**DNA/RNA Radiolabeling Protocol**

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**The Center for Genome Editing and Recording**

Meredith Triplet

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**ATTACHMENTS**

Radiolabeling_CasX_DNA_substrates.pdf

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**PROTOCOL CITATION**

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**KEYWORDS**

Radiolabeling, DNA, RNA, CasX, TS, NTS

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**GUIDELINES**

**CasX TS/NTS with non-hydrolysable spacers:**

**TS:**

5'-CGCTAGCTACG

MW: 15664.2 g/mol
Concentration: X μM
Safety warnings:

* = phosphothioate, **bold letters** = PAM, *italic letters* = spacer

For 10 pmol of TS: X µl of stock

NTS:


MW: 15749.3 g/mol
Concentration: X µM

* = phosphothioate, **bold letters** = PAM, *italic letters* = spacer

For 10 pmol of NTS: X µl of substrate

Labelling reaction setup:

*TS:*

XX µl DNA or RNA (10 pmoles)
2.5 µl 10x PNK buffer
0.5 µl PNK enzyme
1.5 µl P32-gamma-ATP
XX mL dH2O (DEPC for labeling RNA) to 25 µl

*NTS:*

XX µl DNA or RNA (10 pmoles)
2.5 µl 10x PNK buffer
0.5 µl PNK enzyme
1.5 µl P32-gamma-ATP
XX mL dH2O (DEPC for labeling RNA) to 25 µl

Materials

<table>
<thead>
<tr>
<th>NAME</th>
<th>CATALOG #</th>
<th>VENDOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 Polynucleotide Kinase (3' phosphatase minus) - 200 units</td>
<td>M0236S</td>
<td>New England Biolabs</td>
</tr>
<tr>
<td>10X T4 PNK Reaction Buffer</td>
<td></td>
<td>New England Biolabs</td>
</tr>
<tr>
<td>ATP [γ-32P]- 3000Ci/mmol 10mCi/ml Lead 100 µCi (P32-gamma-ATP)</td>
<td>NEG002A100UC</td>
<td>Perkin Elmer</td>
</tr>
<tr>
<td>HiTrap Desalting columns with Sephadex G-25 resin</td>
<td>29048684</td>
<td>Ge Life Sciences</td>
</tr>
</tbody>
</table>

Safety warnings:

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

1. Set up labeling reaction:

<table>
<thead>
<tr>
<th>µl</th>
<th>DNA or RNA (10 pmoles)</th>
<th>10x PNK buffer</th>
<th>PNK enzyme</th>
<th>P32-gamma-ATP</th>
<th>dH2O (DEPC for labeling RNA) to 25 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td></td>
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<tr>
<td>2.5</td>
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<tr>
<td>0.5</td>
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</tr>
<tr>
<td>1.5</td>
<td></td>
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</tbody>
</table>


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Mix the DNA, buffer, enzyme, and H₂O at the bench, and then add the DNA/enzyme mixture to ATP-filled tubes in a radioactive use area.

2. Incubate at \(37 \, ^\circ\mathrm{C}\) for \(00:30:00\).

3. Heat inactivate the PNK at \(65 \, ^\circ\mathrm{C}\) for \(00:20:00\).

4. Prepare G25 columns (from GE, green box): vortex thoroughly, twist cap \(\frac{1}{4}\) turn, snap off bottom, spin for \(00:01:00\) at \(3000 \, \text{rpm}\) to get rid of liquid.

5. Add \(50 \, \mu\text{l}\) H₂O to a labeled eppendorf tube, place G25 column in it.

6. Add \(25 \, \mu\text{l}\) H₂O to each labeling reaction after heat inactivation is done.

7. Apply entire reaction (now 50 \(\mu\text{l}\) total) to G25 column resin.

8. Spin for \(00:02:00\) at \(3000 \, \text{rpm}\).

9. Since 50 \(\mu\text{l}\) H₂O were in bottom of tube and you add your 50 \(\mu\text{l}\) reaction, you should end with up to 100 \(\mu\text{l}\) of \(100 \, \text{Nanomolar (nM)}\) labeled DNA/RNA.

10. Measure \(1 \, \mu\text{l}\) of each reaction with the black rad counter on shelf to get cpm readings.