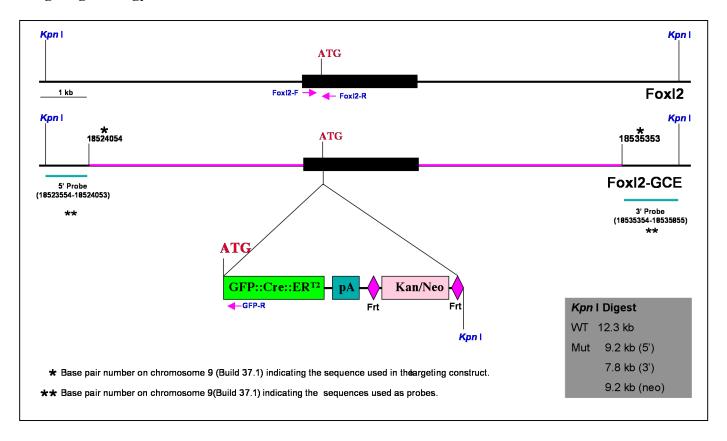
Fox12

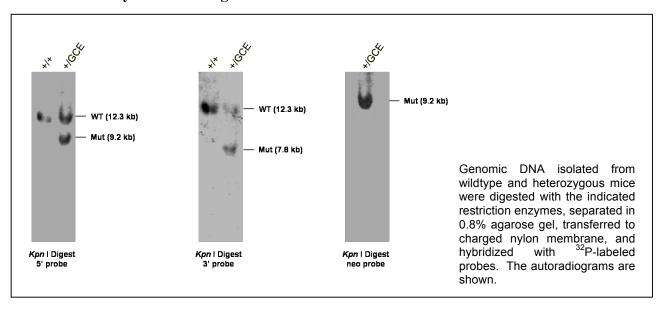
A. Rationale

Foxl2 (Forkhead box L2) is expressed in a population of granulosa cell precursors located in the medulla of the ovary and may be involved in gonadal determination. A strain of mice carrying eGFPCreERT2 knocked into the Foxl2 locus was generated by the GUDMAP consortium to investigate ovarian development.

B. Targeting Strategy



C. Southern Blot Analysis of the Targeted Allele in Mice



D. PCR Genotyping

a. Primers

Foxl2-F: 5' agagaagagagtgagagccg 3' Foxl2-R: 5' gagcgccacgtacgagtacg 3' GFP-R: 5' gtccagctcgaccaggatgg 3'

b. Expected Band Sizes

Foxl2-F + Foxl2-R: 335 bp Foxl2-F + GFP-R: 221 bp

E. Relevant Sequences

a. Genomic clone used for targeting construct

ggcagactttcacaaaatggaattcccctcacttaaagcaatgacttgggaagccatgaagtgggagggcacgctg qcqtqaaqaqacttqqaqtctaaaaqccqatttaacacacttctactaaaatqctcaqaqtctqaacaaatatttqcaaca agatgttgtgtggatgcttaagttgtaggtgtgaagctggaggtccagtagggacataagtcccctttgaagcgctcctct acactagectgectegegtggeggteegggtgtttetgeggeagetgeaggaageteggeeegggagaaaageatettea ccgqqqatccqcqacttqctcacqtaqqcqqcqqtcqaqacttcqqaqcqttaqaccaqcttqctqaqaqqcttctqctct gtccctggctccccggcaaggcatgggcaggcatttctctagaggcacaaggagctggaggcgctgaggaatcctgaaaac cccqqccaqaqaaacacqqaqttcctccqcctqqctcaqqaccqaqqcctctqccactaaqtqqatqqttcttaaccttqc cagaatgaaacgggtgaaagttgtgggggccgcacaacggggccgcacaggcctagaggaacagaatcctactatagtcga agtgcctgctctatccacctactgctgtaccaccgaacaggcaagcacattcgtcccagtcttcttagctctggctgctca cccagggtctggaaggtgatccctcggggcgccctgcagttgtgtttcccagccttgtgcgcacaagaggcactctttctc cacctgggattagggtgctgatcctgcaaaggacagcaagaagtcagttcccaggaatgtgtgactcctatttccactcag acacctcqqqctccttaaaqactqaaaaqqqaqccqacaqaqqaattcaqqqaaqcqatqacacatqcaacaattctqtca cacctggctgctgaggaggagctgagcggccggaaatggagccgtggagggggtgggggatgagacccagggtcagcttgg ccgaccaaagccagatatttttcccctgcccagaaccaagcagactcagatccagacccataacctgggcatgcctgatgg tgaggaagaagacaaacacctgcaggcatgctcagaaaacagacaagtattgaagaagtgctgtttgtctcttgagtgtgc tcaqqqcqqtqaqqqataqcttaqaaaaacatqqaqqctacttqqqqatactqtcaataaqqqctaqatqtqqqtatttct cagagaaatttagagaagcctacctcccttgtatcctggtggttaggccgggtttgcctggctgacctctctgtagtcatc atctcaaaqtqqqaaqcacaaqcacacactqtagataaatggtcttcggaccgctggagccactggccagtgtgggctcgt qqcattctqqaqaactcttaaaqtccaaqcaacctqaaaqaaqaqqtaqaaacaqqcactqqqataqccacctaqatqtq tgactggcacacatcattcattcagaccattatccttcacaacgtgtctgatcactcttcctcagcctctctcacacttcc ttttctattttcggagatgtaactggatccccaccagacttcggctattatcttaaaaacaaggggaaagactactgtcca qatacaaccatqtactctqqtqcttqattcatacacctcactaaqcaaaataaqcaaatacaaqtctcacacattqtaca tatataagcatacctgggtttctgaacagcacacttgcgcagtatcacgtgtgtctttaagaacagatttttgccccgtgc cttccacgcgtgacacttgtgcacgcaacagtccccaaagcccctcagcaacatcctcgtctgtgtccaagccagagtaca agggataccacttgatctttaaaacacacacccaaattgtgggattgcaaggcgagttccaaggagaaagctggtccctac atccacctcagcacagagaagcgaaacgtccgatgccagtgagcggtactcctgggtgtctctttcccagcgatcctctccc cagcctcaattqqqctcaqaqaqctcqaqqqacaqaqcaqaccqaaqcttctqccttccqtqacacqaqctctqaqctqqt ttacctccaqctqccqcaaaqttcqqccactccaccccqqtccqqccccttqccatctcqcccttacctaaqcctcaaaq ggatcacctctcctcgctggggtcagcattcccggggcactggcagggcgcagcgagccccaaccagggcttttcggcgaa aaqqccqqtccaaqttctqccqqccqtttaaaaaaaaaqqaqacttaqaqatqaactcqcccqtqcqctqctqqccc cgctataggggcgaaggcccctgacgcaagcggaactctgcggagcccatacgaatcagaacggagcgaggctcctggcgc





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b. The final construct (excluding plasmid backbone and the negative selection marker)

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Start of Fox12 transcription

Fox12-F

Start of GFP

GFP-R

CGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGAC
CACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCC
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End of GFP Start of Cre

End of Cre ER

CCATCTGCTGGAGACATGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACGCTCTAAGAAGAACAGCCTGGCC

Start of ER

End of ER Start of SV40 polyA

End of SV40 polyA Frt

Start of Kan/Neo

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Fox12-R

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c. 5' probe

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