

Supplements

Cell migration leads to spatially distinct but clonally related airway cancer precursors

Christodoulos P Pipinikas¹, Theodoros S Kiropoulos^{1, 2}, Vitor H Teixeira¹, James M Brown¹, Aikaterini Varanou¹, Mary Falzon³, Arrigo Capitanio³, Steven E Bottoms¹, Bernadette Carroll⁴, Neal Navani^{1, 4}, Frank McCaughan^{5, 6}, Jeremy P George⁴, Adam Giangreco¹, Nicholas A Wright^{7, 8}, Stuart A C McDonald^{7, 8}, Trevor A Graham^{7, 9}, Sam M Janes^{1, *}

Table S1. Primer Details

Gene	Exon	^A Primer	First Round	Product size (bp)	Second Round	Product size (bp)
<i>TP53</i>	5	F	CACTTGTGCCCTGACTTTCA	307	TCTGTCTCCTTCCTCTTCCTACA	245
		R	GAGCAATCAGTGAGGAATCAGA		AACCAGCCCTGTCGTCTCT	
	6	F	AGAGACGACAGGGCTGGTT	237	CAGGCCTCTGATTCCTCACT	182
		R	TGGAGGGCCACTGACAAC		CTTAACCCCTCCTCCCAGAG	
	7	F	TGCTTGCCACAGGTCTCC	236	CTTGGGCCTGTGTTATCTCC	161
		R	GGTCAGAGGCAAGCAGAGG		GTGTGCAGGGTGGCAAGT	
	8	F	TTTTTAAATGGGACAGGTAGGA	258	GCCTCTTGCTTCTCTTTTCC	191
		R	CACCCTTGGTCTCCTCCAC		GCTTCTTGTCTGCTTGCTT	
<i>CDKN2A (p16)</i>	2A	F	GCTTCCTTTCCGTCATGC	300	CCTGGCTCTGACCATTCTGT	245
		R	CAGGTACCGTGCGACATC		CAGCTCCTCAGCCAGGTC	
	2B	F	CTGTTCTCTCTGGCAGGTCA	394	CTTCCTGGACACGCTGGT	234
		R	TGTGCTGGAAAATGAATGCT		TGGAAGCTCTCAGGGTACAAA	
<i>K-RAS</i>	-	F	GAGTTTGTATTAAGGTAAGTGGTGGG	272	TTTGATAGTGTATTAACCTTAT	210
		R	ATCAAAGAATGGTCCTGCAC		TATTAACAAGATTTACCTC	

^A Dried desalted primers were from Sigma-Aldrich (Dorset, UK)

Table S2. PCR Amplification Reactions

First Round PCR							
	<i>TP53-5</i>	<i>TP53-6</i>	<i>TP53-7</i>	<i>TP53-8</i>	<i>p16-2A</i>	<i>p16-2B</i>	<i>K-RAS</i>
	^A 1/5/55	2/5/60	1/5/60	2/5/60	2/5/60	2/5/60	2/5/60
Template	2.00	2.00	2.00	2.00	2.00	2.00	2.00
^B Buffer	2.50	2.50	2.50	2.50	2.50	2.50	2.50
^C MgCl₂	1.00	2.00	1.00	2.00	2.00	2.00	2.00
^D dNTP Mix	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Q-solution	5.00	5.00	5.00	5.00	5.00	5.00	5.00
^E Forward primer	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Reverse primer	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Taq	0.20	0.20	0.20	0.20	0.20	0.20	0.20
H₂O	13.30	12.30	13.30	12.30	12.30	12.30	12.30
Second Round PCR							
	1/5/60	1/0/60	1/5/60	2/0/60	2/5/60	2/5/60	2/5/55
Template	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Buffer	2.50	2.50	2.50	2.50	2.50	2.50	2.50
MgCl₂	1.00	1.00	1.00	2.00	2.00	2.00	2.00
dNTP Mix (10mM)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Q-solution	5.00	0.00	5.00	0.00	5.00	5.00	5.00
Forward primer	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Reverse primer	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Taq	0.20	0.20	0.20	0.20	0.20	0.20	0.20
H₂O	13.30	18.30	13.30	17.30	12.30	12.30	12.30

^A Numbers refer to volume of MgCl₂ and Q-solution in the reaction and PCR annealing temperature (°C), respectively

^B Promega 10x reaction buffer without MgCl₂ used at the supplied concentration (Southampton, UK)

^C MgCl₂, Q-solution and Taq DNA polymerase are all from Qiagen (Crawley, UK)

^D dNTP mix working concentration was 10mM (Invitrogen, Paisley, UK)

^E Primer working concentration was 20µM

Table S3. Microsatellite primer details for multiplex <i>LoH</i> analysis				
^A Marker	Location	Sequence (5'-3')	Expected product size (bp)	Reaction Conditions
D17S 1678 (F)	17p	[6FAM] TTTGGGTCTTTGAACCCTTG	120	^B Multiplex PCR Mix: 25µl 10x Primer Mix (2µM each primer): 5µl RNase Free-H ₂ O: 18µl Template DNA: 2µl Annealing Temperature: 57°C
D17S 1678 (R)		CCACAACAAAACACCAGTGC		
D17S 1506 (F)	17q	[HEX] TGTGGGATGGGGTGAGATTTTC	150	
D17S 1506 (R)		CTGTTGGTCGGTGGGTTG		
D17S 1881 (F)	17p	[6FAM] CCCAGTTTAAGGAGTTTGGC	210	
D17S 1881 (R)		TAGGGCAGTCAGCCTTGTG		

^A Primers were HPLC purified and were reconstituted in TE buffer at a final stock concentration of 100µM.

^B For each microsatellite marker, 10µl of the 100µM stock were mixed together and the volume was adjusted to 500µl using distilled H₂O. The final concentration of each primer in the working primer mix was 2µM. The final concentration of each primer used in the amplification reaction was 0.2µM.

