Isolation of deletion alleles of C. elegans by G4-DNA induced mutagenesis

CURRENT STATUS: POSTED

Daphne Pontier
Department of Toxicogenetics, Leiden University Medical Center, The Netherlands

Evelien Kruisselbrink
Department of Toxicogenetics, Leiden University Medical Center, The Netherlands

Victor Guryev
Hubrecht Institute - Koninklijke Nederlandse Academie van Wetenschappen, University Medical Center Utrecht, Utrecht, The Netherlands

Marcel Tijsterman
Department of Toxicogenetics, Leiden University Medical Center, The Netherlands

DOI:
10.1038/nprot.2010.60

SUBJECT AREAS
Genetics *Biological techniques*

KEYWORDS
G4 DNA, mutagenesis, deletion allele

Introduction

Metazoan genomes contain thousands of sequence tracts that match the guanine-quadruplex (G4) DNA signature G3NxG3NxG3NxG3, a motif that is intrinsically mutagenic, probably because it can form secondary structures during DNA replication. We and others have previously shown that in C. elegans, the gene dog-1 is required to prevent deletion formation at these G4 sites (Chueng et al, Kruisselbrink et al). Here we provide a protocol to show how this feature can be used to isolate deletion alleles of many Caenorhabditis elegans genes.

Site selection

Consult Wormbase or the complete list of G4 DNA sites (available as Supplemental Table with Pontier et al, 2009) whether your gene of interest is close (preferably within 2 kb) to a G4 DNA site.

Primer design

When designing primers, take into account that G4 DNA-induced deletions always occur in one particular orientation: the deleted DNA is always upstream of the G4 sequence. Thus, primers are chosen such that one primer is close to the 3' side of the G4 tract, whereas the other primer is $_{1\ kb}$ upstream (Figure 1). Nested PCRs increase sensitivity, and we had the best results with product sizes of 1200 bp for the (wild-type) outer product, and ~ 1000 bp for the inner product. Primers can be chosen further apart, but this may hamper detection of smaller deletions.

Reagents

dog-1 mutant strain (allele gk10 or pk2247)

NGM culture plates

Equipment

96-well and/or 384 well PCR thermal cycler

Procedure

1. Clone out 96 single dog-1 animals onto 96 6 cm petridishes, and grow these plates till starvation.
2. **Optional**: Rinse off half the plate and freeze the animals. Keep these as a backup for this or for future isolations of (other) G4 deletion alleles.
3. Rinse off half of the worms in M9 buffer and transfer these to 96 well PCR plates in

duplicate. Remove supernatant.

4. Add 50 µL lysis buffer (50 mM KCl; 2.5% mM MgCl2,; 10 mM Tris-HCl (pH=8); 0.45% Nonidet P40; 0.45% Tween-20; 0.01% gelatin and 200 µg ml-1 proteinase K) to the worm pellet.
5. Lyse worm pellets at 60oC for 60 minutes, and inactivate proteinase K for 30 minutes at 95oC. Some non-dissolved pellet may remain.
6. Perform nested PCRs on 1 µL lysis mix in a total volume of 10 µL. As a control, also perform the PCR on lysed wild type (N2) worms and on a blank (no DNA) sample. PCR conditions are as follows: o PCR reaction: 1x PCR buffer (50 mM KCl, 10 mM Tris-HCl pH8.3 and 1.5 mM MgCl2); 0.5 mM dNTPs, 0.5 µM of each primer and 0.025 U ml-1 Taq polymerase

o PCR Program: 1 minute 95oC; 35 cycles of 20 seconds 95oC, 40 seconds 58oC, 1 minute 72oC; 3 minutes 72oC.

o Nested PCR: Transfer 0.2 µL mix from the first PCR (primers 1 and 4) into the second PCR (primers 2 and 3) as a template (e.g. by using a 384-well hedge).

7. Load 5 µL of the second PCR on a 1% agarose gel, where duplicates run next to each other. Search for duplicates that have a band of identical size. This is called a “positive population” and contains a candidate germline mutation.
8. Chunk the plate corresponding to the positive population into 12 pieces onto fresh 6 cm plates. Grow till starvation and repeat the procedure from step 3-7. As a positive control, include the lysis DNA from the previous, positive round.
9. If one or more of these plates result in a deletion product of identical size to the original positive sample, then transfer ~10 worms of this plate to 48 new 6 cm plates. Grow till starvation and repeat step 3-7. If several samples are positive for the germline deletion, proceed to step 10. If no samples are positive, return to the

original (frozen) plate and start the procedure again. Increasing the number of populations should also increase deletion allele isolation efficiency.

10. Return to the plate out of 48 that contains the positive population, and clone out 48 single worms from this plate on fresh plates. Grow till starvation and repeat step 4-8. Alternatively, grow a few days, then pick the mother into 10 µL lysis buffer and perform PCRs on this sample.
11. A positive population from step 10, initiated from a single animal, may still be heterozygous for the deletion allele. To verify this, perform the nested PCR on ~15 individual animals from the positive population. Only if all individuals are positive for the deletion, the population is likely homozygous (this can be confirmed by designing primers that are within the deleted segment and verify that this DNA is lost from these animals). If not, clone out >6 animals from the positive population and perform the nested PCR on 12 F1 progeny animals for each of these.
12. The remaining 5 µL from the PCR reaction can be used to sequence the deletion.
13. Cross the new strain several times back to N2 to remove dog-1(gk10) and possible secondary mutations.

Timing

3-4 weeks

References

Chueng I, Schertzer M, Rose and Lansdorp PM, Disruption of dog-1 in Caenorhabditis elegans triggers deletions upstream of guanine-rich DNA, Nat Genet 2002 Aug;31(4):405-9

Kruisselbrink E., Guryev V, Brouwer K, Pontier DB, Cuppen E and Tijsterman M, Mutagenic capacity of endogenous G4 DNA underlies genome instability in FANCJ-defective C. elegans.Curr Biol 2008 Jun 24;18(12):900-5

Pontier DB, Kruisselbrink K, Guryev V and Tijsterman M, Isolation of deletion alleles by G4 DNA-induced mutagenesis, Nat Methods. 2009 Sep;6(9):655-7

Figures

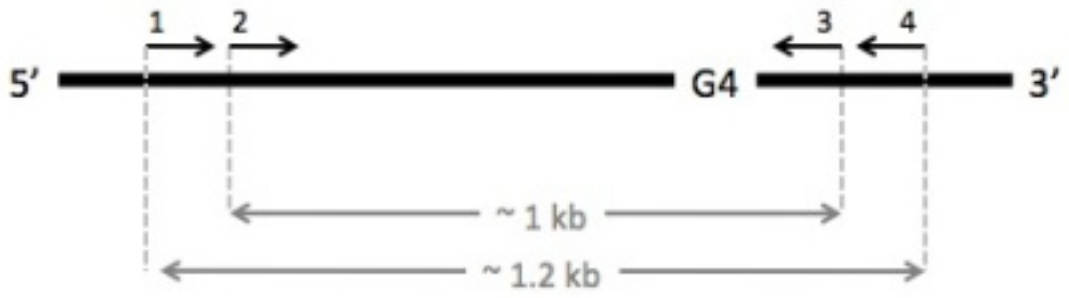

Figure 1

Scheme for primer design for nested PCRs to detect G4-DNA induced deletions

Isolation of deletion alleles by G4 DNA-induced mutagenesis

by Daphne B Pontier, Evelien Kruisselbrink, Victor Guryev, +1

Nature Methods (22 May, 2012)