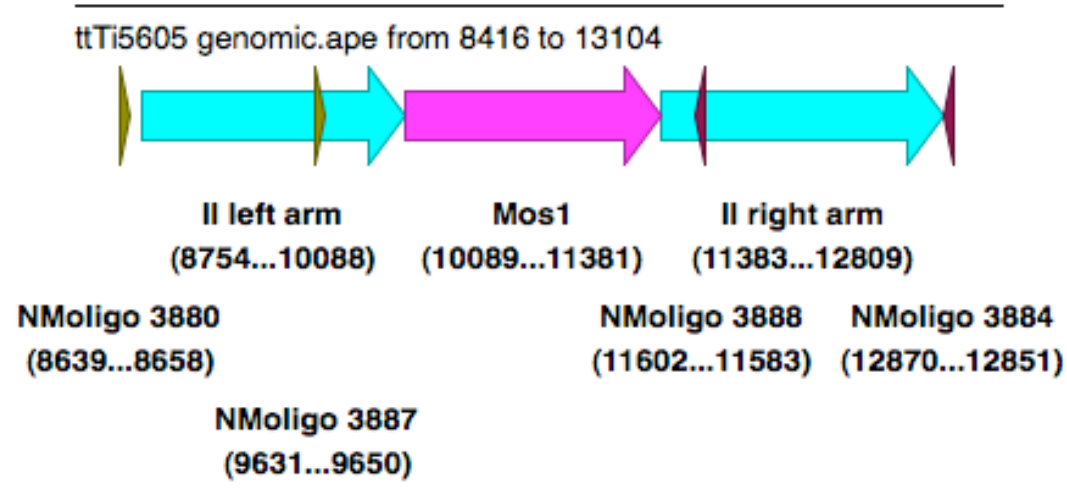
A PCR approach to characterizing MosSCI

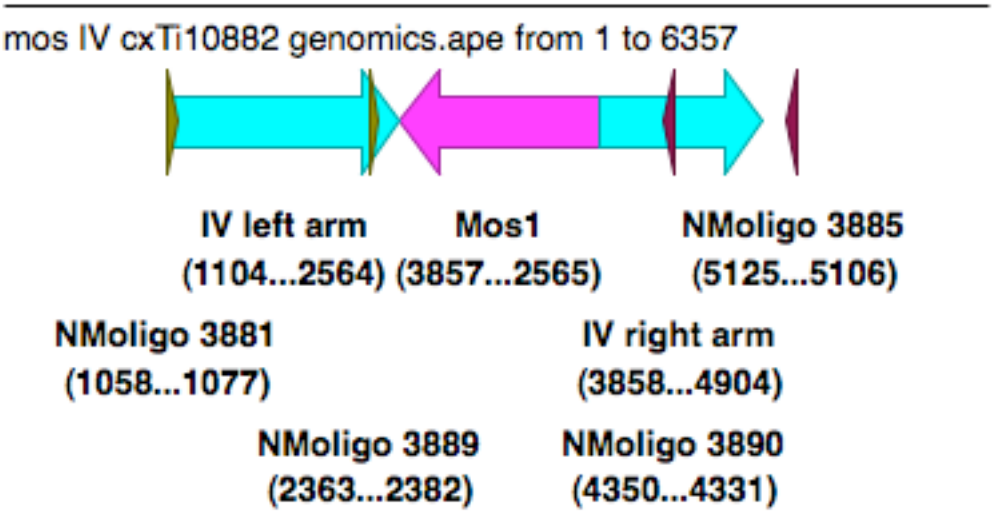
Make high quality genomic DNA. Our protocol for doing this can be found at http://neurosci.wustl.edu/nonetlab/resources.

We use PCR to amplify up inserts

for II
NM3880 5' AGGCAGAATGTGAACAAGACTCG
NM3887 5' ACCGGAAACCAAAGGACGAGAG
NM3888 5' ACGCCCAGGAGAACACGTTAG
NM3884 5'ATCGGGAGGCGAACCTAACTG

for IV
NM3881 5'CAAACGGAGCACCAGGAAAGC
NM3885 5' AAACTCCAAACACACCAGTCAC
NM3889 5' CCAAACAAGTGTCGTTGACCCAG
NM3890 5' CATATCCGCCAAGGACGCTC





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PCR reactions are performed using NEB LongAmp polymerase under the following conditions

Reactions
5 ul 5 X LongAmp Buffer
0.75 ul 10mM dNTPs
0.5 ul 3884 oligo 10 uM
0.5 ul 3880 oligo 10 uM
5 ul template (of a 1:50 dilution of our Genomic DNA prep ~ 100 ng total)
Water to 25 ul
1 ul LongAmp polymerase.

PCR conditions
30 second 95°C
35 cycles of
94°C for 10 seconds
60°C for 50 seconds
65°C for 12 minutes +10 second per cycle

65 for 10 minutes
We use an MJ Research PTC100 thermocycler using the EXTEND function to increase the extension time each cycle. I doubt this is really needed.

Samples	PCR size bp	
	3880/3884	3880/3888
1 N2	2942	1674
2 EG4322	4235	2967
3 EG4887 myo2 cherryH2B unc543'	8453	7185
4) NM2237 plasmid multicopy insertion	-----	6105
5) NM2237 plasmid single copy insertion	7373	6105
6) NM2237 plasmid single copy insertion #2	7373	6105

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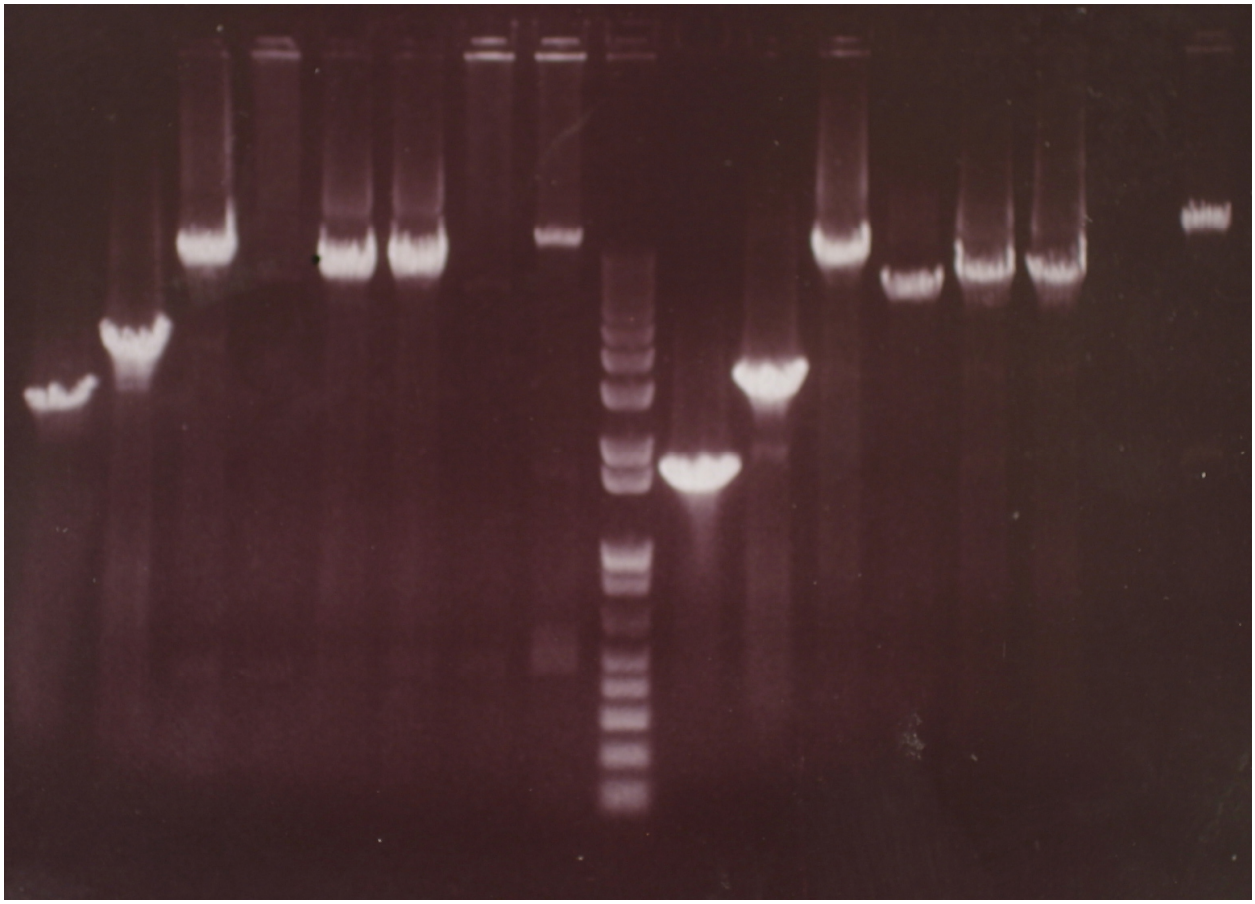
7) NM2215 plasmid insertion of unidentified structure	-----	----
8) NM2215 plasmid single copy insertion	15871	14603

5 ul of each PCR reaction loaded. Markers are 300 ng (total) of 1kb ladder/

3880/3884 oligo pair 3880/3888 oligo pair

1 2 3 4 5 6 7 8 1 2 3 4 5 6 7 8



Notes:

1) We use restriction digestion to make sure there are not obvious deletions or insertions in the insertions. These PCR reactions have been robust enough to simply digest the products via addition of ½ ul of restriction enzyme to 5 ul of product (sometime 5 ul is too much product). DraI, EcoRV, HindIII, HincII, PvuII and PstI all digest well in the 1X LongAmp buffer (a tris-SO4 buffer at pH9.0). Of course, one could also sequence the insert.

2) By performing PCR with oligos outside the arms (3880/3884) as well as one oligo in the arm and one oligonucleotide outside the arm (3880/3888 & 3884/3887) we have been able to show that multicopy inserts have correct structure on both arms (not shown) even though we cannot amplify across the insertion. (The product in lane 4 is actually the same size as lane 5 and 6, but 5 and 6 are so overloaded they are migrating slower).

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3) The lane 7 and lane 8 integrants actually look indistinguishable under the microscope looking at GFP fluorescence, but it is likely that the #7 has some unusual structure. This approach can distinguish such irregularities.

4) We also have PCRed using oligos in vector sequences and shown that the multicopy insertion amplifies with oligos in the Amp and Ori, while all the other strains do not.

5) One could also look for the presence of Mos1 transposons that may have hopped back in using oligos. This is short range PCR and does not require a high template quality.

6) We have not succeeded in PCRing up from single worm lysis (or multi-worm lysis) for these long range PCR reactions.

7) We have not attempted to optimize the oligonucleotides for long range PCR. Barnes (1994 PNAS 91, 2216) claims that longer oligonucleotides that anneal at 68°C work more robustly for long range PCR. For LongAmp taq which recommends 65°C extensions, oligos which can permit the melding of the anneal and extend steps may improve performance further.

8) We have tried the following polymerases: ExTaq (works up to ~ 10kB well) using 60°C anneal, 72°C 10 minute extensions. KlenTaq LA is less effective than ExTaq using their pH9.0 buffer and recommended annealing and extension times.

9) You may think of using LongAmp for other purposes since it gives such robust products. Beware we have found the error rate is extremely high!!

10) If you don't get a product using either approach (accross the whole insert, or from one arm to outside, one can still confirm there is an integration using one one oligo outside the arm and an oligo in unc-119 (one one side) and one oligo outside the arm and one oligo in your gene for the other side. These PCRs can be designed to create small product (<2 Kb) and done on single worms before high quality genomic DNA is even prepared.