



Sample Preparation Guidelines

Regular Sequencing: Plasmids

Sample Type	Concentration	Volume
Plasmid < 6 kb	50-150 ng/ul	8 ul
Plasmid > 6 kb	100-200 ng/ul	8 ul
Primer	5 uM	5 ul

Regular Sequencing: PCR Products (Purified or Unpurified)

Sample Type	Concentration	Volume
Unpurified PCR Product*	N/A	8 ul
Purified PCR Product < 1 kb	10-20 ng/ul	8 ul
Purified PCR Product > 1 kb	20-50 ng/ul	8 ul
ExoSAP-it Treated PCR Product*	N/A	5 ul
Primer	5 uM	5 ul

• For plasmid and PCR samples we recommend providing extra volume in case troubleshooting or a repeat reaction is needed.

* When submitting ExoSAP-it treated or unpurified PCR products, the concentrations are not required. Please **DO NOT** dilute the samples.

Regular Sequencing: Premixed (Sample + Primer)

Sample Type	Concentration	Amount
Plasmid	50-150 ng/ul	250 ng
PCR < 1 kb	10-20 ng/ul	40 ng
PCR > 1 kb	20-50 ng/ul	80-100 ng

• Please provide **8 ul** total volume per reaction.

• Within this 8 ul, please add 1 ul of primer (Primer concentration should be 5 uM or 5 pmol/ul).

• Please add H₂O to make the final volume 8 ul.

• Please indicate on your order form if your sample is premixed.

Reminder: Premixed samples do not qualify for free repeats.



Direct Sequencing (DSC)

Used for culture samples, picked colonies, and agar plates

- Culture: **Submit 5-10 ul** of culture in single tubes or 96 well plates.
- Picked Colony: **Submit in 5-10 ul** of water in single tubes or 96 well plates.
- Agar Plate**: **Label colonies of interest on the plate(s) or include instructions indicating colonies you'd like us to pick.**

****Make sure to keep a replica plate for your own record.**

Special Sequencing

Used for samples with difficult templates, secondary structures, or high GC content

Sample Type	Concentration	Amount
Plasmid < 6 kb	100-200 ng/ul	100-200 ng/ul
Plasmid > 6 kb	150-250 ng/ul	150-250 ng/ul

- Please submit samples and primers separately (not premixed).
- Special Sequencing reactions are not eligible for free repeats.

Sample Storage at Eton

Samples are stored for two weeks only in a 4°C refrigerator in our lab. Once the two weeks are up, we will properly discard of your samples. If you have a special situation in which you need the samples stored for longer than two weeks, please notate it on your order in the special instructions and also contact our support team.

Troubleshooting and Repeats

If you were not satisfied with your results, please contact our customer service department by email (support@etonbio.com) or phone (800-758-1630) and we will be happy to troubleshoot your order. Upon review of the data, we can set up one-time repeats free of charge for failed reactions or reactions with unsatisfactory quality. The original reaction will still be charged. In other words, we charge once and only once for both the original and repeat reactions. To qualify for free repeats samples must meet concentration requirements and must not be premixed.

Additional Recommendations

- Please make sure your sample contains only DNA and dH₂O and is free of salts, EDTA, alcohol, protein, RNA, detergents, cesium and phenol.
- Please make sure you have sufficient DNA in your sample for sequencing.
- It is preferred that you have a unique DNA product in your sample. If you have a plasmid product, make sure all bacterial genomic DNA is removed.
- In the following situations we recommend you submit only a few samples to test out your conditions prior to submitting a large order***:
 - You suspect a problem with your template.
 - You are testing a new template preparation method.
 - You are using untested primers.

*****We also recommend that you submit a control template and primers to facilitate troubleshooting should any problems arise.**

- It is important that the A₂₆₀/A₂₈₀ ratio of your DNA sample is between 1.8 and 2.0. Otherwise, there is a good chance that your reaction will fail or have poor quality due to contamination or improper concentration.
- We highly recommend that you run your templates on agarose gel before submission. There should be one clearly defined band on the gel representing a particular template.
- For PCR products, remove or disable and unincorporated dinucleotides.
- Certain sequence motifs and secondary structures may prevent high quality results. In such situations, we can work with you to formulate a particular sequencing strategy that will yield the best possible results.