



Certificate of analysis

SFC840-03-03-PITX3-GFP

Operator:

Date:

Supervisor:

Date:

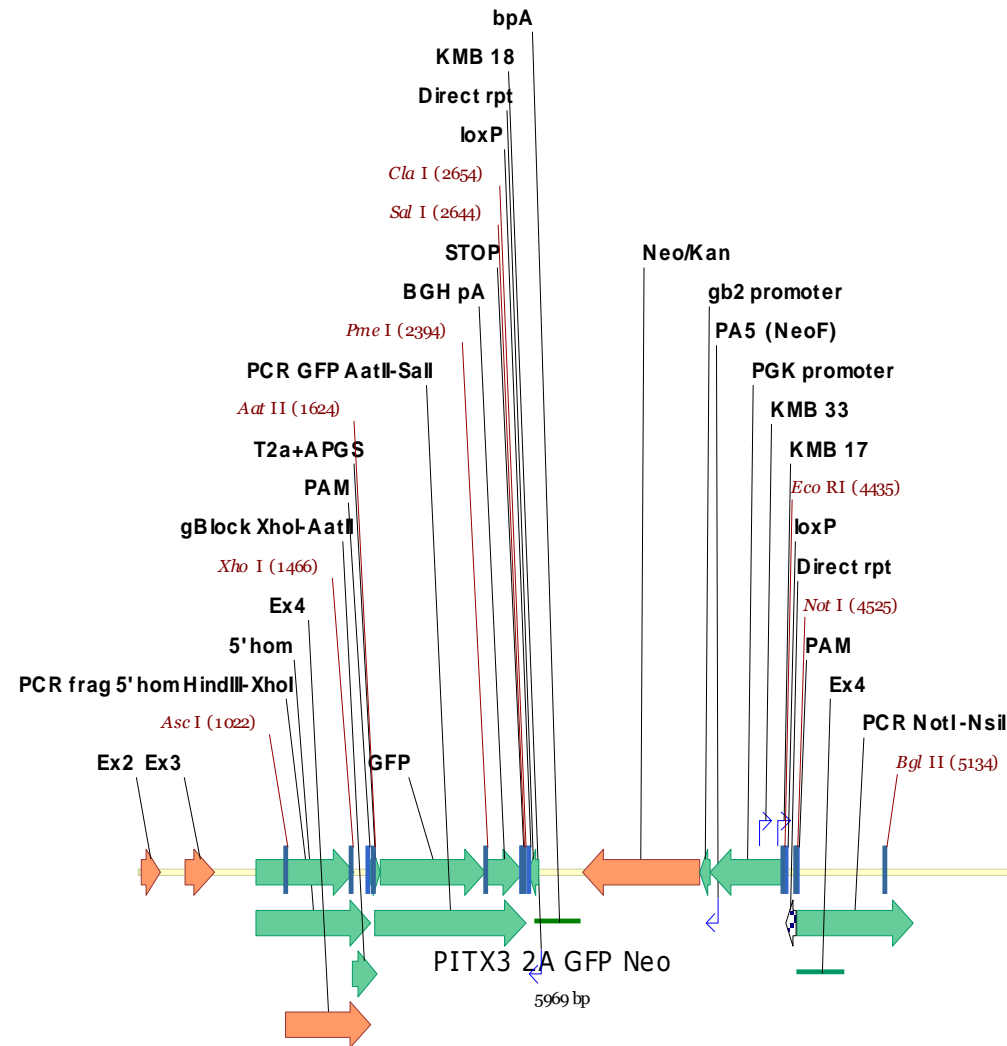
Signature:

HDR(CRISPR/Cas9)-mediated generation of PITX3-GFP reporter iPS line

Source of parental iPSc line and reprogramming information:

- SFC840-03-03 StemBANCC cell line was used in reprogramming.
- SFC840-03-03 QC passed.
- Reprogrammed using CRISPR/Cas9 (HDR) method.
- Reprogramming was done at Passage 12.
- First PITX3-GFP tagging was done and then Neo cassette was removed from the clones.

Locus overview after integration of reporter gene 5' of 3'UTR and stop: PITX3 reporter iPS line



Guide RNA sequences:

gRNA's

PITX3 Cr1 F1

ACCGcgtggaaaggcccgtatgag

PITX3 Cr1 R1

AAACctcatacggggcctttccacg

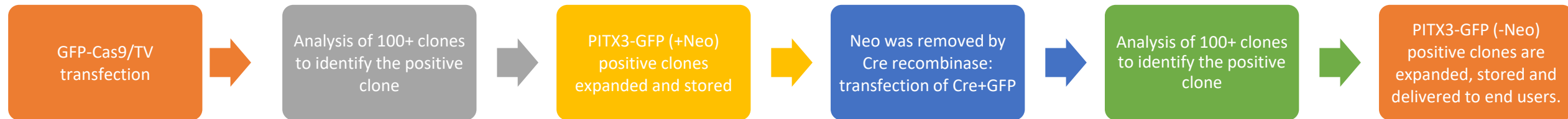
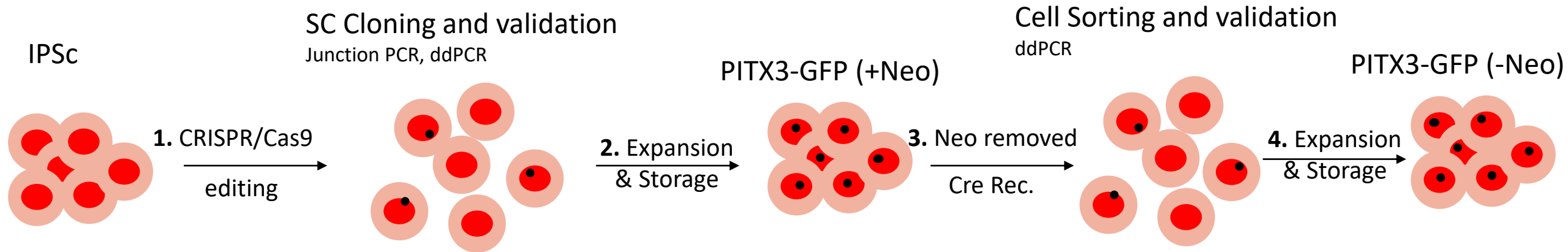
PITX3 Cr2 F2

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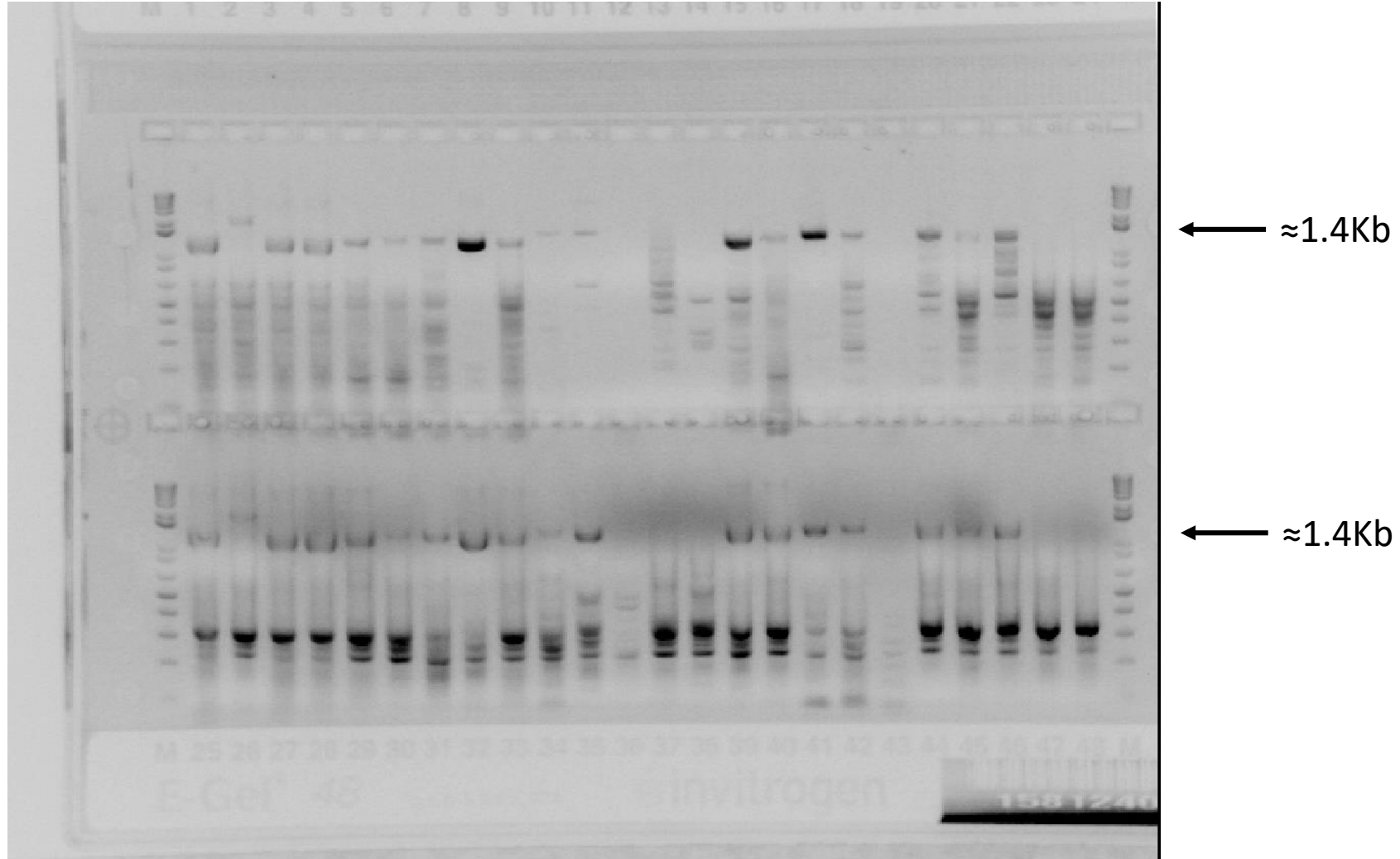
PITX3 Cr2 R2

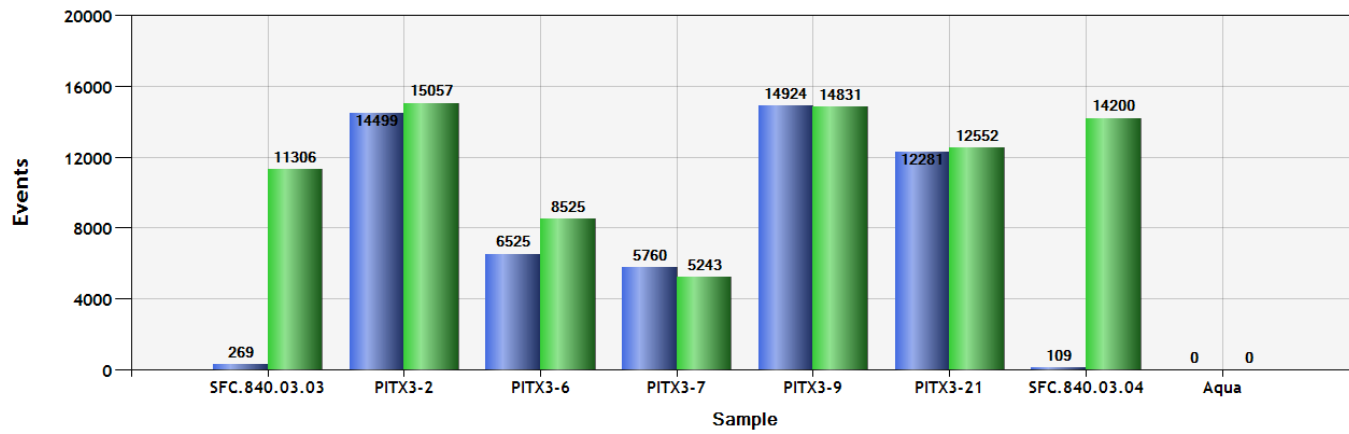
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Strategy & Experimental Plan:

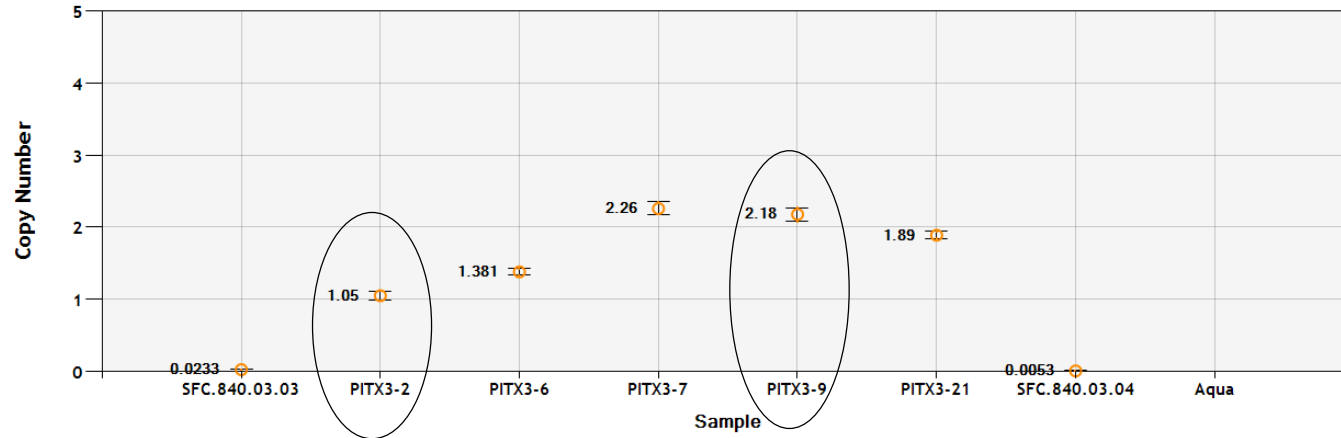
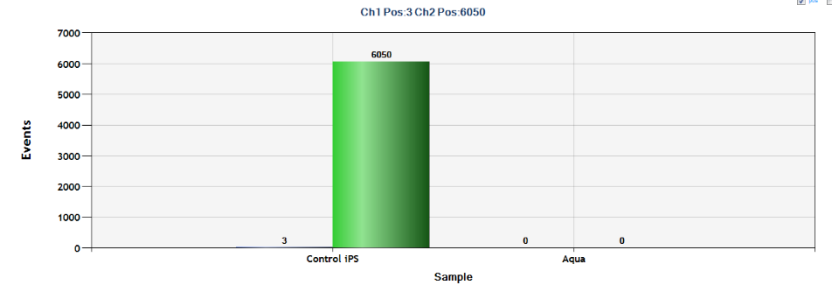
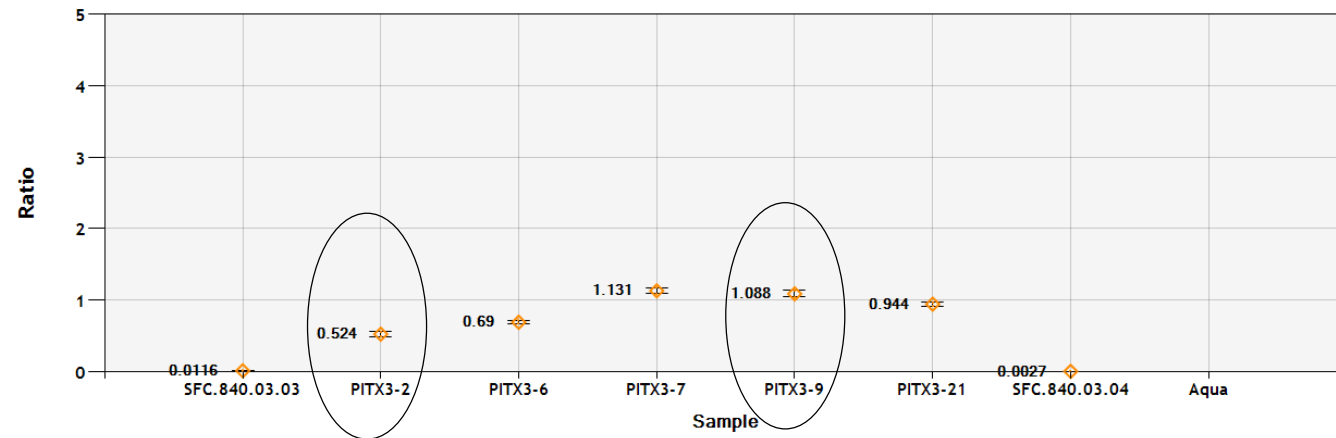


Junction PCR data for PITX3-GFP (+Neo) positive clones:





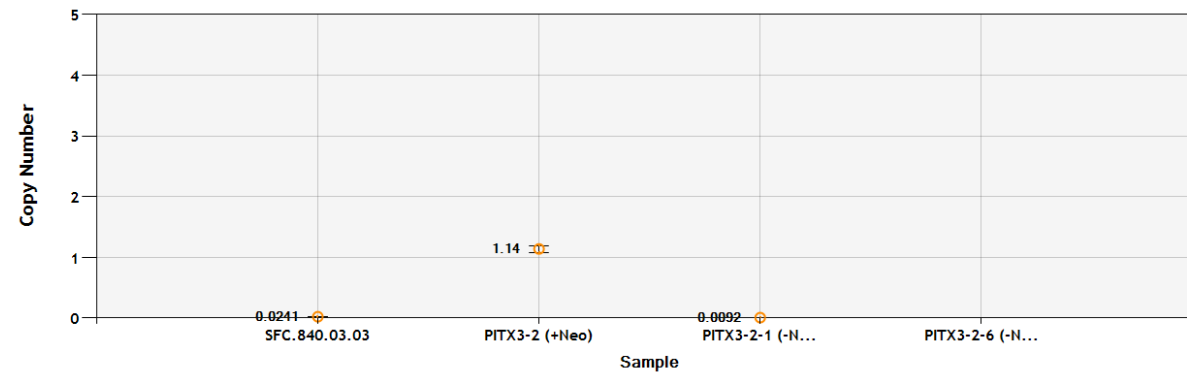
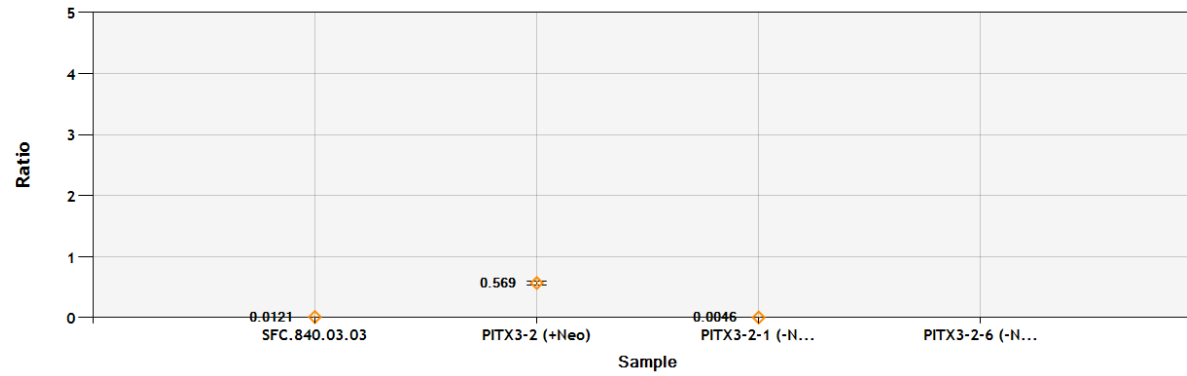
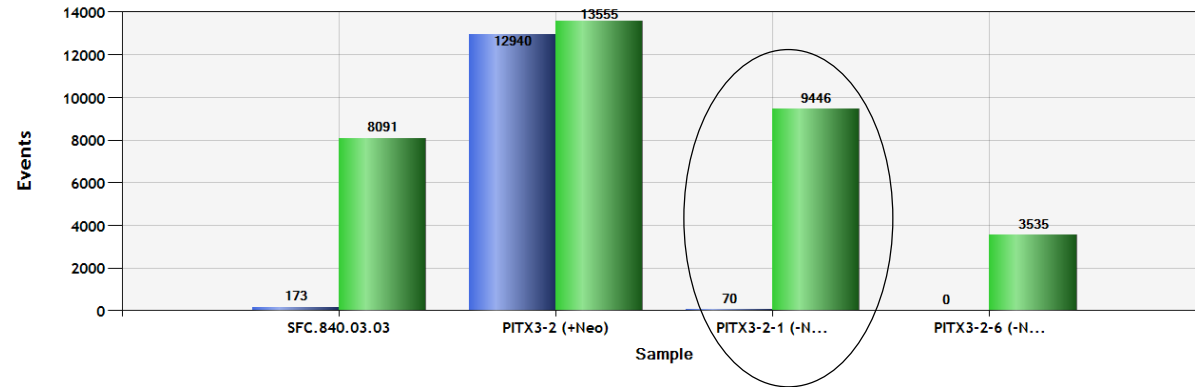
ddPCR data for Nkx6.1 (+Neo) clones:



Nkx6.1 (+Neo) clones proceeded for removing Neo cassette:

1. PITX3-GFP-2 (single copy)

ddPCR data for PITX3-GFP-2 (-Neo) clones:

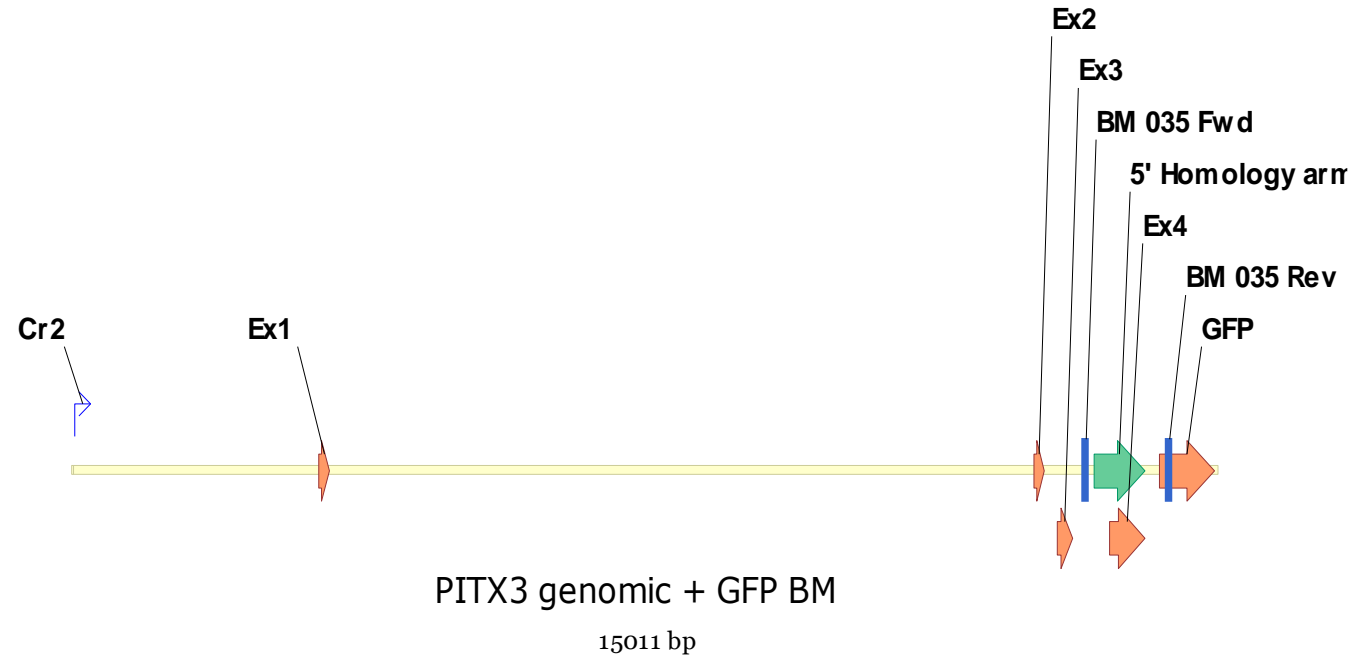


Sequencing data

PITX3

- **Full name:** Homo sapiens paired like homeodomain 3 (PITX3).
- Present in **Chromosome 10.**
- NCBI Reference Sequence: NG_008147.1.
- Here, PITX3 has a GFP tag and BM 035 Rev primer is designed with in GFP tag.

Primer design



VectorNTI link:



C:\Users\kmcj479\
top\PITX_AmpSeq

Primer	Sequence	Amplicon size (bp)
BM_035_FWD	TGCTCCCTACTACTCGGACC	1112
BM_035_REV	CCGGTGGTGTCAGATGAACTT	

BM 035 product seq

BM 035 PCR product (1112 bp)>>

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For editable Sequence please notice the word file



Microsoft Word
Document

Sequencing results: Paired Analysis

4/8/2017

QBio: Amplicon Sequencing Reports

PITX3_GFP

Sample	Frequency	Sequence	Variant depth	Read depth	Variant
Reference	1	GGCGCTCTTCTGGCCCTGACACGGTCTCTCTCCCTCACACCCCTCTCAACCCCA	80		
PITX_clone_9-18	0.012	GGCGCTCTTCTGGCCCTGACACGGTCTCTCTCCCTCACACCCCTCTCAACCCCA	2212	182109	Deletion@PITX3_GFP:890-692
PITX_clone_9-2	0.012	GGCGCTCTTCTGGCCCTGACACGGTCTCTCTCCCTCACACCCCTCTCAACCCCA	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	GGCGCTCTTCTGGCCCTGACACGGTCTCTCTCCCTCACACCCCTCTCAACCCCA	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	GGCGCTCTTCTGGCCCTGACACGGTCTCTCTCCCTCACACCCCTCTCAACCCCA	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	61	TCTACTACTGCCGAGACCTTGGCGCATAGCTCGTCCAGCGCCCGCCCTTCTGT	120		
PITX_clone_9-18	0.012	TCTACTACTGCCGAGACCTTGGCGCATAGCTCGTCCAGCGCCCGCCCTTCTGT	2212	182109	Deletion@PITX3_GFP:890-692
PITX_clone_9-2	0.012	TCTACTACTGCCGAGACCTTGGCGCATAGCTCGTCCAGCGCCCGCCCTTCTGT	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	TCTACTACTGCCGAGACCTTGGCGCATAGCTCGTCCAGCGCCCGCCCTTCTGT	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	TCTACTACTGCCGAGACCTTGGCGCATAGCTCGTCCAGCGCCCGCCCTTCTGT	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	121	TCTGACGCTCCGGACTCGTGGCGCTTCTCCCTCACACCCCTCTCAACCCCA	180		
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PITX_clone_9-2	0.012	TCTGACGCTCCGGACTCGTGGCGCTTCTCCCTCACACCCCTCTCAACCCCA	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	TCTGACGCTCCGGACTCGTGGCGCTTCTCCCTCACACCCCTCTCAACCCCA	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	TCTGACGCTCCGGACTCGTGGCGCTTCTCCCTCACACCCCTCTCAACCCCA	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	181	CGTTTATGACCGAGGCCCCGGCTGGAAGCCGCTGGGGTGGCCGCTCTCAACCG	240		
PITX_clone_9-18	0.012	CGTTTATGACCGAGGCCCCGGCTGGAAGCCGCTGGGGTGGCCGCTCTCAACCG	2212	182109	Deletion@PITX3_GFP:890-692
PITX_clone_9-2	0.012	CGTTTATGACCGAGGCCCCGGCTGGAAGCCGCTGGGGTGGCCGCTCTCAACCG	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	CGTTTATGACCGAGGCCCCGGCTGGAAGCCGCTGGGGTGGCCGCTCTCAACCG	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	CGTTTATGACCGAGGCCCCGGCTGGAAGCCGCTGGGGTGGCCGCTCTCAACCG	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	241	CTGGGACCCCGCCCGGCTGACCGCTTCTCTCCGTCGCGCAGGTGTGGTCAAGA	300		
PITX_clone_9-18	0.012	CTGGGACCCCGCCCGGCTGACCGCTTCTCTCCGTCGCGCAGGTGTGGTCAAGA	2212	182109	Deletion@PITX3_GFP:890-692
PITX_clone_9-2	0.012	CTGGGACCCCGCCCGGCTGACCGCTTCTCTCCGTCGCGCAGGTGTGGTCAAGA	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	CTGGGACCCCGCCCGGCTGACCGCTTCTCTCCGTCGCGCAGGTGTGGTCAAGA	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	CTGGGACCCCGCCCGGCTGACCGCTTCTCTCCGTCGCGCAGGTGTGGTCAAGA	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	301	ACCGGCGCCCAATGGCGAAGCGCGAGCGAGCCAGCAGCGCGAGCTATGCAAGGCA	360		
PITX_clone_9-18	0.012	ACCGGCGCCCAATGGCGAAGCGCGAGCGAGCCAGCAGCGCGAGCTATGCAAGGCA	2212	182109	Deletion@PITX3_GFP:890-692
PITX_clone_9-2	0.012	ACCGGCGCCCAATGGCGAAGCGCGAGCGAGCCAGCAGCGCGAGCTATGCAAGGCA	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	ACCGGCGCCCAATGGCGAAGCGCGAGCGAGCCAGCAGCGCGAGCTATGCAAGGCA	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	ACCGGCGCCCAATGGCGAAGCGCGAGCGAGCCAGCAGCGCGAGCTATGCAAGGCA	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	361	CTTTCGGGCGCTGGGGGGCTGGTGGCCGCTACGAGGAGGTGTACCCGGCTACT	420		
PITX_clone_9-18	0.012	CTTTCGGGCGCTGGGGGGCTGGTGGCCGCTACGAGGAGGTGTACCCGGCTACT	2212	182109	Deletion@PITX3_GFP:890-692
PITX_clone_9-2	0.012	CTTTCGGGCGCTGGGGGGCTGGTGGCCGCTACGAGGAGGTGTACCCGGCTACT	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	CTTTCGGGCGCTGGGGGGCTGGTGGCCGCTACGAGGAGGTGTACCCGGCTACT	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	CTTTCGGGCGCTGGGGGGCTGGTGGCCGCTACGAGGAGGTGTACCCGGCTACT	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	421	CGTACGCAACTGGCCGCTTGGCCGCTTGGCCGCTGCGCAGACCTTTCAT	480		
PITX_clone_9-18	0.012	CGTACGCAACTGGCCGCTTGGCCGCTTGGCCGCTGCGCAGACCTTTCAT	2212	182109	Deletion@PITX3_GFP:890-692
PITX_clone_9-2	0.012	CGTACGCAACTGGCCGCTTGGCCGCTTGGCCGCTGCGCAGACCTTTCAT	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	CGTACGCAACTGGCCGCTTGGCCGCTTGGCCGCTGCGCAGACCTTTCAT	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	CGTACGCAACTGGCCGCTTGGCCGCTTGGCCGCTGCGCAGACCTTTCAT	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	481	TCCCTTCAACTCGTCAACGTGGGCTTGGCTTGGCAGCCGCTTCTCGCACCA	540		
PITX_clone_9-18	0.012	TCCCTTCAACTCGTCAACGTGGGCTTGGCTTGGCAGCCGCTTCTCGCACCA	2212	182109	Deletion@PITX3_GFP:890-692
PITX_clone_9-2	0.012	TCCCTTCAACTCGTCAACGTGGGCTTGGCTTGGCAGCCGCTTCTCGCACCA	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	TCCCTTCAACTCGTCAACGTGGGCTTGGCTTGGCAGCCGCTTCTCGCACCA	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	TCCCTTCAACTCGTCAACGTGGGCTTGGCTTGGCAGCCGCTTCTCGCACCA	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	541	GCTCCATCGCCCTTCCATGTTGCTTCCGCGCGCTGCGCCGCGAGCTGCGAGCG	600		
PITX_clone_9-18	0.012	GCTCCATCGCCCTTCCATGTTGCTTCCGCGCGCTGCGCCGCGAGCTGCGAGCG	2212	182109	Deletion@PITX3_GFP:890-692
PITX_clone_9-2	0.012	GCTCCATCGCCCTTCCATGTTGCTTCCGCGCGCTGCGCCGCGAGCTGCGAGCG	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	GCTCCATCGCCCTTCCATGTTGCTTCCGCGCGCTGCGCCGCGAGCTGCGAGCG	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	GCTCCATCGCCCTTCCATGTTGCTTCCGCGCGCTGCGCCGCGAGCTGCGAGCG	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	601	CTGGGCGCTGAGGCTGGCGGGGCCCCGGGCTGGCTCCGGCGCCGCTGTCT	660		
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PITX_clone_9-2	0.012	CTGGGCGCTGAGGCTGGCGGGGCCCCGGGCTGGCTCCGGCGCCGCTGTCT	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	CTGGGCGCTGAGGCTGGCGGGGCCCCGGGCTGGCTCCGGCGCCGCTGTCT	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	CTGGGCGCTGAGGCTGGCGGGGCCCCGGGCTGGCTCCGGCGCCGCTGTCT	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	661	CCGGGCGCTGCTGCTTATGCTTCCGCGCGCGCGCGCGCGCTTCT	720		
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PITX_clone_9-2	0.012	CCGGGCGCTGCTGCTTATGCTTCCGCGCGCGCGCGCGCGCTTCT	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	CCGGGCGCTGCTGCTTATGCTTCCGCGCGCGCGCGCGCGCTTCT	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	CCGGGCGCTGCTGCTTATGCTTCCGCGCGCGCGCGCGCGCTTCT	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	721	CCCCATGCTATCGGACCGGTGAAGTCAAGCTGAGCTGCGCTCAAGCA	780		
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PITX_clone_9-2	0.012	CCCCATGCTATCGGACCGGTGAAGTCAAGCTGAGCTGCGCTCAAGCA	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	CCCCATGCTATCGGACCGGTGAAGTCAAGCTGAGCTGCGCTCAAGCA	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	CCCCATGCTATCGGACCGGTGAAGTCAAGCTGAGCTGCGCTCAAGCA	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	781	AACAGCAGCTCTTCAAGTACCCTGCTGACAGGCGCCGCGAGCAACTTA	840		
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PITX_clone_9-2	0.012	AACAGCAGCTCTTCAAGTACCCTGCTGACAGGCGCCGCGAGCAACTTA	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	AACAGCAGCTCTTCAAGTACCCTGCTGACAGGCGCCGCGAGCAACTTA	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	AACAGCAGCTCTTCAAGTACCCTGCTGACAGGCGCCGCGAGCAACTTA	1680	145212	SNP:T@PITX3_GFP:583-583

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Sample	Frequency	Sequence	Variant depth	Read depth	Variant
Reference	841	GTCCTGCAAGTACGCCGTGAAAGCCGTAAGAGGAGAGGAGTCTGAACATGCG	900		
PITX_clone_9-18	0.012	GTCCTGCAAGTACGCCGTGAAAGCCGTAAGAGGAGAGGAGTCTGAACATGCG	2212	182109	Deletion@PITX3_GFP:890-692
PITX_clone_9-2	0.012	GTCCTGCAAGTACGCCGTGAAAGCCGTAAGAGGAGAGGAGTCTGAACATGCG	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	GTCCTGCAAGTACGCCGTGAAAGCCGTAAGAGGAGAGGAGTCTGAACATGCG	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	GTCCTGCAAGTACGCCGTGAAAGCCGTAAGAGGAGAGGAGTCTGAACATGCG	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	901	GTCAGCTGAGGAAATCTGGCCAGCCGGGATCCATGCTGAGCAGGGCGAGGAGC	960		
PITX_clone_9-18	0.012	GTCAGCTGAGGAAATCTGGCCAGCCGGGATCCATGCTGAGCAGGGCGAGGAGC	2212	182109	Deletion@PITX3_GFP:890-692
PITX_clone_9-2	0.012	GTCAGCTGAGGAAATCTGGCCAGCCGGGATCCATGCTGAGCAGGGCGAGGAGC	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	GTCAGCTGAGGAAATCTGGCCAGCCGGGATCCATGCTGAGCAGGGCGAGGAGC	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	GTCAGCTGAGGAAATCTGGCCAGCCGGGATCCATGCTGAGCAGGGCGAGGAGC	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	961	TGTTCCAGCGGGTGGTCCATCTGTCAGCTGACGCGACGTAACGCCCAAGT	1020		
PITX_clone_9-18	0.012	TGTTCCAGCGGGTGGTCCATCTGTCAGCTGACGCGACGTAACGCCCAAGT	2212	182109	Deletion@PITX3_GFP:890-692
PITX_clone_9-2	0.012	TGTTCCAGCGGGTGGTCCATCTGTCAGCTGACGCGACGTAACGCCCAAGT	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	TGTTCCAGCGGGTGGTCCATCTGTCAGCTGACGCGACGTAACGCCCAAGT	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	TGTTCCAGCGGGTGGTCCATCTGTCAGCTGACGCGACGTAACGCCCAAGT	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	1021	TGAGCTGTCGCGGAGGCGAGGGGATGACCTACGCAAGCTGACCTGAAGTCA	1080		
PITX_clone_9-18	0.012	TGAGCTGTCGCGGAGGCGAGGGGATGACCTACGCAAGCTGACCTGAAGTCA	2212	182109	Deletion@PITX3_GFP:890-692
PITX_clone_9-2	0.012	TGAGCTGTCGCGGAGGCGAGGGGATGACCTACGCAAGCTGACCTGAAGTCA	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	TGAGCTGTCGCGGAGGCGAGGGGATGACCTACGCAAGCTGACCTGAAGTCA	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	TGAGCTGTCGCGGAGGCGAGGGGATGACCTACGCAAGCTGACCTGAAGTCA	1680	145212	SNP:T@PITX3_GFP:583-583
Reference	1081	TCTGACACCCGG	1093		
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PITX_clone_9-2	0.012	TCTGACACCCGG	1949	168579	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.014	TCTGACACCCGG	2420	170680	Deletion@PITX3_GFP:890-692
PITX_clone_9-8	0.012	TCTGACACCCGG	1680	145212	SNP:T@PITX3_GFP:583-583

Conclusion:
In all 3 clones the frequency of other variants are <1%, which indicate variabilities might result from errors introduced during sequencing.