DPC-Seq: Investigating the genome-wide distribution of DNA-Protein Crosslinks in time

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Method Article

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Abstract

DNA-protein crosslinks (DPCs) arise from enzymatic intermediates, metabolism or exogenous chemicals like chemotherapeutics. DPCs are highly cytotoxic as they interfere with DNA-based processes such as replication and transcription. To investigate the genome-wide distribution of DPCs, we have adapted previously published K-SDS precipitation protocols for analysis by quantitative PCR or next generation sequencing to determine the distribution of DPCs throughout the genome. After the induction of DPCs, the DPCs are isolated and subsequently the DPC-associated DNA is purified. This DNA can be analysed by real-time quantitative PCR or next generation sequencing. By performing this assay at different timepoint after damage induction, genomic regions that are preferentially repaired can be identified. This protocol can be used in wild-type and compared with cells deficient for repair proteins to study their involvement during DPC repair.

Introduction

Reagents

Equipment

Procedure

This procedure has been adapted from Zhitkovich and Costa, 1992 and Stingele et al, 2016[1, 2].

In our experiments, we have used adherent human SV40 transformed MRC-5 cells, in which DPCs were induced with formaldehyde (FA). This protocol is expected to also be compatible with other cell types and with other types of DPC-inducing agents, but this has not been tested.

Treatment and harvesting of cells

1. Grow cells to ~70% confluence in an appropriate culture dish in an appropriate culture medium. If the DPC-associated DNA is analysed by PCR, a 6- well plate (Greiner, Cat No. 657160) is sufficient, while for DPC-Seq requires a 10 cm dish. Cells can also be pretreated with inhibitors.

2. Treat cells with freshly made 1 mM FA (Pierce, 28906) for 30 min.

3. Wash the cells with culture medium containing 10% FBS 3 times to quench and remove the FA.

4. For the “0 hr” sample, cells were immediately harvested, while other samples are harvested at later time points to assess repair in time.

5. Wash the cells twice in PBS.

6. Lyse the cells in 900 µl lysis buffer (2% SDS, 10 mM Tris-HCl (pH 7.5)).

7. Collect the cell lysate in an 2 ml Eppendorf tube (Cat. No. 0030120094) and store at -20°C after snap-freezing in liquid nitrogen until further processing.

Isolation of DPCs

1. Thaw the samples in a water bath or heat block at 55°C.

2. Shear the DNA by passing the lysates through a 23-gauge needle 5 times.

3. Sonicate at **room temperature** with high amplitude and 30 cycles of 30 sec ON and 30 sec OFF (Bioruptor Plus, Diagenode, Cat. No. B01020001).

4. Incubate the samples at 55°C for 10 min.

5. Add an equal volume (900 µl) of precipitation buffer (400 mM KCl, 20 mM Tris-HCl, pH 7.5) and incubate at 4°C for 6 min to complete precipitation.

6. Spin at 20,000 x g for 5 min at 4°C.

7. Collect the supernatant and transfer to a new tube. (This is the **free DNA fraction**).

8. Wash the pellets at 55°C for 10 min with 1 ml of wash buffer (200 mM KCl, 20 mM Tris-HCl, pH 7.5).

9. Incubate for 6 min on ice.

10. Spin at 20,000 x g for 5 min at 4°C.

11. Repeat steps 7-10 twice.

12. Combine all supernatants (collected in step 7) for free DNA measurement.

13. Resuspend the DPC associated pellets in 400 µl resuspension buffer (0.2 mg/ml proteinase K and 0.2 mg/ml RNAse A in 100 mM KCl, 20 mM Tris-HCl, and 10 mM EDTA) by vortexing.

14. Incubate the DPC associated pellets at 50°C for 3 hr.

15. Cool the samples on ice for 6 min.

16. Spin at 20,000 x g for 10 min at 4°C to remove debris.

17. Collect the supernatant, which contains the DPC associated DNA.

18. Purify DNA, from both the free DNA fraction and DPC associated pellets, using a gel extraction kit (Qiagen, Cat. No. 20051).

Depending on the research question, DPC levels were quantified by RT-qPCR or the relative distribution was determined by next generation sequencing.

Troubleshooting

Time Taken

Anticipated Results

References

1. Zhitkovich A, Costa M. A simple, sensitive assay to detect DNA-protein crosslinks in intact cells and in vivo. Carcinogenesis. 1992;13(8):1485-1489. doi:10.1093/carcin/13.8.1485

2. Stingele J, Bellelli R, Alte F, et al. Mechanism and Regulation of DNA-Protein Crosslink Repair by the DNA-Dependent Metalloprotease SPRTN. Mol Cell. 2016;64(4):688-703. doi:10.1016/j.molcel.2016.09.031

Figures

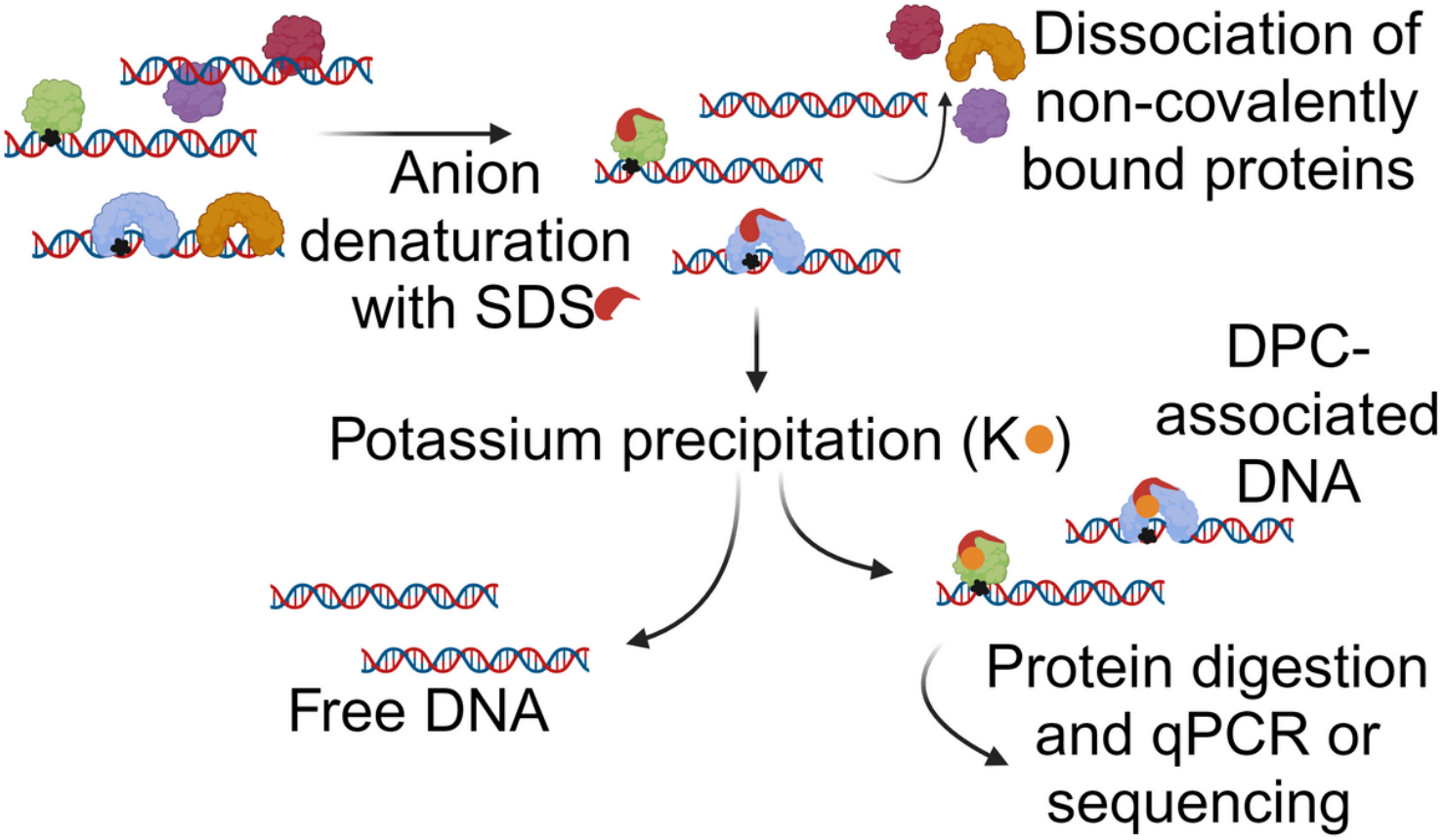

Figure 1

Overview of DPC isolation