Contents

Template Preparation

** Quality templates will result in quality data. Purity and concentration of templates are crucial factors in sequencing success.*

Plasmids

- Recommended host strains for sequencing DH1, DH10B, DH5α, JM109, XL- Blue, and MV1190. Not recommended: JM101, HB101, TG1 & TG2.

- Use commercially available plasmid prep kits for preparing plasmid templates.
  Suggested kits: Promega Wizard Miniprep Kit
  Qiagen QuickLyse Miniprep Kit
  Qiagen Plasmid Midi Kit
  *Maxi preps are not recommended because they tend to yield unpredictable sequencing data.

- **Elute in water only.** Do not use TE buffer or other buffers containing EDTA.
  *EDTA is a problem because it chelates the magnesium in a sequencing reaction.

- When using ETOH precipitation, ensure your pellet is completely dry before resuspending. *ETOH lowers signal strength resulting in poor sequencing data.

- Verify purity by UV spec measurement and agarose gel. A good quality DNA sample should have an A260/A280 ratio of 1.7-2.0 and an A260/A230 ratio of > 1.5. RNA or genomic DNA contaminates not fully detected by UV spec can be detected by the differing migration band pattern on an agarose gel.

PCR Products

- PCR products must be clean. All traces of original PCR primers must be removed as well as unincorporated nucleotides, enzymes, and salts. Gel-purified products that produce a single band yield the best sequencing data.

- Recommended kits: Qiagen QIAquick Gel Extraction Kit
  Qiagen QIAquick PCR Purification Kit
  Promega Wizard® SV Gel and PCR Clean-Up System
  * Ultrafiltration devices alone are not recommended.

Quantitation by UV Spec and Agarose Gel

- To ensure accuracy read an A_{260} AND run the template on an agarose gel alongside standards of similar size and known concentration.

- Using a spectrophotometer alone to read an A_{260} is **not** recommended because contaminants such as genomic DNA, RNA, salts, and proteins all display some absorbance at 260nm. If these contaminants are present at high levels they will contribute to an increased A_{260} reading. A gel is not affected by these contaminants in the same way. RNA or genomic DNA contaminants can be detected by the differing migration band pattern. Using this combined approach, UV spec and gel electrophoresis, is the best method for measuring accurately the purity and concentration of a template, which is critical for sequencing success.

Primer Considerations

- Tm should be between 52-56°C with at least 50% GC content. Avoid a Tm < 50°C.
- Avoid strings of four or more of the same base.
- Do not use primers that can self hybridize.
- Design primers at least 30-50 bases away from the region of interest.
- Submit primers at micro molar concentrations [μM] or pmol/μl. When [primer] are in mass units please convert using the following:

$$[\text{Primer}]\ \mu g \times \frac{10^6\ pg}{1\mu g} \times \frac{pmol}{330pg} \times \frac{1}{n} = \frac{pmol}{} \quad \text{or} \quad \frac{[\text{Primer ng}] \times 1000}{330 \times n} = pmol$$

n=number of nucleotides in the oligo; 330 = average molecular weight of a nucleotide

Standard 1:1

- Log on to the NAC LIMS site (http://uthscsa.genesifter.net/login) and fill out a Standard 1:1 Sequencing Form.

Reaction Requirements

Template Type	Amount	Concentration
Plasmid	1μg / rxn	0.1μg / μl
PCR Product	100ng / rxn	10ng / μl
Primers	3.2pmol / rxn	1pmol / μl = 1μM

** Submission amounts are dependent on the desired read length of your template. Amounts should be calculated using 850bp as the average read length for one rxn*

1. Review the table above and provide the correct amount of template and primer in separate 1.5ml tubes. *Template* *Primer* [1µM]

2. Clearly label tubes with template names on the side of the tube and the template number on the tube lid. Numbers should correspond to the line number on the Sample Information section of the online order form. (See below) Label primer tube lids or tube side with the primer name.

Sample Information

Fill Down | Fill Down Plus | Clear Column | Highlight Column

Position	*Template	*Primer	Primer [C] (uM)	*Template Conc. (ng/µL)	Read Length	Sample Comment	Edited Data	OD260 (*lab use only)
1	Mouse 1	T7						

3. Place templates and primers in separate Ziploc bags. (The NAC can provide these if needed, just inquire)

4. Be aware, both primers and templates should be submitted at the recommended concentrations. Incorrect concentration submission will delay your sequencing project. If concentration corrections (dilution or drying) are required to be done by the NAC a $1.oo fee will be charged for each correction. Primers must be submitted in pmol / µl or µM units. ✱Submitting primers with mass units listed will result in delayed processing and users will be asked to use the conversion calculation before a project can be processed.

5. Print your online order form and follow delivery instructions below.

Standard Many: Many

- Log on to the NAC LIMS site (http://uthscsa.genesifter.net/login)and fill out a Standard M:M Sequencing Form.

Reaction Requirements

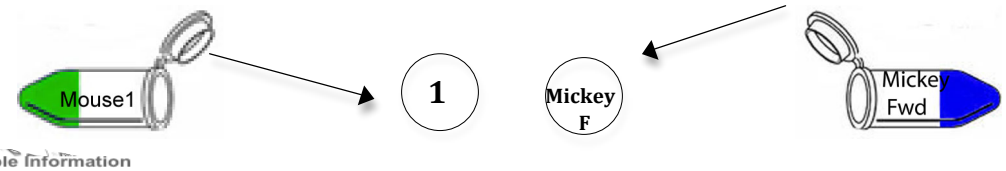
Template Type	Amount	Concentration
Plasmid	1µg / rxn	0.1µg / µl
PCR Product	100ng / rxn	10ng / µl
Primers	3.2pmol / rxn	1pmol / µl = 1µM

**Submission amounts are dependent on the desired read length of your template. Amounts should be calculated using 850bp as the average read length for one rxn*

1. Review the table above and provide the correct amount of template and primer in separate 1.5ml tubes.  *Template*  *Primers* [1µM]

   All primers must be submitted at 1µM concentration.

2. Clearly label tubes with template names on the side of the tube and the template number on the tube lid. Numbers should correspond to the line number on the Sample Information section of the online order form. (See below) Label primer tube lids or tube side with the primer name.



Sample Information

Fill Down | Fill Down Plus | Clear Column | Highlight Column

Position	*Template	*Template Conc. (ng/µL)	Read Length	Sample Comment	Edited Data	OD260 (lab use only)
1	Mouse 1					
2						

3. Place templates and primers in separate Ziploc bags. (The NAC can provide these if needed; please inquire.)

4. Print the online form and follow delivery instructions below.

HT ½ or Full Plate

- Log on to the NAC LIMS site (http://uthscsa.genesifter.net/login) and fill out a HT ½ or Full Sequencing Form.

- HT services dictate additional user responsibility; follow the NAC guidelines carefully. If you have any questions please contact the NAC lab staff prior to submission.

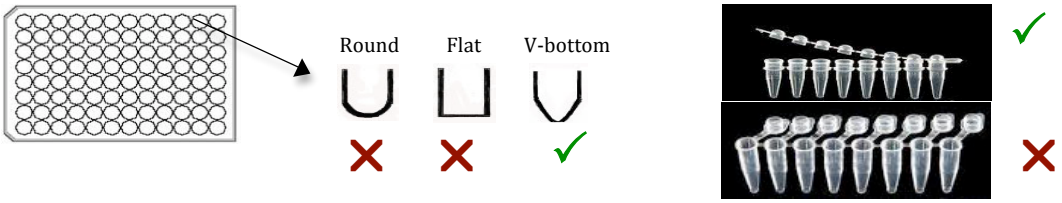
Reaction Requirements

Template Type	Amount	Max Volume
Plasmid	500ng / rxn	5.0 µl
PCR Product	50ng / rxn	5.0 µl
Primers	2.0pmol / rxn	2.0 µl
Total Submission Volume:		**7.0 µl**

** Number of rxns needed should be calculated using 850bp as the average read length for one rxn. If >850bp quality read length is required, multiple reactions are recommended.*

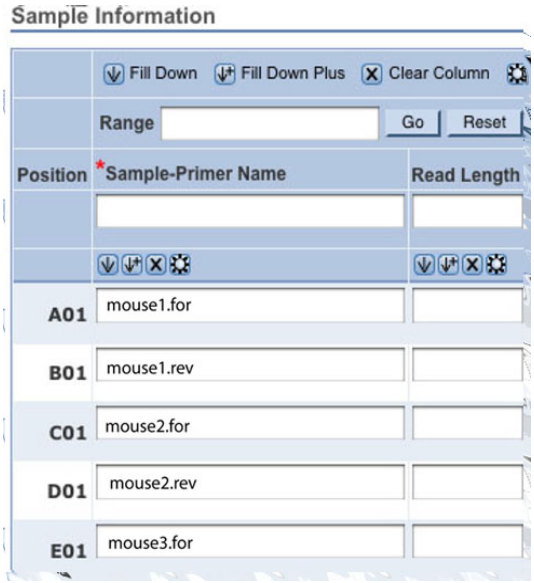
1. Review the table above and provide the correct amount of template and primer for each reaction required.

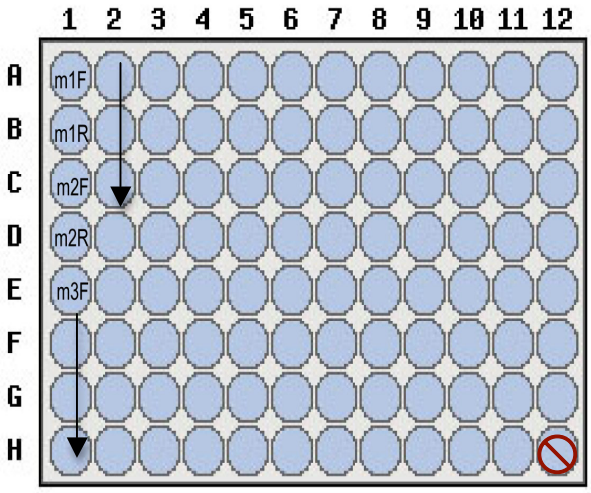
2. Use a V-bottom 96-well plate or 0.2ml 8-strip PCR tubes with strip caps.



✱ Components submitted in non-recommended plates or PCR tubes will result in a delay in processing, and you will be asked to re-submit using the appropriate plate or tubes.

3. Aliquot the appropriate amount of both template and primer for each reaction per well or tube. Orientation of reaction components in the plate or strip tubes should match the Sample Information section of the online HT Sequencing Form (see below).
*Please do not use well H12. This well is used for our control reaction.





4. Seal plates with a plate sealer and label the side of the plate with the plate name. When using strip tubes, securely tighten strip caps and orient strips in a labeled retainer plate. **The NAC can provide retainers if needed, just inquire.*

5. Print the online order form and follow the delivery instructions below.

✱ Final reminder: Users assume all responsibility for accurate reaction component concentrations and amounts. Submission volume must be 7μl.

R2R ½ or Full Plate

- Log on to the NAC LIMS site (http://uthscsa.genesifter.net/login)and fill out a Run Ready ½ or Full Plate Sequencing Form.

- Run Ready (R2R) sequencing services dictate the highest level of responsibility for the user. Please use the BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol (http://micro.uthscsa.edu/dna/bdv31_manual.html) for instruction on reaction set up, clean up, and prep for loading on the 3130. Please contact the NAC staff prior to submission if you have any questions regarding any aspect of the guidelines.

- The NAC receives a wide range of templates for full service sequencing, and because of this, we require a robust protocol that will fit the needs of all or most templates submitted. Our reaction guidelines can be used, but you may find it beneficial to follow the table below and the BigDyev3.1 Protocol for optimization of your own reactions.

NAC Reaction Guidelines: Plasmid 500ng
PCR 40-50ng
Primer 3.2pmol

- The table below indicates the quantity requirements for one sequencing reaction and is taken from ABI BigDye Terminator v3.1 Cycle Sequencing Kit Protocol.

Template Type	Quantity
PCR Product: 100-200 bp 200-500 bp 500-1000 bp 1000-2000 bp >2000 bp	 1-3 ng 3-10 ng 5-20 ng 10-40 ng 20-50 ng
Single-stranded	25-50 ng
Double-stranded	150-300 ng
Cosmid, BAC	0.5-1.0 µg
Bacterial genomic DNA	2-3 µg

Preparing Samples for Submission

1. A control reaction must be submitted with each R2R sequencing order. The standard pGem control DNA with M13F-20 primer supplied with the BigDye kit should be used for this reaction.

   Control Locations:

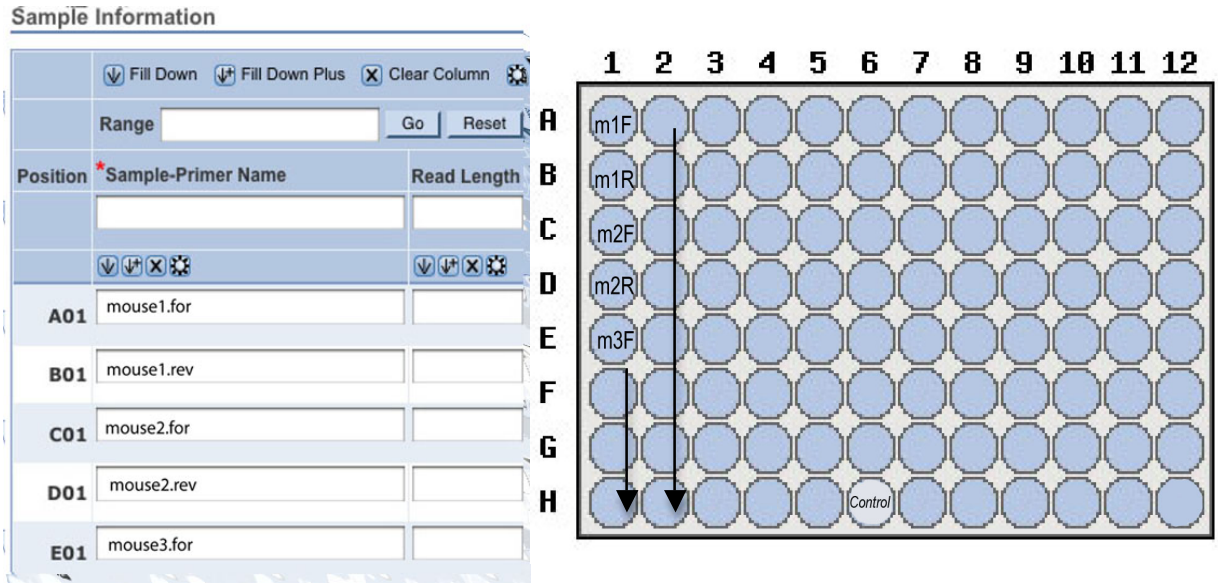
   - **Half Plate (47samples, 1control) and Full Plate (95samples, 1control)**: aliquot in well **H6***;* this well will be inactive on the online form and a control label will be automatically entered after submission**.**

- **<Half Plate**: aliquot into the well following the last aliquoted reaction well and designate this well as a control well on the Sample Information section of the R2R online form.
- **<Full Plate**: aliquot in well **H6***;* this well will be inactive on the online form and a control label will be automatically entered after submission**.**

**It is very important to add a control to your plate in the appropriate well. If the aliqouted plate does not match the online form information, your data cannot be extracted correctly and this will result in mismatched data files.*

2. Please use an AB 3130 compatible V-bottom 96-well plate and resuspend samples in 10-20uL of HiDi Formamide.

3. Ensure that orientation of sequencing samples in the plate matches the Sample Information section of the online R2R Sequencing Form of column by column (see below).



4. Seal plates with a plate sealer and label with the plate name and date on the side of the plate.

5. Print the online form and follow the delivery instructions below.

Delivering Samples to the NAC

Main- Campus

- Bring a printed online form along with your samples to the NAC located in the Basic Science Building Rm 4.059V.
- After hours or if NAC staff is not available use the Sequencing Drop Box located in the refrigerator across from the lab. Please enclose your order form and samples in a large NAC sequencing bag, which may be found in the Sequencing Drop Box.

Off Main Campus

- Enclose a printed online order form along with your samples in a 9"X12" envelope labeled:

  Nucleic Acids Core Facility
  Dept of Microbiology Rm 4.059 V

- Send through campus mail. Keep in mind that campus mail is usually only delivered once daily. Inquire with HSC mail services for drop off locations and delivery schedules at 7-5992.

** **R2R** users should deliver using the below method or hand deliver to the lab. Campus mail is not recommended as HiDi Formamide is considered a hazardous chemical. If limited to campus mail please call the NAC for specific instructions regarding allowable plate prep.*

Non-UTHSCSA

- Use a small disposable cooler and disposable ice pack and enclose samples.
- Place a protected online form (in a plastic bag) inside the cooler with the samples.
- Use a carrier of choice and send to:

  UTHSCSA
  Attention: Nucleic Acid Core Facility
  Dept of Microbiology Rm 4.059 V
  7703 Floyd Curl Dr.
  San Antonio, TX 78229-3900

**Please allow the appropriate time for selected shipping method. You may check receipt of your samples online by logging in to the LIMS and navigating to the View Orders page.*