Supplementary Figures and Tables

Mutations in Pericentrin cause Seckel syndrome with defective ATR-dependent DNA damage signalling.

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Supplementary Figure 1 :
Clinical details of PCNT-Seckel patients

(a) A 13 year-old individual with PCNT-Seckel, showing markedly reduced stature and micro-cephaly, compared with her younger unaffected 11 year-old sister. **(b)** Clinical details of probands from families 1-3. Growth parameters expressed in standard deviations from the relevant age and sex-related mean. Actual measurements in brackets. Wgt, weight; Hgt, height, OFC, occip-itofrontal circumference. Previous reported growth parameters in an unselected cohort of Seckel patients [ref.1], were mean birth weight, 1.54kg (range, 1.0-2.06 kg), and height at exami-nation of –7s.d. (+/- 2.1 s.d.), and a mean OFC of –8.8 s.d (range –5.1 to –13.3 s.d.).

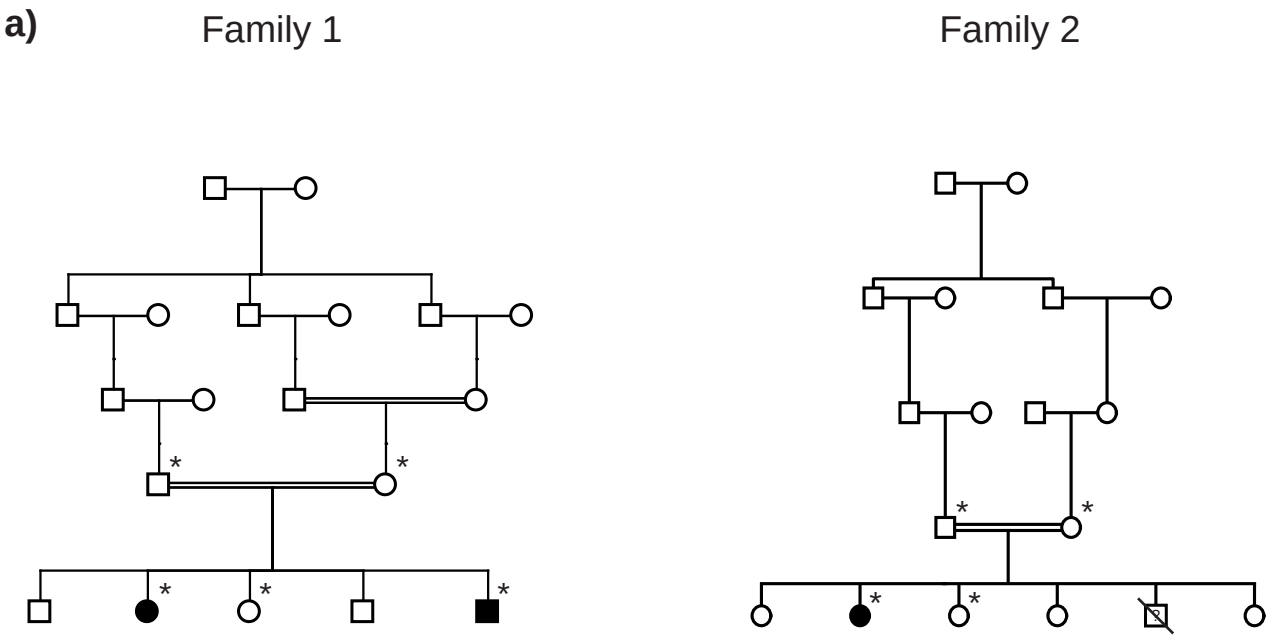
a)

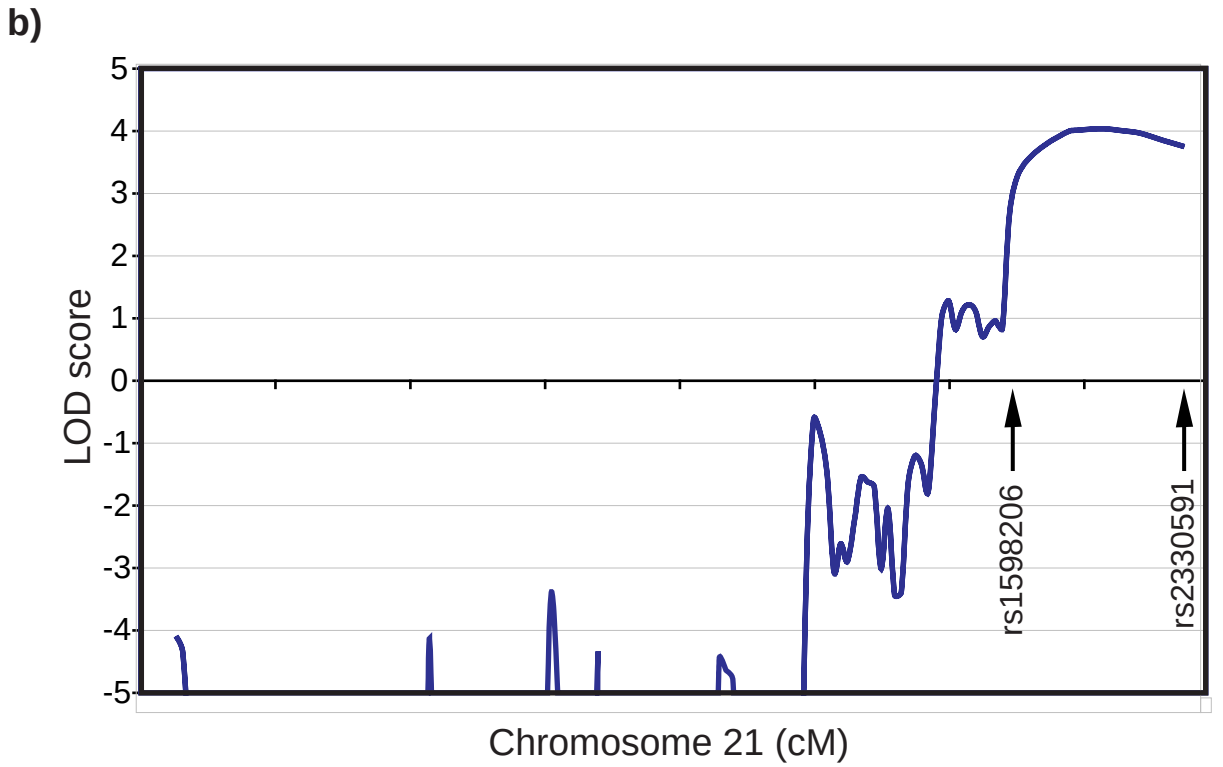
b)

				Birth				At Examination				Other Comments
Mutation	**Family**	**Sex**	**Country of origin**	**Gestation (wks)**	**Length**	**Wgt**	**OFC**	**Age (yrs)**	**Hgt**	**Wgt**	**OFC**	
E220X	**Family 1 1st/ 2nd cousin**	**Male**	**Saudi Arabia**	**33**	**-2.7 (40cm)**	**-3.0 (1.1kg)**	**-2.8 (27cm)**	**3**	**-9.6 (61cm)**	**-10.2 (5.4kg)**	**-5.8 (41cm)**	**Normal skeletal survey. Receding forehead, high nasal bridge, prominent nose, retrognathia. 5th finger clinodactyly. Proportionate short stature. Motor delay. MRI: thin corpus callosum, increased extra-axial spaces. Reduced white matter. 46XY.**
E220X	**Family 1**	**Female**	**Saudi Arabia**	**33**	**NA**	**-3.5 (0.94kg)**	**NA**	**13**	**-8.5 (96.5cm)**	**-6.2 (17.5kg)**	**-8.4 (43.5cm)**	**Receding forehead, peaked prominent nose, microretrognathia. Short neck, low posterior hairline. Some areas of skin hyperpigmentation. Significant learning difficulties. Dysplastic hips, unequal femur length. Myopia. Short mid-phalanges. Diabetes Mellitus. 46XX.**
S629fs	**Family 2 1st cousin**	**Female**	**Kuwait**	**37**	**NA**	**-5.5 (0.87kg)**	**NA**	**13**	**-8.9 (94cm)**	**-8.5 (13kg)**	**-5.8 (47cm)**	**Prominent nose, small ears, receding forehead and chin. Short philtrum. Deep voice, small 'broken' teeth. No abnormal pigmentation. Proportionate short stature. Normal skeletal survey with minor scoliosis. Slightly advanced bone age. 46XX. IQ 44. Growth Hormone levels normal.**
C1190fs	**Family 3 1st cousin**	**Male**	**Morocco**	**35**	**NA**	**-3.9 (1.08kg)**	**-2.4 (27cm)**	**3**	**-8.4 (66cm)**	**-5.9 (5.3kg)**	**-8.4 (39cm)**	**Sloping forehead, prominent nose, retrognathia. Normal skeletal xrays, though significantly retarded bone age. IQ 50. Normal MRI brain.**

Supplementary Figure 2 : Identification of the Sckl4 locus at Chromosome 21q22.3

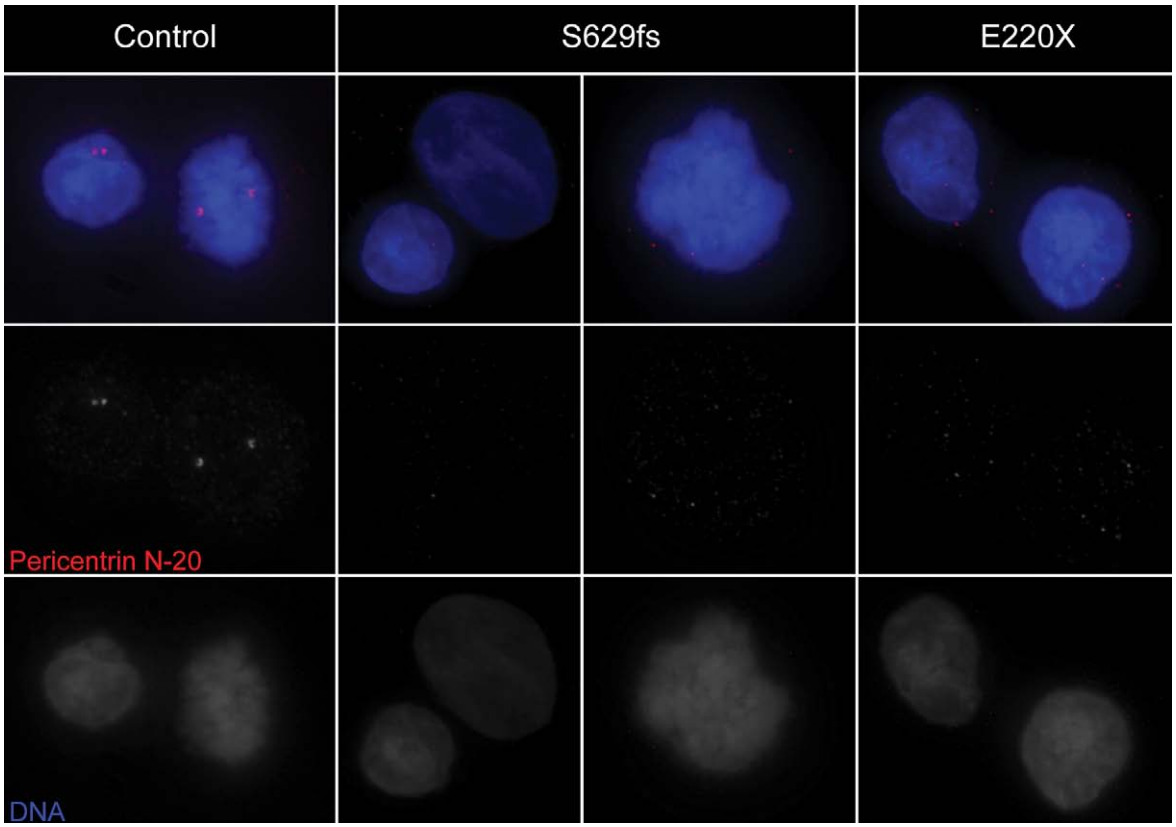
(a) Pedigrees of Families 1 and 2. Asterisk indicates individuals included in genome-wide SNP genotyping and linkage analysis. **(b)** Total multipoint LOD scores for families 1 and 2 for chromosome 21. Distance in centiMorgans, Decode genetic map. Critical region defined by overlapping homozygous segments in the three affected individuals indicated by arrows (rs1598206, 64.25cM; rs2330591, 77.9cM).





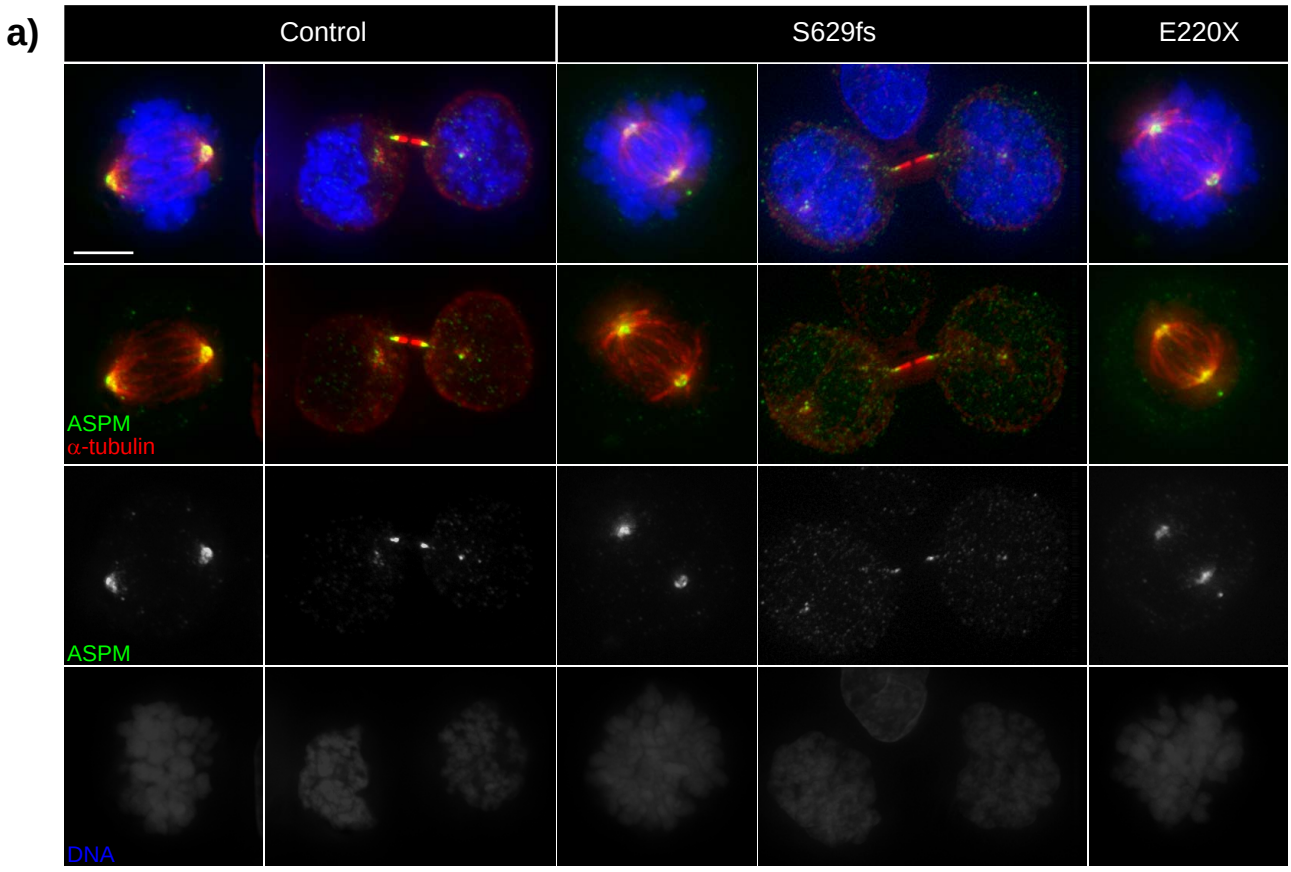
Supplementary Figure 3 :
Pericentrin localisation in PCNT-Seckel cells with the N-20 antibody

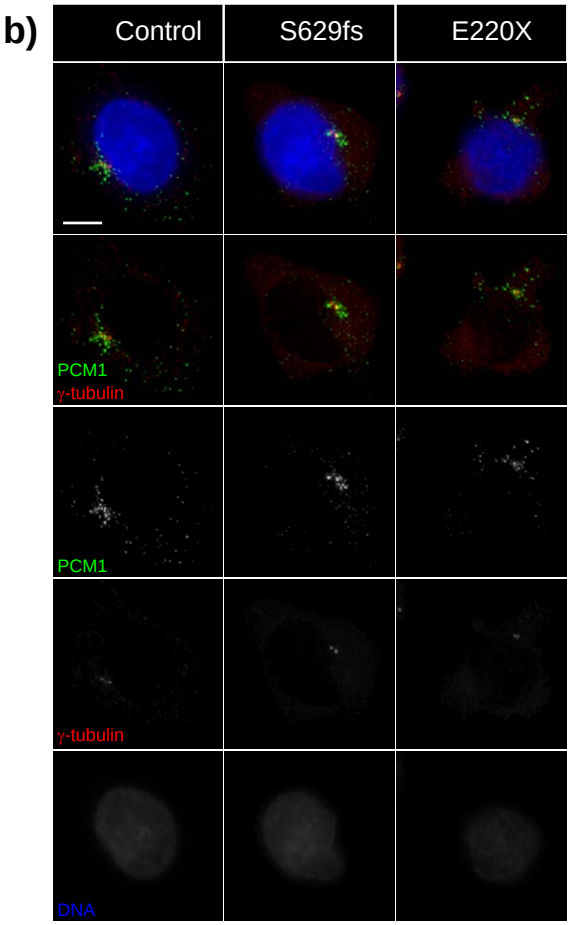
Pericentrin is not detectable at the centrosomes in PCNT-Seckel LCLs using the PCNT N-20 antibody. Control, heterozygote relative, $PCNT^{220X/+}$.

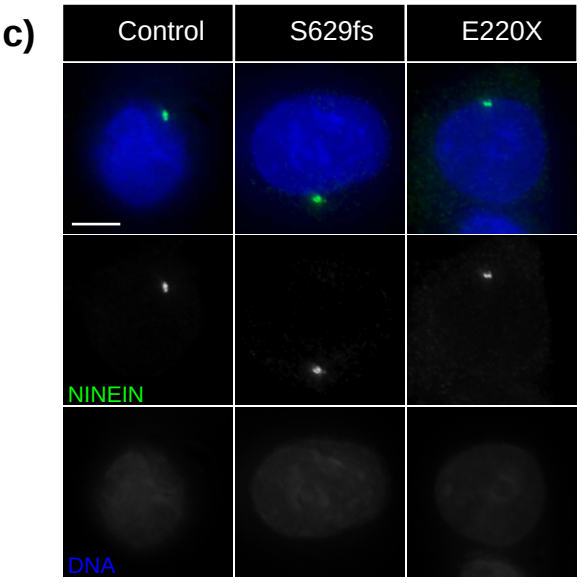


Supplementary Figure 4 : Other centrosomal proteins' localisation in PCNT-Seckel LCLs

ASPM **(a)**, PCM1 **(b)**, and Ninein **(c)** are normally localised in PCNT-Seckel LCLs. A normal mitotic spindle and midbody is formed in the PCNT-Seckel cells shown **(a)**. Control, heterozygote relative, $PCNT^{220X/+}$. Scale bar 5µm.

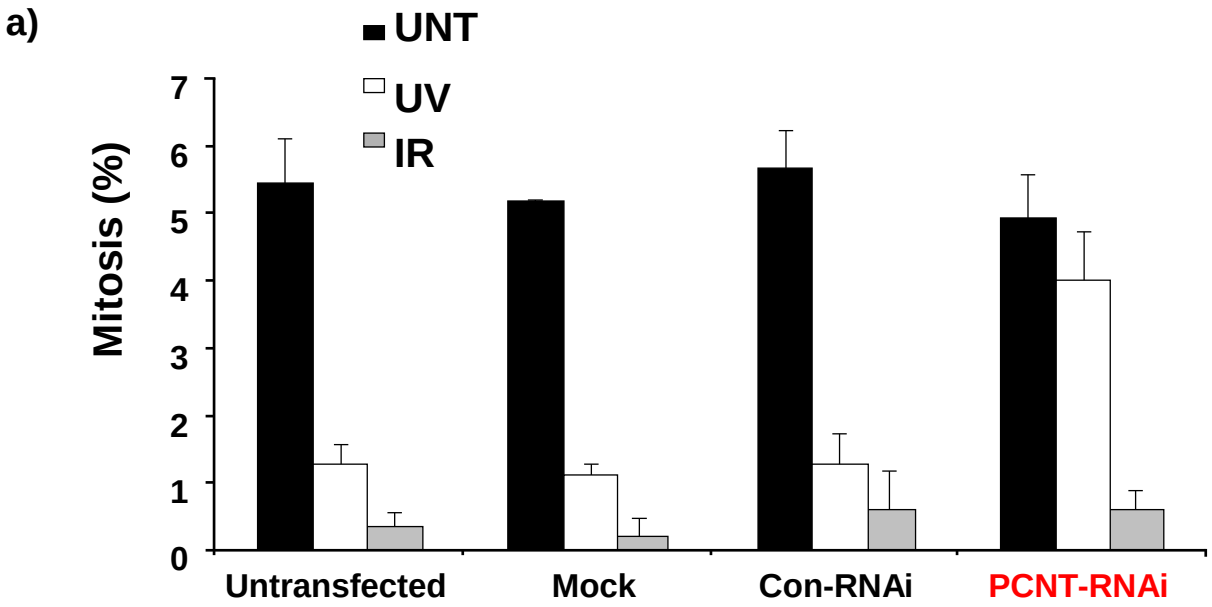


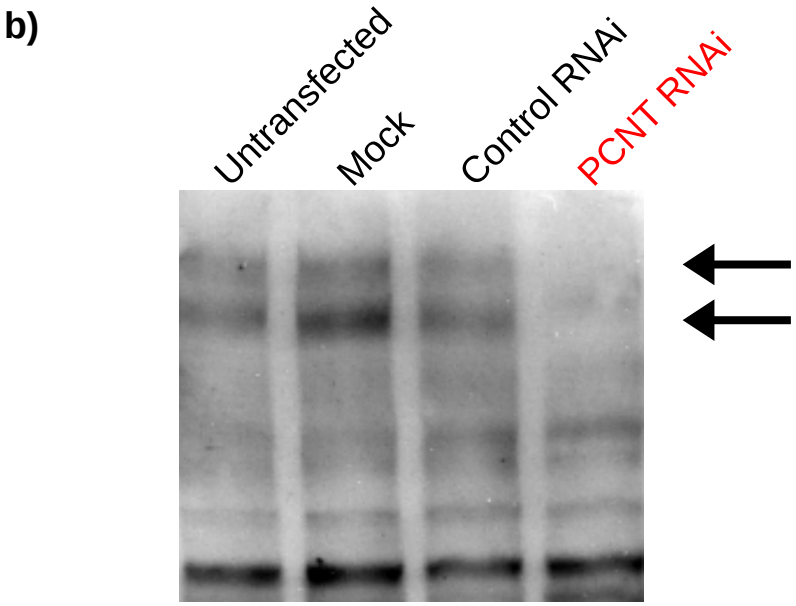




Supplementary Figure 5 :
RNAi Depletion of PCNT in HeLa cells impairs ATR-dependant G2/M arrest

RNAi depletion of PCNT in HeLa cells significantly impairs G2/M arrest following ATR (UV), but not ATM (IR) dependent DNA damage. **(a)** The mitotic index was examined 2hrs after treatment with UV (5J m^{-2}), Ionizing Radiation (IR, 2Gy) or no treatment (UNT). Mock, cells treated with transfection agent NeoFX^{TM} only; Con-RNAi, cells treated with control siRNA duplex (On-TargetPlus siControl non-targetting siRNA#2, Dharmacon); PCNT-RNAi, cells treated with PCNT specific siRNA duplex. Error bars s.d., PCNT-RNAi versus Control-RNAi, p=0.005. **(b)** Western Blot of cell lysates from HeLa treated with siRNAs showing depletion of two isoforms of PCNT (arrows) on treatment with PCNT specific siRNA.





Supplementary table 1: Primer and siRNA oligonucleotide sequences

Primers used for PCNT PCR and Sequencing

Exon		**Primer sequence**	**PCR conditions** Faststart Taq (Roche)
4	F	AGGACGTGCGTCGTCAGTTC	54ºC / 40 cycles
	R	AAAGGAGATGGCAGCGCCC	
12	F	AGCAGGAAACACCTTTGAGGG	54ºC / 40 cycles
	R	TTCACGGAGGACTTGGATCG	
18	F	CGAGGTGTGCAAACTGGTGG	60ºC / 35 cycles
	R	CCAATACGGAGGCTCCTCTCAG	

siRNA oligonucleotide sequences

PCNT (exon 3)

sense:	UCACAAUCAGUGACCACCAACCGGA
anti-sense:	UCCGGUUGGUGGUCACUGAUUGUGA