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# Heterologous expression of the human polybromo-1 protein in the methylotrophic yeast *Pichia pastoris*

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ABSTRACT

The human polybromo-1 protein (BAF180) is a known driver mutation in clear cell renal cell carcinoma, where it is mutated in approximately 40% of cases. BAF180 is the chromatin-targeting subunit of the PBAF complex. BAF180 has six bromodomains, two BAH domains, and one HMG box. Bromodomains are known to recognize acetylated-lysines on histones and play a role in nucleosome recognition. BAH domains are required for ubiquitination of PCNA, a key regulator of DNA damage. The putative HMG box, if functional, may be involved in DNA-binding. While the binding specificities of individual bromodomains have been studied by our lab and others, the results have failed to reach a consensus. The acetyl-histone binding features of the full-length protein is unknown and is the motivation for this work. The hypothetical HMG and BAH domains have not been studied and the actual function of these regions is currently unknown. Thus, the precise interactions of this large and complex protein are not well-studied. Advances in understanding this large protein have been hindered by the inability to express and purify recombinant full-length BAF180 protein. Currently, only phenomenological studies using BAF180 expressed in mammalian cells have been conducted. Here, we report the successful expression, purification of full-length biologically active BAF180 protein using the GAP promoter in the heterologous host *Pichia pastoris*. The ability to express full-length and mutated BAF180 will allow for biophysical binding studies. Knowledge of the binding interactions is critical for us to understand the role of BAF180 in cancer development and its progression.

## 1. Introduction

Since Varela et al. reported that *PBRM1* is mutated in 41% of clear cell renal cell carcinoma (ccRCC) cases [1], the human polybromo-1 protein (gene: *PBRM1*; protein: BAF180) has garnered the attention of medical researchers. *PBRM1* is the second most mutated gene in ccRCC (the first being *VHL*) and a known driver mutation of that cancer [2,3]. It has been found to be mutated (at lower frequencies) in other cancers, such as cholangiocarcinomas [4], metastasizing pleomorphic adenomas [5], esophageal squamous cell carcinoma [6], and breast cancer [7,8]. At last count, COSMIC (Catalogue of somatic mutations in cancer) has more than 1100 unique *PBRM1* mutations in its database [9]. According to the database, missense mutations are the most common, closely followed by frameshift deletions and nonsense mutations. Many of the nonsense mutations eliminate one or more domains. In extreme cases, the mutated *PBRM1* may only code for 2 or 3 domains [1]. The tumor suppressor properties of BAF180 likely stems from its many roles in DNA repair. BAF180 is critical for proper centromeric cohesion, which is important during recombinatorial repair [10].

BAF180, the chromatin-remodeling subunit of the PBAF complex, is a complicated protein with 1634 amino acids (isoform 2) and 9 domains: 6 bromodomains, 2 BAH (bromo-adjacent homology) domains, and 1 HMG (high mobility group) box (Fig. 1). The BAH domains of BAF180 are found to be critical for proper ubiquitination of PCNA during post-replication repair (PRR) [11]. The HMG box, though not well-studied in this protein, has been suggested to promote non-homologous end-joining (NHEJ) by its interactions with DNA [12–14]. Therefore, mutant BAF180 cancer cells could be compromised with regards to DNA repair in 3 ways: the chromosomes are further apart and have less cohesion, which leads to genomic instability and prevents recombinatorial DNA repair. Mutant BAF180 without BAH cannot ubiquitinate PCNA, which obstructs most of the PRR pathway; deletion of the HMG box likely hinders NHEJ and transcription because BAF180 can no longer bind to DNA.

*Abbreviations:* BRD, Bromodomain; BAH, Bromo-adjacent homology domain; HMG, High mobility group; NHEJ, Non-homologous end-joining; PRR, Post-replication repair; AEX, Anion exchange chromatography; GAP, Glyceraldehyde-3-phosphate dehydrogenase

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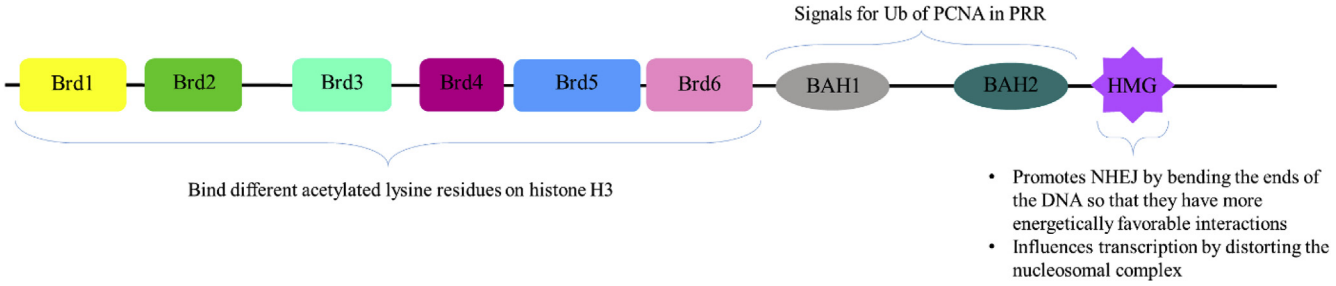


**Fig. 1.** Diagram of the 9 domains of BAF180. Sizes of the domains and distances between are accurate for isoform 2 (GenBank accession no. AAG48933.1). The overall size is 1634 amino acids.

**Table 1**
The primer combinations used for confirming integration of the BAF180 sequence into the genome of Pichia pastoris.

| Fragment: | Domains Covered | Primers |
|---|---|---|
| 1 | BRD1 - BRD3 | Fwd: CCAAGCAGGAAAAGGAGGA<br>Rev: GGTGGCTGAAGAGATCATGC |
| 2 | BRD3 - BRD5 | Fwd: GCACTTCAATATGGCTCA<br>Rev: CTCTTGAATCAGCAAAGTCACATT |
| 3 | BRD3 - BRD6 | Fwd: GCACTTCAATATGGCTCA<br>Rev: TGTGGTATAGCTGAGTGCCG |
| 4 | BRD6-BAH2 | Fwd: CCAAATGTGACTTTGCTGATTC<br>Rev: GGCCCTCCTTCTGAGGAACAATTG |
| 5 | BRD4 - BRD6 | Fwd: AGCATGATCTCTTCAGCCAC<br>Rev: TGTGGTATAGCTGAGTGCCG |
| 6 | BRD6 - BAH1 | Fwd: CCAAATGTGACTTTGCTGATTC<br>Rev: GCGCCTGGGCATAACTTAAAGTATTCCTT |

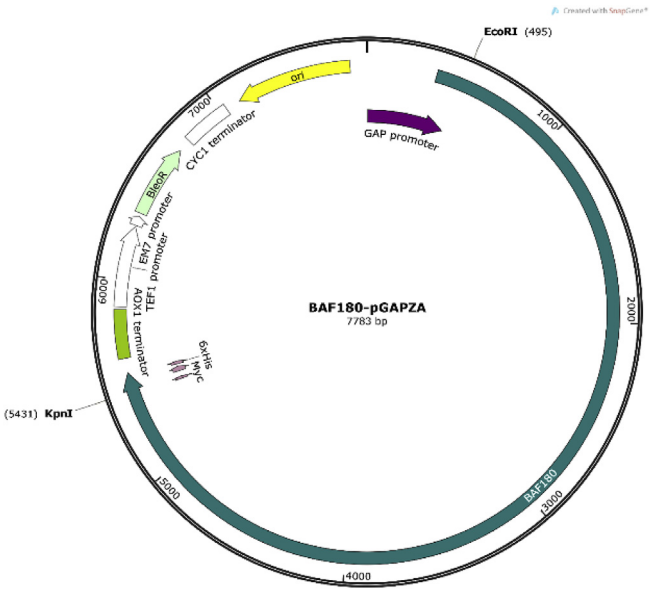


**Fig. 2.** Construct of BAF180-pGAPZA. BAF180 was ligated into pGAPZA vector via EcoRI and KpnI. Prior to transformation, the *plasmid* was linearized with AvrII, located within the GAP promoter.

The precise interactions of BAF180 with the nucleosome are unknown because the recombinant full-length BAF180 protein has not been available for investigations; only phenomenological studies using BAF180 expressed in mammalian cells have been conducted. The ability to study distinct interactions is crucial to increasing our understanding of the role of BAF180 in cancer development and its progression. In studying the exact mechanisms by which BAF180 performs its many roles, we could begin to develop better treatment options for cancer patients. Here, the first successful expression of full-length BAF180 in a heterologous host, using the yeast *Pichia pastoris* is described.

## 2. Materials and methods

### 2.1. Materials

*Pichia pastoris* wild-type strain X33 and pGAPZA vector were purchased from Invitrogen (Carslbad, CA). *E. coli* subcloning strain DH5α was used for propagation of vector and construct. Zeocin (cat. ant-zn-1p) was purchased from Invivogen (San Diego, CA). The BAF180 gene sequence (pBabepuroBAF180) was a gift from Ramon Parson's lab [7]. Primers were purchased from Integrated DNA Technologies (IDTDNA: Coralville, IA). Q5 polymerase (M0491), T4 ligase (M0202), all restriction enzymes, TriDye 1 kb ladder (N3272), Color Prestained Broad Range Protein Marker (P7712) were purchased from New England BioLabs (Ipswich, MA). Tryptone, peptone, yeast extract, and dextrose were purchased from Amresco (VWR: Radnor, PA). Lyticase from *Arthrobacter luteus*, phenol:chloroform:isoamyl alcohol (25:24:1), chloroform:isoamyl alcohol (24:1), sodium acetate, and 100% ethanol were purchased from Sigma Aldrich (St. Louis, MO). Zirconia/silica disruption beads (0.5 mm) were purchased from Research Products International Corp. (Mt. Prospect, IL). DNA purification kits (Zyppy™ Plasmid Miniprep Kit, Zymo Gel Extraction Kit, and Zymo DNA Clean & Concentrator™) were purchased from Zymo Research Corp. (Irvine, CA). 0.45 µm PVDF membrane (cat. 88585), blotting paper (cat. 8860), NuPAGE Tris-Acetate gels 3–8% (cat. EA0375), NuPAGE LDS Sample Buffer 4x (cat. NP0007), NuPAGE Tris-acetate running buffer (LA0041), NuPAGE transfer buffer (cat. NP0006), NuPAGE antioxidant (cat. NP0005), NuPAGE reducing agent (cat. NP0004) and Pierce Fast Western Blot Kit, ECL substrate (cat. 35050), and anti-His-tag antibody (cat. PA1-983B) were purchased from ThermoFisher (Waltham, MA). Anti-*PBRM1* antibody (cat. 12563-1-AP, rabbit polyclonal) was purchased from Proteintech (Rosemont, IL). Western blot transfer was performed using a Hoefer TE22 transfer tank (Holliston, MA). Dialysis membranes were purchased from Spectrum Labs (Rancho Dominguez, California): Spectra/Por 1 (6–8 kD MWCO membrane; part 132660) and Spectra/Por 6 (50 kD MWCO membrane; part 132544). Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-100 membrane (cat. UFC910024, MilliporeSigma Billerica, MA). SDS, glycine, Tris hydrochloride, Tris base, glycerol, guanidine hydrochloride, and Protease Inhibitor Cocktail (cat. 97063–970) were purchased from Amresco (VWR: Radnor, PA). Bio-Scale Mini Macro-Prep High Q (cat. 7324122) strong anion exchange cartridge was purchased from Bio-Rad, Inc. (Hercules, CA). MODified Histone Peptide Array (cat. 13005) and MODified Array Labeling Kit (cat. 13006) were purchased from Active Motif (Carlsbad, CA). The MODified Array Labeling Kit included Blocking Buffer, 10x Wash Buffer, c-myc mouse monoclonal antibody, anti-mouse-HRP conjugated secondary antibody, anti-rabbit-HRP-conjugated secondary antibody, and ECL Reagents A and B.