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***In vitro* determination of temperature-dependent DNA-binding of the Evening Complex using Electrophoretic Mobility Shift Assays**

Stephanie Hutin^a, Philip A. Wigge^{b,c} and Chloe Zubieta^{a*}

^[a] Laboratoire Physiologie Cellulaire et Végétale, Univ. Grenoble Alpes, CNRS, CEA, INRAE, IRIG-DBSCI-LPCV, 17 Avenue des Martyrs, 38054, Grenoble, France.

^[b,c] Leibniz-Institut für Gemüse- und Zierpflanzenbau, Großbeeren, Germany and Institute of Biochemistry and Biology, University of Potsdam, Potsdam, Germany.

*Address correspondence to Chloe Zubieta (chloe.zubieta@cea.fr)

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Abstract

Electrophoretic mobility shift assays (EMSAs) of DNA-binding proteins and labelled DNA allow the qualitative and quantitative characterization of protein-DNA complex formation using native (nondenaturing) polyacrylamide or agarose gel electrophoresis. By varying the incubation temperature of the protein-DNA binding reaction and maintaining this temperature during electrophoresis, temperature-dependent protein-DNA interactions can be investigated. Here, we provide examples of the binding of a transcriptional repressor complex called the Evening Complex, comprising the DNA-binding protein LUX ARRITHMO (LUX), the scaffold protein EARLY FLOWERING 3 (ELF3) and the adapter protein ELF4, to its cognate DNA and demonstrate direct detection and visualization of thermoresponsive binding *in vitro*.

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As negative controls we use the LUX DNA-binding domain and LUX full length protein, which do not exhibit temperature dependent DNA-binding.

Keywords electrophoretic mobility shift assay, protein-DNA binding, temperature dependence, native polyacrylamide gel electrophoresis, native agarose gel electrophoresis, Evening Complex, LUX ARRHYTHMO, EARLY FLOWERING 3, EARLY FLOWERING 4

1. Introduction

Proper gene regulation relies on the specific binding of different transcription factors (TF) and transcriptional complexes to their cognate DNA. In multicellular organisms, 6-8% of protein coding genes encode TFs. Understanding the interplay between TFs, DNA binding and gene regulation as a function of environmental variables, including temperature, is a fundamental challenge in biology (1). *In vivo* techniques such as chromatin immunoprecipitation followed by polymerase chain reaction (ChIP-PCR) or deep sequencing ChIP-seq have been commonly used to identify TF binding sites (TFBS) *in vivo* (2, 3). *In situ* techniques including Cleavage Under Targets and Release Using Nuclease (CUT&RUN) (4–6) and Cleavage Under Targets and Tagmentation (CUT&Tag) (7) have further refined the identification of *in vivo* DNA binding sites by investigating TF binding in the native chromatin environment and can be performed under different growth conditions including different temperatures. However, these techniques have distinct challenges including the need for specific antibodies or the generation of transgenic lines expressing a tagged version of the target TF, dependence on the chromatin environment and accessibility of putative binding sites, competition with other DNA-binding proteins, the presence or lack of cofactors, poor signal to noise ratios due to low endogenous TF expression levels or artefacts due to TF overexpression if a 35S transgene is used, for example. In order to simplify the complexity of *in vivo* systems

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and determine the molecular mechanisms of protein-DNA interactions, *in vitro* techniques are often used. *In vitro* genome-wide techniques include DNA-affinity purification and sequencing (DAP-seq) in which purified immobilized TF or TF complexes and fragmented genomic DNA (gDNA) are mixed and bound DNA fragments are sequenced and mapped to the genome (8–13). Generation of a binding site model using systematic evolution of ligands by exponential enrichment and sequencing (SELEX-seq) is a similar approach in which a synthetic randomized DNA library is used in the pull-down step (14). Often these *in vivo* and *in vitro* techniques are used to identify important putative target genes and specific DNA binding sites for a given TF. In order to explore the specificity of DNA-binding and perform systematic variation of binding conditions, purified TFs and a single DNA binding site contained in a short DNA oligomer are commonly used.

Examination of binding to a single region by a given TF or TF complex and quantification of the interaction is often carried out to validate direct binding and identify the exact nucleotide sequence responsible for high affinity site-specific interactions. Various methods are available to examine, quantitatively and qualitatively, these protein-DNA interactions including isothermal titration calorimetry (15), thermal shift assays (16), microscale thermophoresis (17), size exclusion chromatography (18), fluorescence anisotropy (19) and, most commonly, electrophoretic mobility shift assays (EMSAs) (20). EMSAs, or band shift assays, provide a simple, rapid and sensitive method to determine protein-DNA interactions both qualitatively and quantitatively. These experiments require nanomolar concentrations of labelled DNA and low micromolar concentrations of protein and rely on equipment present in most biochemistry laboratories. The TF or TF complex is generally recombinantly overexpressed, purified and incubated with a short fluorescently labelled dsDNA fragment containing a known binding sequence or motif. The TF-DNA complex is then run on a polyacrylamide or agarose gel under non-denaturing conditions, allowing the

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separation of unbound and bound DNA, with the TF-DNA complex exhibiting a slower migration than the unbound DNA. The fluorescently tagged DNA allows for visualization of the different DNA-containing species (unbound DNA, DNA-TF complex(es)). As different factors, including additive concentration, ionic strength, pH and temperature will affect protein-DNA interactions, these variables can be systematically changed to examine their effect on protein-DNA interactions. In this protocol, we will focus on the effects of temperature on TF-DNA binding.

Temperature is a critical variable affecting plant growth and development. Changes in temperature alter the transcriptome by tuning the activity of thermosensitive transcription factors and complexes. One example of a transcriptional complex that exhibits thermosensitive binding *in vivo* and *in vitro* is the Evening Complex, a component of the circadian clock and a regulator of thermosensitive growth and flowering (**21, 22**). The EC is comprised of three proteins- LUX ARRHYTHMO (LUX), a Myb domain DNA-binding protein, EARLY FLOWERING 3 (ELF3), a plant-specific scaffold protein and EARLY FLOWERING 4 (ELF4), a small alpha-helical adaptor protein (**23**). At lower temperatures the EC binds its cognate DNA and represses its target genes, while at higher temperatures DNA binding is reduced, resulting in derepression of EC target genes such as *PHYTOCHROME INTERACTING FACTOR 4 (PIF4)*, a hub of stem/hypocotyl elongation and seedling thermomorphogenic growth (**24, 25**). In order to determine whether the EC acts as a direct thermosensor and exhibits changes in DNA-binding affinity as a function of temperature (**26**), *in vitro* studies of its temperature-responsive DNA binding are required. Here, we provide a detailed protocol using EMSAs to characterize the temperature-dependent DNA binding of the EC over a physiologically relevant temperature range in which the EC exhibits differential DNA binding. This protocol is generally applicable to any TF or TF complex of interest that may exhibit temperature-dependent DNA-binding.

2. Materials

Freshly prepare all solutions using ultrapure water and analytical grade reagents. This protocol uses fluorescently labelled DNA for visualization of DNA-containing complexes. Radiolabeled DNA probes may be used in place of fluorescently labelled DNA with attention to all applicable safety and waste disposal regulations. The protocol specifies purified TFs and TF complexes and urea denaturation and refolding steps for EC formation; however, this may not be necessary depending on the TF or TF complex of interest. Binding buffers for TF-DNA complexes will vary depending on the TF of interest and the buffer detailed in the protocol should be considered as a “starting point”. Factors including salt concentration, pH, additives (detergents, glycerol, BSA, competitor DNA) must be systematically varied and empirically tested in order to obtain an optimized TF-DNA binding buffer.

2.1. Protein production and purification

1. Bacterial Strains for protein expression: Rosetta™ 2(DE3)pLysS Competent Cells or similar (*see Note 1*).
2. Expression media and antibiotics: LB-medium (Roth), for 1 L of LB, weigh out 20 g of LB powder into a 2 L Erlenmeyer flask, add 1L ultrapure water and sterilize by autoclaving. Prepare 10 ml of antibiotic stock solutions 50 mg/ml kanamycin stock solution in ultrapure water. Prepare 60 µg/ml chloramphenicol in ultrapure water. Prepare 10 ml of a 1 M solution of IPTG in ultrapure water and filter sterilize.
3. Protein purification buffers for ELF3-mCherry purification under denaturing conditions: Resuspension buffer: 200 mM N-cyclohexyl-3-aminopropanesulfonic acid (CAPS), pH to 9.7, 500 mM NaCl, 1 mM Tris(2-carboxyethyl)phosphine (TCEP) and 1x cComplete™, EDTA-free Protease Inhibitors or similar. Denaturing resuspension buffer : 8

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M Urea, 20 mM Imidazole, 1 mM TCEP. Denaturing elution buffer: 8 M Urea, 250 mM Imidazole, 1 mM TCEP.

4. Protein purification buffers for LUX and ELF4 under nondenaturing conditions: Lysis buffer: 100 mM Bis-Tris-propane (BTP) pH 9.4, 500 mM NaCl, 20 mM imidazole, 1 mM TCEP and 1x cComplete™, EDTA-free Protease Inhibitors or similar. Wash buffer: 100 mM (BTP) pH 9.4, 500 mM NaCl, 20 mM imidazole, 1 mM TCEP. High salt wash buffer: 100 mM BTP pH 9.4, 1 M NaCl and 1 mM TCEP. Elution buffer: 100 mM BTP pH 9.4, 500 mM NaCl, 300 mM imidazole and 1 mM TCEP. Dialysis and SEC-buffer: 100 mM BTP pH 8.0, 500 mM NaCl and 1 mM TCEP.

5. Size exclusion chromatography buffer: 50 mM BTP pH 8, 500 mM NaCl, and 1 mM TCEP.

6. Ni-NTA FastFlow resin (Qiagen) or similar.

7. 30 ml disposable column (BioRad) or similar.

8. Dialysis buffers for complex reconstitution: Buffer 1: 6 M Urea, 1 mM TCEP. Buffer 2: 4 M Urea, 1 mM TCEP. Buffer 3: 2 M Urea, 50 mM sodium phosphate pH 7.3, 150 mM NaCl and 1 mM TCEP. Buffer 4: 1 M Urea, 50 mM sodium phosphate pH 7.3, 150 mM NaCl and 1 mM TCEP.

2.2. Protein-DNA binding reaction and EMSAs

1. 10x binding buffer: 100 mM Tris, pH 7.5, 10 mM MgCl₂, 10 mM TCEP, 60% glycerol, 280 ng/μl herring sperm DNA, 200 μg/ml BSA, 25% CHAPS and 12.5 mM spermidine.

2. 10x TBE: 121 g Tris base, 61.8 g boric acid, 7.4 g EDTA, add water to a final volume of 1 L. This gives a final concentration of 1.0 M borate, 1.0 M Tris base and 20 mM EDTA.

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3. 5 % polyacrylamide non-denaturing gels: mix 5 mL of a 30% polyacrylamide (37.5:1) with 3 ml 10x TBE, 300 μ L ammonium persulfate (10% stock solution) and 24 μ L TEMED and 22 mL H₂O (sufficient for 4, mini protein gels 8.3 cm x 7.3 cm x 1 mm), in a 50 ml Falcon tube. Invert the tube 5x to mix and immediately pour the gel solution between the glass plates of the casting system to the top of the smaller plate and place a 10-well comb between the plates. Allow the gels to polymerise for at least 1 h. before use. Gels may be stored at 4 °C by covering with a 1x TBE soaked paper towel and wrapped in plastic wrap to avoid evaporation and drying of the gel (*see Note 2*).
4. 2 % nondenaturing agarose gels: Weigh out 2 g of molecular biology grade agarose and add 100 ml of 1x TBE. Microwave to dissolve the agarose and immediately cast the gel. Let the gel cool for at least 1 hour before use.

2.3 Additional reagents and equipment required:

1. Tobacco etch virus protease (TEV), 1 mg/ml stock.
2. Nuclease-free water (1 mL).
3. Three gels of 12 %, 15% and 18% polyacrylamide SDS or similar.
4. Instant Blue Coomassie Protein Stain or similar.
5. Precision Plus Protein dual color standard (BioRad) or similar.
6. Synthetic DNA oligomers labelled with an appropriate fluorophore (*i.e.*, 5'- Cy5-ATGATGTCTTCTCAAGATTCGATAAAAATGGTGTTG-3') and (CAACACACCATTTTTATCGAATCTTGAGAAGACATCAT-3'). The LUX/EC binding site is shown in bold (*see Note 3*).
7. Recombinant plasmid DNA with the genes of interest (*i.e.*, pESPRIT vectors encoding 6x His-ELF3-mCherry, 6x His-LUX and 6x His-ELF4).
8. Beckman Coulter Avanti J-26 XP centrifuge with the rotor JLA 8.1000 or similar.

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9. Beckman Coulter Avanti J-26 XP centrifuge with the rotor JA-25.25 or similar.
10. Sonicator (Branson Sonifer 250) or similar.
11. Spin concentrators (Amicon Ultra 4, Ultracel -10 k or similar).
12. Dialysis tubing MWCO 3500 Dalton (Spectra/Por™) or similar.
13. Superdex 200 Increase 10/300 GL or similar (Cytiva) or similar.
14. AktaPure or Biorad FPLC protein purification system or similar.
15. Polyacrylamide gel rig.
16. Agarose gel rig.
17. NanoDrop microvolume spectrophotometer (ThermoFisher) or similar.
18. ChemiDoc (ThermoFisher) or similar.

3 Methods

All protein-DNA binding reactions should be carried out using buffers equilibrated to the target temperature of interest unless otherwise specified. Pipette tips, Eppendorf tubes and liquid media should be sterilized by autoclaving and all buffers and stock solutions filtered prior to use.

3.1 Protein expression

1. Transform 50 μ l of BL21(DE3) Rosetta cells with the genes of interest (*ELF3*, *LUX* and *ELF4*) in the pESPRIT expression vector according to the manufacturer's instructions (*i.e.*, gently thaw an aliquot of cells in a 1 ml Eppendorf tube on wet ice and add 1 μ l of purified plasmid DNA at 20-50 ng/ μ l, tap the side of the tube to mix and keep on ice for 15 min. Perform a heat shock at 42 °C for 45 s in a heating block or PCR machine followed by 2 min on ice and a 60 min incubation at 37 °C in 500 μ l of LB medium). Plate the transformed cells onto an agar

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plate supplemented with 50 µg/ml kanamycin and 60 µg/ml chloramphenicol and incubate overnight at 37 °C (*see Note 2*).

2. In a 500 ml Erlenmeyer flask, prepare 100 ml of sterile LB media supplemented with 50 µg/ml kanamycin and 60 µg/ml chloramphenicol. Select a single colony from the agar plates of transformed cells (step 1) by gently touching with a sterile wand and swishing through the LB media. Perform this step for each individual construct to be expressed.
3. Grow the bacteria overnight at 37 °C in a shaker at 150-180 rpm.
4. Prepare 3x 1 L of LB media per target construct (1 L of LB in a 2 L or larger Erlenmeyer flask) and supplement with a final concentration of 50 µg/ml kanamycin and 60 µg/ml chloramphenicol. Inoculate each flask with 20 ml of the overnight culture from step 3.
5. Grow the bacteria at 37 °C, 150 rpm until on optical density $A_{600} = 0.6-0.8$ is reached. Reduce the temperature to 18 °C and induce protein expression by the addition of 1 mM IPTG (final concentration). Grow the culture overnight.
6. Transfer the liquid culture to a 1 L centrifuge container and pellet the cells at 4500 x g for 20 min.

3.2 Native protein purification (LUX and ELF4)

7. Resuspend the bacterial pellets in 50-100 mL lysis buffer.
8. Sonicate the sample in an ice bucket with an ice water mixture (1s pulse, 1 s pause 5 min total processing time, 70% power).
9. Spin the pellets at 45000 x g for 30 min.
10. Prepare the Ni-NTA column by adding 2 mL of Ni-NTA slurry (75% w/w Ni resin) for a final column volume of 1-1.5 mL to a 30 mL gravity column. Wash the resin with 50 column volumes (CV) of ultrapure water and equilibrate in 50 CV of wash buffer.
11. Load the supernatant onto the nickel affinity column pre-equilibrated with lysis buffer.

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12. Wash with 50 CV wash buffer.
13. Wash with 50 CV high salt wash buffer.
14. Elute the protein in 500 μ L steps in elution buffer and collect each fraction in a 2 ml tube.
15. Run a denaturing SDS gel in Laemmli buffer and stain with Instant Blue or Coomassie stain or similar to visualise the eluted protein fractions.
16. Combine the fractions of interest and determine the concentration of protein using a NanoDrop spectrophotometer or Bradford assay.
17. Dialyse the fractions of interest against dialysis buffer.
18. Optional: Add 1:10 w/w TEV and incubate overnight to cleave the 6x His tag.
19. Optional: Perform a TEV-cleaved and uncleaved protein depletion by running the protein/TEV solution over a preequilibrated Ni-NTA column, collect the flow through, add 3 ml of dialysis buffer and collect in the same tube. This will be 6x His-tag cleaved protein depleted in TEV protease.
20. Concentrate the protein to 5-10 mg/ml using a spin concentrator.
21. Equilibrate a Superdex 200 in Dialysis SEC-buffer.
22. Inject the protein onto the column and collect 1 ml fractions using an automatic fraction collector.
23. Run a denaturing 15 % SDS gel of the peak fractions in Laemmli buffer and stain with Instant Blue or Coomassie or similar to assess the purity of the sample.
24. Select the fractions of interest and concentrate the proteins to 5-10 mg/ml. The proteins can be stored at 4 °C overnight or at -80 °C for long term storage.

3.3 ELF3-mCherry purification from inclusion bodies

25. Resuspend the bacterial pellets in nondenaturing resuspension buffer.

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26. Sonicate the sample in an ice bucket with an ice water mixture (1s pulse, 1 s pause, 5 min total processing time, 70 % power).
27. Spin the pellets at 45000 x g for 30 min.
28. Decant the supernatant.
29. Resuspend the pellet in 30 mL lysis buffer.
30. Spin the pellets at 45000 x g for 30 min.
31. Decant the supernatant und resuspend the pellet in 20 ml of denaturing resuspension buffer.
32. Spin the pellets at 45000 x g for 30 min.
33. Load the supernatant onto a 1 ml nickel affinity column pre-equilibrated in denaturing resuspension buffer.
34. Wash with 50 CV denaturing resuspension buffer.
35. Elute the protein in 500 µL steps in denaturing elution buffer and collect each fraction in a 1.5 ml tube.
36. Run a denaturing 12 % SDS gel in Laemmli buffer and stain with Instant Blue or Coomassie or similar to assess the purity of the sample.
37. Combine the ELF3-mCherry containing fractions, (they should be pink) (*see Note 4*). Determine the concentration using a NanoDrop spectrophotometer or Bradford assay.

3.4 EC reconstitution

38. Mix ELF3-mCherry : LUX : ELF4 in a ratio of 1:1:1.2 to a final volume of at least 100 µl (*see Note 5 and Note 6*).
39. Dialyse the proteins against 250 ml complex reconstitution buffer 1 for at least 1 h at 4 °C, with slow stirring.

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40. Dialyse the proteins against 250 ml complex reconstitution buffer 2 for at least 1 h at 4 °C, with slow stirring.
41. Dialyse the proteins against 250 ml complex reconstitution buffer 3 for at least 1 h at 4 °C, with slow stirring.
42. Dialyse the proteins against 250 ml complex reconstitution buffer 4 for at least 1 h at 4 °C, with slow stirring.
43. Pierce the dialysis membrane with a micropipette tip and pipette the protein solution into a fresh 1.5 ml reaction tube.

3.5 Annealing of single stranded DNA oligos

44. Resuspend Cy5-labeled single-stranded DNA oligos to a concentration of 200 µM in nuclease-free water.
45. Mix equimolar amounts of the forward and reverse oligomers in a reaction tube, seal the tube with parafilm, cover the sample with aluminium foil to reduce damage to the fluorophore by ambient
46. Boil 500 ml water in a 1 L glass beaker in the microwave, and place it onto a heating plate. Place the DNA sample into a floating microtube rack and heat it in the beaker for 5 min.
47. Switch of the heating plate and allow the 500 ml of water to cool to room temperature on the benchtop. The double-stranded DNA (dsDNA) stock solution can be stored at -20 °C.
48. Dilute the dsDNA to 10 µM. This will be used for preparing the EMSA reaction.

3.6 Protein-DNA complex binding reaction

49. Prepare 200 µl of 2x EMSA binding reaction buffer by combining 40 µl of 10x binding buffer with 0.8 µl of dsDNA probe and 159.2 µl of nuclease-free water.

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50. Mix 10 μl of the 2x EMSA binding reaction buffer mix with varying amounts of protein or protein complex (*i.e.*, 0.5 μM , 1 μM , 2 μM) to a final volume of 20 μl (enough for running 2 nondenaturing gels in parallel).
51. Incubate 1 h at the desired temperatures (*i.e.*, 4 $^{\circ}\text{C}$ and 20 $^{\circ}\text{C}$).

3.7 Electrophoretic mobility shift assay using nondenaturing polyacrylamide gels

52. Rinse the wells of the 5% nondenaturing polyacrylamide gel with 1x TBE
53. Pre-run the gel in 1x TBE at 30 V for at least 30 min (up to 1.5 h) at the desired temperature with buffer at the same experimental temperature (*i.e.*, 4 $^{\circ}\text{C}$ and 20 $^{\circ}\text{C}$).
54. Load 9 μl of the reaction mix into each gel (*i.e.*, if running 2 gels in parallel at 2 different temperatures, divide the reaction mix equally between the gels for comparison) (*see Note 7*).
55. Run the gel at 100 V for 5 min. to ensure the samples enter the gel (Fig. 1).
56. Run the gel at 75 V for 75 min. at 4 $^{\circ}\text{C}$ or 55 min at 20 $^{\circ}\text{C}$. Adjust times to result in similar migration distances (*see Note 8*).

3.8 Electrophoretic mobility shift assays using nondenaturing agarose gels

57. Load 9 μl of the reaction mix into each well of the nondenaturing agarose gel.
58. Run the gel at 50 V for 60 min. at 4 $^{\circ}\text{C}$ or 30 min at 20 $^{\circ}\text{C}$. Adjust times to result in similar migration distances (Fig. 2).

3.9 Visualisation and quantification of DNA-binding

59. Place the gel directly on the tray of the ChemiDoc or similar fluorescence imager and image the gel at the appropriate excitation wavelength (*i.e.*, 650 nm for Cy5) (*see Note 9*).

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60. Optional: Built in ChemiDoc software such as Image Lab software may be used for quantification. Alternatively, ImageJ may be used for band quantification and K_d calculation provided the protein concentrations were determined for each reaction (*see Note 10*).

Notes

1. Choice of cell line for bacterial expression is important depending on the presence of rare codons. We have found that using cell lines with additional tRNAs such as Rosetta(DE3) (Novagen), BL-21 Codon Plus (Agilent) can be helpful in the expression of plant proteins. Alternatively, synthetic genes encoding the TF of interest with codons optimized for bacterial expression may be used.
2. When preparing non-denaturing gels, it is important to use equipment that has not been in contact with SDS, since small amounts of SDS carry-over from denaturing SDS-PAGE can interfere with the EMSA.
3. HPLC-purified DNA oligomers may be ordered with a covalently attached fluorophore directly from the manufacturer. These are generally of high quality and may be used directly without further purification. Alternatively, DNAs may be ordered unlabeled and derivatization with a fluorophore of interest. Generally, this requires a separate HPLC purification step prior to use to remove excess free dye.
4. mCherry is not fully denatured in 8 M urea and the mCherry tagged protein will generally be pink during purification. Longer term storage at 4 °C in 8 M urea will result in gradual reduction of the pink color due to unfolding of the mCherry. This is also observable for other fluorescent protein tags such as GFP.
5. The concentration of EC will be limited by the concentration of ELF3-mCherry. We have found that ELF3-mCherry does not concentrate well using spin concentrators due to

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adherence to the membrane. Use the most concentrated fraction from the Ni-NTA column elution and calculate LUX and ELF4 ratios accordingly.

6. Low volume dialysis cassettes may be used to dialysis 100 μ l samples. Alternatively, an Eppendorf tube may be cut to remove as much of the conical tube as possible leaving the top of the rim intact, the sample applied to the lid well, covered with dialysis tubing and the tube cap closed and snapped into place, sealing the dialysis membrane.

7. The glycerol in the reaction buffer will allow the protein-DNA complex to settle to the bottom of the gel well. Loading gels is difficult as no colored stain is added. Pipette slowly to minimize sample diffusion from the well.

8. Samples will migrate faster with increasing temperature. If similar migration distances are desired for the same samples run at different temperatures, running times will need to be adjusted with longer times required for low temperature experiments. Generally, for 20 °C differences in running temperature, times will need to be increased 1.5x for the lower temperature experiment at the same running voltage.

9. If the migration of the protein-DNA complex is not sufficient, the gel may be returned to the gel rig and a voltage applied for a longer running time.

10. Due to the denaturation and refolding steps for EC formation and difficulties in quantifying complex and free LUX concentrations, EC-DNA experiments are performed using a master mix of all components divided into separate reactions and incubated at the desired temperatures with all gels run at the same time and in parallel to allow for binding comparisons at different temperatures.

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Acknowledgements

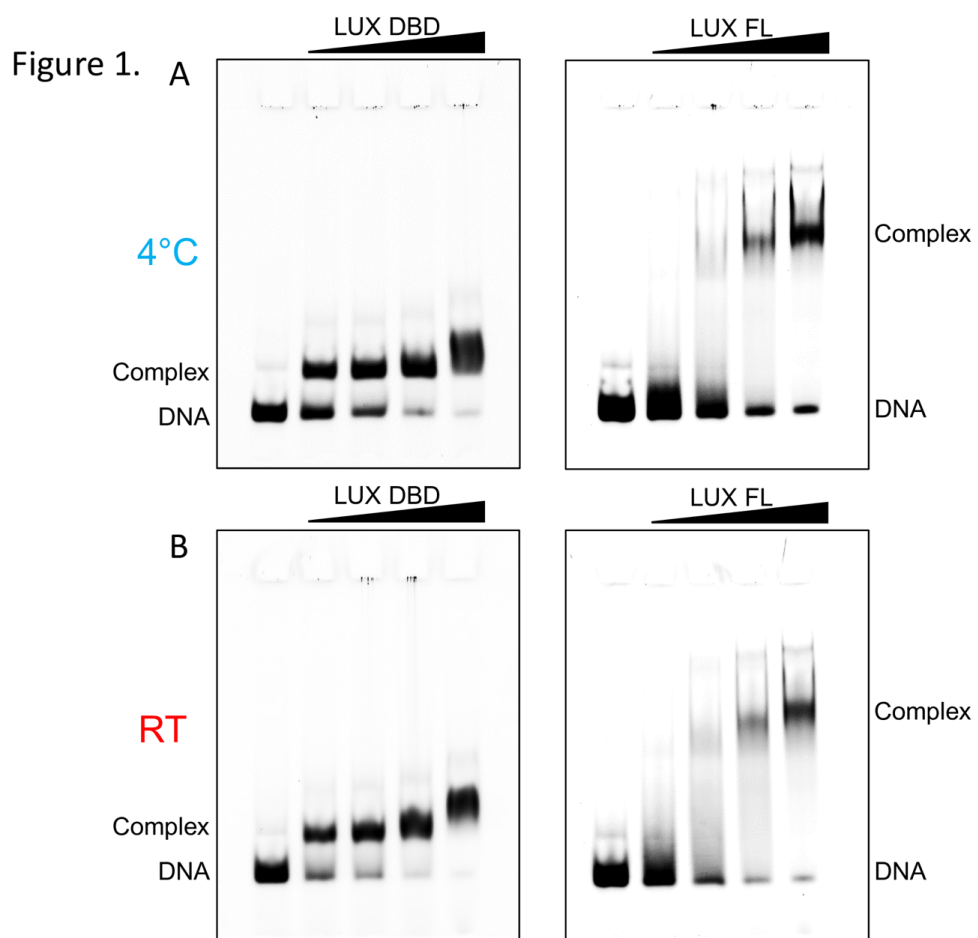
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Figure Captions

Figure 1. Polyacrylamide EMSA of LUX DNA binding domain (DBD) and LUX full length (FL) protein. **A.** Left, EMSA run at 4 °C with increasing concentrations of LUX DBD from 0 to 1 μM. Right, EMSA run at 4 °C with increasing concentrations of LUX FL from 0 to 1 μM nM. **B.** Reactions as per A run at room temperature (RT). No temperature responsiveness in DNA binding is detected between the two experiments for LUX DBD and LUX FL. Free DNA and protein bound DNA are indicated.

Figure 2. Agarose EMSA of EC performed at two different temperatures. Free DNA, LUX full length and the reconstituted EC (lanes 4-6) at increasing concentrations were run on a 2 % agarose gel. At 4 °C a band corresponding to EC-DNA is visible, whereas at room temperature (RT) this band no longer appears. All reactions were from the same master mix, equally divided and incubated at the gel running temperature for 1 h. LUX-DNA appears as a smear due to the agarose gel which allows diffusion of the small complex. EC-DNA appears as a distinct band.

EMSAs of temperature dependent transcription factor complexes



EMSAs of temperature dependent transcription factor complexes

Figure 2.

