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Coupling an amino-functionalized oligo to Sepharose beads for tRNA purification

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COMMENTS 0

ABSTRACT

For this protocol we will be coupling a DNA oligo with a 3' amino-modification to N-Hydroxysuccinimidyl-Sepharose® 4 Fast Flow beads (H8280, SIGMA) for use in the purification of specific tRNA isoacceptors. Although this is a general protocol, it can typically be used for coupling any 3' amino-modified DNA oligo to your Sepharose beads.

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Basic Information Regarding tRNA and DNA oligo of Interest

1 Depending on your tRNA of interest, you will need to order one of the following DNA oligos from IDT or a similar company. When you order the oligos from IDT or a similar company, you should order each oligo at an amount of 5 µmoles and have it purified using a standard desalting procedure. If we have not designed an oligo for your tRNA isoacceptor of interest, you will have to design an oligo to purify your tRNA before proceeding.

A	B	C	D	E	F	G
E. coli						
tRNA-aa, anticodon	Oligo Name	Sequence	Modification Type	ε260 (M-1 cm-1)	Tm (50 nM NaCl)	
Gln, UUG	Ec-Gln-UUG	5'-CAAAAACCGGTGCCTTACCGCTTGGCG-3'/3AmMC	AMINE	248300	66.0	
Val, TAC	Ec-Val-TAC	5'-GTAAGGGAGGTGCTCTCCCAGCTGAGC-3'/3AmMC	AMINE	256800		

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A	B	C	D	E	F	G
S. cerevisiae						
tRNA-aa, anticodon	Oligo Name	Sequence	Modification Type	ε260 (M-1 cm-1)	Tm (50 nM NaCl)	
Arg, 1CU	Yeast-Arg-1CU-amine	5'-AGAAGTCAGACGCGTTGCCATTACG-3'/3AmMO/	AMINE	247100	61.2	
Trp, CCA	Sc-Trp-CCA-amine	5'-TTTGGAGTCGAAAGCTCTACCATT-3'/3AmMO/	AMINE	228700	56.3	
Arg, ICG	Sc-Arg-ICG	5'-CGTAGCCAGACGCCGTGACCATTGGG-3'/3AmMO/	AMINE	246800	66.8	
Pro, AGG (IGG ?)	Sc-Pro-AGG	5'-CCTAAGCGAGAATCATACCTCTAGAC-3'/3AmMO/	AMINE	255100	55.0	
Gly, CCC	Sc-Gly-CCC	5'-GGGAAGCATGAATTCTAACCACAGAAC-3'/3AmM(	AMINE	275300	57.9	
Pro, UGG	Sc-Pro-UGG	5'-ACCCAAAGCGAGAATCATACCACT-3'/3AmMO/	AMINE	239900	58.3	

Table of Amino-Modified DNA oligos that we have used for our *in vitro* translation work as of 3/26/2021.

2 Once you have chosen an appropriate oligo to purify your tRNA of interest, record the information related to the oligo, the tRNA species that you are attempting to purify, the species that you will be purifying the tRNA from, and the anti-codon of your tRNA below:

Date of Bead Preparation:

tRNA Species:

tRNA Source Species:

tRNA Anti-Codon:

DNA Oligo Sequence:

Buffer Preparation

3 Before beginning the protocol, you must prepare the following buffers. The preparation of each buffer is described in detail in the "Buffer Recipes" document in the General Protocols directory. To retain maximum binding capacity of the pre-activated Sepharose medium prior to the coupling step, use cold (0°C to 4°C) solutions. The time interval for all washing steps must be minimized. The coupling pH depends on the ligand to be coupled, normally pH 6 to 9. Be aware that the NHS-ester groups rapidly hydrolyze at higher pH.

A	B
Buffer	Components
Coupling Buffer	0.2 M NaHCO3, 0.5 M NaCl (pH 8.3)
Beads-wash solution	1 mM HCl (ice-cold)
Quench (Q) buffer	0.1 M Tris-HCl (pH 8.5)
Wash buffer 2	0.1 M Na-acetate, 0.5 M NaCl (pH 5.0)

Brief description of the buffers used in this protocol and their composition.

Coupling Procedure

4 The Coupling Procedure can roughly be broken into three steps:

1). Bead Washing - the Sepharose medium that you use for this procedure comes stored in 100% isopropanol and must be washed thoroughly before the coupling procedure can be done.
2). DNA Oligo Preparation - the DNA oligo that you use for this procedure will come as a lyophilized pellet that must be dissolved in your Coupling Buffer before use. It is important to accurately determine the concentration of your oligo and how much you will be adding to the Sepharose medium so that you can determine how much of the oligo binds to the medium after the procedure is complete.
3). Coupling of DNA Oligo to Sepharose medium - the coupling of the DNA oligo to the Sepharose medium is itself quite simple but does take some time so make sure to have a couple of days to complete the procedure.

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4.1 Amine Oligo Preparation:

1. Begin by briefly spinning the tube that contains the amino-functionalized oligo to ensure that the lyophilized flake of oligo is gathered at the bottom of the tube.
2. Then dissolve the oligo by adding 1 mL of Coupling Buffer to the tube and mix briefly by vortexing.
3. Then determine the concentration of the oligo (using Coupling Buffer as a blank):

OligoConcentrationCalculator.xlsx

4.2 Amine Oligo Bead Preparation:

- Then prepare the N-Hydroxysuccinimidyl-Sepharose 4 Fast Flow (H8280, Sigma) beads by pipetting 20 mL of beads from the storage container into a 50 mL conical tube. Note that we really only need 15 mL of packed beads, but since the volume of the bead solution will compress into a smaller packed volume, we are going to use 20 mL of bead suspension at first.
- Spin these beads down for 5 minutes at 3,000 rcf and then dispose of the supernatant, making sure to not disturb the pelleted beads.
- Then wash at least 15 times with 15 mL of ice-cold Beads Wash Solution by spinning down with the centrifuge as described above. Pipette off the supernatant after each wash, making sure not to disturb the pelleted beads.
- Then wash the beads with 15 mL of Coupling Buffer 5 times. To wash the beads, once again spin down the beads for 5 minutes at 3,000 rcf and then remove the supernatant without disturbing the pelleted beads.
- After the fifth wash with Coupling Buffer, record the volume of packed beads that you have in your 50 mL conical tube:

- Beads Volume (mL):

- Supernatant Volume Removed (mL):

4.3 Amine-Oligo Coupling:

- Next we want to couple the oligo to the beads. To do this, we want to ideally add at least 500 nmol of the oligo to the beads. Using the total nmols (as calculated above), determine the volume of the resuspended oligo that you need to add to the beads so as to have at least 500 nmol of oligo mixed with the beads.
- Add amino-functionalized oligo to the beads and mix gently by vortexing. Incubate for 2 hours at room temperature and then overnight (12 - 16 hours) at 4 C.

- Volume of oligo added to beads:

- Total nmols of oligo added to beads:

Blocking and Washing the Beads

5 Now that we have coupled the amino-functionalized oligo to the beads, we want to wash off any excess / unbound oligo from the beads and block any reactive groups on the beads that did not get coupled to an oligo during the overnight incubation.

5.1 Attach your 50 mL conical tube containing the beads and oligo to a vacuum filtration apparatus. Then perform the following wash steps, making sure to minimize the time between each wash step.

- NOTE: Store all of the following washes at 4 C for analysis. All of the following washes are carried out according to the manufacturers recommended protocol. DO NOT DEVIATE.

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5.2

A	B
Wash Step	Instructions
Wash #1	Vacuum down supernatant as much as possible. This will contain oligo that did no
Wash #2	Wash with 30 mL of Quench Buffer
Wash #3	Wash with 30 mL of Quench Buffer
Wash #4	Wash with 30 mL of Quench Buffer
Wash #5	Resuspend beads in 30 mL of Quench Buffer and incubate at room temperature fo
Wash #6	Wash with 30 mL of Quench Buffer
Wash #7	Wash with 30 mL of Quench Buffer
Wash #8	Wash with 30 mL of Quench Buffer
Wash #9	Wash twice with 15 mL of Wash Buffer 2
Wash #10	Wash twice with 15 mL of Wash Buffer 2
Wash #11	Wash twice with 15 mL of Quench Buffer
Wash #12	Wash twice with 15 mL of Quench Buffer
Wash #13	Wash twice with 15 mL of Wash Buffer 2
Wash #14	Wash twice with 15 mL of Wash Buffer 2
Wash #15	Wash twice with 15 mL of Quench Buffer
Wash #16	Wash twice with 15 mL of Quench Buffer
Wash #17	Wash twice with 15 mL of Wash Buffer 2
Wash #18	Add 15 mL of Wash Buffer 2 to the beads and incubate overnight at 4 C. Always ca
Wash #19	Wash twice with 15 mL of Quench Buffer
Wash #20 - X	Wash twice with 15 mL of Quench Buffer until A260 value is minimal

5.3 After all of the above washes have been performed, measure the A260 values using the respective buffers as blanks and record the values in the below excel document:

Wash_Concentration_Calculator.xlsx

Estimate Amount of Oligo Attached to the Beads

6 For this step we are going to estimate the number of nmols of amino-functionalized oligo still bound to your beads by precipitating the unbound oligo from Washes #1 - #4 and measuring the A260 value of each wash. We will use these washes as they contain most of the unbound oligo from the original incubation step. We will then subtract the number of nmols contained in Washes #1 - #4 from the original number of nmols that we incubated with the beads to determine how much of the oligo remained on the beads following the washes.

6.1 To ethanol precipitate the oligo from Washes #1 - #4, do the following:

- Mix 500 uL of flow through from Washes #1, #2, #3 and #4 (four individual samples) with 50 uL of 20% KAc (pH 5.5) and 1375 uL of 200 proof ethanol.
- Incubate the above samples at -80 C for 1 hour to precipitate the DNA.
- Spin the samples at 13,000 rpm for 30 minutes to pellet the precipitated DNA.
- Remove all supernatant from each sample and dry the pellet at room temperature. You can either let the pellet air dry or you can use a vacuum chamber to dry the pellet. The pellet should be visible at this point.

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- Dissolve the dried pellet in 500 uL of 10 mM Tris-HCl (pH = 7.6).

- Measure the oligo-amine concentration and record your results in the following table:

Determination of oligo-bead concentration.xlsx

6.2 If the amount of oligo bound to the beads is satisfactory, then this concludes the protocol for the Amine-Oligo bead preparation. You can now proceed to the tRNA isolation portion of the protocol. All excel sheets are included in the following excel document:

Calculations for Amine Bead Preparation.xlsx

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