Sanger Sequencing Information

Various helps for Sanger Sequencing.

- Sanger Sample Submission Guidelines
- Primer Sequences
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Sanger Sample Submission Guidelines

PCR Products

- Quantitate using a gel (nanodrop isn’t reliable with PCR products)
- For small templates use 50ng PCR product (please clean it up first as left over primers can mess up sequencing) and 4 picomoles of primer (4uL of a 1uM aliquot)
- For larger templates, it is recommended to go high as 150ng (for approx. 2kb). Otherwise somewhere between the minimum and this will likely work. Use 4 picomoles of primer (4uL of a 1uM aliquot).

Plasmids

- For small samples (2-6kb) 600ng of product and 4 picomoles of primer (4uL of a 1uM aliquot) is recommended.
- For larger samples use more template/product (7-8kb: 700-800ng recommended, 9-10kb: 900-1000ng recommended) with 4 picomoles of primer (4uL of a 1uM aliquot). This is based on the manufacturer’s original recommendations and they also add if the larger amounts for large plasmids don’t work, drop to 600ng and 3.2 pmoles of primer and try that.

Primer Sequences

These are the sequences of the primers that we have aliquots of here in the core for our users to make use of.

M13F(-21)	GTAAAACGACGGCCAGT 3'
M13R	CAGGAAACAGCTATGAC 3'
T7prom	TAATACGACTCACTATAGG 3'
T7term	GCTAGTTATTGCTCAGCGG 3'
T3prom	ATTAACCCTCACTAAAGGG3'
SP6	GATTTAGGTGACACTATAG 3'
CMV-F	CGCAAATGGGCGGTAGGCGTG 3'
CMV-R	AGTAGGAAAGTCCCGTAAGG
EGFP-C	CATGGTCCTGCTGGAGTTCGTG
EGFP-N	CGTCGCCGTCCAGCTCGACCAG

All users are welcome to come get an aliquot of 50uL of 2uM primer dilutions for use on samples here through our core. Please do NOT re-freeze the aliquots. They will store fine in the fridge. Repeated freeze/thaw cycles of unconcentrated primers can be problematic is Sanger sequencing.

Sequencing Drop Off Sites

We currently have three sequencing drop off sites around campus.

HCI - End of the bench 4Y, 4th floor Research North Building. PICKUP TIME 11:00AM

Biology Building, Bldg 84, Room 202A (Mailroom in the small fridge). PICKUP TIME 11:30AM

DNA Sequencing Core Laboratory, Building 585 Room 110 (small white fridge on the counter next to Derek's office)

After hours drop off location: Building 585 Room 114 (Walk in fridge just down the hall from the DNA Sequencing Lab. Please note: The building locks down at night until early morning and I cannot get people access to the building for drop off purposes. If you already have access, this location is available when we are not open. Please drop samples in the Core when we are here. Thank you.

NOTE: If there are areas that are significantly distant from these locations, we would be willing to consider adding more. To do so, we would need a lab willing to host the drop off location that would allow access to other users in your area to come drop samples off during the week. This could include a walk in fridge, a lab fridge, etc. We would just need a small space that we can clearly mark and leave a few racks for samples to be placed in.