# Chromosome Conformation Capture (3C) (alternate technique) (PROT31)

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## Introduction

*This is an alternative protocol for 3C that has been adopted by the author's lab. Much of it is identical to the previous version (PROT5). The major differences are in the amounts of DNA used at different steps. We reliably get the same results as previously, but also a greater yield of 3C material. The original yields are ample for real-time PCR analysis, but greater yields are required if the 3C material is going to be processed further.*

The 3C (Chromosome Conformation Capture) technique generates a population average measurement of juxtaposition frequency between any two genomic loci, thus providing information on their relative proximity in the nucleus (Dekker *et al*., 2002). Cells are fixed with formaldehyde which forms DNA-protein and protein-protein cross-links between regions of the genome in proximity (see figure 1). Subsequent restriction enzyme digestion and intra-molecular ligation produces novel junctions between restriction fragments in proximity in the nucleus. Novel ligation products can be detected by PCR. We adapted the 3C assay (Dekker *et al*., 2002) to determine the conformation of mouse chromosome 7 and in particular the co-localization of actively transcribed genes in transcription factories (Osborne *et al*., 2004). The 3C assay can also be used to reveal proximity between active genes and distal genomic elements (Tolhuis *et al*., 2002).

An important consideration in the interpretation of 3C data is the understanding that not all pairs of restriction fragments that provide a positive result (i.e. generate a novel PCR product) are necessarily engaged in a functional interaction in the nucleus. For example, compare results of Tolhuis *et al*., 2002, with Carter *et al*., 2002 in which the higher order structure of the mouse hbb locus was assayed by two different methods, 3C and RNA TRAP. Clearly, distal fragments can be cross-linked by formaldehyde simply because they are near each other in the nucleus, and presumably can "bump into" each other during the fixation process (Osborne *et al*., 2004). Therefore fixation conditions are critical in the 3C assay since increased fixation leads to greater cross-linking resulting in the detection of chromatin fragments that may be in proximity in the nucleus but not necessarily engaged in a specific intermolecular interaction with implied function.

## Procedure

### Fixation and digestion

1. Dissect anaemic mouse spleen and kidney and strain through 70µm strainer into a chilled petrie dish in cold D-MEM + 10% FBS. Transfer to 50ml Falcon and make up to 50ml with cold medium;
2. Centrifuge at 1300 rpm for 8 minutes at 4°C. Remove supernatant and resuspend cells in trace of liquid;
3. Make up to 40ml with room temperature medium + 2% formaldehyde (2.16ml 37% formaldehyde + 37.84ml medium), and fix for 5 minutes at room temperature on rocker;
4. Quench with 5.7ml cold 1M glycine, and centrifuge at 1300 rpm for 8 minutes at 4°C;
5. Wash with 50ml cold PBS and centrifuge at 1300 rpm for 8 minutes at 4°C;
6. Make up to 50ml in cold lysis buffer and incubate on ice for 30 minutes with occasional mixing;
7. Centrifuge at 1800 rpm for 5 minutes at 4°C and resuspend nuclei in trace of liquid;
8. Dilute two to three-fold with 1.2x NEB3, and take $1x10^7$ nuclei aliquots. Pulse centrifuge to visually check nuclei amount, and raise each $1x10^7$ nuclei aliquot in 500µl 1.2x NEB3;
9. Add 7.5µl 20% SDS and incubate for 1 hour at 37°C, 950 rpm;

10. Add 50µl 20% Triton-X100 and incubate for 1 hour at 37°C, 950 rpm;
11. Add 30µl/1500U Bgl II and incubate overnight at 37°C, 950 rpm. Optional: Add 10µl/500U Bgl II in the morning, and incubate for a further 1-2 hours at 37°C, 950 rpm.

## Ligation

12. Add 40µl 20% SDS and incubate for 25 minutes at 65°C, 950 rpm;
13. Transfer to 15ml Falcon with 7ml 1.1x ligation buffer;
14. Add 375µl 20% Triton-X100 and incubate for 1 hour at 37°C, mixing occasionally;
15. Add 2µl/800U T4 DNA ligase and incubate for 4 hours in a 16°C water bath, then for 30 minutes at room temperature. Optional: An aliquot can be used as a no ligase control (all subsequent treatments are the same);
16. Add 90µl/900µg proteinase K and incubate overnight at 65°C.

## DNA purification

17. Cool to room temperature and add 3µl/300µg RNase A. Incubate at 37°C for 1 hour;
18. Transfer to 50ml Falcon, add 10ml phenol and vortex. Centrifuge at 3500 rpm for 15 minutes at room temperature;
19. Take upper phase and extract as previously with 7ml chloroform;
20. Take upper phase and add 700µl 2M NaOAc, pH 5.2, and 17.5ml ethanol. Precipitate for 1-3 hours at -20°C;
21. Centrifuge at 3500 rpm for 30 minutes at 4°C and remove supernatant;
22. Vortex pellet with 20ml 70% ethanol and centrifuge at 3500 rpm for 30 minutes at 4°C;
23. Air dry pellet for 5 minutes at room temperature and 3 minutes at 37°C (with lid loosely on), then add 100µl water and incubate for 2 hours at 65°C;
24. Rigorously pipette and fully resuspend DNA overnight at 37°C.

## 3C validation

25. Quantitate DNA by PicoGreen assay.

Check for 3C products by PCR (two rounds of 35 cycles with nested primers).

## Materials & Reagents

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| **lysis buffer** | 10mM Tris-HCl, pH 8 (500µl 1M)<br>10mM NaCl (100 µl 5M)<br>0.2% NP-40 (100 µl)<br>1 tablet complete protease inhibitors |
| **ligation buffer** | 33 mM Tris-HCl, pH 8 (1.65ml 1M per 50ml)<br>11mM $MgCl_2$ (550µl 1M per 50ml)<br>11mM DTT (550 µl 1M per 50ml)<br>1.1 mM ATP (1.1ml 50mM per 50ml) |

## Figures

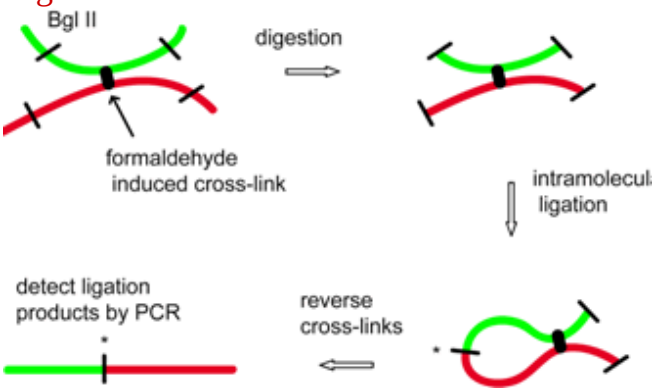


**Figure 1.**
An overview of the 3C technique. Fixation with formaldehyde is followed by BglII digestion and intra-molecular ligation. Cross-links are reversed and novel ligation products are detected by PCR (adapted from Figure 1A. Dekker *et al.*, 2002).

## References


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