# Submission of samples for Fragment Analysis.

## Purpose

To help the Customer when submitting samples to the SegoliP Unit for Fragment Analysis on the ABI 3730 Genetic Analyser.

## Before Submission the Client should:

1. Ensure that the samples are placed in either a 96-well or a 384-well PCR plate that is compatible with the 3730 Genetic Analyser. The client should check on the compatibility of their plates with the Segolip unit staff.
2. Ensure the Dye set used with your samples are compatible with those used in the calibrations of the 3730 genetic analysers. The dye set used in the unit are appended below:

**Dye set G5 (6FAM, VIC, NED, PET) – with Liz as an internal size standard.**

**Dye set F (5-FAM, JOE, NED, and ROX).**

## Materials

- Fluorescently labelled PCR products prepared for fragment analysis.
- GeneScan™ 500 LIZ® Size Standard (Applied Biosystems catalog # 4322682). This can be purchased from the SegoliP Unit. Store at 2-8°C.
- Hi-Di™ Formamide (Applied Biosystems catalog # 4311320). This can be purchased from the SegoliP Unit. Store at -20°C.
- MicroAmp® Optical 96-well Reaction Plate (Applied Biosystems catalog # N8010560) or a Thermo-Fast 96 Non-Skirted plate (ABGene catalog # AB-0600) or MicroAmp® Optical 384-well Reaction Plate (Applied Biosystems catalog # 4343370).
- 3730 Sample Sheet - 96-well Plate or 3730 Sample Sheet - 384-well Plate.
- Clear adhesive film (Applied Biosystems catalog # 4306311) or a full plate cover (Applied Biosystems catalog # N8010550).
- Autoclaved, de-ionised or distilled water

## Procedure

### Multi-well plate compatibility

All samples submitted to the SegoliP Unit for fragment analysis on the ABI 3730 Genetic Analyser must be put into either a 96-well or a 384-well plate. Plates that are compatible with the ABI 3730 Genetic Analyser include those from Applied Biosystems (MicroAmp® Optical 96-well Reaction Plate catalog # N8010560 or MicroAmp® Optical 384-well Reaction Plate catalog # 4309849) and ABGene (AB-0600 Thermo-Fast® 96 Non-Skirted or AB-1310 Thermo-Fast® 384 PCR Plate). If you are using any other supplier of 96-well plates please check with the SegoliP Unit staff on their compatibility.

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## The order of your samples on a 96 well plate

The 3730 Genetic Analyser loads and runs the first 48 samples from odd numbered columns (purple columns), then loads and runs samples from even numbered columns (white background) – see Figure 1. Consequently, samples must be put into particular wells according to the total number of samples (see below), and the positions of your samples must be recorded on a 3730 Sample Sheet - 96-well Plate (see below).

**Figure 1**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A01 | A02 | A03 | A04 | A05 | A06 | A07 | A08 | A09 | A10 | A11 | A12 |
| B01 | B02 | B03 | B04 | B05 | B06 | B07 | B08 | B09 | B10 | B11 | B12 |
| C01 | C02 | C03 | C04 | C05 | C06 | C07 | C08 | C09 | C10 | C11 | C12 |
| D01 | D02 | D03 | D04 | D05 | D06 | D07 | D08 | D09 | D10 | D11 | D12 |
| E01 | E02 | E03 | E04 | E05 | E06 | E07 | E08 | E09 | E10 | E11 | E12 |
| F01 | F02 | F03 | F04 | F05 | F06 | F07 | F08 | F09 | F10 | F11 | F12 |
| G01 | G02 | G03 | G04 | G05 | G06 | G07 | G08 | G09 | G10 | G11 | G12 |
| H01 | H02 | H03 | H04 | H05 | H06 | H07 | H08 | H09 | H10 | H11 | H12 |

## Instructions for loading different numbers of samples are given below:

### 1-48 samples

For 1 - 48 samples use a 96-well plate and arrange your samples in either the purple coloured wells (Figure 1) and then complete the yellow fields on the 3730 Sample Sheet - 96-well Plate as follows:

a. Enter your PlateName in the two yellow fields indicated. This must match the name of the 96-well plate submitted to the SegoliP Unit.

b. Enter your name in the field marked “Owner”.

c. Enter your sample names in the “Sample Name” column within the fields: **Sample 01 - 48**. Ensure the position of the sample on the plate corresponds to the “Well” number on the Sample Sheet.

d. Rename the Excel sheet tab name highlighted with XXXXXX to the name of your plate. *To do this, place cursor over the tab, right click, go to “Rename”, then change the name.*

Please contact the SegoliP Unit staff if you have any problems creating the Sample Sheet.

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### 49-96 samples

For 49 - 96 samples use a 96-well plate and arrange your samples in both the purple **AND** white coloured wells (Figure 1) and then complete the yellow fields on the 3730 Sample Sheet - 96-well Plate as follows:

a. Enter your PlateName in the two yellow fields indicated. This must match the name of the 96-well plate submitted to the SegoliP Unit.

b. Enter your name in the field marked "Owner".

c. Enter your sample names in the "Sample Name" column within the fields: **Sample 01 - 96**. Ensure the position of the sample on the plate corresponds to the "Well" number on the Sample Sheet.

d. Rename the Excel sheet tab name highlighted with XXXXXX to the name of your plate. *To do this, place cursor over the tab, right click, go to "Rename", then change the name.*

Please contact the SegoliP Unit staff if you have any problems creating the sample sheet.

### 97-384 samples

For 97-384 samples use a 384-well plate and complete the yellow fields on the 3730 Sample Sheet - 384-well Plate as follows:

1. Enter your PlateName in the two yellow fields indicated. This must match the name of the 384-well plate submitted to the SegoliP Unit.
2. Enter your name in the field marked "Owner".
3. Enter your sample names in the "Sample Name" column within the fields: **Sample 01 - 384**. Ensure the position of the sample on the plate corresponds to the "Well" number on the Sample Sheet.
4. Rename the Excel sheet tab name highlighted with XXXXXX to the name of your plate. *To do this, place cursor over the tab, right click, go to "Rename", then change the name.*

Please contact the SegoliP Unit staff if you have any problems creating the sample sheet.

### Preparation of the Fragment Analysis Run Plate

1. If you are submitting a Fragment Analysis request to the SegoliP Unit for the first time we recommend that you try out a few samples at different dilutions so as to determine the optimum dilution factor for your samples. (Overloading and underloading of samples will result in poor data being obtained).
2. Prepare the **internal size standard** by adding 15 µl of GeneScan™ 500 LIZ® Size Standard to 1 ml of Hi-Di™ Formamide. This should be prepared on the day your samples will be submitted to the SegoliP Unit. Any unused size standard/Hi-Di formamide should be stored at 4°C for up to one week.
3. Prepare an appropriate dilution of the PCR products in distilled water (for example 1:4 dilution for SSRs). The ideal sample concentration should be 50ng/µl.

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4. To each of the wells of the reaction plate add 9 μl internal size standard mixture (see step 2). Add 1 μl of the diluted PCR product into the wells according to "The order of your samples on a 96 well plate" above.

   **NOTE:** Do not leave any wells of the reaction plate empty. An empty well may lead to capillary damage during the run. To empty wells add duplicates or other dilutions of your PCR products or just neat Hi-Di Formamide (10 μl).

   **NOTE**: Add the internal size standard/Hi-Di formamide mixture to your samples on the day you bring them to the SegoliP Unit as formamide degrades DNA.

5. Seal the plate with a clear adhesive film or a full plate rubber cover. For external clients, strictly use strip caps to cover samples to avoid cross contamination.
6. Clearly label the skirt of the reaction plate (the label must correspond to the Plate Name on the 3730 Sample Sheet).
7. Prepare the Sample Sheet and upload to the Segolip services website.
8. Bring the plate to the SegoliP Unit (Lab 4, Bay A) and leave it in the fridge labeled "Customer Samples".

   **NOTE**: For external customers, kindly ship your samples to the address provided below. Ensure the wells are properly sealed with strip caps to avoid cross contamination.

   **The Segolip Unit,**

   **International Livestock Research Institute (ILRI)**

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

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

## Results

Results will be uploaded in the Segolip website. A link will be shared for downloading purposes.

## Summary

- Download, complete and upload the 3730 Sample Sheet for Fragment Analysis according to your sample size. (i.e.48,96 and 384 samples.)
- Add **internal size standard/Hidi** to wells of the 96/384 well plate*
- Add your samples onto the 96/384 well plate.
- Seal and label plate. *
- Deliver plate to SegoliP Unit or ship the plate to the address provided *

*Do on same day.

Any plates submitted to the SegoliP Unit that is not claimed within two weeks will be discarded. The plates will be in the fridge labeled "Customer Samples" in the SegoliP Unit (Lab 4, Bay A) until then.