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SEQUENCING FAQs

1.Q: What is high throughput sequencing?

A: High Throughput sequencing is a procedure that is very useful for sequencing many different templates of DNA with any number of primers. It is very quick and economical.

2. Q: How much DNA do I need?

A: Samples should be submitted in a total volume of 10 µl of DNA along with the primers.

PCR product (bp)	Concentration	Amount
<200	5ng/ul	50 ng
300 – 500	10ng/ul	100ng
600 – 1000	20ng/ul	200ng
Plasmid DNA		500 ng
SsDNA		100 - 200 ng
BACs and Cosmids		1 - 2 µg
Bacterial Genomic DNA		2 - 3 µg

3. Q: How should I quantify my DNA?

A: Using a spectrophotometer like the NanoDrop is the quick and easy way to quantitate samples. But it is always best to run the samples on an agarose gel to verify concentration.

4. Q: How much primer do I need?

A: 5 to 20 pmol

5. Q: Are primers provided by the Sequencing Facility?

A: Yes, we have the following primers we offer with no charge:

Universal Prime Name	Primer Sequence
M13 Universal Primer(-20) Forward	5'-GTAAAACGACGGCCAGT-3'
M13 Universal Primer (-40) Forward	5'-GTTTTCCCAGTCACGAC-3'
M13 Reverse Primer (-24)	5'-GGAAACAGCTATGACCATG-3'
M13 Reverse Primer (-48)	5'-AGCGGATAACAATTTCACACAGGA-3'
SP6 Sequencing Primer	5'-GATTTAGGTGACACTATAG-3'
T3 Sequencing Primer	5'-AAATTAACCCTCACTAAAGG-3'
T7 Sequencing Primer	5'-TAATACGACTCACTATAGGG-3'
T7 Terminator Primer	5'-GCTAGTTATTGCTCAGCGGT-3'

6. Q: In what type of tube should I send the sample?

A: Samples should be submitted in 1.5ml microfuge tube.

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7. Q: How do I submit a sequencing request?

A: Sequencing request should be submitted on our Ocimum Biosolutions website, at http://www.ocimumbio.com/web/BioResearch/sequencing

8. Q: What is a User Prepared sample?

A: Generally we get the samples and primers then process it for cycle sequencing reaction, clean up and electrophoresis. User Prepared sample is where the customer does the cycle sequencing reaction and clean up then send the sample to Ocimum Biosolutions for reading on the sequencer.

9. Q: What is the time line for the Sequencing?

A: All the samples for direct sequencing will be completed within five working days.

Samples require presequencing process like plasmid/PCR purification need additional days. On receipt of the samples the schedule will be intimated to the customers.

10. Q: When will I get my data?

A: The data will be placed on FTP server by 5.00 p.m everyday (Monday through Friday).

11. Q: How do I retrieve my data?

A: Sequencing data can be downloaded from our website.

For more information contact your sales person.

12. Q: How do I view my data? The data can be viewed with any DNA analysis program (chromas).

13. Q: My samples failed, Why?

A: The #1 problem with samples that fail is template concentration. To get accurate quatitation use Nanodrop. Contamination is another reason why samples fail. Contamination can be in the form of the following and in either your template, primer or both:

- Two templates present in one sample
- RNA
- Proteins

To fix this problem you should do one or all of the following:

- Run your sample out on a gel to make sure that there is only one template present.
- Check your primer sequence and make sure there aren't two primer binding sites on your template.
- Clean up your sample again to try and get rid of any contamination.

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14. Q: Do you send an email when my samples fail?

A: We send out emails to give you troubleshooting advice and to help pinpoint problems with sequencing. We do this with every user.

15. Q: How can I add a universal primer?

A: When you fill out your sequencing order online just be sure to check on radio button under "Primer". Also, make sure you click on one of the primers listed in the prime drop down menu. These are the primers provided free of cost..

16. Q: How long of sequence should I get?

A: With quality samples over 800 bases is common. When the sample preparation is good greater than 1000 bases may be obtained.

17. Q: I am not getting good results what should I do?

A: Make sure you are using enough DNA, we request 500 nanograms for plasmids of 5kb size. Low DNA concentration is the most common reason for sample failure.

18. Q: Will my samples be rerun if the results are not good?

A: We rerun samples that we believe to improve the sequencing data. Samples can also be rerun by request (failed reactions are charged).

19. Q: Who should I contact if I am having trouble with sequencing?

A: Your respective sales person. If required, he will connect you to the Sequencing lab.

20. Q: How should I purify my DNA?

A: Most of the DNA purification kits work fine. Make sure the sample is resuspended in water, not TE. EDTA can cause lower signal intensities. Also for Qiagen, do the optional wash to help get rid of any contaminates. Make sure the sample is dry before elution to prevent the presence of ethanol.

21. Q: What are the best primer conditions?

A: The primer conditions as follows

- Primers should be at least 18 bases long to ensure good hybridization.
- Avoid runs of an identical nucleotide, especially runs of four or more Gs.
- Keep the G-C content in the range 30 - 80%, preferably 50 - 55%.
- Tm between 45 and 60°C is best. Primers with Tm>45°C produce better results than primers with lower Tm.

- Primers with a G-C content less than 50%, it may be necessary to extend the primer sequence beyond 18 bases to keep the Tm>45oC.
- Use of primers longer then 18 bases also minimizes the chance of having a secondary hybridization site on the target DNA.
- Avoid primers that can hybridize to form dimmers or palindromes.
- The primer should be as pure as possible, preferably purified by HPLC and resuspended in dH2O.

22. Q: What if I want to submit two samples with my own primer and two samples that I want the universal primers?

A: You need to select the respective universal primers in the popup menu (in the sequencing order form) under respective sample/primer.

23.Q: How do I get my samples back?

A: We return the samples on your request mentioned in the sequencing order form.

24.Q: How do I address problems with the report?

A: You can write to your contact sales person. You have to address your queries within five days after the receipt of the report and the sequencing results.

25.Q: What is primer walking?

A: DNA fragments longer than 2000 bases have always been difficult to convert or sequence in a read. Sequencing methods such as Primer Walking are pivotal for these long DNA fragments. Instead of long reads, this method provides short consecutive stretch. In Primer Walking, the primers first get annealed at the start of DNA fragment and then get extended. Ultimately, this generates short stretch reads using chain termination method. The resulting short reads act as primer for generating the next read. In this fashion, primer walking generates sequence data in small reads.

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26.Q: What is SNP profiling?

A: A SNP (single nucleotide polymorphism) is a single base substitution of one nucleotide with another and the frequency in the general population at greater than 1%. SNP are becoming the markers of choice due to their high frequency in the genome and low mutation rate compared with microsatellites. Sequence based identification of SNP helps to narrow down the genes associated with disease conditions.

We offer the following services

- SNP screening
- SNP discovery

27.Q: What is Shot Gun Sequencing (BACs/Cosmids)?

A: Bacterial artificial chromosomes (BAC)/cosmids are highly stable, specific markers and preferred constructs for high-throughput genomic sequencing of organisms with long genomes. BAC end terminals provide highly specific signature/markers, but due to the large size of BAC DNA (>100 Kbp), the sequencing process becomes difficult and is more inclined to secondary structure, inaccurate base calling and bad read length.

28.Q: What is Bacterial identification?

A: Bacterial identification is to determine the species of specific bacterium based on the 16S rRNA. Since 16S rRNA is conserved within the species, sequencing this gene helps us to identify the organism and also to construct the phylogenetic relationship.