Fred's plasmids.

pFC1 Contains a 1kb KpnI fragment from the mouse H19 gene cloned blunt at the HincII site of pTW101. This KpnI fragment is located far downstream from the H19 promoter. I was supposed to clone a HindIII-KpnI fragment located upstream of the promoter but this cloning didn't go the predicted way... Plasmid was discarded. See experiment 2.

pFC2 Contains a 1867 bp KpnI-AvrII fragment from pBSH19 14/1 made by CLH cloned blunt at the HincII site of pTW101. This KpnI-AvrII is located from 1.7 to 3.5kb upstream of the mouse H19 gene. The KpnI junction was checked by DNA sequencing. Both orientations of the fragment were cloned, only one was named. See experiment 2.

pFC3 Contains a 1617 bp AvrII-EcoRV fragment from pBSH19 14/1 made by CLH cloned blunt at the HincII site of pTW101. This fragment is located immediately upstream of the H19 gene. Both junctions were checked by DNA sequencing. Both orientations of the fragment were cloned, only one was named. See experiment 2. Forms R-loops upon transcription with T3 (see experiment 3).

pFC4 Corresponds to pTW101 in which a H19, PCR-derived, fragment obtained using FC1 and FC2 as primers was cloned blunt at the HincII site. Junctions checked by DNA sequencing. T7 promoter is in physiological orientation. See experiment 5. Forms R-loops upon transcription with T3 (see experiment 6).

pFC5 Error during cloning, plasmid not useful (corresponds to pFC6). See experiment 5.

pFC6 Corresponds to pTW101 in which a H19, PCR-derived, fragment obtained using FC5 and FC6 as primers was cloned blunt at the HincII site. Junctions checked by DNA sequencing. T3 promoter is in physiological orientation. See experiment 5.

pFC7 Corresponds to pTW101 in which a human SNRPN, PCR-derived, fragment obtained using FC11 and FC12 as primers was cloned blunt at the HincII site. One junction checked by DNA sequencing. T3 promoter is in physiological orientation. See experiment 8.

pFC8 Corresponds to pTW101 in which a human SNRPN, PCR-derived, fragment obtained using FC13 and FC14 as primers was cloned blunt at the HincII site. T3 promoter is in physiological orientation. This corresponds to the major G-rich island. See experiment 8. Forms R-loops upon transcription with T3 (see experiment 9).

pFC9 Corresponds to pTW101 in which a human SNRPN, PCR-derived, fragment obtained using FC13 and FC14 as primers was cloned blunt at the HincII site. This corresponds to the major G-rich island. One junction checked by DNA sequencing. Contrary to pFC8, T7 promoter is in physiological orientation. See experiment 8. Forms R-loops upon transcription with T7 (see experiment 9).

pFC10 Corresponds to pTW101 in which a human SNRPN, PCR-derived, fragment obtained using FC15 and FC16 as primers was cloned blunt at the HincII site. One junction checked by DNA sequencing. T3 promoter is in physiological orientation. It looks like this plasmid carries a deleted FC1516 fragment. See experiment 8. Forms R-loops upon transcription with T3 (see experiment 9).

pFC11 Corresponds to pTW101 in which a human KvLQT1 (LIT1), PCR-derived, fragment obtained using FC24 and FC25 as primers was cloned blunt at the HincII site. T7 promoter is in physiological orientation. See experiment 14.

pFC12 Corresponds to pTW101 in which a human KvLQT1 (LIT1), PCR-derived, fragment obtained using FC26 and FC27 as primers was cloned blunt at the HincII site. However, this highly GC-rich and repetitive insert deleted. See experiment 14.

pFC13 Corresponds to pTW101 in which a human SNRPN fragment corresponding to the 571 bp moiety of FC1516 PCR fragment digested with StyI was cloned blunt at the HincII site. See experiment 14.

pFC14 Corresponds to pTW101 in which a human KvLQT1 (LIT1) fragment corresponding to the 489 bp SacI-StuI from FC2627 was cloned blunt at the HincII site. See experiment 14. Forms R-loops upon transcription with T3 (see experiment 15).

pFC15 Corresponds to pTW101 in which a human KvLQT1 (LIT1) fragment corresponding to the 447 bp StuI from FC2627 was cloned blunt at the HincII site. See experiment 14. Forms R-loops upon transcription with T3 (see experiment 15).

pFC16 Corresponds to pTW101 in which a human KvLQT1 (LIT1) fragment corresponding to the 447 bp StuI from FC2627 was cloned blunt at the HincII site. Same as pFC15 except opposite orientation. See experiment 14.

pFC17 Corresponds to pTW101 in which a human KvLQT1 (LIT1), PCR-derived, fragment obtained using FC28 and FC29 as primers was cloned blunt at the HincII site. T3 promoter is in physiological

orientation See experiment 14. Forms R-loops upon transcription with T3 (see experiment 15).

pFC18 Corresponds to pFC11 in which a ~350bp EcoRI-HindIII fragment was deleted. See experiment 15.

pFC19 Corresponds to pCLH22B/H in which the HindIII-KpnI fragment (luc) was deleted and replaced by the HindIII-KpnI fragment from pFC8. This episome has the human SNRPN gene in the wrong orientation with respect to the RSVLTR promoter. See experiment 22.

pFC20 Corresponds to pCRTopo2.1 in which the FC1516 PCR fragment was cloned full length. T7 promoter is in physiological orientation. See experiment 14.

pFC21 Corresponds to pCRTopo2.1 in which the FC1516 PCR fragment was cloned full length. T7 promoter is in anti-physiological orientation. See experiment 14.

pFC22 Corresponds to pCRTopo2.1 in which the FC2627B PCR fragment was cloned full length. T7 promoter is in physiological orientation. See experiment 15.

pFC23 Corresponds to pCRTopo2.1 in which the FC2627B PCR fragment KvLQT1-LIT1) was cloned full length. T7 promoter is in anti-physiological orientation. See experiment 15.

pFC24 Corresponds to pCRTopo2.1 in which the FC3738R PCR fragment (IGF2R) was cloned full length. T7 promoter is in anti-physiological orientation. See experiment 35.

pFC25 Corresponds to pCRTopo2.1 in which the FC3738R PCR fragment (human IGF2R) was cloned full length. Same as pFC24 except T7 promoter is in physiological orientation. See experiment 35.

pFC26 Corresponds to pCRTopo2.1 in which the FC3841 PCR fragment (IGF2R) was cloned full length. T7 promoter is in anti-physiological orientation. See experiment 35.

pFC27 Corresponds to pCRTopo2.1 in which the FC3841 PCR fragment (IGF2R) was cloned full length. T7 promoter is in anti-physiological orientation. See experiment 35.

pFC28 Corresponds to pTW101 in which a human IGF2R, PCR-derived, fragment obtained using FC38 and FC41 as primers was cloned blunt at

the HincII site. T7 promoter is in physiological orientation. See experiment 35.

pFC29 Corresponds to pTW101 in which a human IGF2R, PCR-derived, fragment obtained using FC38 and FC41 as primers was cloned blunt at the HincII site. T3 promoter is in physiological orientation. See experiment 35.

pFC30 Corresponds to pCLH22 in which the HindIII-KpnI fragment (luc) was deleted and replaced by the HindIII-KpnI fragment from pFC9. This episome has the human SNRPN gene in the physiological orientation with respect to the RSVLTR promoter. See experiment 22.

pFC31 Corresponds to pCLH22 in which the HindIII-KpnI fragment (luc) was deleted and replaced by the EcoRI fragment from pFC25, containing IGF2R. This episome is suspiciously missing a XhoI site in the insert. See experiment 22.

pFC32 Corresponds to pTW101 in which a human IGF2R, PCR-derived, fragment obtained using FC37 and FC38R as primers was cloned blunt at the HincII site (FC3738R fragment is coming from pFC24 cut by EcoRI-Klenow). T3 promoter is in physiological orientation (Experiment 36)

pFC33 Corresponds to pTW101 in which a human IGF2R, PCR-derived, fragment obtained using FC37 and FC38R as primers was cloned blunt at the HincII site (FC3738R fragment is coming from pFC24 cut by EcoRI-Klenow). T7 promoter is in physiological orientation. (Experiment 36)

pFC34 Corresponds to pREP4 in which the small HindIII-Asp718 fragment was replaced by the HindIII-Asp718 fragment from pFC8 containing the human SNRPN gene. SNRPN is in physiological orientation with regards to the RSVLTR promoter. (Experiment 36)

pFC35 Corresponds to pREP4 in which the small HindIII-Asp718 fragment was replaced by the HindIII-Asp718 fragment from pFC9 containing the human SNRPN gene. SNRPN is in anti-physiological orientation with regards to the RSVLTR promoter. (Experiment 36)

pFC36 Corresponds to pREP4 in which the small BamHI-NheI fragment was replaced by the BamHI-XbaI fragment from pFC32 containing the human IGF2R gene. IGF2R is in physiological orientation with regards to the RSVLTR promoter. (Experiment 36)

pFC37 Corresponds to pREP4 in which the small BamHI-NheI fragment was replaced by the BamHI-XbaI fragment from pFC33 containing the human IGF2R gene. IGF2R is in anti-physiological orientation with regards to the RSVLTR promoter. This plasmid is very prone to deletion and contains a deleted molecule (<5%). (Experiment 36)

pFC38 Corresponds to pTW101 in which a mouse Snrpn, PCR-derived, fragment obtained using FC17 and FC21 as primers was cloned between Asp718 and HindIII after initial cloning into pCR2.1. T7 promoter is in physiological orientation. (Experiment 37)

pFC39 Corresponds to pTW101 in which a human SNRPN, PCR-derived, fragment obtained using FC50 and FC51 as primers was cloned between Asp718 and HindIII after initial cloning into pCR2.1. T3 promoter is in physiological orientation (Experiment 37)

pFC40 Corresponds to pREP4 in which a human SNRPN, PCR-derived, fragment obtained using FC50 and FC51 as primers was cloned between Asp718 and HindIII after initial cloning into pCR2.1. Fragment is in physiological orientation with respect to RSV promoter. (Experiment 37)

pFC41 Corresponds to pTW101 in which a mouse Snrpn, PCR-derived, fragment obtained using FC54 and FC55 as primers was cloned between Asp718 and HindIII after initial cloning into pCR2.1. T7 promoter is in physiological orientation. (Experiment 37)

pFC42 pREP4 + H19 anti phys.

pFC43 pREP4 + 9.9 from c-myc phys.

pFC44 Corresponds to pTW101 in which a mouse Snrpn, PCR-derived, fragment obtained using FC56 and FC20 as primers was cloned between Asp718 and HindIII after initial cloning into pCR2.1. T7 promoter is in physiological orientation. (Experiment 37)

pFC45 Corresponds to pTW101 in which a mouse Snrpn, PCR-derived, fragment obtained using FC56 and FC20 as primers was cloned between Asp718 and HindIII after initial cloning into pCR2.1. T3 promoter is in physiological orientation. (Experiment 37)

pFC46 Corresponds to pTW101 in which a human SNRPN, PCR-derived, fragment obtained using FC50 and FC53 as primers was cloned between Asp718 and BamHI after initial cloning into pCR2.1. This construct is the "spliceable" version of human SNRPN containing both exons 1 and 2. Compare to pFC39, which only possesses exon 1 or

pFC8/9, which have none. T3 promoter is in physiological orientation. (Experiment 37)

pFC47 Corresponds to pREP4 in which a human SNRPN, PCR-derived, fragment obtained using FC50 and FC53 as primers was cloned between Asp718 and BamHI after initial cloning into pCR2.1. This construct is the "spliceable" version of human SNRPN containing both exons 1 and 2. Compare to pFC40, which only possesses exon 1 or pFC30, which has none. RSV promoter is in physiological orientation. (Experiment 37)

pFC48 Corresponds to pREP4 in which a mouse Snrpn, PCR-derived, fragment obtained using FC56 and FC20 as primers was cloned between XhoI and HindIII after initial cloning into pCR2.1. This insert in this construct comes from pFC44. RSV promoter is in physiological orientation. (Experiment 37)

pFC49 Corresponds to pREP4 in which a mouse Snrpn, PCR-derived, fragment obtained using FC56 and FC20 as primers was cloned between XhoI and HindIII after initial cloning into pCR2.1. This insert in this construct comes from pFC45. RSV promoter is in anti-physiological orientation(Experiment 37)

pFC50 Corresponds to pTW101 in which a mouse Snrpn, PCR-derived, fragment obtained using FC17 and FC21 as primers was cloned between Asp718 and HindIII after initial cloning into pCR2.1. T3 promoter is in physiological orientation. (Experiment 37)

pFC51 Corresponds to pTW101 in which a mouse Snrpn, PCR-derived, fragment obtained using FC54 and FC55 as primers was cloned between Asp718 and HindIII after initial cloning into pCR2.1. T3 promoter is in physiological orientation. (Experiment 37)

pFC52 Corresponds to pTW101 in which a mouse Igf2r, PCR-derived, fragment obtained using FC57 and FC58 as primers was cloned between Asp718 and HindIII after initial cloning into pCR2.1. T3 promoter is in physiological orientation. (Experiment 40)

pFC53 Corresponds to pTW101 in which a mouse Igf2r, PCR-derived, fragment obtained using FC59 and FC60 as primers was cloned between Asp718 and HindIII after initial cloning into pCR2.1. T3 promoter is in physiological orientation. (Experiment 40)

pFC54 Corresponds to pCLH29 in which a human SNRPN, PCR-derived, fragment obtained using FC13 and FC14 as primers was cloned

between Asp718 and HindIII. The fragment was from pFC8. CMV is in anti-physiological orientation. (Experiment 43)

pFC55 Corresponds to pCLH29 in which a human SNRPN, PCR-derived, fragment obtained using FC13 and FC14 as primers was cloned between Asp718 and HindIII. The fragment was from pFC9. CMV is in physiological orientation. (Experiment 43)

pFC56 Corresponds to pCLH29 in which a mouse Igf2r, PCR-derived, fragment obtained using FC59 and FC60 as primers was cloned between Asp718 and HindIII. RSV is in anti-physiological orientation. (Experiment 43)

pFC57 Corresponds to pFC34 in which the small SalI fragment was flipped. (Experiment 43)

pFC58 Corresponds to pCLH22 in which the small Asp718-HindIII from pFC44 was cloned in place of luciferase. This fragment corresponds to the murine Snrpn IC (FC5620) and is cloned in the anti-physiological orientation. (Experiment 42)

pFC59 Corresponds to pCLH22 in which the small Asp718-HindIII from pFC45 was cloned in place of luciferase. This fragment corresponds to the murine Snrpn IC (FC5620) and is cloned in the physiological orientation. (Experiment 42)

pFC60 Corresponds to pCLH22 in which the FC5960 PCR fragment was cloned between Asp718-HindIII in place of luciferase. This fragment corresponds to the murine Igf2R/Air IC. (Experiment 42)

pFC61 Corresponds to pCLH22B/H in which the FC5960 PCR fragment was cloned between Asp718-HindIII in place of luciferase. This fragment corresponds to the murine Igf2R/Air IC. Transcription is in anti-physiological orientation (Experiment 42)

pFC62 Corresponds to pFC30 in which the annealed FC63/FC64 oligos were cloned between the Asp718-HindIII sites in place of SNRPN. FC63/FC64 corresponds to a multiple cloning site. (Experiment 43)

pFC63 Corresponds to pFC19 from which the BamHI-HindIII fragment corresponding to the RSV promoter was deleted. (Experiment 43)

pFC64 Corresponds to pFC62 in which the Asp718-HindIII fragment from pFC39 (FC5051 = hSNRPN IC) was cloned between Asp718 and HindIII. Transcription is in physiological orientation (Experiment 44)

pFC65 Corresponds to pFC62 in which the Asp718-BamHI fragment from pFC46 (FC5053 = hSNRPN IC) was cloned between Asp718 and HindIII. Transcription is in physiological orientation (Experiment 44)

pFC66 Corresponds to pFC62 in which the FC5960 PCR fragment (murine Igf2r/Air IC) was cloned between Asp718-HindIII. Transcription is in physiological orientation (Experiment 44)