

SUPPLEMENTAL FIGURE LEGENDS

Figure 1. Results of SAAB assays performed with bHLH048-V5 or a mixture of LD and bHLH048-V5.

Western blot using T7-Ab against the LD, demonstrating that complexes of LD and bHLH048-V5 form under SAAB assay conditions. 500ng of LD and 2ug of bHLH048-V5 were pre-incubated, then incubated with magnetic beads bound to V5-Ab. Beads were pelleted and the supernatant was collected (Natant). Four sequential washes with binding buffer were performed to remove unbound LD (Washes 1-4). Finally, SDS-PAGE loading buffer was added to the beads to elute bound proteins (Bead Eluate). Collected supernatants were separated on an SDS-PAGE polyacrylamide gel and blotted to nitrocellulose. Incubation with T7-Ab, which recognized the T7 tag on LD, detected a strong signal in the Natant lane, corresponding to unbound LD. Progressive washes recovered diminishing amounts of LD, demonstrating that unbound LD was effectively removed. LD was eluted from the beads (Bead Eluate lane), confirming that LD was complexed with bHLH048-V5.

Figure 2. Alignment of unique oligonucleotide sequences identified in the SAAB assay. **(A)** Sequences recovered with bHLH048-V5 contained a G-box motif, although efficiency of this DNA-binding was very low. The palindromic G-box motifs have dyad symmetry, indicating that bHLH048-V5 likely binds as a homodimer (1,2). **(B,C)** Two discrete populations of sequences were recovered in SAAB reactions containing LD and bHLH048-V5. **(B)** Oligonucleotides containing G-box motifs with dyad symmetry likely corresponded to binding by bHLH048-V5 homodimers, as seen in **(A)**. **(C)** At a lower frequency (6 of 16 unique oligonucleotides), the LBD motif was also recovered, suggesting that when complexed to bHLH048-V5, the LD is able to recognize the LBD motif. The low complexity of the sequences indicates that this binding is at a greatly reduced efficiency relative to LD alone, consistent with EMSA results.

SUPPLEMENTAL MATERIALS & METHODS

Recombinant bHLH048-V5 Protein

bHLH048 coding sequence was amplified (see 'Supplemental Material – Oligonucleotide Sequences' for primer sequences), cloned into pET101/D/TOPO (Invitrogen, Carlsbad, CA) and sequenced to confirm integrity. Plasmids were transformed into *E. coli* BL21(DE3) cells for protein overexpression according to manufacturer's protocols (Stratagene, La Jolla, CA). Bacteria were disrupted by sonication and His-tagged proteins were purified from soluble fractions using a Ni²⁺ column (Novagen, Madison, WI). Eluted proteins were desalted through a Sepharose column (Amersham Biosciences Corp, Piscataway, NJ) in 1x EMSA buffer (25 mM Tris HCl pH 8.8, 50 mM KCl, 1 mM DTT, 2 mM EDTA, 10 mM MgCl₂, 20% glycerol, 0.5% NP-40). Desalted proteins were quantified on a Coomassie Blue-stained denaturing gel compared to a bovine serum albumin (BSA) standard (EMD, San Diego, CA).

SAAB Assays

SAAB assays were performed essentially as described in the main text, with minor modifications. 500ng of LD or 2ug of bHLH048-V5 were incubated with magnetic beads bound to either T7-Ab (Novagen, Madison, WI) or V5-Ab (Invitrogen, Carlsbad, CA), respectively. To assay DNA-binding properties of LD-bHLH048-V5 complexes, 500ng of LD and 2ug of bHLH048-V5 were pre-incubated for 15 minutes at room temperature before addition to magnetic beads bound to V5-Ab. Washes, magnetic bead aliquots and PCR reactions were performed as previous.

Western Blot

Protein fractions corresponding to Natant, Washes 1-4 and Bead Eluate were collected, separated on a 12% SDS-PAGE polyacrylamide gel, followed by transfer to Immobilon-P nitrocellulose membrane (Millipore, Bedford, MA) at 40v for 3h. Mouse monoclonal T7 1^o Ab (Novagen, Madison, WI) was used at a 1:10,000 dilution, and Goat anti-Mouse IgG (H+L) Phosphatase-Labelled 2^o Ab (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was used at a 1:5,000 dilution. NBT and BCIP (Roche Diagnostics, Indianapolis,

IN) were used as substrate to detect the 2^o Ab, and the color reaction was stopped with a solution of 10mM Tris-HCl and 1mM EDTA.

Oligonucleotide Alignments

Oligonucleotide sequences were aligned using the publicly available Multalin software (<http://www-archbac.u-psud.fr/genomics/multalin.html>; (3)).

SUPPLEMENTAL REFERENCES

1. Pabo, C.O. and Sauer, R.T. (1984) Protein-DNA recognition. *Annu. Rev. Biochem.*, **53**, 293-321.
2. Kumar, V. and Chambon, P. (1988) The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. *Cell*, **55**, 145-156.
3. Corpet, F. (1988) Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Res.*, **16**, 10881-10890.