

Fox Chase Sequencing Facility

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Location: 382

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Function

The faculty provides Fox Chase investigators with computer-readable sequences of their DNA templates in a timely and cost effective manner.

[Description](#)

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Description

The DNA Sequencing Facility provides Fox Chase investigators with computer-readable sequences of their DNA templates in a timely and cost effective manner. The facility includes one ABI 377XL gel-based analyzer and one ABI 3100 capillary genetic analyzer. Besides sequencing, analysis of DNA polymorphisms, important in genetic mapping studies, is also available using ?GeneScan? software. The facility performs taq cycle sequencing reactions on user-supplied templates and routinely generates sequences of 700?800 bases, with an average of 1?2% ambiguities. The 377 analyzes up to 96 samples, while two 96 well plates can be loaded on the 3100. Short PCR product sequencing or GeneScan runs are done during the day, with most sequencing run overnight. The facility provides sequencing primers for all routine vectors. Investigators are provided with a printout of their sequence, including electropherogram traces. Additionally, sequence files are available from a networked file server so that investigators can re-analyze their data on local computers in their laboratories and import the sequence data into software of their choosing for further analysis. All sequence analysis samples are processed using a plate-based setup, providing rapid sample throughput.

General Overview

The reactions are processed in the order received. A brief outline of steps involved in processing the samples are:

set-up of reactions
 3 hr cycle sequencing
 post-cycling processing which includes a precipitation step
 loading on a gel which then runs 10 to 15 hrs
 computer analysis of results
 printing and distribution of results to folders on file server

The current cost per lane is \$12.

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Sequencing Plasmids

Primers used by the facility:

m13 forward b	TTT CCC AGT CAC GAC GTT G	RH399
m13 reverse b	CAC ACA GGA AAC AGC TAT GAC	RH402
T7a	TAA TAC GAC TCA CTA TAG GGA GA	RH403
T7b	CCA GTG AAT TGT AAT ACG ACT C	RH404
Sp6b	CGC CAA GCT ATT TAG GTG ACA	RH406
newT7	AGC TGT AAT ACG ACT CAC TAT AGG	RH500
L-T3	AAT TAA CCC TCA CTA AAG GG	RH501
L-T7	GTA ATA CGA CTC ACT ATA GGG C	RH502

The following is required per lane of sequence:

5 μ l of 3 μ M (or greater concentration) Primer diluted in water (No TE or EDTA!) unless using one of the above primers.

At least 0.5 µg DNA diluted in a maximum of 10 µl water (No TE or EDTA!).

[Order sheet](#) (can be printed out from web or obtained in ICR282) with sample and primer names which match those written on the tubes. Please be sure to label the tubes with the concentrations of either the primer or DNA.

Place the DNA and primers in the yellow rack in the freezer, located under the middle bench on the left side. Place the order sheet on Anita's desk on the right side of the middle bench in the back.

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Sequencing PCR Products

Some purification kits used by other labs:

Promega	Wizard® PCR Preps DNA Purification System	50 preps	A7170	\$ 77
Qiagen	QIAquick PCR Purification Kit	50 Columns	28104	\$65
5 Prime to 3 Prime	PCR Select Columns (3 sizes available) Call 800-533-5730			

The following is required per lane of sequence:

5 µl of 3 µM (or greater concentration) Primer diluted in water (No TE or EDTA!)

At least 200 ng DNA diluted in no more than 10 µl of water (No TE or EDTA!).

[Order sheet](#) (can be printed out from web or obtained in ICR282) with sample and primer names which match those written on the tubes.

Place the DNA and primers in the yellow rack in the freezer, located under the middle bench on the left side. Place the order sheet on Anita's desk on the right side of the middle bench in the back.

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