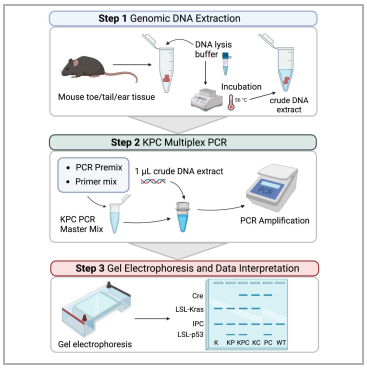

MAR 30, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.eq2ly7bxrlx9/v1

External link:
https://cihwang.com

Protocol Citation: Shou Kitahara, Shounak Ranabhor, Thae Su Thu, Neha Ramesh, Chang-il Hwang 2023. Multiplex Genotyping PCR for the KPC Pancreatic Cancer Mouse Model. **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2ly7bxrlx9/v1

Protocol status: Working
We use this protocol and it's working

Created: Mar 30, 2023

Last Modified: Mar 30, 2023

PROTOCOL integer ID:
79723

Multiplex Genotyping PCR for the KPC Pancreatic Cancer Mouse Model

Shou Kitahara[1], Shounak Ranabhor[1], Thae Su Thu[1], Neha Ramesh[1], Chang-il Hwang[1]

[1]University of California Davis

Shou Kitahara: SK and SR contributed equally to this protocol.
Chang-il Hwang: Corresponding author

Chang-il Hwang

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Keywords: pancreatic cancer, PDAC, KPC, genotyping, multiplex, Kras, Trp53, Pdx1-Cre

ABSTRACT

Genetically engineered mouse model (GEMM) is one of the most important pre-clinical models in cancer research. In pancreatic cancer research, *Kras*$^{+/LSL\text{-}G12D}$; *Trp53*$^{+/LSL\text{-}R172H}$; *Pdx-1-Cre* (KPC) mouse model has been widely used in the laboratory setting, since it faithfully recapitulates the progression of human pancreatic ductal adenocarcinoma. Polymerase Chain Reaction (PCR) is commonly used to genotype GEMMs. However, genotyping one gene at a time is inefficient and labor intensive. To simplify the genotyping process, we multiplexed three separate genotyping PCR protocols for a single PCR reaction. In addition, we provided the optimized PCR protocol for determining heterozygous or homozygous status of *Trp53*$^{LSL\text{-}R172H}$allele. Overall, this protocol offers cost-efficient and accurate genotyping results for KPC mice.

ATTACHMENTS

Appendix1_KPC Simple Protocol.pdf

Appendix2_p53 Simple Protocol.pdf

p53-het-homo.pdf

KPC-multiplex.pdf

GUIDELINES

Introduction

Genetically engineered mouse models (GEMMs) are critical preclinical models that allow researchers to investigate disease progression and therapeutic responses *in vivo*. In cancer specifically, GEMMs allow researchers to study the means of tumor progression in patients, including tumor initiation, metastasis, and mutational heterogeneity, by aptly recapitulating human cancer characteristics[1]. To use GEMMs in the laboratory setting, it is necessary to cross individual genetically modified alleles and maintain the desired genotypes.

Mutations in *KRAS* (Kirsten rat sarcoma viral oncogene homolog) and *TP53* are the two most frequently mutated genes in cancers[2].For pancreatic ductal adenocarcinoma (PDAC), *KRAS* mutations are present in about 95% of cases and *TP53* are co-present in about 70% of cases[3]. Therefore, mice with $Kras^{+/LSL\text{-}G12D}$; $Trp53^{+/LSL\text{-}R172H}$; *Pdx1-Cre* (KPC) are widely used because this model faithfully recapitulates human pancreatic cancer pathogenesis[4].

It is common practice to isolate genomic DNA from toes, tails, or ears and perform molecular methods like PCR (polymerase chain reaction), Southern blot or dot blot techniques[5]. In general, the genotyping methods should be rapid and reproducible, and allow for the analysis of large numbers of mice. However, it is still labor-intensive to genotype multiple alleles individually. Mis-genotyping of the alleles could lead to irrevocable consequences[6].

Here, we have provided a simple, rapid, and cost-effective multiplex genotyping protocol for KPC mice with crude DNA extracts from tail, toes, or ear biopsy. In addition, we refined the protocol to distinguish the homozygous (homo) alleles for $Trp53^{LSL\text{-}R172H}$ from heterozygous (het). This protocol requires a single PCR reaction for three alleles of K ($Kras^{LSL\text{-}G12D}$), P ($Trp53^{LSL\text{-}R172H}$) and C (*Cre*) as well as an internal positive control without any commercial genomic DNA isolation kit.

MATERIALS

1. Crude genomic DNA isolation

- Lysis buffer
  i. 10 mM Tris-HCL
  ii. 50 mM KCl
  iii. 2.5 mM $MgCl_2$

iv.0.45 % NP-40 Buffer
v.0.45 % Tween 20
vi.60 µg/mL Proteinase K (20 mg/mL Proteinase K, NEB, #P8107)

●ThermoMixer (Eppendorf, Cat #5382000023) or water bath

2. Polymerase Chain Reaction for KPC Genotyping

●Platinum hot start PCR 2X mix (Thermo Fisher Scientific, Cat # 13000012),
●Template DNA (retrieved from toe/tail DNA isolation)
●7 primer sequences as detailed in Table 1 and 2
●UltraPure™ DNase/RNase-Free Distilled Water(Thermo Fisher Scientific (Invitrogen), Cat # 10977015)

3. Polymerase Chain Reaction for *Trp53 Het/Homo* Genotyping

●MiniAmp Thermal Cycler (Thermo Fisher Scientific)
●Template DNA (retrieved from toe/tail DNA isolation)
●10 mM Deoxynucleotide (dNTP) Solution Mix (NEB, #N0447S)
●10 mM primer (4 primers)
●NEB Taq polymerase (NEB, #M0273) and Standard buffer
●UltraPure™ DNase/RNase-Free Distilled Water(Thermo Fisher Scientific (Invitrogen), Cat # 10977015)

4. Gel Electrophoresis

●100 bp ladder (NEB, N3231)
●Ethidium bromide (10 mg/mL, Sigma, E1510)
●Agarose
●1X TAE Buffer
i.242g Tris free base (Thermo Fisher Scientific, Cat # BP152-5)
ii.18.61g Disodium EDTA (Thermo Fisher Scientific, Cat # BP120-1)
iii.57.1 ml Glacial Acetic Acid (Thermo Fisher Scientific, Cat #A38-212)
iv.Double-distilled H_2O to 1L
v.Dilute 50X to desired volume of 1X with Double-distilled H_2O
●Gel casting mold and combs
●Electrophoresis apparatus
●6X Loading Dye (NEB, #B7025)
●Power Supply

Table 1. KPC Primers

Primer Type	Sequence 5′ → 3′
Internal Positive Control Forward	CTG TCC CTG TAT GCC TCT GGT CGT A
Internal Positive Control Reverse	AGA TGG AGA AAG GAC TAG GCT ACA ACT TAC
Cre Forward	GGA TCG CCA GGC GTT TTC TG
Cre Reverse	CCA GCC ACC AGC TTG CAT GA
LSL-Forward	AGC TAG CCA CCA TGG CTT GAG TAA GTC TGC A
Kras Reverse	CCT TTA CAA GCG CAC GCA GAC TGT AGA
Trp53 Reverse	CTT GGA GAC ATA GCC ACA CTG

Table 2. *Trp53 Het/Homo* Primers

Primer Type	Sequence 5′ → 3′
Internal Positive Control Forward	CAA ATG TTG CTT GTC TGG TG
Internal Positive Control Reverse	GTC AGT CGA GTG CAC AGT TT
Trp53 1loxp Forward	AGC CTG CCT AGC TTC CTC AGG
Trp53 1loxp Reverse	CTT GGA GAC ATA GCC ACA CTG

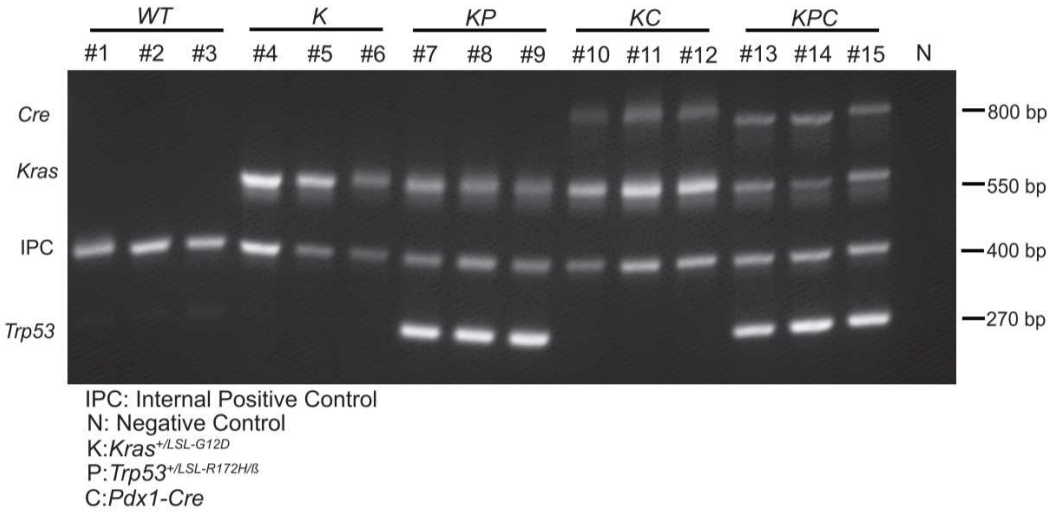

Figure 1. Representative gel image of KPCgenotyping results. PCR was used to amplify *Kras*$^{LSL\text{-}G12D}$ *~ 550 bp. Trp53*$^{LSL\text{-}R172H}$ ~270 bp, *Pdx1-Cre*~800 bp, Internal positive control ~ 415 bp, and negative control - no band.

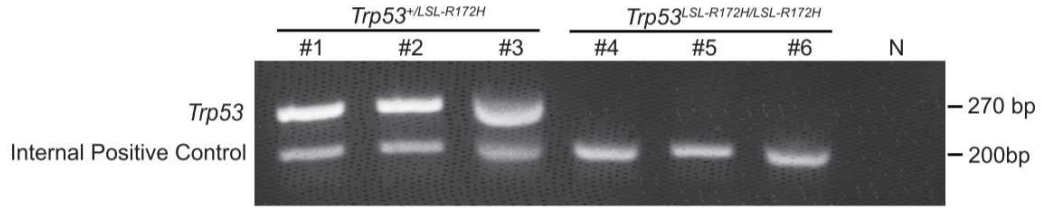

Figure 2. Representative gel image of genotyping of heterozygous *vs.* homozygous *Trp53*$^{LSL\text{-}R172H}$alleleprotocol. PCR was used to amplify Heterozygous *Trp53*$^{+/LSL\text{-}R172H}$~270 bp, Homozygous *Trp53*$^{LSL\text{-}R172H/LSL\text{-}R172H}$~ no band, Internal positive control ~ 200 bp and negative control - no band.

Crude genomic DNA isolation

1 Prepare Lysis Buffer (Recipe for 10 mL)
- 0.0121 g Tris-HCl pH 8.0
- 0.0373 g KCl
- 12.5 µl 2M $MgCl_2$
- 45 µL NP-40
- 45 µL Tween-20
- 9.9 mL Ultrapure Distilled Water (DW)

2 Add 3 µl Proteinase K (20 mg/mL) to 1 mL of Lysis buffer prior to use (Store in -20°C)

3 Add 30 µl Lysis buffer with Proteinase K to cut tissue in 1.5 mL tube

4 Incubate at 56°C for 1.5 hours

5 Place on ice briefly and spin down briefly in a tabletop centrifuge

6 Incubate at 96°C for 10 min in ThermoMixer

Polymerase Chain Reaction for KPC

7 Prepare PCR master mix for KPC in 1.5mL centrifuge tubes
1. 10 µL Platinum hot start PCR Mix
2. 0.4 µL 10 mM primer each (7 primers)
3. 6.2 µL DW

8 Aliquot 19 µL of master mix into each PCR tube

9 Add 1 µL DNA template to individual PCR tubes

10
1. Perform PCR using the following conditions:
   a. 94°C 3 min
   b. 94°C 1 min/ 65°C 2 min/ 72°C 1 min (40 cycles)
   c. 72°C 3 min / 4°C ∞

Polymerase Chain Reaction for Trp53 Het/Homo

11
1. Prepare PCR master mix for Trp53 het/homo in 1.5mL centrifuge tubes
2. 2 µL 10X NEB polymerase buffer
3. 0.4 µL 10mM dNTP
4. 0.4 µL 10mM primer (4 primers)
5. 0.1 µL Taq polymerase
6. 14.9 µL DW

12 Aliquot 19 µL of master mix into each PCR tube

13 Add 1 µL DNA template to individual PCR tubes

14 1. Perform PCR using the following conditions:
a.94°C 3 min
b.94°C 30sec/ 60°C 30sec/ 72°C 30sec (40 cycles)
c.72°C 3 min / 4°C ∞

Gel Electrophoresis

15 Make 2% agarose gel in TAE buffer

16 Add 2 µL ethidium bromide (10 mg/ml stock concentration) to 100 ml melted agarose. Swirl to mix.

17 Pour into the casting mold with combs inserted to set up wells.

18 Remove combs and casting mold from agarose gel after the cast harden (~ 30 min)

19 Place the gel into electrophoresis apparatus

20 Add 1X TAE buffer to electrophoresis apparatus until the gel is submerged

21 Add 4 µL 6X loading dye to each 20 µL PCR product and load 10 µL of it into the wells

22 Load 3 µL of 100 bp DNA ladder mixed with loading dye to 1st well of each lane

23 Run gel at 120V until loading dye is visualized 75-80 % down the gel

24 Remove the gel from the electrophoresis apparatus and visualize it under UV light

25 Analyze images to determine the genotype for each mouse