Denaturation	95 °C	30 s	35
Annealing/extension/data acquisition	60 °C	45 sec	35
Melt curve analysis	60-95 °C		

34l Add the appropriate DNA copies for each standard when setting up the qPCR plate in the qPCR program, as follows:

Standard	**dsDNA molecules/µl**
Standard 1	$1.2x10^7$
Standard 2	$1.2x10^6$
Standard 3	$1.2x10^5$
Standard 4	$1.2x10^4$
Standard 5	$1.2x10^3$
Standard 6	$1.2x10^2$

35l Analyze qPCR results. Multiply the average of duplicate values by the dilution factor and by the five-fold dilution factor of the qPCR reaction, as follows: Total copies/µl = # * dilution factor.

36l Pool library for MiSeq. Pool all the samples in one library at equimolar concentrations. 1X pooled library should be in a total volume of 5 µl, ~ 8×10^9 molecules.

37l Denature the pooled library (~ 8×10^9 molecules) by adding 5 µl of NaOH 0.2N and incubate at room temperature for 5 min. Then, add 940 µl of Hyb buffer (supplied with MiSeq® Reagent Kit v3 (600 cycle)).

38l Prepare the Phix control V3 (PhiX Control V3 KIT) as follows: mix 2 μl of 10 nM PhiX control with 3 µl of Tris-HCl 10 mM + 0.1% Tween-20, denature with 5 µl of NaOH 0.2N and incubate at room temperature for 5 min. Add 990 µl of Hyb buffer, to generate 20 pM PhiX. Then, make a 12.5 pM PhiX dilution, by mixing 375 µl of the 20 pM PhiX with 225 µl of Hyb buffer. Add 100 µl of the 12.5 pM Phix to the denatured library.

39l Clean the Flow Cell (supplied with MiSeq® Reagent Kit v3 (600 cycle)) with ultra-pure water, dry with lens tissues, followed by cleaning with alcohol wipes and lens tissue.

40l Load and sequence library using a MiSeq 600-cycle v3 kit according to manufacturer's instructions using MiSeq system. Sequencing is performed with 150 bp paired-end reads and 8 bp dual-index reads.

41l After sequencing, copy the demultiplexed output FASTQ files to a location accessible to CIRCLE-seq/CHANGE-seq analysis pipeline.