ChIP Primer Design by Ethan Ford

1. Go to UCSC Genome Browser home page http://genome.ucsc.edu/
2. Click ‘Genome Browser’ button (upper left side of page)
3. Select Genome (e.g. mouse) and assemble (e.g. Feb. 2006 (NCBI36/mm8))
4. Type position into ‘position or search term’ field (format example chr12: 57,612,909-57,630,019) or type Gene Symbol into ‘gene’ field.
5. Zoom in so that only the region you want to amplify is shown.
6. Click ‘DNA’ button at top of browser.
7. Click ‘Get DNA’ button
8. Copy DNA sequence
9. Open new browser window with NCBI’s Primer Blast page.
10. Set parameters according to attached page. If you are working in mouse change species to ‘Mus musculus (taxid:10090)’
11. Click ‘Get Primers’
12. Choose a primer set that is unique and does show homology to other regions of the genome.
13. Open a new browser window with UCSC’s In Silico PCR page
14. Select the species and genome build you are working with, e.g. mouse and Feb. 2006 (NCBI36/mm8).
15. Copy past your primers into the appropriate boxes and click the submit button.
16. If primers melting temp comes out more then 1˚ C different from each other remove 5’ nucleotides (relative to the primer NOT the amplified DNA sequence – that's the beginning of each primer sequence when written 5’ to 3’) from the primer with the higher melting temp until they are within 1˚ C.
17. Record the primer melting temp given by the in silico PCR results and the product size.
18. Name the primers according to (1) the chromosomal location of the end of the amplified DNA fragment (which is given by the In Silico PCR results). (2) Also add an F for the forward primer and a R for the reverse primer. (3) if working in mouse add 'mm' to the beginning of each primer name, for example ‘mm chr7 3939309 F’