Long-Range PCR Screening of targeted ES cells

Negative control:
Wt ES cell genomic DNAs

Positive Control: wt genomic DNA spiked stoichiometrically with one of below
Intermediate BAC if made by BAC recombineering or
Targeting vector with bigger arms than your targeting vector

Important Parameters for long-range PCR (3~10kb in length):

Genomic DNA: 100~500ng of high quality genomic DNA

Primer design: use free primer design software called **primer 3** (http://frodo.wi.mit.edu/) to design 2 to 3 primers on both sides of amplicon. Test combinations to pick the best pair.

a. 25-35 primer in length
b. 40~60% GC content
c. Primer pairs with similar Tm
d. Avoid complementarity of 2 or 3 bases at the 3' end of primer pairs
e. Avoid runs of 3 or more G/C at the 3' end
f. Avoid a 3' end T
g. Blast primer sequence against mouse genome to make sure there is no non-specific binding

PCR conditions: Use enzymes specialized for a long-range genomic DNA PCR!

a. Amplification condition: follow the instruction from manufacturers
b. Primer concentration: 400nM each
c. dNTP concentration: 500uM of each
d. MgCl2: 2~2.5mM
e. DMSO or betaine to relax secondary structure of gDNAs
f. Hot start can reduce non-specific bands greatly
g. 35 or more cycles