3059

Other information

- Clients are advised to collect unused samples and primers within 60 days.
- Uncollected samples will be destroyed according to the biosafety protocol established in ILRI Biosciences.
- Sequencing Data will be uploaded on the website for the client to download.
- Optimally, 500-700 bases are obtained per plasmid reaction.

Segolip M13 Primer Sequences

M13 forward	TGT AAA ACG ACG GCC AGT
M13 reverse	TCA CAC AGG AAA CAG CTA TGA C