Preparation of NGS libraries for Amplicon Sequencing
Working protocol for amplify-on library prep

Principle: This is our current working protocol for preparation of NGS libraries from cells edited at any specific gene. It starts with a rough extraction of gDNA from the cells, and then goes through 2 PCRs, the second PCR attaches adaptors ("amplify-on library preparation"). It is simple and highly amenable to multiplexing.

I. Primers:
PCR 1: (Amplicon primers with short stub):

Forward Primer: GCTCTTCCGATCTNNNNNNNNNNNNNNN
Reverse Primer: GCTCTTCCGATCTNNNNNNNNNNNNNNN

To design your own primers, simply add the stub (in red) to the 5' of each of your sequences.

1. ***PCR1***

Master mix (per sample)

FP, 100 µM:	0.15 µL
RP, 100 µM:	0.15 µL
5X GXL buffer:	10 µL
GXL dNTP mix:	4 µL
PCR grade water:	32.7 µL
GXL Polymerase:	1 µL
gDNA extract	2-5 µL

PCR cycling:
30 cycles of:
98ºC 10 sec
60ºC 15 sec
68ºC 30 sec
Purification: 1.8X SPRI purification using Beckman's instructions (90 µL SPRI beads to 50 µL PCR). Elute from beads in 20 µL.
Gel: 2% Agarose gel of selected samples (e.g. 2 per row per plate)
Quantitation: Qubit using DNA HS assay, and manufacturer's instruction. Nanodrop should also work, since the DNA should be relatively pure.
Note: Qubit can be readily performed using a fluorescent plate reader, and identical sample preparation instructions.

2. PCR 2: Submit to CTG for rest of Library prep and Sequencing…….