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Modifying HIC to Affinity Chromatography by Adding HA Tag to pARA-R

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ABE Master Teacher Fellowship Program

AMGEN Biotech Experience

Scientific Discovery for the Classroom

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The projects designed by the 2024–25 ABE Master Teacher Fellows are a compilation of curricula and materials that are aligned with the Amgen Biotech Experience (ABE) and further support teachers and students in their biotechnology education. These projects were created over the course of a 1-year Fellowship in an area of each Fellow's own interest. Each is unique and can be adapted to fit the needs of your individual classroom. Objectives and goals are provided, along with expected outcomes. Projects can be used in conjunction with your current ABE curriculum or as an extension.

As a condition of the Fellowship, these classroom resources may be downloaded and used by other teachers for free. The projects are generally not edited or revised by the ABE Program Office for content, clarity, or language except to ensure safety protocols have been clearly included where appropriate.

We are grateful to the ABE Master Teacher Fellows for sharing their work with the ABE community. If you have questions about any of the project components, please reach out to us at ABEInfo@edc.org, and we will be happy to connect you with the author and provide any assistance needed.

AMGEN Biotech Experience

Scientific Discovery for the Classroom

Greater Los Angeles

Modifying HIC to Affinity Chromatography by Adding HA Tag to pARA-R

TIME FRAME: 3 class periods for thought experiment, 6 class periods to complete the wet labs once primers are designed, ordered and received.

SUGGESTED AGE RANGE: High School Seniors

SUGGESTED COURSE OR CONTENT AREA: Biotechnology

CONNECTIONS DESCRIPTIONS:

- Project or problem-based learning
- Professional skills in STEM/Profiles in STEM

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PROGRAM SITE: ABE Greater Los Angeles

Overview

Commercial affinity columns are available through numerous vendors with multiple affinity tags available. Scaling up the ABE protocol to produce more purified RFP is the next step in the commercial process of going from proof-of-concept prototype to commercial-scale production. The first step in this approach would be to reengineer the pARA-R plasmid to include a hemagglutinin tag after the RFP sequence. In this unit, students will use online bioinformatics tools to include a HA-tag for purification and restrictions enzyme sequences to incorporate the HA-tagged RFP to pARA-R. This lesson is designed to be a thought experiment in how you could incorporate an HA-tag into the existing pKAN/pARA-R rationale utilized in the ABE protocol.

Learning Goals

- To understand sequence analysis utilizing various online tools and databases, including BLAST and GenBank
- To develop a rationale to add the HA tag sequence to existing pARA-R plasmid in the correct location and orientation

Assessed Outcome

Submission of a successful rationale to include the HA-tag using engineered PCR primers that include both the HA-tag and the appropriate restriction sites to clone the HA-tag into the pARA-R plasmid.

Key Vocabulary

- Plasmid
- Restriction Endonuclease
- Hemagglutinin
- Affinity Chromatography
- Bioinformatics

Materials and LabXchange Pathway(s)

- Nucleotide Base Pairing
- Sorting Out DNA
- Restriction Fragment Analysis of the TAS2R38 Gene
- Using PCR to Amplify the TAS2R38 Gene

Teacher Preparation:

1. This lesson is designed to be a thought experiment in how you could incorporate an HA-tag into the existing pKAN/pARA-R rationale utilized in the ABE protocol. This approach would follow the rationale below.
2. Gather all sequences (pKAN-R, BamHI, HindIII, rfp, HA-tag): The purpose of this is to add an HA-tag to the rfp portion of the pKAN-R plasmid. We will use PCR with primers that include BamHI, HindIII, HA-tag and RFP sequence).
3. Primer design: Choice to design HA tag on N-term end or C-term end of RFP gene. If N-term, Fwd is BamHI-HA-tag-RFP gene start, Rvs is HindIII-RFP gene end. If C-term Fwd is HindIII-HA-tag-RFP end, Rvs is BamHI-RFP gene start, as below:
    a. N-term:
        i. Fwd **5'-GGA-TTC-TAC-CCA-TAC-GAT-GTT-CCA-GAT-TAC-GCT-AAA-GAG-TTC-ATG-3'**
        ii. Rvs **5'-AAG-CCT-AAT-GAA-CAT-GTC-GAG-CAG-GTA-CGG-CAT-GTC-CTT-GTC-CAC-3'**
    b. C-term:
        i. Fwd **5'-AAG-CCT-TAC-CCA-TAC-GAT-GTT-CCA-GAT-TAC-GCT-AAT-GAA-CAT-GTC-3'**
        ii. Rvs **5'-GGA-TTC-ATG-GTG-AGC-AAG-GGC-GAG-GAG-GTC-ATC-AAA-GAG-TTC-ATG-3'**
    c. PCR conditions: PCR conditions:
        i. Initial Denaturation: 98°C for 5 minutes
        ii. Cycling Denaturation: 98°C for 30 seconds
        iii. Annealing: Primer™ = 67°C and 70°C, Annealing should be 5°C lower than Tm, 62°C for 45 seconds
        iv. Extension: 72°C for 60 seconds, 30 cycles
        v. Final Extension: 72°C for 15 minutes

4. Predict Length, purify, and gel verify PCR product (pKAN-R rfp = 702 bp, BamHI/HindIII = 6 bp, HAtag = 27 bp) = 735 bp total on gel.
5. Digest pARA with BamHI/HindIII,purify and gel verify. pARA plasmid - BamHI/HindIII segment = 4058 bp
6. Ligate BamHI/HindIII digested pARA and rfpHA amplicons. Digested pARA and rfpHA amplicons from PCR above both have BamHI/HindIII ends.
7. Gel Verify pARA-RHA plasmid = 5302 bp (pARA-R) + 27 bp (HA-tag) = 5329 bp

Lab Safety Considerations: PCR thermocycler surfaces may be hot.

Sequence of Activities

Activity Description	***Time***	***Materials***
1. Gather all sequences (pKAN-R, BamHI, HindIII, rfp,HA-tag)	90 min	BamHI and HindIII RFP (AY678268.1) HA tag
2. Design primers (N-term vs C-term tag) and PCR conditions: ● **Initial Denaturation:** 98°C for 5 minutes ● **Cycling Denaturation:** 98°C for 30 seconds ● **Annealing:** Primer ™ = 67°C and 70°C, Annealing should be 5°C lower than Tm, 62°C for 45 seconds ● **Extension:** 72°C for 60 seconds, 30 cycles ● **Final Extension:** 72°C for 15 minutes	90 min	PCR Primer Stats
3. Predict Length, purify, and gel verify PCR product (pKAN-R rfp = 702bp, BamHI/HindIII = 6pb, HAtag = 27bp)	90 min	Run 1% TAE gel with 1kb ladder to verify PCR product length = 735bp
4. Digest pARA with BamHI/HindIII = 4058bp and purify.	90 min	Run 1% TAE gel with 1kb ladder to verify successful BamHI/HindIII digestion = 4058bp. Excise from gel and purify cut product.
5. Ligate BamHI/HindIII digested pARA and rfpHA amplicons.	90 min	DNA ligase reaction with digested pARA and rfpHA amplicons (both with BamHI/HindIII ends)
6. Gel Verify pARA-RHA plasmid = 5302bp (pARA-R) + 27bp (HA-tag) = 5329bp	90 min	Run 1% TAE gel with 1kb ladder to verify successful ligation product = 5329bp