

Protocol for submitting Bulk Sequence Requests in a 96 well format for reduced pricing

1. You must submit a minimum of 48 reactions.
2. Wells should contain template, primer and water to a volume of 11.6 uL based on the following guidelines.
3. Plates containing less than 96 reactions should follow the proper layout as described below.
4. Plates should be sealed with an adhesive foil cover to prevent evaporation and spillage and should be frozen. Please label the plate with your name, Investigator and date.
5. Login to your iLab account. Find the 'Request for Services' tab, and then locate 'Sample Submission and Laboratory Services' heading. Open the VCC DNA Analysis Facility Folder.
6. Fill out the [Sequencing] - Bulk Sequence (min. 48 samples) Request.

Cycle Sequence Reactions:

The success of your sequence depends on the proper ratio of template and primer. Please do not vary these conditions!

Template Type	Template Concentration	Final Template amount in reaction	Template (uL vol to use)	Primer @2uM (uL vol to use)	H2O qs to 11.6uL
Double strand plasmid	50 ng/uL	375 ng	7.5	1.2	2.9
Large plasmid	50 ng/uL	525 ng	10.4	1.2	0
Single strand plasmid	25 ng/uL	75 ng	3	1.2	7.4
PCR 0-200 bp	5 ng/uL	10 ng	2	1.2	8.4
200-500 bp	5 ng/uL	20 ng	4	1.2	6.4
500-1000 bp	5 ng/uL	40 ng	8	1.2	2.4
>1000 bp	5 ng/uL	45 ng	9	1.2	1.4
PCR (Exo-Sap)	(5uL PCR + 2uL ExoSapIT)		1.5	1.2	8.9
Bac and gDNA	1ug/uL	1-2 ug	1.5	10pmol	q.s to 20uL

Please remember to put a single primer and template in a well.

Proper layout of the 96 well plate:

Samples should be filled into the plate vertically starting with A1 through H1 as indicated below.

This is the CORRECT layout:

	1	2	3	4	5	6	7	8	9	10	11	12	
A	#1	#9											A
B	#2	#10											B
C	#3	#11											C
D	#4	#12											D
E	#5	#13											E
F	#6	#14											F
G	#7	#15											G
H	#8	#16											H

This is the INCORRECT layout:

	1	2	3	4	5	6	7	8	9	10	11	12	
A	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	A
B	#13	#14	#15	#16	#17	#18	#19	#20	#21	#22	#23	#24	B
C													C
D													D
E													E
F													F
G													G
H													H

